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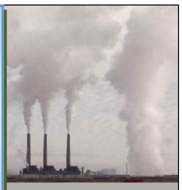

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# Modelling the Effect of Heavy Metal on the Growth Rate of an SDS-degrading *Pseudomonas* sp. strain DRY15 from Antarctic soil

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**ABSTRACT**

The SDS-degrading bacterium *Pseudomonas* sp. strain DRY15 was strongly inhibited by heavy metals especially mercury. Growth of the SDS-degrading bacterium at various concentrations of mercury shows a sigmoidal pattern with lag periods ranging from 7 to 10 h. As the concentration of mercury was increased, the overall growth was inhibited with 1.0 g/L causing an almost cessation of bacterial growth. The modified Gompertz model was utilized to obtain growth rates at different concentrations of mercury. The growth rates obtained from the modified Gompertz model was then modelled according to the modified Han-Levenspiel, Wang, Liu, modified Andrews, the Amor and Shukor models. Out of the five models, only the Shukor, 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. The best model was Shukor 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, which are $S_m$, $\mu_{max}$ and $n$ which represent critical heavy metal ion concentration (mg/L), maximum growth rate ($h^{-1}$) and empirical constant values were 6.0 (95%, confidence interval from 5.87 to 6.14), 0.09 (95%, confidence interval of 0.086 to 0.096) and 4.2 (95%, confidence interval from 3.1 to 5.2), respectively.

## INTRODUCTION

The setting up of many bases and the increase in the numbers of tourists and tourist's ships in the cold region has introduced anthropogenic pollutants in Antarctica. Anionic surfactant such as sodium dodecyl sulphate has been reported to occur in the Antarctic Maxwell Bay and its adjacent sea areas at concentrations of up to 1.0 mg $l^{-1}$ [1] and biodegradation of the surfactant by sea water bacteria has been reported [2]. Detergents are known to have detrimental effects to aquatic life [3–5]. According to literature data, anionic surfactants give toxic effects to various aquatic organisms at concentrations from 0.0025 to 300 mg $l^{-1}$ [6]. Another study indicated that exposure to SDS has a detrimental effect on oyster digestive gland, resulting in the perturbation of the metabolic and nutritional functions, and having a direct influence on oyster survival [7]. Toxicity towards invertebrates and crustaceans could occur as the considerable number of anionic surfactants released into water. The life cycle of aquatic animals has also been influenced by the anionic surfactants, modified the behavior of the fish such as erratic movements, muscle spasms and body torsion [8]. Due to this, remediation of SDS is of vital importance.

Microbes are known for their ability to degrade organics including SDS [9–15], and their use as bioremediation agents is important for economical removal of xenobiotic pollutants. Biodegradation of anionic surfactant under aerobic conditions by the bacterium *Pseudomonas* sp. strain C12B was among the first to be studied [16], and to date quite a number of SDS-degrading bacteria have been isolated and characterized [10,11,17–29].

Works on cold-adapted microbes with ability to degrade SDS are rare [11,30]. We have previously reported the isolation of such a bacterium and found that the degradation of detergent is strongly affected by heavy metals—zinc especially. In this work we model the inhibitory effect of SDS on the growth rate of this bacterium using several models.

## MATERIALS AND METHODS

### Growth and maintenance of bacterium

The bacterium was sourced from our inhouse culture collection unit. The bacterium was grown and maintained on an SDS enrichment media in a 250 ml conical flask, incubated at 15 °C with shaking at 150 rpm on an orbital shaker. The basal salts (BS) medium contained the followings: $KH_2PO_4$, (1.36 g $l^{-1}$), $Na_2HPO_4$, (1.39 g $L^{-1}$), $KNO_3$, (0.5 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 $L^{-1}$). The medium also contained the following trace elements: $ZnSO_4.7H_2O$ (0.01 g $L^{-1}$), $MnCl_2.4H_2O$ (0.01 g $L^{-1}$), $H_3BO_4$ (0.01 g $L^{-1}$), $CoCl_2.6H_2O$ (0.01 g $L^{-1}$), $FeSO_4.2H_2O$ (0.01 g $L^{-1}$), $CuCl_2.2H_2O$ (0.01 g $L^{-1}$) and $Na_2MoO_4.2H_2O$ (0.01 g $L^{-1}$) [18]. Filter-sterilized sodium dodecyl sulphate was added into the medium as a carbon source at the final concentration of 1.0 g $l^{-1}$. Maintenance of the bacterium every fortnight was carried out on agar plate (1.5% agar) supplemented with solid sodium dodecyl sulphate. The growth rate of the bacterium at 0.1% SDS (w/v) in the presence of various concentrations of zinc of up to 6 mg $L^{-1}$ was monitored under the same optimum conditions previously reported [12].

### Primary growth modelling on SDS

The maximum specific growth rate on SDS was modelled according to the modified Gompertz model as this model is routinely used in modelling the growth of microorganisms on xenobiotics [31–37]. The equation (**Eqn. 1**) is as follows;

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

The value obtained from this primary modelling exercise was then used to model the effect of metal as follows;

### Effect of metal on growth rate of on SDS

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

**Table 1.** Metal inhibition models.

| Models | Equation | Ref |
|---|---|---|
| Modified Han-Levenspiel | $r = u_{max}\left(1 - \frac{C}{C_{crit}}\right)^m$ | [38] |
| Wang | $r = \frac{u_{max}}{1 + \left(\frac{C}{K_C}\right)^m}$ | [39] |
| Liu | $r = \frac{u_{max}K_C}{K_C + C}$ | [40] |
| Modified Andrews | $r = \frac{u_{max}C}{K_s + C + \left(\frac{C^2}{K_i}\right)}$ | [41] |
| Shukor | $r = v_{max}\left(1 - \left(\frac{C}{S_m}\right)^n\right)$ | (Shukor 2019) |

### 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

The quality of fit of the models to the experimental data was evaluated statistically using the Root-Mean-Square Error (RMSE) (**Eqn. 2**), adjusted coefficient of determination ($R^2$) (**Eqn. 3**), corrected AICc (Akaike Information Criterion) (**Eqn. 4**), bias factor (BF) (**Eqn. 5**) and accuracy factor (AF) (**Eqn. 6**) as carried out in previous works [42–46].

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(Pd_i - Ob_i)^2}{n - p}} \quad \textbf{(Eqn. 2)}$$

$$Adjusted\left(R^2\right) = 1 - \frac{(1 - R^2)(n - 1)}{(n - p - 1)} \quad \textbf{(Eqn. 3)}$$

$$AICc = 2p + n\ln\left(\frac{RSS}{n}\right) + 2(p+1) + \frac{2(p+1)(p+2)}{n-p-2} \quad \textbf{(Eqn. 4)}$$

$n$ and $p$ represent the number of data points and the number of parameters, respectively. A model is more likely to be correct if it has the smallest AICc value compared to other models [47].

$$\text{Bias factor} = 10^{\left(\sum_{i=1}^{n}\frac{\log(Pd_i/Ob_i)}{n}\right)} \quad \textbf{(Eqn. 5)}$$

$$\text{Accuracy factor} = 10^{\left(\sum_{i=1}^{n}\frac{\log|(Pd_i/Ob_i)|}{n}\right)} \quad \textbf{(Eqn. 6)}$$

## RESULTS AND DISCUSSION

Zinc shows an inhibitory effect to the growth of this bacterium and increasing the concentration of this toxicant further inhibited growth with a cessation of growth was observed at the zinc concentration of 6 mgL-1 (**Fig. 1**). The growth of this bacterium was successfully modelled using the modified Gompertz model with visually acceptable fit to the datapoint was obtained (**Fig. 2**). The specific growth rates obtained from this modelling exercise was then plugged into the above heavy metal inhibition kinetics models. Two of the models; Liu (**Fig. 5**) and Andrews (**Fig. 6**) show poor modelling with the Andrew models exhibiting some problem with the equations as the fitted data needs to pass through the origin, probably due to the $K_s$ and $K_i$ terms in the equation, and this might indicate the unsuitability of the Andrews model originally included in the works of Gopinath et al [48] in modelling the effect of heavy metals to the specific growth rate in general.

The best model was Shukor 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**). The parameters obtained from the Shukor model, which are $S_m$, $\mu_{max}$ and $n$ which represent critical heavy metal ion concentration (mg L $^{-1}$), maximum growth rate (h$^{-1}$) and empirical constant values were 6.0 (95%, confidence interval from 5.87 to 6.14), 0.09 (95%, confidence interval of 0.086 to 0.096) and 4.2 (95%, confidence interval from 3.1 to 5.2), respectively.

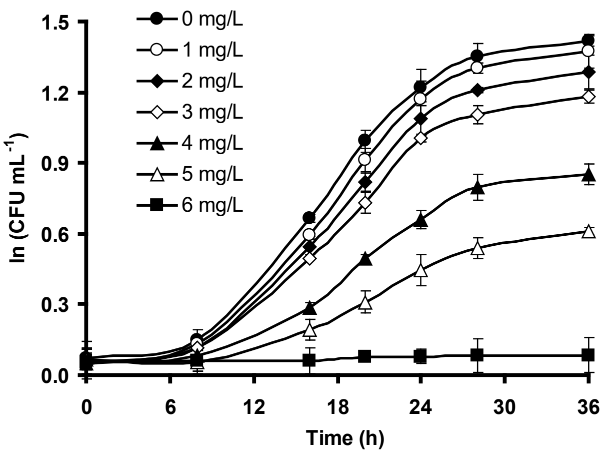


**Fig. 1**. The effect of increasing concentrations of zinc to the growth of *Pseudomonas* sp. strain DRY15 on SDS. Data represent mean standard deviations (three replicates).

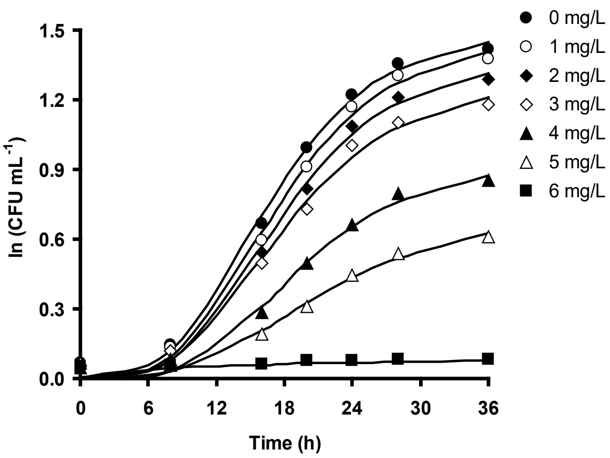


**Fig. 2**. The effect of increasing concentrations of zinc to the growth of *Pseudomonas* sp. strain DRY15 on SDS as fitted to the modified Gompertz model.

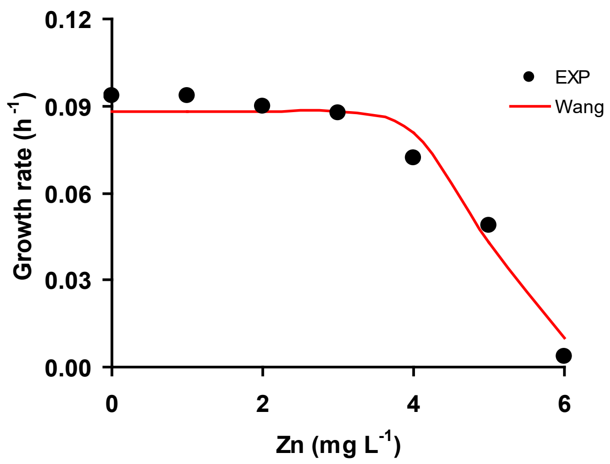


**Fig. 3**. The effect of increasing concentrations of zinc to the maximum specific growth rate of *Pseudomonas* sp. strain DRY15 on SDS as fitted to the Wang model.

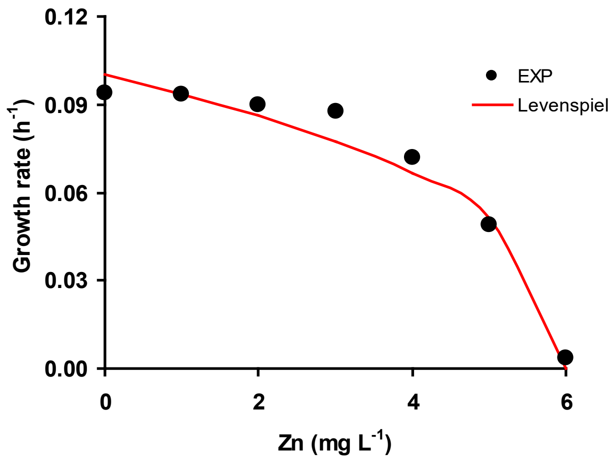


**Fig. 4**. The effect of increasing concentrations of zinc to the maximum specific growth rate of *Pseudomonas* sp. strain DRY15 on SDS as fitted to the Hans-Levenspiel model.

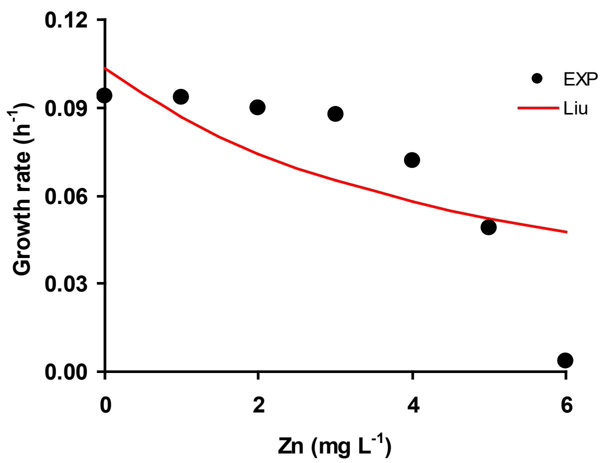


**Fig. 5**. The effect of increasing concentrations of zinc to the maximum specific growth rate of *Pseudomonas* sp. strain DRY15 on SDS as fitted to the Liu model.

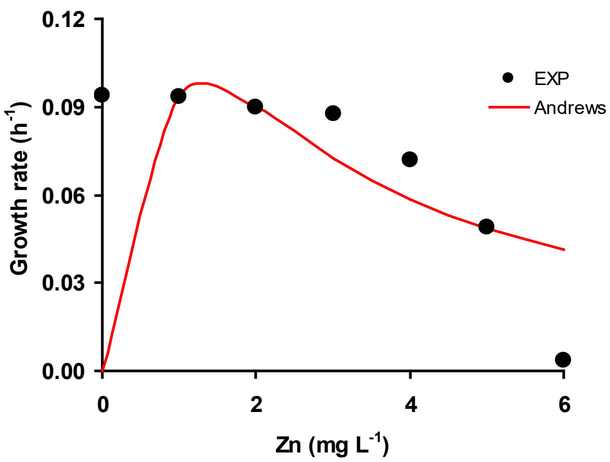


**Fig. 6**. The effect of increasing concentrations of zinc to the maximum specific growth rate of *Pseudomonas* sp. strain DRY15 on SDS as fitted to the Andrews model.

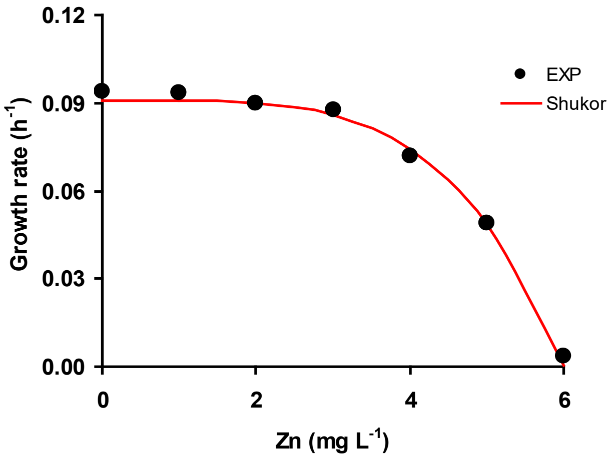


**Fig. 7**. The effect of increasing concentrations of zinc to the maximum specific growth rate of *Pseudomonas* sp. strain DRY15 on SDS as fitted to the Shukor model.

**Table 2**. Error function analysis of the effect of increasing concentrations of zinc to the maximum specific growth rate of *Pseudomonas* sp. strain DRY15 on SDS as fitted to various secondary models.

| Model | *p* | RMSE | *adR²* | AF | BF | AICc |
|---|---|---|---|---|---|---|
| Wang | 3 | 0.01 | 0.92 | 1.06 | 0.98 | -38.56 |
| Levenspiel | 3 | 0.01 | 0.94 | 1.05 | 0.98 | -39.08 |
| Liu | 2 | 0.02 | -0.90 | 1.14 | 0.92 | -36.19 |
| Andrews | 4 | 0.06 | -4.14 | 2.03 | 0.49 | 32.57 |
| Shukor | 3 | 0.00 | 0.99 | 1.02 | 0.99 | -51.71 |

Note:
RMSE Root mean Square Error
*p* no of parameters
$adR^2$ Adjusted Coefficient of determination
BF Bias factor
AF Accuracy factor
AICc Adjusted Akaike Information Criterion

In a similar study, zinc inhibits the biodegradation of Congo red by *Pseudomonas* sp. mutant [48] where there was an observable increase in lag period with increasing Zn (II) concentrations. Similarly, the specific growth rate decreasing gradually with increasing Zn (II) concentrations up to 200 mg $L^{-1}$ of Zn (II) and later surged rapidly towards zero. In another work, the inhibition constants ($K_i$) for the Andrews model in modelling the effect of zinc on the growth rate of *Bacillus* sp. and *Pseudomonas* sp. on monoaromatics such as ethylbenzene, o-xylene and toluene ranged from 20 to 26 mg $L^{-1}$ [49] indicating that the growth rate on SDS is severely inhibited by zinc. To combat this issue, soil amendments technique such as the addition phosphate, calcium carbonate, manganese oxide, and magnesium hydroxide to reduce the solubility of zinc and to allow bioremediation to proceed [50,51]. Alternatively, immobilization of the SDS-degrading bacterium could offer some protection to combat the toxicity metal ions in general [52].

## CONCLUSION

The biodegradation of SDS by an Antarctic bacterium was found to affect the growth rate on SDS. The best model to study this inhibition to the growth rate was Shukor of which the model predicted accurately the concentration of zinc that caused a complete cessation of growth rate.

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