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Mathematical Modeling of The Biodegradation of Phenol from Industrial Effluents Using Immobilized *Pseudomonas putida*

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ABSTRACT

Synthetic chemicals are extremely harmful, particularly those man-made ones. Models are used to describe the behavior of microorganisms under different physical or chemical conditions such as temperature, pH, and water activity. Phenol is one of the potentially hazardous synthetic industrial contaminants capable of causing deteriorating effects in humans. In this paper, for the first time we present different kinetics models such as Von Bertalanffy, Baranyi-Roberts, modified Schnute, modified Richards, modified Gompertz, modified Logistics and the most recent Huang were used to get values for the above kinetic constants or parameters from simultaneous biodegradation of phenol from industrial effluents using immobilized *Pseudomonas putida.* All the curves present the best models with highest adjusted R^2 value with the lowest RMSE and AICc value. The Accuracy and Bias Factors values were close to unity (1.0). Nearly all of the models best fit the curves indicating that *Pseudomonas putida* growth on phenol can be described mathematically the modelling parameters obtained can be utilized for predicting bioremediation of phenols in batch culture and perhaps in the future will be valuable in modelling growth eon industrial effluent containing phenol.

INTRODUCTION

Synthetic chemicals are extremely harmful, particularly those man-made ones. Overall, more than 80,000 chemicals were synthesized in the United States for industrial use, and others were introduced into the atmosphere without adequate health monitoring. While the toxicity of all natural and synthetic chemicals cannot be compared, it is worth noting that the Earth's five most toxic chemicals are all naturally found[1]. The phenol industrial contaminant is between the most common, potentially hazardous substances come in essence as a result of industrialization[2].

Models are used to describe the behavior of microorganisms under different physical or chemical conditions such as temperature, pH, and water activity. These models allow the prediction of microbial safety or shelf life of products, the detection of critical parts of the production and distribution process, and the optimization of production and distribution chains [3]. Bacterial growth curves have exerted a great deal of interest on microbiologists, as Frederick Neidhardt eloquently summed up in his short commentary 'Bacterial growth: constant fixation with dN/dt,' published nearly 20 years ago [4]. When supplied with a defined mixture of salts, sugar, vitamins and trace elements, a population of bacterial cells contained in the liquid medium can grow and replicate in a highly reproducible way at a constant rate. This observed regularity poses fundamental concerns about how the cellular processes that turn nutrients into biomass are organized [5]. Usually, the bacterial growth curve showed a sigmoidal pattern, starting with the lag section just after t = 0, followed by the logarithmic section, and then the bacteria enter the stationary phase and finally move to the death phase or decrease in bacterial growth. A comparison of different sigmoidal functions has been made in order to explain bacterial growth curve, such as Von Bertalanffy, Baranyi-Roberts, modified schnute, modified Richard, modified

Gompertz, modified logistics and Stannard [5]. They were compared statistically using a comprehensive model (Schnute model), which is a model that encompasses all other models. All sigmoidal functions have been updated to include all biologically important parameters. The Stannard, Schnute and Richards models appeared essentially to be the same [6]. The Gompertz equation was statistically adequate in the experiments to describe the growth data for caffeine [7]. The value of the growth curve is the maximum specified growth rate (μ_{max} or μ_m), delay time and asymptotic values. For secondary models to analyze the effects of the substrate, temperature, pH, and product on growth rate, a maximum specific growth rate (μ_m) value can be utilized. While the two categories can exist side by side, in fact, most bacterial growth models are between mechanistic and empirical features [8]. In this study, the use of primary models to model the phenol biodegradation of coke oven effluents using immobilized *Pseudomonas putida* is discussed for the first time.

MATERIALS AND METHODS

Data from Fig 1. from Singh et al [9] was processed using the software Webplotdigitizer 2.5 [10] which digitizes the scanned figure and has been utilized by many researchers and acknowledged for its reliability [11,12].

STATISTICAL ANALYSIS

Statistical significant difference between the models was calculated through various methods including the adjusted coefficient of determination (R^2), accuracy factor (AF), bias factor (BF), Root-Mean-Square Error (RMSE) and corrected AICc (Akaike Information Criterion) as before [11].

FITTING OF THE DATA

Fitting of the bacterial growth curve using various growth models (Table 1) was carried out using the CurveExpert Professional software (Version 1.6) by nonlinear regression utilizing the Marquardt algorithm. μ_{max} of estimation was carried out by the steepest ascent rifle of the curve while the crossing of this line with the x-axis is an estimation of λ. The highest growth was chosen for the modelling exercise.

RESULTS AND DISCUSSION

Out of the eight different models analysed it was shown that all the models show acceptable model fittings (fig 2 to 8) which were effective and relevant for the biodegradation of phenol present in coke-oven effluent using immobilized *Pseudomonas putida*. The best performance was measured using the lowest value for RMSE, AICc and the highest value for adjusted R^2. The AF and BF values were also excellent for the model with their values were the closest to 1.0.

Table 1: Growth models used in this study.

Model	p	Equation
Modified Logistic	3	$y=\frac{A}{\left\{1+\exp\left[\frac{4\mu_m}{A}(\lambda-t)+2\right]\right\}}$
Modified Gompertz	3	$y=A\exp\left\{-\exp\left[\frac{\mu_m e}{A}(\lambda-t)+1\right]\right\}$
Modified Richards	4	$y=A\left\{1+v\exp(1+v)\exp\left[\frac{\mu_m}{A}(1+v)\left(1+\frac{1}{v}\right)(\lambda-t)\right]\right\}^{\left(\frac{-1}{v}\right)}$
Modified Schnute	4	$y=\left(\mu_m\frac{(1-\beta)}{\alpha}\right)\left[\frac{1-\beta\exp(\alpha\lambda+1-\beta-\alpha t)}{1-\beta}\right]^{\frac{1}{\beta}}$
Baranyi-Roberts	4	$y=A+\mu_m x+\frac{1}{\mu_m}\ln\left(e^{-\mu_m x}+e^{-h_0}-e^{-\mu_m x-h_0}\right)-\ln\left(1+\frac{e^{\mu_m x+\frac{1}{\mu_m}\ln\left(e^{-\mu_m x}+e^{-h_0}-e^{-\mu_m x-h_0}\right)}-1}{e^{(y_{max}-A)}}\right)$
Von Bertalanffy	3	$y=K\left[1-\left(\frac{A}{K}\right)^3\right]\exp^{-\left(\mu_m x/3K^{\frac{1}{3}}\right)^3}$
Huang	4	$y=A+y_{\max}-\ln\left(e^{A}+\left(e^{Y\max}-e^{A}\right)e^{-\mu_m B(x)}\right)$ $B(x)=x+\frac{1}{\alpha}\ln\frac{1+e^{-\alpha(x-\lambda)}}{1+e^{\alpha\lambda}}$
Buchanan Three-phase linear model	3	Y = A, IF X < Lag Y=A + K(X–λ), IF λ ≤ X ≥ X_{MAX} Y = Y_{MAX}. IF X > X_{MAX}

Note:
A= Microorganism growth lower asymptote;
y_{max}= Microorganism growth upper asymptote;
u_{max}= maximum specific microorganism growth rate;
v= affects near which asymptote maximum growth occurs.
λ=lag time
e = exponent (2.718281828)
t = sampling time
α,β,k = curve fitting parameters
h_0 = a dimensionless parameter quantifying the initial physiological state of the reduction process. The lag time (h^{-1}) or (d^{-1}) can be calculated as $h_0=\mu_{max}$

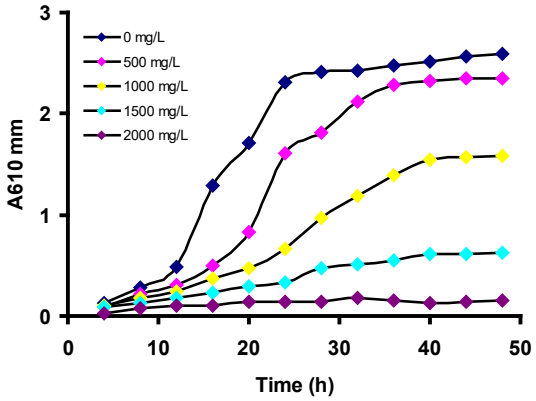

Fig 1. Growth of *Pseudomonas putida* (NAUN-16) in nutrient broth (NB) supplemented with various concentrations of phenol.

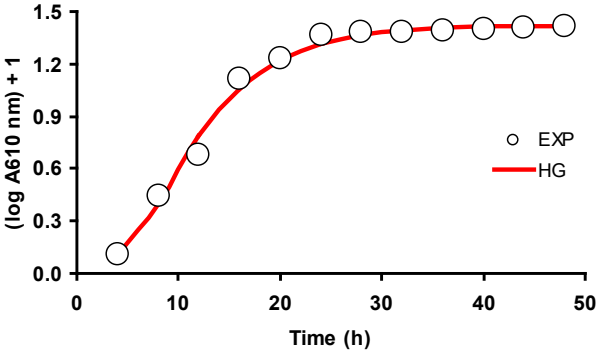

Fig. 2. Growth of *Pseudomonas putida* as modelled using the Huang model.

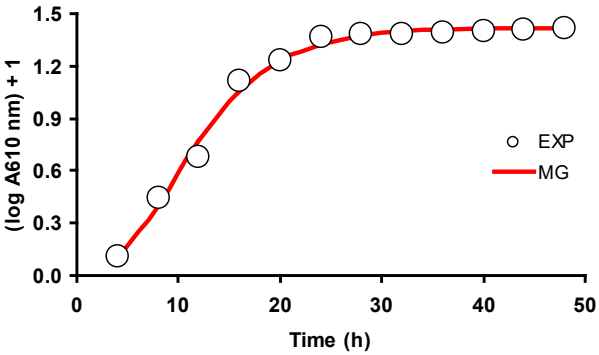

Fig. 3. Growth of *Pseudomonas putida* as modelled using the modified gompertz model**.**

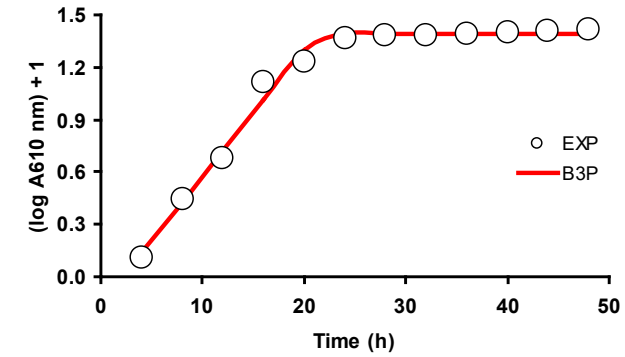

Fig. 4. Growth of *Pseudomonas putida* as modelled using the Buchanan-3-phase model.

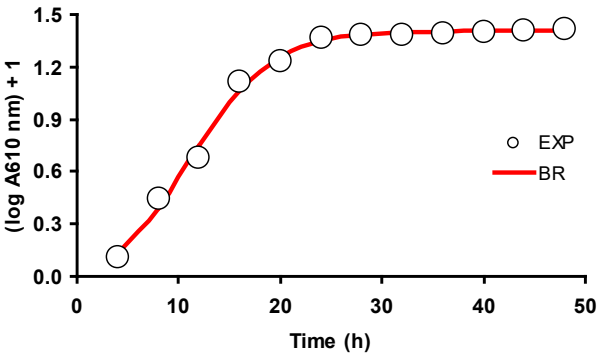

Fig. 5. Growth of *Pseudomonas putida* as modelled using the modified Richard model.

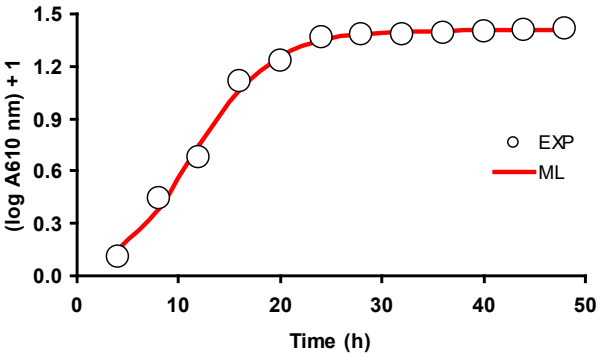

Fig. 6. Growth of *Pseudomonas putida* as modelled using the modified Logistics model.

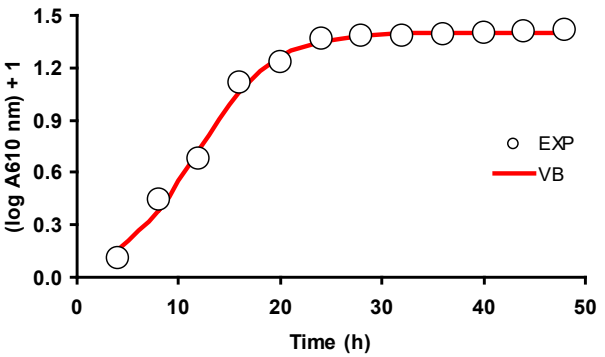

Fig. 7. Growth of *Pseudomonas putida* as modelled using the von Bertalanffy model

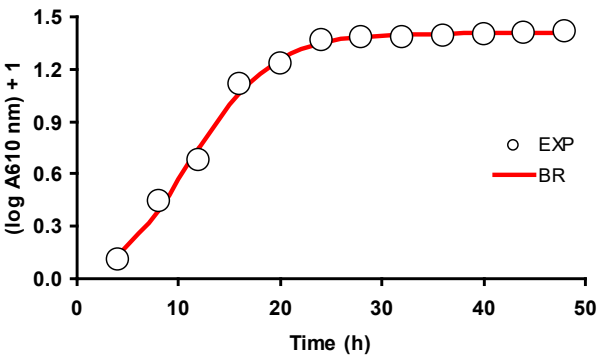

Fig. 8. Growth of *Pseudomonas putida* as modelled using the Baranyi-Roberts model.

Table 2 Statistical tests for the various models utilized in modelling the growth curve of microorganism.

Model	*p*	RMSE	R^2	adR^2	AF	BF	AICc
Huang	4	0.050	0.991	0.986	1.033	0.998	-48.97
Baranyi-Roberts	4	0.037	0.995	0.992	1.039	1.007	-56.01
modified Gompertz	3	0.041	0.993	0.990	1.044	0.992	-60.13
Buchanan-3-phase	3	0.046	0.991	0.988	1.044	1.015	-57.73
modified Richards	4	0.039	0.994	0.991	1.051	1.015	-54.84
modified Schnute	3	0.039	0.994	0.991	1.051	1.015	-54.84
modified Logistic	3	0.037	0.994	0.992	1.058	1.021	-62.81
von Bertalanffy	4	0.049	0.990	0.987	1.064	0.971	-56.34

Note: p is no of parameter

The Gompertz model is well-known and commonly used in many fields of biology. This has also been used to explain the growth of animals and plants, as well as the amount or volume of bacteria and cancer cells [13]. The logistic function also fits a sigmoid curve, but the modified model adds a lag time λ to account for a latency phase, as in the modified Gompertz model[14]. Huang's model is compared with Baranyi and Roberts' model in terms of the capacity to estimate microbial growth under dynamic temperature conditions, when evaluated by general estimate behavior, bias factor, precision and root-mean-squared error[15]. Baranyi model also has acceptable practical identifiability properties in the presence of realistic data, which means that the confidence intervals on the parameter values are reasonable[16]. On the other hand, von Bertalanffy curve is used to model mean length from age in animals, The function is commonly applied in ecology to model fish growth, however it is now used in all organism including biodegradation by bacteria [17].

Parameters obtained from the fitting exercise were maximum growth rate (μ_{max}), lag time (δ) and maximal growth (Y_{max}). In basic research, these mechanistic models are used and are meant to reach a better understanding of the biological, chemical and physical processes that lead to the growth profile seen. All other things being equal, mechanistic models are more powerful since they tell you about the fundamental procedures driving patterns. They are more probable to work properly when concluding beyond the observed conditions [18].

CONCLUSION

In conclusion, the use of mathematical models to model the biodegradation of synthetic environmental chemical toxicants is not very common, though very important. In our study the use of immobilized bacteria (*Pseudomonas putida)* for the biodegradation of phenol present in coke-oven was modelled, and all the models best fitted the curves.

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