

Mathematical Modelling of the Growth of an Antarctic Bacterium *Rhodococcus* sp. strain ADL36 on Palm Oil

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Introduction

Spills of vegetable oily waste especially palm oil as a result of ballast water discharge from vegetable oil tankers in temperate waters are of environmental concern because they cause serious effects on marine life and coastal environments (El-Masry et al., 2004). The ongoing reclassification of oil palm ballast waste as a hazardous substance by the European Union will seriously affect the Malaysian economy. The use of bioremediating bacteria to degrade the vegetable oily ballast waste water before discharge requires cold- and salinity tolerant bacteria. This study aims to mathematically model the growth of *Rhodococcus* sp. strain ADL36 previously isolated from Antarctica on processed palm oil. The growth of the bacterium under simulated conditions of oily ballast waste water was modelled using eight primary growth models including Logistic Gompertz, Richards, Schnute, Baranyi-Roberts, Von Bertalanffy, Buchanan three-phase and the Huang model.

The bacterium utilized in this study was obtained from the Bioremediation, Biomonitoring and Ecotoxicology Lab, Universiti Putra Malaysia and can grow on diesel as a carbon source (Habib et al., 2018). Growth studies were achieved using Mineral Salt Medium (MSM) of Zajic and Supplisson (1972) which consists of $\text{NH}_4\text{CL} - 4 \text{ g/L}$, $\text{K}_2\text{HPO}_4 - 1.8 \text{ g/L}$, $1.2 \text{ g KH}_2\text{PO}_4$, $4.0 \text{ g NH}_4\text{Cl}$, $0.2 \text{ g MgSO}_4 \cdot 7\text{H}_2\text{O}$, $0.01 \text{ g FeSO}_4 \cdot 7\text{H}_2\text{O}$ and 15 g of agar (for solid media) in 1 L of distilled water; pH 7. The medium was supplemented with 0.1% trace elements ($\text{MnSO}_4 \cdot 7\text{H}_2\text{O} 0.1 \text{ g/L}$, $\text{CuCl}_2 0.025 \text{ g/L}$, $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O} 0.025 \text{ g/L}$, $\text{Co} (\text{NO}_3)_2 \cdot 6\text{H}_2\text{O} 0.025 \text{ g/L}$, $\text{ZnCl} 0.025 \text{ g/L}$, $\text{NH}_4\text{NO}_3 0.01 \text{ g/L}$, and $(\text{NH}_4)_6\text{MO}_7\text{O}_{24} \cdot \text{H}_2\text{O} 0.025 \text{ g/L}$) (Bouchez et al., 1999). Fitting of the primary modelling utilizes the Marquardt algorithm that minimizes the residuals (difference between observed and predicted) sums of square. The curve fitting software CurveExpert Professional software (Version 1.6) was utilized in this work. Estimation of μ_m (maximal bacterial growth rate) was carried out utilizing the steepest ascent search of the curve amongst four datum points. Estimation of λ (lag period) was carried out by determining the intersection of this line with the x axis. Finally, estimation for the asymptote (A) (maximum amount bacterial growth) was carried out by taking the final datum point. Statistically significant of the various primary and secondary models was assessed according to various methods that take into account on the number of parameters used and include bias factor (BF), accuracy factor (AF), the corrected AICc (Akaike Information Criterion), Root-Mean-Square Error (RMSE), and adjusted coefficient of determination (R^2) were utilized (Motulsky and Brown, 2006).

The bacterial growth from this bacterium was sigmoidal in shape with a lag phase of about 1 to 2 days and reaching maximum bacterial growth at approximately day 7 of aerobic incubation. The highest amount of growth was consistently produced using palm oil concentration of 2% (v/v) (**Fig. 1**). Hence, this concentration was utilised to fit to eight different primary models. The resultant fitting (**Figs. 1**) shows visually acceptable fitting for all models. The best performance was modified Gompertz model with the lowest value for RMSE, AICc and the highest value for adjusted R^2 (**Table 1**). The AF and BF values were also excellent for the model with their values were the closest to 1.0. The poorest performance was modified Richards; a four-parameter model with the lowest score for most of the statistical tests. The coefficients for the modified Gompertz model at various palm oil upon analysis shows that the lag period increased with increasing concentration of substrate (**Table 2**) although not linearly, probably indicating that higher substrate concentration is inhibitory to the growth of palm oil.

The modified Gompertz model was the best in modeling the bacterial growth on palm oil based on statistical assessments such as bias factor (BF), accuracy factor (AF), the corrected AICc (Akaike Information Criterion), Root-Mean-Square Error (RMSE), and adjusted coefficient of determination (R^2).

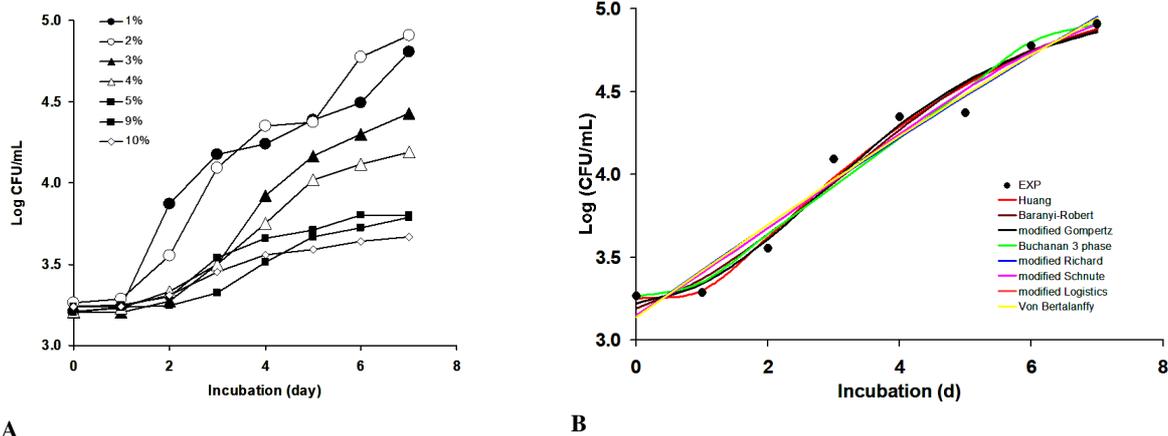


Fig. 1. A. The bacterial growth curves of *Rhodococcus* sp. strain ADL36 at various concentrations of palm oil over time after RSM optimization. **B.** Growth profile of *Rhodococcus* sp. strain ADL36 on palm oil fitted to various growth model.

Table 1. Statistical analysis of the various fitted models.

Model	p	RMSE	R^2	adR^2	AF	BF	AICc
Huang	4	0.11	0.98	0.96	1.01	1.0003	7.54
Baranyi-Roberts	4	0.14	0.97	0.93	1.02	1.0000	11.14
modified Gompertz	3	0.03	1.00	1.00	1.02	1.0000	-33.47
Buchanan-3-phase	3	0.12	0.97	0.95	1.02	1.0011	-10.19
modified Richards	4	0.15	0.97	0.92	1.03	1.0003	12.21
modified Schnute	3	0.10	0.98	0.96	1.03	1.0003	6.19
modified Logistics	3	0.14	0.97	0.94	1.03	1.0004	-7.86
von Bertalanffy	4	0.14	0.96	0.94	1.03	1.0004	-7.65

Note:
 p no of parameters
 adR^2 Adjusted Coefficient of determination
 BF Bias factor
 AF Accuracy factor
 RMSE Root Mean Square Error
 AICc Akaike Information Criteria with correction

Table 2. Bacterial growth coefficients at various palm oil as modelled using the modified Gompertz model (\pm standard error).

Parameters	1%	2%	3%	4%	5%	9%	10%
Y_0 (Log CFU/mL)	4.371 \pm 0.580	4.797 \pm 0.172	4.567 \pm 0.019	4.320 \pm 0.020	4.272 \pm 0.012	3.438 \pm 0.021	3.235 \pm 0.012
Lag (day)	-0.197 \pm 2.181	0.872 \pm 0.840	2.201 \pm 0.125	1.893 \pm 0.178	2.461 \pm 0.167	1.646 \pm 0.244	1.426 \pm 0.199
Y_{max} (Log CFU/mL)	6.145 \pm 0.294	6.637 \pm 0.283	5.826 \pm 0.039	5.394 \pm 0.052	4.860 \pm 0.027	4.000 \pm 0.025	3.668 \pm 0.015
μ_{max} (day ⁻¹)	0.297 \pm 0.054	0.380 \pm 0.044	0.355 \pm 0.015	0.284 \pm 0.012	0.214 \pm 0.011	0.137 \pm 0.017	0.132 \pm 0.008

The constants obtained from this modelling exercise (**Table 2**) can be used in further studies to model the effect of substrate, pH and temperature on growth rates.

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