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Kinetics Modelling of Tributyltin Toxicity on The Growth of *Bacillus subtilis*

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ABSTRACT

Since the 1970s, tributyltin (TBT) has been used as a biocide in antifouling paints to prevent the growth of bacteria. In 2003, the Marine Environmental Protection Committee (MEPC) proposed that TBT be banned internationally due to the negative environmental impacts of the substance. However, even though BTs are banned, they may be found in large quantities in seawater, bottom sediments, and the biota, all of which are contaminated with them. To prevent the adhesion of fouling organisms to the surface of ships and boats, tributyltin (TBT) has been widely employed as an antifouling agent in marine paints for many years. Tributyltin has been discovered to be very persistent, particularly in sediment, and to be extremely harmful to species other than those targeted. *Bacillus subtilis* growth was intensely inhibited by tributyltin (TBT). As the TBT concentration increases, the overall specific growth rate was inhibited. The growth rates obtained were then modelled according to the modified Han-Levenspiel, Amor, Wang, Liu, Shukor and modified Andrews. Among the five models, the Andrew and Amor models show poor fittings. Results of the statistical analysis showed that the Shukor model was the best model based on the lowest values for RMSE and AICc, highest adjusted correlation coefficient (AdR^2) and values of AF and BF closest to unity. The parameters obtained from the Shukor model were C_{crit} 742.32 nM (95%, C.I., 303.35 to 1181.29), μ_{max} 1.20 h^{-1} (95% C.I., 1.08 to 1.319) and m 0.507 (95% C.I., 0.308 to 0.832). The findings of this study can be utilized for further bioremediation works.

INTRODUCTION

Hazardous chemicals are found to have an inhibitory impact on bacterial development. Due to the presence of heavy metals in the environment, biodegradation may be hindered, which in turn can impede the bioremediation process. The reason for this is because, in contrast to a number of other inhibitors, heavy metal ions cannot be destroyed and, if accumulated by microbes to a hazardous level, cause a decrease in the rate of development in the microorganism under consideration. There may be substantial variations in the sensitivity to metals of microorganisms depending on the microorganism, even across different strains of the same species, and even between different activities of the same microbial species. When soils with similar physical and chemical properties were compared to one another, it was discovered that the sensitivity of the microbiome that is responsible for acetate mineralization in soils with no history of exposure to elevated metal concentrations differed by orders of magnitude between soils with varying physical and chemical characteristics [1].

A large number of studies have shown that the addition of trace quantities of heavy metals to the surroundings of microbial cells may promote the development of the bacteria. However, the concentration at which increased microbial activity is seen leads in a substantial reduction in growth rate as well as an increase in the lag time for the occurrence of the event (due to the higher lag time). A slew of studies has shown that heavy metals are harmful to microorganisms, especially sulfate-reducing bacteria, and that these effects are widespread [1–10].

The start of enhanced metabolic activity is delayed as a consequence of elevated heavy metal concentrations, and the rate of oxygen mass transfer is reduced as a result of elevated heavy metal concentrations, both of which are detrimental. Since the discovery that bacteria, like all other forms of life, are highly

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susceptible to heavy metal exposure, researchers have been working on this problem for many decades. Chemistry was employed in some of the first efforts to control microbes, including the use of copper chloride in plants as a fungicide and mercury salts in the treatment of some infectious illnesses, among other things. In the treatment of fungal infections, copper chloride was employed, and mercury salts were used in the detection of some infectious illnesses, to name only a few of examples. A number of these metals are components or cofactors of enzyme systems. A number of variables, including the organism, the metal, and the chemical and physical composition of the metal, are known to have an effect on the average concentrations at which the activities take place in a particular environment. Heavy metals are very weakly interacted with by the vast majority of organisms, and such reactions occur at lower concentrations [11–2] .

The bioamplification of butyltins in the marine environment has been observed by scientists. Animal and human research have shown that organotin compounds are harmful. In BTs' testing, toxicity on immune system cells (as shown by thymus shrinkage, decreased spleen weight, and cytotoxicity to bone marrow and red blood cells) was discovered, particularly in tests performed on cells and tissues [21–23]. Tributyltin is extremely persistent in sediment, and it's harmful to all species, including those that are not the target of the toxin [24]. High concentrations of the chemical may persist in fresh water and sediments for up to 30 years. Although the international maritime organization (IMO) banned the use of tbt as a marine biocide in 2008, its residue has been detected in numerous locations around the world, including South Africa, Portugal, Malaysia's strait of Johore, and Australia [25–27].

Organotin used in industries have also been detected in terrestrial environment [28]. Bacteria have been well-known to degrade organic pollutants [29]. Martin et al. [30] studied the interactions between *Bacillus stearothermophilus* and *bacillus subtilis* with different TBT concentrations (0, 100, 200, 300, 400 and 500 nM). The growth of both bacteria was severely inhibited as the TBT concentration increases. The effect of TBT on the growth rate of *B. stearothermophilus* been previously modelled using several toxicant inhibition kinetics models such as modified Han-Levenspiel, Amor, Wang, Liu, Shukor and modified Andrews with Wang as the best model [8]. The aim of this work is to model the effect of tributyltin on the growth rate of *Bacillus subtilis* through the use of the same inhibition models.

MATERIALS AND METHODS

Data source

Data from Table 1 from Martins et al [30] was processed using the software Webplotdigitizer 2.5 [31] which digitizes the scanned figure and has been utilized by many researchers and acknowledged for its reliability [32,33].

Effect of metal on growth rate of on SDS

Six models for tributyltin inhibition to the growth rate of this bacterium is available (**Table 1**).

Table 1. Growth inhibition models.

Models	Equation	Authors
Modified Han-Levenspiel	$r = u_{max}\left(1 - \frac{C}{C_{crit}}\right)^m$	[34]
Wang	$r = \frac{u_{max}}{1 + \left(\frac{C}{K_C}\right)^m}$	[35]
Liu	$r = \frac{u_{max}K_C}{K_C + C}$	[36]
Modified Andrews	$r = \frac{u_{max}C}{K_s + C + \left(\frac{C^2}{K_i}\right)}$	[37]
Shukor	$r = v_{max}\left(1 - \left(\frac{C}{S_m}\right)^n\right)$	[38]
Amor	$r = \frac{u_{max}C}{C + \left(\frac{C^2}{K_i}\right)}$	[39]

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) were utilized as before [40].

RESULTS AND DISCUSSION

The growth rates at various concentrations of TBT was then modelled using the available metal inhibition models. Out of the five models, only Wang, modified Han-Levenspiel and the Liu models were able to fit the curve, whilst the modified Andrews and Amor models were unable to fit the curves (**Figs. 1** to **5**). Both the Wang and modified Han-Levenspiel models show acceptable fitting while the Liu model shows poor fitting. Results of the statistical analysis showed that the Wang model was the best model 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 of the effect of increasing concentrations of tributyltin to the maximum specific growth rate of *Bacillus subtilis* as fitted to various secondary models.

Model	*p*	RMSE	adr^2	AF	BF	AICc
Wang	3	0.04	0.94	1.05	1.00	-14.40
Han-Levenspiel	3	0.06	0.85	1.04	1.00	-9.29
Liu	2	0.94	-157.81	197.38	0.01	14.85
Andrews	4	0.70	-5.68	1.03	0.99	67.05
Shukor	3	0.03	0.96	1.04	1.00	-17.12

Note:
p no of parameter
adR^2 adjusted correlation coefficient
RMSE root mean square error
AF accuracy factor
BF bias factor

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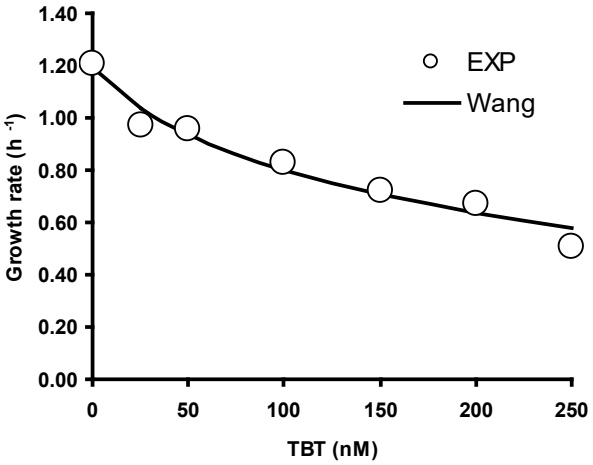

Fig. 1. The effect of increasing concentrations of TBT to the specific growth rate of *B. subtilis* as fitted to the Wang model.

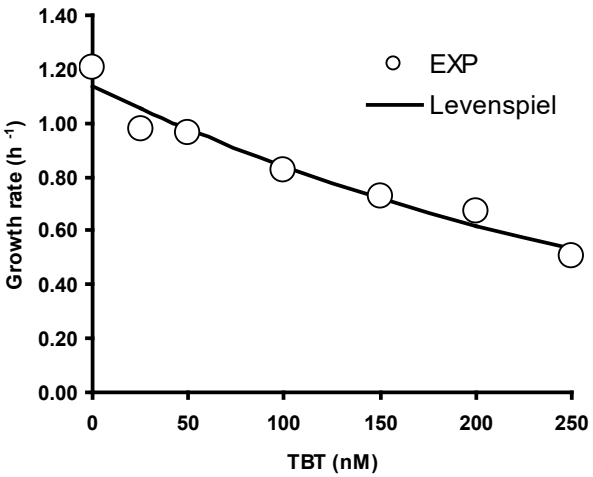

Fig. 2. The effect of increasing concentrations of TBT to the specific growth rate of *B. subtilis* as fitted to the Han-Levenspiel model.

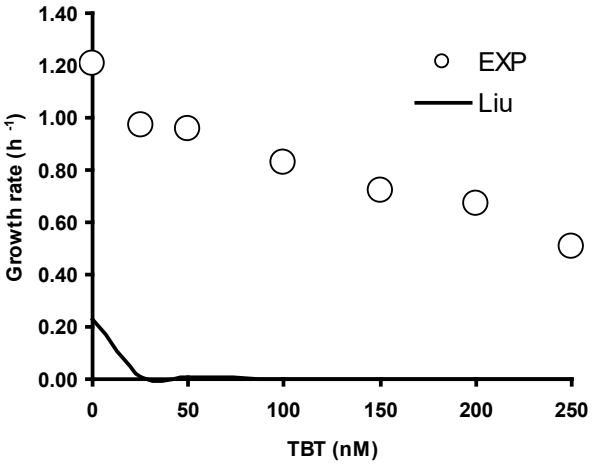

Fig. 2. The effect of increasing concentrations of TBT to the specific growth rate of *B. subtilis* as fitted to the Liu model.

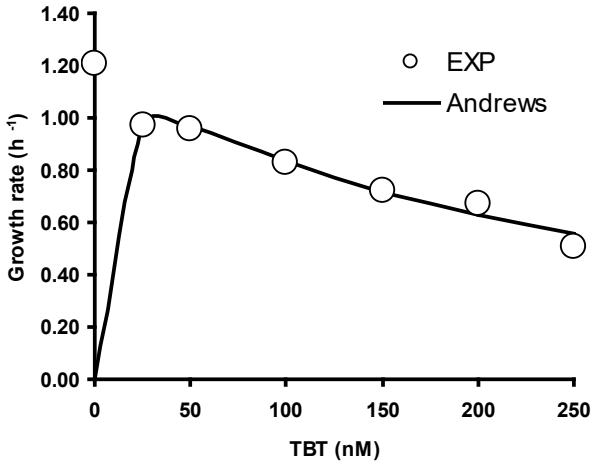

Fig. 4. The effect of increasing concentrations of TBT to the specific growth rate of *B. subtilis* as fitted to the Andrew model.

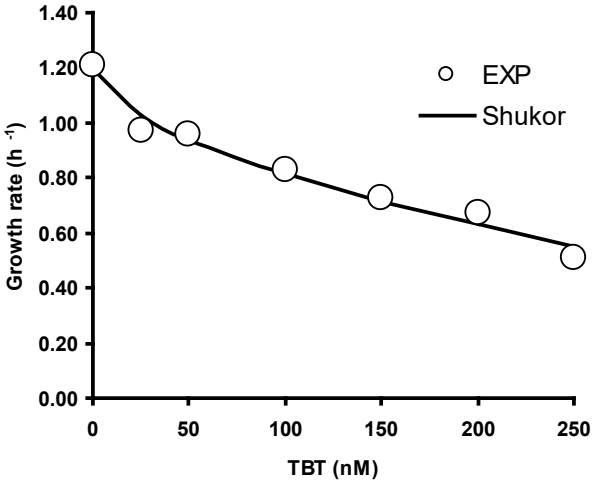

Fig. 5. The effect of increasing concentrations of TBT to the specific growth rate of *B. subtilis* as fitted to the Shukor model.

In a previous publication, the parameters obtained from the Wang model that govern TBT inhibition of *Bacillus stearothermophilus* which are *Ccrit,* μ_{max} and *m* which represent critical TBT concentration (nM), maximum growth rate (h^{-1}) and empirical constant values were 177.99 (nM), 2.41 and 2.76, respectively. The parameters obtained from the Shukor model (**Table 3)** were *Ccrit* 742.32 nM (95%, C.I., 303.35 to 1181.29), μmax 1.20 h^{-1} (95% C.I., 1.08 to 1.319) and *m* 0.507 (95% C.I.,0.308 to 0.832), indicating that TBT is more toxic to *Bacillus stearothermophilus.* Because of its ability to forecast the threshold concentrations that will fully limit bacterial growth, the Shukor model has been widely used to model the inhibitory effect of metals to growth rate of microorganisms on xenobiotics [38,41–44].

In a specific example, using a batch photobioreactor, researchers investigated the removal of Cu(II) by *Nostoc muscorum*, a cyanobacterium isolated from a hazardous metal-polluted site in Meghalaya, with the goal of elucidating the removal mechanism and the impact on nitrate absorption by the cyanobacterium in the process. The Han-Levenspiel and Andrew models were the most closely matched to the experimental data.

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The Han-Levenspiel constant; the critical Cu(II) concentration was determined to be 32.5 mg/L [5]. In another study, using a mutant of the bacteria *Pseudomonas* sp., it was determined that there is an inhibitory effect by heavy metal ions on the biodegradation of Congo Red. The critical heavy metals concentrations obtained from the Han–Levenspiel inhibition model for Cr, Zn and Cu were 895, 302 and 204 mg/L, respectively [42].

In the study on the inhibitive effects of heavy metals on Reactive Black 5 decolourization by *Pseudomonas aeruginosa* strain Gb30, the best model modelling the inhibitory effect of heavy metals on the decolourization rate was Han-Levenspiel with C_{crit}, μ_{max} and m values of 3496 mg/L (50 mM), 2.013 h^{-1} and 1.193, respectively, for zinc and 280 mg/L (2.491 mM), 1.991 h^{-1} and 0.882, respectively, for cadmium [45].

Table 3. Parameter values for the Shukor's model.

Parameters	Value	95%, confidence interval
μ_{max} (h^{-1})	1.20	1.08 to 1.31
C_{crit} (nM)	742.32	303.35 to 1181.29
m	0.57	0.308 to 0.832

Note
C_{crit} critical heavy metal ion concentration (mg/L)
μ_{max} maximum growth rate (g/L.h)
m empirical constant

However, despite the fact that heavy metals and organic pollutants are both ubiquitously present in polluted waters, the use of metal inhibition models is underrepresented in the literature. Few studies have investigated the effect of heavy metals on the growth rate of bacteria that are growing in the presence of a toxic substance. According to one study, zinc and nickel inhibited the biodegradation of monoaromatic hydrocarbons by *Bacillus* sp. and *Pseudomonas aeruginosa* significantly, and the effect of these heavy metals on the degradation rate was successfully modelled using the Andrews model [39]. Metals such as gold and silver interact with functional groups in enzymes such as the sulfhydryl group, which is frequently found at the active sites of enzymes, and this is most likely the mechanism of inhibition [42].

Metals can have bactericidal or bacteriostatic effects on microorganisms, which can be positive or negative in nature. A range of biochemical and morphological effects have been observed when delivered at sub-lethal levels. Copper has been demonstrated to turn *E. coli* into spherical forms, whilst platinum has been shown to transform *E. coli* into extremely long filamentous forms [2,46]. According to preliminary observations, inhibitive metal interference appears to be generating abnormalities in the processes of cell wall production and cell division. Several species develop in the presence of cobalt or copper, and their biochemical makeup varies due to an altered ratio of macromolecular elements, particularly nucleic acids, and a decreased activity of certain oxidative enzymes, particularly porphyrin-containing enzymes, among other variables. According to the offered theory, metals may be damaging to human health because they establish strong interactions with numerous ionic groups on the surface of our cells [11,14,47–51].

Increased toxicity is caused by the metal becoming more electronegative, which boosts its bonding and binding strength. Because metals can form complexes with metal-binding molecules in cells, this notion explains why metals can be harmful to cells.

The toxicity of a medium containing such substances is significantly lowered when cells grow and develop in it. The amino acids histidine and cysteine, both efficient complexing agents, are useful in reversing the bacterium's growth inhibition [52,53].

To account for this, cells grown in the presence of cobalt in basic glucose-salts growth media have a 1000-fold higher toxicity than cells produced in the presence of organic acids. In a similar way, cobalt and copper have been demonstrated to reverse their inhibitory effects on a variety of bacteria when exposed to organic ligands such as citrate, glutamate, and ethylenediamine tetraacetic acid. Chelation has the potential to minimise metal toxicity in natural ecosystems, which would be advantageous. It is considered to be capable if a bacterium that would ordinarily be hampered by metals can survive and develop in ground water or marine sediments containing complexing agents. Recent study indicates that certain species may release complexation organic acids into the surrounding environment, which has the effect of detoxifying the environment [6,7,10,53–55].

Metals can be removed from solutions in order to make them non-toxic, if desired. In this application, the precipitation of insoluble metal sulphides in the presence of H_2S is the most conspicuous phenomenon observed. The addition of inorganic ions such as phosphate and thiosulfate to the growth medium resulted in a substantial reduction in the toxicity of copper [56,57]. When manganese is present, cobalt toxicity is reversed in humans; when calcium and copper are present, cobalt toxicity is reversed in yeast; and when zinc is present in lactic acid bacteria, zinc toxicity may be counteracted by the presence of either manganese, magnesium, or calcium, respectively [3]. It is still unclear how these connections function at this time. Many scientists, on the other hand, believe that they represent a direct competition between the inhibitory minerals, such as manganese and magnesium, and the required minerals, such as manganese and magnesium, for enzyme activation sites in the cell [9].

After all is said and done, metal inhibition models are only utilised in a few scenarios to simulate the impact of metal ions on bacterial growth rates on dangerous chemicals, which is unfortunate because this knowledge is important to a properly functioning biological system. Because bacteria must be able to withstand the toxicity of both types of toxicants, the rate of development is likely to be greatly delayed when heavy metals are present in the environment. When metal contamination in co-polluted areas is sought, the findings of this study may have a substantial impact on the bioremediation field trial operations.

CONCLUSION

In conclusion, the use of inhibition models to model the effect of toxicity on the growth rate of bacteria is very rare and largely ignored despite the importance of such study. In this study the effect of TBT toxicity on the growth of *B. subtilis* bacterium was modelled according to several inhibition models, with the Shukor model discovered as the best model. The Shukor model allows for the prediction of the critical TBT concentration which can completely inhibited the bacterial growth. It is expected that in the presence of TBT, the growth rate on toxic substance will be even strongly affected as the bacteria have to resist the toxicity of both kind of toxicants at the same time. The results from this study can be very important in field trial works where TBT bioremediation is required.

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