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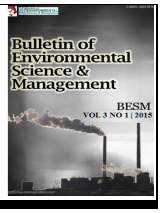

Short Communication

Outlier Analysis of the Buchanan-three-phase Model used in fitting the Growth of *Moraxella* sp. B on Monobromoacetic acid

Shukor, M.S.[1], Masdor, N.A.[2*], Shamaan, N.A.[3], Gunasekaran, B.[4] and Shukor, M.Y.[1,4]

[1]Snoc International Sdn Bhd, Lot 343, Jalan 7/16 Kawasan Perindustrian Nilai 7, Inland Port, 71800, Negeri Sembilan, Malaysia.
[2]Biotechnology Research Centre, MARDI, P. O. Box 12301, 50774 Kuala Lumpur, Malaysia
[3]Faculty of Medicine and Health Sciences, Universiti Sains Islam Malaysia, 13th Floor, Menara B, Persiaran MPAJ, Jalan Pandan Utama, Pandan Indah, 55100 Kuala Lumpur, Malaysia.
[4]Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, UPM 43400 Serdang, Selangor, Malaysia.

*Corresponding author:
Noor Azlina Masdor
Biotechnology Research Centre, MARDI, P. O. Box 12301,
50774 Kuala Lumpur, Malaysia
Email: azlina@mardi.gov.my
Tel: +603-8943 5942
Fax: +603-8945 6037

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ABSTRACT

Monohalogenated acetic acids have been widely used in industry and agriculture as precursor to chemicals and as herbicides, respectively. Monobromoacetic acid (MBA) is a chemical intermediate for the manufacturing of various chemicals with application in agriculture and pharmacy. Bioremediation of monobromoacetic acid, has been touted as a more economical and feasible method compared to physical and chemical approaches. Previously, we model the growth of growth of *Moraxella* sp. B on monobromoacetic acid from published literature to obtain vital growth constants. We discovered that the Buchanan-three-phase model via nonlinear regression utilizing the least square method was the very best model to explain the growth curve. However, the use of statistical tests to choose the best model relies heavily on the residuals of the curve to be statistically robust. More often than not, the residuals must be tested for the presence of outliers (at 95 or 99% of confidence). In this work, the Grubb's test to detect the presence of outlier in the growth model was carried out.

INTRODUCTION

Monohalogenated acetic acids include compounds such as monobromoacetic acid (MBA), monofluoroacetic acid (MFA) and monochloroacetic acid (MCAA). They are widely used in industry and agriculture. They have been used as herbicides as they are toxic to plants [1,2]. The growth curve exhibited sigmoidal properties, but the authors did not capitalize the existence of various primary growth models in order to obtain important growth constants that can be used in further modelling. The growth profile of the strain showed inhibition of growth at elevated concentrations of MBA. Modelling of the growth curves can yield important parameters that could be used for further secondary modelling exercise such as the inhibitory effect of substrate on growth.

Previously, we have utilized several growth models to model the growth of *Moraxella* sp. B on monobromoacetic acid. It was discovered that the Buchanan-three-phase model via nonlinear regression utilizing the least square method was the best model to describe the growth curve [3]. Nevertheless, the use of statistical tests to select the best model relies greatly on the residuals of the curve to be dispersed normally, of equal variance (homoscedastic) and random. More often than not, the residuals must be tested for the presence of outliers [at 95 or 99% of confidence). This is normally done using the Grubb's test.

METHODOLOGY

Conversion of published data from graphs to raw data was carried out by scanning and electronically processing the scanned grapsh using WebPlotDigitizer 2.5 [4]. The software helps to digitize scanned plots into table of data with good enough precision [5]. Data were acquired from the works of Torz et al. [2] from Figure 1 and then replotted (**Fig. 1,** with permission) [3].

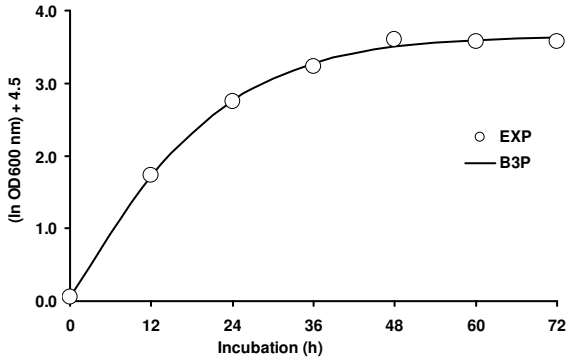

Fig. 1. Growth curves of *Moraxella* sp. B on monobromoacetic acid fitted by the Buchanan-three-phase model.

Grubbs' Statistic

Either data distortions by a single data point the mean or a single data point from a triplicate can lead to gross error in the fitting of a nonlinear curve. Checking for outlier is thus an important part of curve fitting. Grubbs test is used to detect outlier in univariate environment and the data is assumed to be normally distributed [6]. The test can be applied to the maximal or minimal observed data from a Student's t distribution (Eqn. **1**) and to test for both data simultaneously (Eqn. **2**).

$$G_{\min}=\frac{\bar{X}-\min(X)}{s}$$

$$G_{\max}=\frac{\max(X)-\bar{X}}{s} \qquad (\mathbf{1})$$

$$p_G=2n.p_t\left(G\frac{\sqrt{n(n-2)}}{n-1},n-2,1\right)$$

$$G_{\text{all}}=\frac{\max(\bar{X}-\min(X),\max(X)-\bar{X}}{s}$$

$$p_G=n.p_t\left(G\frac{\sqrt{n(n-2)}}{n-1},n-2,2\right) \qquad (\mathbf{2})$$

In the event that there is more than one outlier, then the ROUT method [7] will be used. The method is based on the False Discovery Rate (FDR). Q, a chance of (falsely) identifying one or more outliers will have to be set manually. It is the maximum desired FDR. In the absence of outliers, Q is very similar to alpha. Assuming all data come from a Gaussian distribution.

RESULTS AND DISCUSSION

Statistics of nonlinear regression relies heavily on the use of residuals data. Residuals are the difference between predicted and observed values of a mathematical model. Statistical tests should be carried out to test for the adequacy of the residuals in randomness, does not contain outlier, obeying normality and do not show autocorrelation. As a rule of thumb, the larger the difference between the predicted and observed values, the poorer the model [8]. The residuals for the Buchanan-three-phase model is shown in Table 1.

Table 1. Residuals for the Buchanan-three-phase model utilized in the modelling of the growth of *Moraxella* sp. B on monobromoacetic acid.

Time (h)	Residuals
0	0.00
12	-0.02
14	0.03
15	-0.03
18	-0.02
20	0.00
23	0.04
25	0.04
27	-0.06
29	-0.02
31	0.02
34	0.01
63	-0.02

When the Grubbs' test was applied, there was an absence of outlier for the residual data above. This indicates that the Buchanan-three-phase model was adequate to model the data. Either data distortions by a single data point the mean or a single data point from a triplicate can lead to gross error in the fitting of a nonlinear curve. Checking for outlier is thus an important part of curve fitting [6]. Grubbs' test identifies one outlier at any given time. This outlier is expunged from the dataset and the analyse is iterated till no outliers are discovered. Nevertheless, numerous iterations alter the likelihood of detection, and also the test must not be employed for sample sizes of six or less as it regularly tags the majority of the points as outliers.

Grubbs' test was applied in order to identify the outlier(s). The Grubbs' test statistic identifies the largest absolute deviation from the sample mean in units of the sample standard deviation. In the event the ensuing test statistic g is larger compared to the critical value, the related value is usually considered to generally be an outlier. The Grubbs' test indicated that there was no outlier.

A possible outlier is really an extreme data point that the investigator tags as improbable in view of a few detailed requirements. Much more specifically, an outlier in a sample is really an extreme value that is definitely too severe. For example, the maximum is considered an outlier when it is statistically too big for that distribution on the maximum in the population model (Barnett & Lewis, 1996, Saporta, 2011) [9]. A simple method to label potential outliers in measurements is to use boxplot, while a bit more advanced method is also used such as the Chauvenet's criterion in engineering and the 3-sigma criterion together with the Z-score in chemometrics. Although these methods are simple and quick, a more correct method is to use a statistical test for outlier detection. Specific tests include the Dixon's Q-test or Grubbs' ESD-test for one outlier. The main restriction of the Grubbs test would be that the thought quantity of outliers, k, should be described precisely. If k is not described properly, this could distort the findings of the tests. In the event outliers are multiple or the exact number of outliers are not known, the Rosner's generalized Extreme Studentized Deviate or ESD-test is recommended [10].

In conclusion, the Grubbs' test shows the absence of any outlier. The residuals of the Buchanan-three-phase model were robust enough to be used in all future statistical tests such as normality, runs test, tests for homoscedasticity and presence of autocorrelation. This test is important because data distortions by a single data can lead to gross error in the fitting of a nonlinear curve.

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