



Statistical Diagnostic Tests of the Luong Model in Fitting Molybdenum Reduction from the bacterium *Bacillus* sp. strain A.rzi

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ABSTRACT

Of all heavy metals, molybdenum is one of the very toxic metals ions to ruminants with level as low as several parts per million could cause death. It is an emerging pollutant. It is also toxic to the spermatogenesis process with several animal model showed its toxic property and hence, is of great concern. Previously, a molybdenum-reducing bacterium *Bacillus* sp. strain A.rzi has been isolated and its kinetics of reduction studied with the best model to fit the curve is the Luong model. The use of this and other nonlinear regression model and further statistical analyses to find the best model relies on the facts that the residuals (difference between observed and predicted data) followed a normal distribution and that the data must be free of outliers and the variance homogenous (homoscedasticity). If all of these assumptions are satisfied, the test is said to be robust. In this work we perform statistical diagnostics to the residuals to satisfy the requirements above and found that the residuals conformed to all of the requirements above indicating the Luong model is a robust model for modelling molybdenum reduction in the bacterium.

INTRODUCTION

Molybdenum toxicity to animals have been reported to ruminants with levels as low as several ppm causing scouring and even deaths [1,2]. More recent works have shown that it is also toxic to catfish and mice by inhibiting spermatogenesis at the same concentration level [3–6]. Another similar toxic element, chromium, is intensely being studied in areas of its bioremediation using microbes and plants. As the toxicity of molybdenum is beginning to be reported, research on its removal especially bioremediation would be increasingly carried out. Bioremediation is an economical way to remove toxic metals from the environment [7].

A number of molybdenum reducing bacteria has been reported and most of these studies have been reported through our works from Malaysian soils [7,8,8–23], Antarctica [24] and Pakistani's soils [25]. Previously, a molybdenum-reducing bacterium *Bacillus* sp. strain A.rzi has been isolated and its kinetics of reduction studied with the best model to fit the curve is the Luong model [14]. The nonlinear regression utilizes the ordinary least squares method for mathematically fitting the non linear curve of the Luong model. However, the use of statistical

tests to choose the best model relies heavily on the residuals of the curve to be distributed normally, of equal variance (homoscedastic) and random.

More often that 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. In this work we perform statistical diagnosis tests such as the Wilks-Shapiro, Kolmogorov-Smirnov and D'Agostino-Pearson tests for normality and the Wald-Wolfowitz runs test for detecting residual for nonrandomness.

Method

Data from Othman et al [14] was scanned and electronically processed using Webplotdigitizer 2.5 [25]. Data were then replotted (Fig. 1).

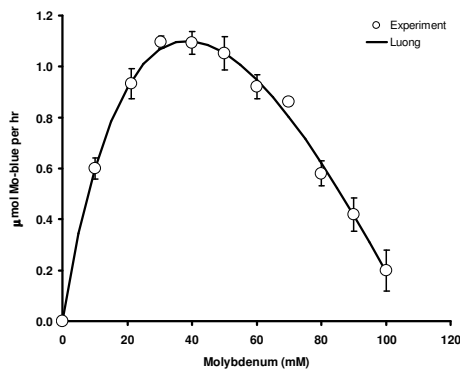


Fig 1. Mo-reduction kinetics fitted with the Luong model.

Grubbs' Statistic

Data distortions by a single data point either 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 [26]. The test can be applied to the maximal or minimal observed data from a Student's t distribution (Equation 1) and to test for both data simultaneously (Equation 2).

$$G_{\min} = \frac{\bar{X} - \min(X)}{s} \tag{1}$$

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

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

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

$$p_G = n \cdot p_i \left(G \frac{\sqrt{n(n-2)}}{n-1}, n-2, 2 \right) \tag{2}$$

Normality test

Residuals from the Buchanan- three phase model were subjected to three normality tests- Kolmogorov-Smirnov [27], Wilks-Shapiro [28] and the D'Agostino-Pearson omnibus K2 test [29]. Two ways to check for normality are through graphical and numerical means. Graphical methods such as the normal quantile-quantile (Q-Q) plots, histograms or box plots are the simplest and easiest way to assess normality of data. The detail mathematical basis of these normality test statistics is extensive and is available in the literature. The normality tests were carried out using the GraphPad Prism® 6 (Version 6.0, GraphPad Software, Inc., USA).

Runs test

This test was carried out to the residuals of the regression in order to detect nonrandomness [30]. This could detect a systematic deviation of over or under estimation sections of the curve when using a specific model [31]. 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. 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

$$Z = \frac{R - \bar{R}}{sR} \tag{3}$$

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;

$$\bar{R} = \frac{2n_1 \cdot n_2}{n_1 + n_2} + 1 \tag{4}$$

$$s^2 R = \frac{2n_1 \cdot n_2 (2n_1 \cdot n_2 - n_1 - n_2)}{(n_1 + n_2)^2 (n_1 + n_2 - 1)} \tag{5}$$

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 non random manner.

Test for equality of variance

Several tests are available to check the equality of variances on residual data from three or more samples and include those of Cochran, Bartlett, Brown-Forsythe and Levene; the F-test is used to check for homogeneity of two variances. In this work, the Bartlett's test would be used to test for homogeneity of variance (homoscedasticity) or equality of variance of the residuals [31,32].

Bartlett's test is used to test the null hypothesis, H_0 that all k population variances are equal against the alternative that at least two are different. If there a k samples with size N_i , sample variance of the ith group S_i^2 and S_p^2 is the pooled variance and the Bartlett's test statistic is;

$$\chi^2 = \frac{(N-k) \ln(S_p^2) - \sum_{i=1}^k (N_i - 1) \ln(S_i^2)}{1 + \frac{1}{3(k-1)} \left(\sum_{i=1}^k \left(\frac{1}{N_i - 1} \right) - \frac{1}{N - k} \right)} \tag{6}$$

Where $N = \sum_{i=1}^k n_i$ and $S_p^2 = \frac{1}{N-k} \sum_{i=1}^k (n_i - 1) S_i^2$ is the pooled estimate for the variance. The test statistics has an approximately a χ^2_{k-1} distribution. Thus the null hypothesis is rejected if $\chi^2 > \chi^2_{k-1, \alpha}$ where $\chi^2_{k-1, \alpha}$ is the upper tail critical value for the χ^2_{k-1} distribution.

RESULTS AND DISCUSSION

Statistics of nonlinear regression relies heavily on the used 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 [33].

Plot of residuals (observed-predicted) were checked and the analysis showed that all of the normality test including the popular D'Agostino & Pearson omnibus K2 test indicated that the residuals conform to a Gaussian distribution (Table 1). Data distortions by a single data point either the mean or a single data point from a triplicate can lead to gross error in the fitting of a nonlinear curve. The Grubbs' test shows the absence of outlier for the residual data. Checking for outlier is thus an important part of curve fitting [26].

Table 1. Numerical normality test for the residual from the Luong model.

Normality test	Analysis
D'Agostino & Pearson omnibus normality test	
K2	0.4958
P value	0.7805
Passed normality test (alpha=0.05)?	Yes
P value summary	ns
Shapiro-Wilk normality test	
W	0.9367
P value	0.4821
Passed normality test (alpha=0.05)?	Yes
P value summary	ns
KS normality test	
KS distance	0.1815
P value	> 0.1000
Passed normality test (alpha=0.05)?	Yes
P value summary	ns
Skewness	-0.4489
Kurtosis	-0.2415

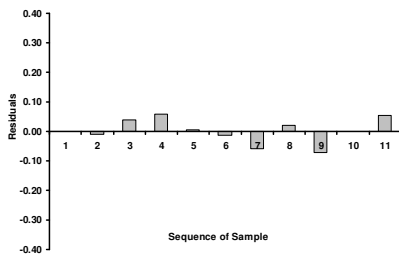


Fig. 2. Residual plot for the Luong model before removal of an outlier. Residual data is presented in terms of sequence of data instead of concentration of molybdenum.

A visual indicator for normality is the Q-Q (Quantile-Quantile) plot. The residual data from the Luong model when plotted on the normal probability Q-Q plot of residuals showed an almost straight line and indicates no underlying pattern indicating conformation to normality (Fig. 3). The resulting histogram of the residuals showed at first a non Gaussian distribution but the normality tests showed that the residuals were indeed conforming to normality [31]. The histogram was

then overlaid with the resulting normal distribution curve (Fig. 4).

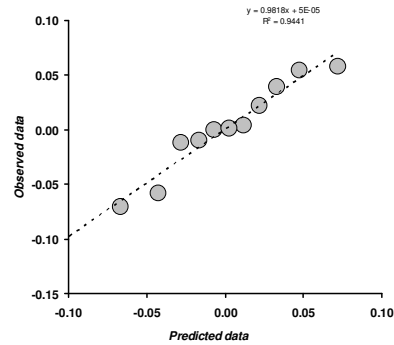


Fig. 3. Normal Q-Q plot for the observed sample against theoretical quantiles.

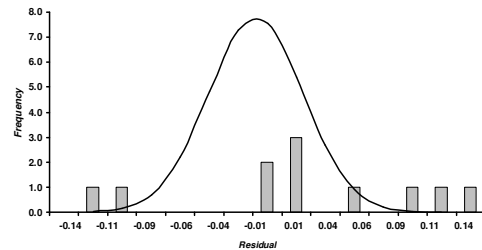


Fig. 4. Histogram of residual for the Luong model overlaid with a normal distribution (mean 0.0140 and standard deviation 0.0534).

In a histogram, the number of bins and samples examined determined the shape of the distribution [31]. The Kolmogorov-Smirnov statistic is a non-parametric numerical test that compares the cumulative frequency of residuals. It calculates the agreement between the model and observed values. It could also be used as a measure between two series of observation. The p value is calculated for the difference between two cumulative distributions and sample size [27,34]. The skewness and kurtosis of the distribution is computed as a method to quantify the difference between the sample distributions to a normal distribution. In the Wilks-Shapiro test [28], a W^2 statistic is calculated based on the expected values of the order statistics between identically-distributed random variables and their independent covariance and the standard normal distribution, respectively. If the test statistics value- W^2 is high, then the agreement is rejected. In the D'Agostino-Pearson normality test method. A p-value from the sum of these discrepancies is then computed. The most often form of the D'Agostino-Pearson normality tests is the omnibus K2 test as D'Agostino developed several normality tests [31].

Runs test

The expected number of runs under the assumption of randomness in the runs tests was 6.45 whilst the number of runs was 6 (Table 2), indicating the series of residuals had adequate runs. The Z-value indicates how many standard errors the observed number of runs is below the expected number of runs, the corresponding p-value indicate how extreme this z-value is [30,31]. The interpretation is the same like other p-values statistics. If the p-value is less than 0.05 then the null hypothesis that the residuals are indeed random can be rejected. Since the p-value 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.

Table 2. Runs test for randomness.

Runs test	Residual data set
Observations	6
Below mean	6
Above mean	5
No of runs	11
E(R)	6.454545
Var(R)	2.429752
StDev(R)	1.558766
Z-value	-0.29161
2 sided p-value	0.770588

If there is a frequent presence of too many of a run sign could indicate the presence of negative serial correlation. On the other hand the presence of too few runs could indicate a clustering of residuals with the same sign or the presence of systematic bias. The runs test calculates the probability for the presence of too many or too few runs of sign. The runs test is an important tool in nonlinear regression to detect nonrandomness of the residuals [30]. The runs test could detect systematic deviation of the curve such as over or under estimation of the sections when using a specific model [31]. 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.

Since the results from this work have shown that the residuals were normally distributed, the Bartlett tests is adequate to test for homogeneity of variance (homoscedasticity) or equality of variance of the residuals [31]. The value of χ^2 was at 3.38 and the critical was 18.31. Using the CHIDIST function from Excel, a probability of 0.98 was obtained (not significant) indicating that there were no real differences between the variances of the residuals. Several tests are available to check the equality of variances on residual data from three or more samples aside from the Bartlett's test such as Cochran, Brown-Forsythe and Levene while the F-test is used to check for homogeneity of two variances.

In conclusion, the various statistical tests for the residuals indicated that the use of the Luong model in fitting of the reduction curve in this bacterium is adequate and all of the reported values. The tests statistics carried out in this work is important since if the results obtained violated Gaussian or normal distribution than non parametric methods such as the Pearson's correlation coefficient either normal or adjusted, root mean square analysis, Kruskal-Wallis (nonparametric ANOVA) test should be used. Another remedy that can be used in the event of nonconformity includes changing to a different model that obeys or fulfills the above robust requirement. These assumptions could avoid errors of the Type I and II errors.

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