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A Study on Kinetic Models for Analysing the Bacterial Growth Rate

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***Abstract**: Four mathematical models for the prediction of bacterial growth rate; Blackman, Monod, Moser, Powell models are examined in this paper. The objectives of this study is to estimate the parameters of these models using Gauss -Newton iteration procedure and to select the best fit model among these models based on an empirical data set of Bacterial growth rate. Matlab version 7.11.0 has been used for the estimation of the parameters. The initial value specifications for the parameters using any iterative methods of estimation have also been identified based on the properties and the definitions of the parameters.The best fit model has been selected on the basis of root mean square error (RMSE) and R^2 value. Based on the result it may conclude that the three parameter Moser model provides better fits than the other models.*

***Keywords**: Kinetic Models, Gauss-Newton iteration method, bacterial growth rate, Parameter estimation.*

I. Introduction

Microbial growth kinetics has been the subject of many scientific studies and has many implications for our society. Combination of mathematical modelling with experimental works provides a meaningful and quantitative interpretation of the experimental results and revealing new aspects of microbial physiology. The modelling of biotechnological processes began with the equation of Blackman in 1905. Blackman [7] gave one of the earliest overall growth descriptions as eq. 1. In a nonmathematical way, he simply assumed that at low substrate concentration the rate of uptake is proportional to substrate concentration and at high substrate concentration both uptake and growth rate are independent of the substrate concentration because some other nutrient or intracellular factor is limiting under those conditions [1]. The Blackman model makes a sharp transition from first order to zero-order when substrate concentration exceeds the half-saturation coefficient. It does not allow for a gradual transition from zero-order to first-order kinetics, i.e. the function is not smooth [17].

$$\mu = \begin{cases} \frac{\mu_m \cdot S}{k_s} & if\ s < k_s \\ \mu_m & if\ s \geq k_s \end{cases} \tag{1}$$

The basis for mathematical modeling of the kinetics of bacterial growth was derived by German biochemist Leonor Michaelis and Canadian physician Maud Menten. Their model, which was published in 1913, describes the enzyme activity depending on substrate concentration [14]. This dependency can be transferred to bacterial growth, because the microbial growth is also an autocatalytic reaction. But not until the 1940th, Monod [11] recognized the non-linear relation between specific growth rate and limited substrate concentration, based on observations of the growth of E. coli at various glucose concentrations. He investigated the growth of bacteria cultures and parallelism to the Michaelis-Menten theory. The Monod equation has the same form as the Michaelis-Menten equation, but differs in that it is empirical while the latter is based on theoretical considerations. For bacterial growth, Monod formulated:

$$\mu = \frac{\mu_m S}{k_s + S} \tag{2}$$

Here, S denotes the concentration of the nutrient in the media at time t and μ is the specific growth rate. Since it is an function of S, this equation increases monotonically with limit μ_m as $S \to \infty$. Therefore μ_m is called the maximum specific growth rate. The maximum specific growth rate is unique for every bacteria culture [2]. According to this model, the specific growth rate increases strongly for low substrate concentration and slowly for high substrate concentration, until a saturation of bacteria is reached. The other constant k_s is called the half-saturation constant because when $\mu = \frac{\mu_m}{2}$; $S = k_s$. The specific growth rate has two limiting form. At high substrate concentrations, $S \gg k_s$, Eq. (2) reduces to a zeroth order dependence on substrate concentration. At low substrate concentrations, $S \ll k_s$, Eq. (2) reduces to a first order dependence on substrate concentration and is proximately linear. The values of μ_m vary with the type of organism and the value of k_s depends on the nature of the substrate [10]. Since μ_m is the maximum specific growth rate therefore $\frac{s}{s+k_s}$ must be less than 1 and which is possible only when k_s is greater than zero. Also the parameter μ_m cannot be negative as it is the maximum specific growth rate. Unlike the enzyme activity described by Michaelis-Menten, in fact μ does not start at zero, due to the degradation of substrate by bacteria for maintenance energy. Thus, the growth cannot start until S reached a certain value [24]. According to Contois [3], the accuracy of the Monod model for pure cultures and simple substrates is very high. The model is appropriate for homogenous cultures, but not for heterogeneous cultures or complex substrates [21]. Pfeffer [12], concluded that the Monod model is unable to describe the degradation of municipal wastes as a complex substrate. The limitation of classical Monod's equation is that it does not account for the fact that cells may need substrate or may synthesize product even when they do not grow [23]. Furthermore, the lag phase is not included in the Monod model. Also A relationship between the growth of cells and substrate concentration is too complex to describe in terms of fundamental physical laws. For this reason, a variety of constitutive equations, in addition to the Monod equation have been proposed to describe microbial growth.

The empirical equation proposed by Hermann Moser [9] was a modified form of Monod equation. Moser upgraded the model of Monod with a parameter n (usually $n > 1$) to integrate effects of adoption of microorganisms to stationary processes by mutation. For $n = 1$ the specific growth rate becomes equal to the Monod model. This model was never very popular but occasionally used in various experimental studies ([17], [6]). The mathematical formulation of the Moser model is given by

$$\mu = \frac{\mu_m S^n}{k_s + S^n} \qquad (3)$$

Where n is an adjustable parameter. This provides a degree of flexibility in fitting data and can predict interesting dynamic behavior in continuous stirred tank reactors [10].

The original Monod equation was modified by Powell [5], introducing the terms of maintenance rate (m) which takes into account of some of the limitation of Monod model. The Powell kinetic model is,

$$\mu = \frac{(\mu_m + m)S}{k_s + S} - m. \qquad (4)$$

Nonlinear models are more difficult to specify and estimate than linear model. But for prediction purpose it is very important to estimate these parameters properly. A lots of method of estimation were developed in various time ([22], [8], [16], [15], [19]). The present paper is aimed to estimate the parameters of the above nonlinear kinetic models using Gauss -Newton iteration procedure and to select the best fit model among these models based on an empirical data set of Bacterial growth rate.

II. Material and methods

The data are used for this study is the relation between bacterial growth rate and concentration of the substrate with glucose as the limiting factor of a culture of *Escherichia coli* [13]. The data were presented in Table 1.

Table 1. Bacterial growth rate data of *Escherichia Coli.*

s (1/h)	5.1	8.3	13.3	20.3	30.4	37	43.1	58	74.5	96.5	112	161	195	266	386
μ (mg/L)	.059	.091	.124	.177	.241	.302	.358	.425	.485	.546	.61	.662	.725	.792	.852

The nonlinear growth models describing are Blackman, Monod, Moser, Powell models. These growth models can be written in the form as

$$\mu_i = f(s_i, \mathbf{B}) + \varepsilon_i, \qquad (5)$$

$i = 1,2,\cdots,n,$ where μ is the response variable, s is the independent variable, $\mathbf{B}$ is the vector of parameters μ_m, k_s, n and m. ε_i is a random error in the model has mean zero and constant variance σ^2. The best fitted growth model is selected based on lowest RMSE.

A. Method of Estimation

The parameters of these models are estimated by minimizing the sum of square residue $S(\boldsymbol{B})$ under the assumption that the $\varepsilon_i's$ are independent $N(0,\sigma^2)$ random variable.

$$S(\boldsymbol{B}) = \sum_{i=1}^{n}[\mu_i - f(s_i,\mathbf{B})]^2 \qquad (6)$$

Since w_i and t_i are fixed observations, the $S(\boldsymbol{B})$ is a function of $\boldsymbol{B}$. Now the eq. (6) is differentiated with respect to $\boldsymbol{B}$ and setting the result to zero, we get

$$f_j = \sum_{i=1}^{n}[\mu_i - f(s_i,\mathbf{B})]\left[\frac{\partial f(s_i,\boldsymbol{B})}{\partial b_j}\right] = 0 \qquad (7)$$

for $j = 1,2,\cdots,m$ and $b_j \in \mathbf{B}$ [20]. This provides a system of m nonlinear equation with m unknown parameters and that must be solved for $\mathbf{B}$ using any iteration method. In this literature, the Gauss Newton procedure in Matlab version 7.11.0 was used to fit the models to the bacterial growth rate data and estimate the parameters. The Gauss Newton method requires specification of the names and starting values of the parameters to be estimated, the model using a single dependent variable, and the partial derivatives of the model with respect to each parameter. The usual statistical tests which are appropriate in the general linear model case are, in general, not appropriate when the model is nonlinear and one cannot use the F statistic to obtain conclusions at any stated level of significance [18]. Hence the models were compared based on the proportion of the unexplained variation.

III. Results and Discussion

A. Initial Value specification

All iteration procedures require initial values of the parameters to be estimates and the better these initial estimates are, the faster will be the convergence to the fitted value. Initial value specification is one of the most difficult problems encountered in estimating parameters of nonlinear model [4]. If the initial estimates are poor, convergence to the wrong final values can easily occur. There is no any general method for obtaining initial estimates. One uses whatever information is available.

Initial Value of $\boldsymbol{\mu_m}$: From the earlier discussion, it is noticed that for the kinetic model, the parameter μ_m is defined as the maximum specific growth rate. Therefore for the bacterial growth, the parameter was specified as the maximum value of μ in the data.

Initial Value of $\boldsymbol{k_s}$: The parameter k_s is defined as the half-saturation constant because when $\mu = \frac{\mu_m}{2}$; $S = k_s$. Therefore for bacterial growth rate, the parameter was specified as the value of s, when the value of μ is half of its maximum value in the data.

The initial value for the parameters n and m, was specified by evaluating the models at the starting position. Table 2 gives expressions used to specify the initial estimates of the parameter n of the Moser model and the parameter m of the Powell kinetic model at the initial stage. Where μ_0 and s_0 are the respective values of μ and s at the initial stage and μ_{m_0} and k_{s_0} are the initial values of the parameters μ_m and k_s respectively.

Table 2. Expressions used to specify initial estimates for the parameter n and m.

Model	Parameter	Expression
Moser	n	$\dfrac{\log \dfrac{\mu_0 k_{s_0}}{\mu_{m_0} - \mu_0}}{\log s_0}$
Powell	m	$\dfrac{\mu_{m_0} s_0 - \mu_0 k_{s_0} - \mu_{m_0} s_0}{k_{s_0}}$

B. Parameter Estimations

The Blackman, Monod, Moser and Powell nonlinear kinetic models have been fitted to the data of the relation between bacterial growth rate and concentration of the substrate with glucose as the limiting factor of a culture of *Escherichia coli.* The parameters of these models have been estimated using Gauss Newton Method of estimation. Estimated parameters for the four fitted models along with their Corresponding 95% Asymptotic Confidence intervals have been presented in the Table 3. Also presented the computed RMSE and R^2 values for each nonlinear models. The null hypothesis $H_0 : b_j = 0$ was rejected when the 95% confidence interval of b_j include zero. It is observed from the Table 3 that the Moser model has the smallest RMSE, $0.01567(1/h)$, the Monod model has the second smallest RMSE, $0.0163(1/h)$ and the Powell model has the third smallest RMSE, $0.0165(1/h)$. The another kinetic model, Blackman model provides a quite unsatisfactory result with the RMSE $0.2053(1/h)$.

The Moser model has produced a slightly smaller RMSE compared to the Monod and Powell models. All models except the Blackman model in Table 3 appear to predict reasonable estimates over the entire range of the data.

Table 3. Estimated parameters along with 95% Asymptotic Confidence interval, RMSE and the R^2 value for each fitted models.

Models	RMSE	R^2	Parameters							
			μ_m	Lower	k_s	Lower	n	Lower	m	Lower
				Upper		Upper		Upper		Upper
Blackman	0.2053	0.4332	0.189	-7.6e06	58.01	-2.2e09	___		___	
				7.6e06		2.2e09				
Monod	0.0163	0.9964	1.077	1.026	92.67	82.38	___		___	
				1.127		103				
Moser	0.0156	0.9969	1.019	0.9309	114.8	74.01	1.077	0.959	___	
				1.107		155.6		1.196		
Powell	0.0165	0.9966	1.065	1.006	88.15	72.46	___		0.011	-0.019
				1.124		103.8				0.041

IV. Conclusion

The main Focus of this paper is to estimate the parameters of these models using Gauss -Newton iteration procedure and to select the best fit model among these models based on an empirical data set of Bacterial growth rate. Good initial estimates are required to estimate the parameters from any iteration method. Also to specify good initial estimates of the parameters one should know the properties of the parameters. This paper developed some expressions to specify the initial values of the parameters based on the definitions and properties of the parameter of the kinetic models. These expressions will be very useful to specify the initial values of the parameters of the kinetic models in the bacterial growth rate data. From the result it is noticed that the Gauss Newton algorithm is a very useful method in case of the kinetic models.

Gauss Newton method of iteration has been investigated for rapid estimation of parameters of Blackman, Monod, Moser and Powell kinetic models. A comparative study has also been made based on a set of well-known bacterial growth rate data. It is noted from the above results that the Moser model produce the best fit for the growth rate data of *E. coli*. The Monod and Powell model also provides a satisfactory result for the data set. But unfortunately the Blackman model is failed to give a satisfactory fit to the data set.

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