



BULLETIN OF ENVIRONMENTAL SCIENCE AND MANAGEMENT

<http://journal.hibiscuspublisher.com/index.php/BESM>



Short Communication

Test of Randomness of Residuals for the Buchanan-three-phase model used in the Fitting the Growth of *Moraxella* sp. B on Monobromoacetic acid (MBA)

Sabullah, M.K.^{1*}, Shukor, M.S.², Masdor, N.A.³, Shamaan, N.A.⁴, and Shukor, M.Y.^{2,5}

¹Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia

²Snoc International Sdn Bhd, Lot 343, Jalan 7/16 Kawasan Perindustrian Nilai 7, Inland Port, 71800, Negeri Sembilan, Malaysia.

³Biotechnology Research Centre, MARDI, P. O. Box 12301, 50774 Kuala Lumpur, Malaysia

⁴Faculty of Medicine and Health Sciences, Universiti Sains Islam Malaysia, 13th Floor, Menara B, Persiaran MPAJ, Jalan Pandan Utama, Pandan Indah, 55100 Kuala Lumpur, Malaysia.

⁵Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, UPM 43400 Serdang, Selangor, Malaysia.

*Corresponding author:

Dr. Mohd. Khalizan Sabullah

Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Jalan UMS,
88400 Kota Kinabalu, Sabah, Malaysia

Email: ejanz_sart2@yahoo.com

Tel: +6088) 320000 Ext: 8875

Fax : +6088 320259

HISTORY

Received: 1st May 2015
Received in revised form: 17th of June 2015
Accepted: 5th of July 2015

KEYWORDS

MAB-degrading
Buchanan-three-phase
Moraxella sp. B
ordinary least squares method
runs test

ABSTRACT

Monobromoacetic acid (MBA) is a chemical intermediate often use in agriculture and pharmaceutical industries. The use of monohalogenated acetic acids including monobromoacetic acids in industry and agriculture has led to numerous environmental issues. Bioremediation of monobromoacetic acid, has been recommended as a cheaper and achievable method when compared with physical and chemical approaches. Formerly, 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, an essential consideration, which has not been pointed out enough, is the residuals of the model have to be random. To ensure that randomness being met we carry out the Wald-Wolfowitz runs test. The results showed that the number of runs was 13, and the expected number of runs under the assumption of randomness was 7.462, indicating the series of residuals had adequate runs. The p-value obtained was greater than 0.05, therefore the null hypothesis is not rejected indicating no convincing evidence of non-randomness of the residuals and they do represent noise.

INTRODUCTION

The compound monobromoacetic acid (MBA) also known as bromoethanoic acid, α -bromoacetic acid, monobromoacetic acid and carboxymethyl bromide has been employed as a chemical intermediate for producing numerous chemical compounds with application in various industries [1]. Some studies have indicated that bacteria can aerobically degrade HAAs either cometabolically [2] or as a sole carbon and energy source [1,3–8]. The strain *Moraxella* sp. B [9] possesses two haloacid dehalogenases, one active with all monohaloacetates and the

other active with MCA and MBA [10]. The growth of this bacterium on monohaloacetates including MBA was studied by Torz et al [7]. It was discovered that the growth curve exhibited sigmoidal properties. However, the authors did not use primary growth models to obtain essential growth constants, which can be used in additional modelling. The growth profile of the strain demonstrated inhibition of growth at elevated concentrations of the compound. Modelling of the growth curves can yield important parameters that may be employed for additional secondary modelling exercise including 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 was the best model to describe the growth curve [11].

The method of mathematically fitting nonlinear curve using the ordinary least squares method relies heavily on the residuals for the curve to be normally distributed of equal variance (homoscedastic), and does not show autocorrelation [12–14]. Aside from this, an important consideration that has not been highlighted enough is that the residuals must be random. In order for randomness to be met we perform the Wald–Wolfowitz runs test [15] statistical diagnosis tests.

The runs test is an important tool in nonlinear regression to detect nonrandomness of the residuals [16]. The runs test could detect systematic deviation of the curve such as over or under estimation of the sections when using a specific model. The runs test look at the sequence of the residuals that are usually positive and negative. A good runs is usually signifies by alternating or a balance number of positive and negative residual values. The number of runs of sign is usually expressed in the form of a percentage of the maximum number possible [15].

METHODOLOGY

Graphs were scanned and electronically processed using WebPlotDigitizer 2.5 [17] which helps to digitize scanned plots into table of data with good enough precision [18]. Data were acquired from the works of Torz et al. [7] from Figure 1 and then replotted (Fig. 1, with permission) [11].

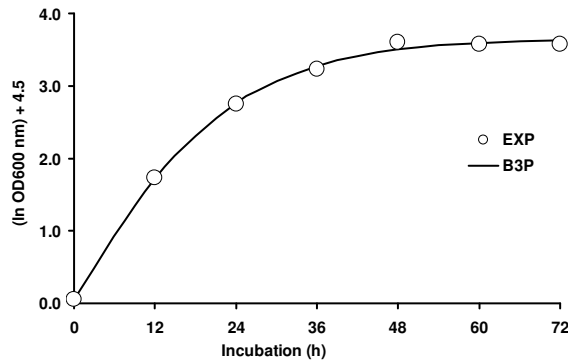


Fig. 1. Growth curves of *Moraxella* sp. B on MAB fitted by the Buchanan-three-phase model (B3P).

Runs test

The runs test [16] was carried out to the residuals of the regression in order to detect nonrandomness. The runs test calculates the probability for the presence of too many or too few runs of sign. The presence of too many of a run sign could indicate the presence of negative serial correlation whilst the presence of too few runs could indicate a clustering of residuals with the same sign or the presence of systematic bias.

The test statistic is

- H_0 = the sequence was produced randomly
 H_a = the sequence was not produced randomly

$$Z = \frac{R - \bar{R}}{sR} \quad (1)$$

Where Z is the test statistic, $\bar{R}$ is the expected number of runs, R is the observed number of runs and sR is the standard deviation of the runs. The computation of the values of $\bar{R}$ and sR (n_1 is positive while n_2 is negative signs) is as follows;

$$\bar{R} = \frac{2n_1n_2}{n_1+n_2} + 1 \quad (2)$$

$$s^2R = \frac{2n_1n_2(2n_1n_2 - n_1 - n_2)}{(n_1+n_2)^2(n_1+n_2-1)} \quad (3)$$

As an example

Test statistic: $Z = 3.0$

Significance level: $\alpha = 0.05$

Critical value (upper tail): $Z_{1-\alpha/2} = 1.96$

Critical region: Reject H_0 if $|Z| > 1.96$

Since the test statistic value (Z) is larger than the critical value then the null hypothesis is rejected at the 0.05 significance level or the sequence was produced in a nonrandom manner.

RESULTS

From Table 2, the number of runs was 13, the expected number of runs under the assumption of randomness was 7.462, indicating the series of residuals had adequate runs. The Z -value signifies the number of standard errors the observed number of runs is below the predicted number of runs, the corresponding p -value point out how severe this z -value is. The meaning is identical like other z -values statistics. If the p -value is less than 0.05 then the null hypothesis that the residuals truly are random can be rejected. Since the p -value was greater than 0.05, meaning that the null hypothesis is not rejected suggesting no persuasive evidence of non-randomness of the residuals and they do signify noise.

Table 2. Runs test for randomness.

Runs test	Residual data set
observations	7
below mean	7
above mean	6
no of runs	13
$E(R)$	7.462
$var(R)$	2.941
$stdev(R)$	1.715
Z -value	-0.269
p -value	0.394

Although the runs test has also been documented to be used for a test for autocorrelation in time-series regression models, simulation studies using Monte Carlo have shown that the runs test resulted in distinctly asymmetrical error rates in the two tails [19]. The investigation is carried out to analyse the empirical properties of the runs test utilizing (a) sample sizes of between 12 and 100 (b) using non-intervention and intervention regression models, (c) utilizing directional and nondirectional tests (d) with three levels of α , and (e) with 19 levels of autocorrelation among the errors. In addition, both directional and nondirectional tests produce no satisfactory results with respect to Type I error. The increase of the ratio of degrees of freedom to sample size to as high as .98 could also not remedy the situation. Hence, the

Durbin-Watson method would be the method of choice to assess autocorrelation.

To conclude, the runs test utilized in this work has shown that the use of the Buchanan-three-phase model in fitting of the growth curve of *Moraxella* sp. B is adequate. The use of runs test has been neglected by many publications, and the data may be nonrandom- an important requirement for all of the parametric statistical evaluation methods. In the event that the diagnostic tests show that the residuals indicate a trend, then various treatments such as nonparametric analysis or changing to a different model should remedy the problem.

ACKNOWLEDGEMENT

This project was supported by a grant from Snoc International Sdn. Bhd.

REFERENCES

- [1] Hamid AAA, Tengku Abdul Hamid TH, Wahab RA, Huyop F. Identification of functional residues essential for dehalogenation by the non-stereospecific α -haloalkanoic acid dehalogenase from *Rhizobium* sp. RC1. J Basic Microbiol. 2015;55(3):324–30.
- [2] Weightman AL, Weightman AJ, Slater JH. Microbial dehalogenation of trichloroacetic acid. World J Microbiol Biotechnol. 1992;8(5):512–8.
- [3] Yu P, Welander T. Growth of an aerobic bacterium with trichloroacetic acid as the sole source of energy and carbon. Appl Microbiol Biotechnol. 1995;42(5):769–74.
- [4] Olaniran AO, Babalola GO, Okoh AI. Aerobic dehalogenation potentials of four bacterial species isolated from soil and sewage sludge. Chemosphere. 2001;45(1):45–50.
- [5] McRae BM, Lapara TM, Hozalski RM. Biodegradation of haloacetic acids by bacterial enrichment cultures. Chemosphere. 2004;55(6):915–25.
- [6] Olaniran AO, Pillay D, Pillay B. Haloalkane and haloacid dehalogenases from aerobic bacterial isolates indigenous to contaminated sites in Africa demonstrate diverse substrate specificities. Chemosphere. 2004;55(1):27–33.
- [7] Torz MS, Yankov DS, Beschkov DN. Biodegradation of mono- and dihaloacetic acids by *Moraxella* sp. B. Comptes Rendus L'Academie Bulg Sci. 2006;59(3):295–300.
- [8] Alomar D, Abdul Hamid AA, Khosrowabadi E, Gicana RG, Lamis RJ, Huyop F, et al. Molecular characterization of monochloroacetate-degrading *Arthrobacter* sp. Strain d2 isolated from Universiti Teknologi Malaysia agricultural area. Bioremediation J. 2014;18(1):12–9.
- [9] Tonomura K, Kawasaki H, Tone N, Yahara H. Plasmid encoding mercury reductase and haloacetate halohydrolyase in drug-resistant bacteria. J Pharmacobiodyn. 1981;4(4).
- [10] Liu J-Q, Kurihara T, Ichiyama S, Miyagi M, Tsunasawa S, Kawasaki H, et al. Reaction mechanism of fluoroacetate dehalogenase from *Moraxella* sp. B. J Biol Chem. 1998;273(47):30897–902.
- [11] Shukor MS. Modelling the growth of *Moraxella* sp. B on Monobromoacetic acid (MBA). Bull Environ Sci Manag. 2015;3(1):1–6.
- [12] Razali NM, Wah YB. Power comparisons of Shapiro–Wilk, Kolmogorov–Smirnov, Lilliefors and Anderson–Darling tests. J Stat Model Anal. 2011;2:21–3.
- [13] Jarque CM, Bera AK. Efficient tests for normality, homoscedasticity and serial independence of regression residuals: Monte Carlo evidence. Econ Lett. 1981;7(4):313–8.
- [14] Snedecor GW, Cochran WG. Statistical methods. 7th ed. Ames Iowa: Iowa State University Press; 1980.
- [15] Motulsky HJ, Ransnas LA. Fitting curves to data using nonlinear regression: a practical and nonmathematical review. FASEB J Off Publ Fed Am Soc Exp Biol. 1987;1(5):365–74.
- [16] Draper NR, Smith H. Applied Regression Analysis. Wiley, New York; 1981.
- [17] Rohatgi, A. WebPlotDigitizer. <http://arohatgi.info/WebPlotDigitizer/app/> Accessed June 2 2014.;
- [18] Halmi MIE, Shukor MS, Johari WLW, Shukor MY. Evaluation of several mathematical models for fitting the growth of the algae *Dunaliella tertiolecta*. Asian J Plant Biol. 2014;2(1):1–6.
- [19] Huitema BE, McKean JW, Zhao J. The runs test for autocorrelated errors: unacceptable properties. J Educ Behav Stat. 1996;21(4):390–404.