Substrate Inhibition Modelling of *Pseudomonas nitroreducens* Growth on Octylphenol Polyethoxylates

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INTRODUCTION

Octylphenol polyethoxylates (OPEs) constitute a class of non-ionic surfactants extensively employed in various industrial applications. However, concerns have arisen regarding the potential environmental and human health impacts of OPEs because of their widespread use and persistence in aquatic environments. Bioremediation of OPE in the environment using OPE-degrading bacterium is appealing as bacterial metabolism converts OPE to harmless carbon dioxide and water as byproducts. In this study, various secondary growth models such as Luong, Yano, Teissier-Edward, Aiba, Haldane, Monod, Han, and Levenspiel were employed to model the inhibitory effect of high OPE concentrations to the growth rate of *Pseudomonas nitroreducens* TX1 the bacterium on OPE. Following thorough statistical analyses such as root-mean-square error (RMSE), adjusted coefficient of determination (adj\(R^2\)), bias factor (BF), and accuracy factor (AF), the Teissier model emerged as the most optimal choice. All of the studied models showed good fittings except Moser, Monod and Hinshelwood which showed the poorest curve fitting. The Teissier model emerged as the most suitable model, as indicated by its remarkably low values for RMSE, AICc, and modified adj\(R^2\). Furthermore, the model's AF and BF values were close to unity (Table 2). The experimental data obtained indicates that OPE is toxic and slows down the rate of growth at higher concentrations. The maximum OPE specific growth rate (\(\mu_{max}\)), half-saturation concentration (\(K_S\)), half inhibition concentration (\(K_i\)) was 0.613 h\(^{-1}\) (95% Confidence Interval or C.I. from 0.519 to 0.707), 2352.8 mg/L (95% C.I. from 1668.8 to 3036.8) and 52,456.7 mg/L (95% C.I. from 38395.0 to 66518.5, respectively. It is possible that these new constants found when modeling could be useful inputs for future modeling efforts.

**KEYWORDS**

Substrate Inhibition Models  
Teissier  
*Pseudomonas nitroreducens*  
Octylphenol polyethoxylates  
OPE-degrading bacterium

**ABSTRACT**

OCTYLPOLYETHOXYLATES (OPEs) are non-ionic surfactants used extensively in different industrial applications. However, concerns have arisen about the potential environmental and human health impacts of OPEs due to their widespread use and persistence in aquatic environments. Biodegradation of OPE in the environment using OPE-degrading bacteria is appealing as bacterial metabolism converts OPE to harmless carbon dioxide and water. In this study, various secondary growth models, such as Luong, Yano, Teissier-Edward, Aiba, Haldane, Monod, Han, and Levenspiel, were employed to model the inhibitory effect of high OPE concentrations on the growth rate of *Pseudomonas nitroreducens* TX1, the bacterium on OPE. Following thorough statistical analyses such as root-mean-square error (RMSE), adjusted coefficient of determination (adj\(R^2\)), bias factor (BF), and accuracy factor (AF), the Teissier model emerged as the most optimal choice. All of the studied models showed good fittings except Moser, Monod, and Hinshelwood, which showed the poorest curve fitting. The Teissier model emerged as the most suitable model, as indicated by its remarkably low values for RMSE, AICc, and modified adj\(R^2\). Furthermore, the model's AF and BF values were close to unity (Table 2). The experimental data obtained indicates that OPE is toxic and slows down the rate of growth at higher concentrations. The maximum OPE specific growth rate (\(\mu_{max}\)), half-saturation concentration (\(K_S\)), and half inhibition concentration (\(K_i\)) were 0.613 h\(^{-1}\) (95% Confidence Interval or C.I. from 0.519 to 0.707), 2352.8 mg/L (95% C.I. from 1668.8 to 3036.8) and 52,456.7 mg/L (95% C.I. from 38395.0 to 66518.5), respectively. It is possible that these new constants found when modeling could be useful inputs for future modeling efforts.
cooling during machining processes by maintaining stable emulsions in water-based fluids [2]. Personal care products, such as shampoos and lotions, may contain OPEs due to their emulsifying and dispersing properties. In adhesives and sealants, OPEs contribute to improved wetting of surfaces, enhancing overall product performance. Furthermore, in polymer industries, OPEs may be utilized as emulsifiers in polymerization processes, aiding in the dispersion of polymer particles and stabilizing reactions. It's important to note that despite their widespread past use, concerns about the environmental and health impacts of OPEs have led to regulatory scrutiny. As a result, alternative, more environmentally friendly surfactants are being explored in various industries [3].

OPEs exhibit toxicity towards aquatic organisms like fish, algae, and invertebrates by disrupting cell membranes and affecting normal functioning. Accumulation in sediments and bioaccumulation in aquatic species pose long-term exposure risks, leading to potential ecological consequences [2,4–6]. A major concern is their capacity to function as endocrine disruptors, particularly with documented estrogenic activity that interferes with the endocrine system of aquatic organisms, resulting in reproductive abnormalities and compromised success in exposed organisms.

Despite well-documented environmental impacts, research also emphasizes potential human health risks associated with OPE exposure. These compounds can enter the human body through various pathways, raising concerns about their role in hormone regulation and the development of specific health conditions. Environmental monitoring and biomonitoring data, reviewed in a study assessing human exposure to nonylphenol (NP), indicate source-specific Margins of Exposure (MOEs) ranging from 2863 to 8.4 × 10^7, well above 1000, suggesting reasonable certainty of no harm for both source-specific and aggregate exposures to NP [1]).

In a study by Baldwin et al. [4], it was observed that nonylphenol polyethoxylate (NPPG) at 5.0 mg/liter inhibits testosterone elimination in Daphnia magna, mirroring the effects seen with its degradation product 4-nonylphenol. Interestingly, NPPG did not induce significant chronic toxicity, suggesting that environmental concentrations of NPPGs may not pose a risk to invertebrates. Another investigation by TenEyck and Markee [5] focused on assessing the toxicity of three phenolic compounds—nonylphenol (NP), nonylphenol monoethoxylate (NP1EO), and nonylphenol diethoxylate (NP2EO)—to Pimephales promelas and Ceriodaphnia dubia. The study involved testing binary and tertiary mixtures, commonly found in surface waters due to wastewater discharges. The fathead minnows exhibited LC50 values of 136, 218, and 323 μg/L for NP, NP1EO, and NP2EO, respectively, indicating potential additive or synergistic effects in mixtures.

Furthermore, Song and Bielefeldt [6] explored the impact of five alkylphenol polyethoxylate nonionic surfactants on the microbial degradation of glucose and pentachlorophenol (PCP) by Sphingomonas chlorophenolicum RA2. The study revealed that surfactants with mid-range hydrophilic−lipophilic balance (HLB) values (13.5–15) were most compatible with substrate degradation. Interestingly, the lowest HLB surfactant inhibited RA2 growth, while the highest HLB surfactant showed inhibitory effects only at concentrations well above its critical micelle concentration (CMC).

The surfactants exhibited more inhibitory effects on RA2's PCP biodegradation compared to glucose, suggesting potential interactions with membrane-associated PCP-degrading enzymes [6]. These findings have practical implications for selecting surfactants in remedial applications involving biodegradation or oil dispersion. In a previous study, an OPE-degrading bacterium was isolated and characterized as an effort to remediate OPEs. The growth rate of the bacterium on OPE showed significant inhibition at high concentration of OPE.

**MATERIALS AND METHODS**

**Data acquisition**

The graphical data extracted from Figure 2 on the biodegradation of OPE by Pseudomonas nitroreducens TX1, was analyzed using the software tool Webplot digitizer. This software is widely acknowledged and embraced within the scientific community [7], for its capacity to convert scanned figures into digital data. Its precision and reliability have been consistently recognized by numerous researchers [8,9]. The data was further analyzed and modeled using Curve Expert Professional software (Version 2.6.5) to elucidate the scientific insights and trends within the dataset, contributing to the robustness of the study's findings. This combination of data digitization and advanced software analysis is a common and essential practice in modern scientific research, ensuring the accuracy and validity of results.

**Fitting of the data**

The Marquardt algorithm was employed for nonlinear regression to fit various bacterial growth models (Table 1) and this analysis was conducted using Curve Expert Professional software (Version 2.6.5). The algorithm aims to find the most optimal method for minimizing the sum of squares between predicted and observed values. In this process, the software can be configured manually or automatically to determine the initial parameter values, and the steepest gradient search between the four data points was utilized to estimate the maximum growth rate (μ_max).

**Statistical analysis**

The statistically significant difference between the models was evaluated using various metrics. The following statistical functions were utilized to determine the best models.

The RMSE allows number of parameters’ penalty and was calculated using Equation 1, where n illustrates the number of experimental data, where else p is the number of parameters calculated by the model and experimental data and values predicted by the model are Ob, and Pa, respectively [20]. With the regression line approaching the data points, the root mean square error (RMSE) reduces due to the reduced error in the model. More accurate predictions are generated by a model that has a lower error rate.

Comparable in magnitude to the dependent (outcome) variable, the RMSE values span an infinite number of positive infinities. The root mean square error (RMSE) can be employed to assess the extent of impression in a statistical model, including regression models. If a value is zero, it signifies that the predicted and actual values are an exact match. The model exhibits superior data fit and generates more precise predictions, as indicated by low RMSE values. In contrast, increased levels indicate a greater magnitude of errors and a reduced number of precise predictions.
Another statistical measure that is founded on information theory is known as the Bayesian Information Criterion (BIC) (Equation 5), which can be compared to the AICc. Models with the lowest Bayesian information criterion (BIC) are typically preferred over those with higher BICs when choosing from a finite number of models. It has close ties to the Akaike information criteria and is partially based on the likelihood function (AIC). This error function imposes a harsher penalty on the number of parameters than the AIC does [22].

$$BIC = n \ln \frac{RSS}{n} + p \ln (n)$$  \hspace{1cm} (Eqn. 5)

The Hannan–Quinn information criterion, often known as the HQC, is an additional error function approach that is based on the information theory (Equation 7). To evaluate how well a statistical model fits data, experts use the Hannan-Quinn information criterion (HQC). It is a common metric to employ when choosing one model over another. In contrast to the LLF, it is connected to Akaike's information criterion. The HQC, like the AIC, includes a penalty function for the total number of model parameters, however it is significantly bigger than the value assigned by the AIC because the equation contains the $\ln \ln n$ term [23];

$$HQC = n \times \ln \frac{RSS}{n} + 2 \times p \times \ln(\ln n)$$  \hspace{1cm} (Eqn. 7)

Both BF and AF were utilized in an effort to evaluate the appropriateness of the models. In order to get a correlation of 1 between the anticipated value and the observed value, the Bias Factor needs to be equal to 1.

The Bias Factor and Accuracy Factor originates from predictive microbiology under the food microbiology field and have found applications in modelling microbial growth that leads to food spoilage [24–31]. A fail-safe model is indicated when the value of the Bias Factor (Equation 8) is greater than 1, and a fail-negative model is indicated when the value of the Bias Factor is less than 1. When compared to 1, a value of Accuracy that is less than 1 indicates a less accurate prediction (Equation 9).

$$Bias\ factor = 10 \left( \frac{\sum_{i=1}^{n} \log \left( \frac{Pd_{i}}{Ob_{i}} \right) }{n} \right)$$  \hspace{1cm} (Eqn. 8)

$$Accuracy\ factor = 10 \left( \frac{\sum_{i=1}^{n} \log \left( \frac{Pd_{i}}{Ob_{i}} \right) }{n} \right)$$  \hspace{1cm} (Eqn. 9)

Another parameter-penalized model is MPSD. The Marquardt’s percent standard deviation (MPSD). This error function distribution follows the geometric mean error which allows for the penalty to the number of parameters of a model (Equation 10).

$$MPSD = 100 \left( \frac{1}{n-p} \sum_{i=1}^{n} \left( \frac{Ob_{i}-Pd_{i}}{Ob_{i}} \right)^{2} \right)$$  \hspace{1cm} (Eqn. 10)

where $p$ is the number of parameters, $n$ is the number of experimental data, $Ob_i$ is the experimental data, and $Pd_i$ is the value predicted by the model.
RESULTS AND DISCUSSION

According to the analysis of the bacterial growth model, as depicted in Figs. 1 to 7, all of the studied models showed good fittings except Moser, Monod and Hinshelwood which showed the poorest curve fitting. The Teissier model emerged as the most suitable model, as indicated by its remarkably low values for RMSE, AICc, and modified adjR². Furthermore, the model's AF and BF values were close to unity (Table 2). The experimental data obtained indicates that OPE is toxic and slows down the rate of growth at higher concentrations. The maximum OPE specific growth rate (µmax), half-saturation concentration (Ks), half inhibition concentration (Ki) was 0.613 h⁻¹ (95% Confidence Interval or C.I. from 0.519 to 0.707), 2352.8 mg/L (95% C.I. from 1668.8 to 3036.8) and 52,456.7 mg/L (95% C.I. from 38395.0 to 66518.5), respectively.

The Teissier model (or Tessier) is an extension of the Monod equation, which describes the rate of a microbiological reaction as a function of substrate concentration. The Teissier model introduces an additional parameter to account for substrate inhibition, a phenomenon where high substrate concentrations lead to a decrease in growth rate [12]. The Teissier model adapted for bacterial growth on toxic substances considers the impact of inhibitory effects of toxic compounds on microbial populations. Research in this area often involves assessing the impact of pollutants on microbial communities in contaminated environments. The Teissier model provides a means to understand how toxic substances influence bacterial growth dynamics, aiding in predicting the behavior of microbial populations under varying environmental conditions. The Teissier model has found excellent applications in other toxic compounds degradation by microorganisms such as in the degradation of nicosulfuron by a Pseudomonas nitroreducens strain NSA02 [32], anionic surfactant (SDS) degradation by immobilized mixed bacteria consortium [33], bioreduction rate of Chromium (VI) by Bacillus subtilis [34], degradation of Bisphenol A by Pseudomonas aeruginosa PAb1 isolated from the effluent of a thermal paper industry [35], degradation of asphaltene utilizing microorganisms isolated from oil samples [36], Staphylococcus aureus growth on Basic Violet 3 [37] and molybdenum reduction by bacterium [38].

Table 2. Statistical analysis of the substrate inhibition models utilized in this study.

<table>
<thead>
<tr>
<th>Model</th>
<th>p</th>
<th>RMSE</th>
<th>adR²</th>
<th>MPSD</th>
<th>AICc</th>
<th>BIC</th>
<th>HOC</th>
<th>BF</th>
<th>AF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luong</td>
<td>4</td>
<td>0.0489</td>
<td>0.9398</td>
<td>19.967</td>
<td>-53.578</td>
<td>-71.659</td>
<td>-74.297</td>
<td>1.020</td>
<td>1.141</td>
</tr>
<tr>
<td>Yano</td>
<td>4</td>
<td>0.0355</td>
<td>0.9432</td>
<td>14.433</td>
<td>-56.995</td>
<td>-75.056</td>
<td>-77.713</td>
<td>1.015</td>
<td>1.106</td>
</tr>
<tr>
<td>Tessier-Edward</td>
<td>3</td>
<td>0.0318</td>
<td>0.9557</td>
<td>12.641</td>
<td>-66.499</td>
<td>-78.758</td>
<td>-80.752</td>
<td>1.004</td>
<td>1.110</td>
</tr>
<tr>
<td>Aiba</td>
<td>3</td>
<td>0.0371</td>
<td>0.9386</td>
<td>15.754</td>
<td>-62.799</td>
<td>-75.059</td>
<td>-77.052</td>
<td>1.001</td>
<td>1.122</td>
</tr>
<tr>
<td>Haldane</td>
<td>3</td>
<td>0.0348</td>
<td>0.9456</td>
<td>15.552</td>
<td>-64.326</td>
<td>-64.586</td>
<td>-78.579</td>
<td>1.023</td>
<td>1.122</td>
</tr>
<tr>
<td>Monod</td>
<td>2</td>
<td>0.1405</td>
<td>1.7543</td>
<td>35.790</td>
<td>-36.289</td>
<td>-44.320</td>
<td>-45.648</td>
<td>1.132</td>
<td>1.472</td>
</tr>
<tr>
<td>Han and</td>
<td>5</td>
<td>0.0587</td>
<td>0.8513</td>
<td>12.954</td>
<td>-35.702</td>
<td>-62.077</td>
<td>-65.399</td>
<td>1.069</td>
<td>1.103</td>
</tr>
<tr>
<td>Levengiel</td>
<td>3</td>
<td>0.1404</td>
<td>-1.0630</td>
<td>36.692</td>
<td>-30.851</td>
<td>-43.111</td>
<td>-45.104</td>
<td>1.092</td>
<td>1.423</td>
</tr>
<tr>
<td>Hinshelwood</td>
<td>4</td>
<td>0.1571</td>
<td>-2.5414</td>
<td>40.015</td>
<td>-21.289</td>
<td>-39.350</td>
<td>-42.007</td>
<td>1.132</td>
<td>1.472</td>
</tr>
<tr>
<td>Webb</td>
<td>4</td>
<td>0.0369</td>
<td>0.9378</td>
<td>16.496</td>
<td>-56.040</td>
<td>-74.101</td>
<td>-76.759</td>
<td>1.023</td>
<td>1.122</td>
</tr>
</tbody>
</table>

Note: p is the number of parameters.

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Fig. 4. Growth of *Pseudomonas nitroreducens TXI* modeled using Aiba.

Fig. 5. Growth of *Pseudomonas nitroreducens TXI* modeled using Haldane.

Fig. 6. Growth of *Pseudomonas nitroreducens TXI* modeled using Monod.

Fig. 7. Growth of *Pseudomonas nitroreducens TXI* modeled using Han-Levenspiel.

Fig. 8. Growth of *Pseudomonas nitroreducens TXI* modeled using Moser.

Fig. 9. Growth of *Pseudomonas nitroreducens TXI* modeled using Webb.
Practically, the biologically significant coefficients derived from this analysis hold substantial value for guiding and optimizing both batch and field experiments. These coefficients provide a valuable tool for researchers and environmental scientists, enabling precise predictions concerning the growth conditions and requirements of *Pseudomonas nitroreducens* TX1 in the context of remediating Octylphenol Polyethoxylates (OPE) in polluted environments.

Notably, employing a substrate inhibition kinetics model to assess the impact of toxic compounds on microbial growth or degradation rates is gaining recognition as a crucial practice. While many studies traditionally lean towards either the Haldane or Monod models for modeling purposes, a select few, including this study, adopt a comprehensive modeling approach to leverage the flexibility offered by alternative models. The utilization of such a comprehensive approach not only yields improved curve fitting results compared to a few popular models but also represents a more nuanced and thorough strategy for understanding the dynamics of microbial responses to toxic compounds.

**CONCLUSION**

In conclusion, after conducting a comprehensive analysis that included various statistical metrics such as the corrected AICc (Akaike Information Criterion), bias factor (BF), adjusted coefficient of determination (R²), and root-mean-square error (RMSE), it has been determined that the Teissier model stands out as the most suitable model for describing the growth of *OPE* in polluted environments.

Fig. 10. Growth of *Pseudomonas nitroreducens* TX1 modeled using Hinshelwood.

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