Testing the Normality of Residuals on Regression Model for the Growth of *Paracoccus* sp. SKG on Acetonitrile

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INTRODUCTION

Acetonitrile, an organonitrile, is extensively utilized in laboratories as a solvent and extractant for HPLC (High Performance Liquid chromatography). Organonitriles are classified as priority pollutants. The global industrial consumption of acetonitrile alone is more than 4 × 10^4 tonne in 2001 [1,2]. Consequently, wastewaters from the various uses of organonitriles often contain high contents of organonitrile compounds. Bioremediation of acetonitrile has been touted as a more economical and feasible method compared to physical and chemical approaches. Santoshkumar et al [3] has isolated a bacterial strain that could grow on acetonitrile. The growth profile of the strain showed inhibition of growth at elevated concentrations of acetonitrile. 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. 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 conformation to normal distribution. In order for these assumptions to be met, we perform statistical diagnosis tests such as the Kolmogorov-Smirnov, Wilks-Shapiro and D'agostino-Pearson tests.

KEYWORDS
acetonitrile-degrading
Buchanan-three-phase
*Paracoccus* sp. SKG
ordinary least squares method
normality test

METHODOLOGY

Graphs were scanned and electronically processed using WebPlotDigitizer 2.5 [4] which helps to digitize scanned plots into table of data with good enough precision [5]. Data were acquired from the works of Santoshkumar et al. [3] from Figure 4 and then replotted, and then assessed using several growth
models where the Buchanan-three-phase model was found to be the best (Fig. 1, with permission) (Shukor, M.S., Masdor, N.A., Shamaan, N.A., Ahmad, S.A., Roslan, M.A.H. and Shukor, M.Y. 2015. The growth of Paracoccus sp. SKG on acetonitrile is best modelled using the Buchanan Three Phase Model. Manuscript in preparation).

![Graph of Paracoccus sp. SKG growth on acetonitrile fitted by the Buchanan-three-phase model.](image1)

**Fig. 1.** Growth curves of Paracoccus sp. SKG on acetonitrile fitted by the Buchanan-three-phase model.

**Normality test**

Residuals from the Buchanan-three-phase model were subjected to three normality tests- Kolmogorov-Smirnov [6,7], Wilks-Shapiro [8] and the D’Agostino-Pearson omnibus K2 test [9]. 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 [10]. The normality tests were carried out using the GraphPad Prism® 6 (Version 6.0, GraphPad Software, Inc., USA).

Residuals are very important in assessing the health of a curve from a particular used model. Mathematically, residual for the \(i\)th observation in a given data set can be defined as follows (Eqn. 1);

\[
e_i = y_i - f(x_i; \beta)
\]

where \(y_i\) denotes the \(i\)th response from a given data set while \(x_i\) is the vector of explanatory variables to each set at the \(i\)th observation corresponding values in the data set.

**RESULTS AND DISCUSSION**

The fit of a statistical model can be diagnosed accurately using tests that use residuals. Residuals are the difference between a predicted and observed quantity using a particular mathematical model. The rule of thumb is that the larger the difference between the predicted and observed values, the poorer the model. Plot of residuals (observed-predicted) were checked and the analysis showed that the data were normal with the exception of the D’Agostino & Pearson omnibus normality test that indicated that the data was too small for the test. In practice, the data is considered to be normally distributed based on the majority of the tests (Table 1). The residuals plot does no indicate data that supported non normal distribution (Fig. 2).

The normal probability Q-Q plot of residuals for Buchanan-three-phase model was almost in a straight line and appears to show no underlying pattern (Fig. 3). The resulting histogram overlaid with the resulting normal distribution curve (Fig. 4) indicates the residuals were truly random and the model used was appropriately fitted.

**Table 1.** Numerical normality test for the residual from the Buchanan-three phase model.

<table>
<thead>
<tr>
<th>Normality test</th>
<th>Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>D’agostino &amp; Pearson Omnibus Normality Test</td>
<td></td>
</tr>
<tr>
<td>K2</td>
<td>n too small</td>
</tr>
<tr>
<td>P Value</td>
<td></td>
</tr>
<tr>
<td>Normality Test (Alpha=0.05)?</td>
<td></td>
</tr>
<tr>
<td>Passed Normality Test (Alpha=0.05)?</td>
<td></td>
</tr>
<tr>
<td>P Value Summary</td>
<td></td>
</tr>
<tr>
<td>Shapiro-Wilk Normality Test</td>
<td></td>
</tr>
<tr>
<td>W</td>
<td>0.8759</td>
</tr>
<tr>
<td>P Value</td>
<td>0.2091</td>
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<tr>
<td>Passed Normality Test (Alpha=0.05)?</td>
<td>Yes</td>
</tr>
<tr>
<td>P Value Summary</td>
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<tr>
<td>Ks Normality Test</td>
<td></td>
</tr>
<tr>
<td>Ks Distance</td>
<td>0.2681</td>
</tr>
<tr>
<td>P Value</td>
<td>&gt; 0.1000</td>
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<tr>
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<td>Yes</td>
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<tr>
<td>P Value Summary</td>
<td>ns</td>
</tr>
</tbody>
</table>

![Graph of residual plot for the Buchanan-three phase model.](image2)

**Fig. 2.** Residual plot for the Buchanan-three phase model.

**Graphical diagnostic of residuals normality**

![Graph of normal Q-Q plot for the observed sample against theoretical quantiles.](image3)

**Fig. 3.** Normal Q-Q plot for the observed sample against theoretical quantiles.
Number of bins and samples examined determined the shape of the distribution. In the Wilks-Shapiro test, 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 [8]. 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 [6,7].

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 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 [9].

In conclusion, normality tests for the residuals used in this work has indicated that the use of the Buchanan-three-phase model in fitting of the growth curve of Paracoccus sp. SKG on acetonitrile was adequate. It is well known that many publications did not elaborate further on the use of statistical diagnostic of the residuals from the model used. This could results in data violating the Gaussian or normal distribution. This assumption is an important requirement for many of the parametric statistical evaluation methods used in non linear regression. Methods such as the Pearson’s correlation coefficient either normal or adjusted, root mean square analysis, F-test and t-test rely on the residuals to be normally distributed. These assumptions could avoid errors of the Type I and II errors. Furthermore, in the event that the diagnostic tests shows that the residuals violated some of the assumptions various nonparametric treatments could be used or changing to a different model can in practice remedy the situation.

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REFERENCES