The Levenberg-Marquardt method to fit parameters in the Monod kinetic model

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Abstract

Mathematical models can describe biochemical processes. Accurately measured data are fundamental for the estimation of the model parameters. This research uses the Monod model describing the bacterial kinetic degradation. The Levenberg-Marquardt method was applied successfully in order to fit the parameters of the model expressing the substrate concentration as a function of time. Hereby the method of steepest ascent and the iterative Gauss-Newton method with its quadratic convergence rate were used to find optimized parameter values of the Monod kinetic model. These results are compared with the results of other minimization methods. Orthogonal error measurement is introduced as uncertainty is present for all variables. This corrected type of error measurement is used to validate the parameter estimations.

Keywords: kinetic; biodegradation; optimization; mathematical modeling; bioprocess engineering; numerical simulation

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1 Introduction

Kinetic equations [4], which describe the activity of an enzyme on a substrate, are crucial in understanding many phenomena in biotechnological processes. Accurate models, based on quantitative experimental data, are required for the design and optimization of biological transformation processes. The main goal of this work was the construction of a valuable nonlinear regression model [1] in this application field of microbiology, inspired by the Monod kinetic model [9] and characterized by an accurate estimation of the parameters. Linear regression is insufficient because an enzyme reaction consists of two timescales with a different behaviour: the initial stage near $t = 0$ when there is little change in

the substrates concentration and a second stage when the substrates concentration changes significantly. Simkins and Robinson [12, 13] already determined whether the variety of shapes of substrate disappearance curves could be modeled with only the variables of substrate concentration and population density and the parameters of classical Monod kinetics. Compared to these previouly published relative papers, the novelty of this contribution is the application of the Levenberg-Marquardt algorithm [6, 7, 10, 3] or damped least-squares method, to obtain a highly accurate model for the considered enzyme kinetic problem.

2 Materials and methods

2.1 Monod kinetic model

In enzyme kinetics the reaction rate is measured to study the chemical reactions that are catalysed by enzymes. The study of the enzyme's kinetics can reveal the catalytic mechanism of this enzyme, its role in metabolism, how its activity is controlled, and how a drug or a poison might inhibit the enzyme. The idea of microbial growth kinetics has been dominated by an empirical model described in Eq. (1) originally proposed by Monod [9]. Monod's model introduced the concept of a growth limiting substrate by an empirical equation or an approximate quantification of reaction kinetics.

$$\mu = \frac{\mu_{max}\, S}{K_s + S} \tag{1}$$

Here μ is the growth rate and μ_{max} is the maximum growth rate specific for the enzyme. S is the substrate concentration and K_s is the substrate saturation constant (i.e. substrate concentration at half μ_{max}). In Monod's model, the microbial growth rate is related to the substrate concentration S of a single growth limiting substrate through the parameters μ_{max} and K_s. In the activity study, a distinction has to be made between resting conditions (no growth) and conditions of growth. The latter is characterized by a S-shaped substrate depletion curve expressing the sigmoidal kinetics [11].

2.1.1 Monod's no growth model

In case no growth is assumed, the rate of change of substrate consumption by a bacterium growing in batch, can be described by [9]

$$\frac{dS}{dt} = -\frac{\mu_{max}\, S}{K_s + S}\, X_0, \tag{2}$$

with the half-saturation constant for growth K_s, the initial biomass concentration X_0 and the maximum specific growth rate μ_{max} as parameters. Following the differential equation Eq. (2), the relation between the substrate concentration S and time t can be written as

$$K_s\, \ln \frac{S}{S_0} + S - S_0 = -X_0\, \mu_{max}\, (t - t_0). \tag{3}$$

In case of the no growth model, the sensitivity equations can be obtained by differentiation of Eq. (3) with respect to μ_{max} and K_s respectively:

$$K_s \frac{S_0}{S} \frac{1}{S_0} \frac{dS}{d\mu_{max}} + \frac{dS}{d\mu_{max}} = -X_0 (t - t_0) \Rightarrow \frac{dS}{d\mu_{max}} = \frac{-X_0 (t - t_0)}{1 + \frac{K_s}{S}} \tag{4}$$

$$\ln \frac{S}{S_0} + K_s \frac{S_0}{S} \frac{1}{S_0} \frac{dS}{dK_s} + \frac{dS}{dK_s} = 0 \Rightarrow \frac{dS}{dK_s} = \frac{-\ln \frac{S}{S_0}}{\frac{K_s}{S} + 1} \tag{5}$$

These sensitivity equations express the rate of change of the concentration S with varying parameters μ_{max} and K_s.

2.1.2 Monod's growth model

In case of the growth model, the rate of change of substrate consumption by a bacterium growing in batch, can be described by [9]

$$\frac{dS}{dt} = -\frac{\mu_{max} S}{K_s + S} \frac{X}{Y} \tag{6}$$

with the yield coefficient Y. The variable X is the biomass concentration and is linked at the substrate concentration by the mass balance relation

$$X = Y(S_0 - S) + X_0, \tag{7}$$

which makes that

$$\frac{dX}{dt} = -Y \frac{dS}{dt} \quad \text{and} \quad Y S_0 + X_0 = X + Y S. \tag{8}$$

The integrated form of Eq. (6) gives

$$C_1 \ln \frac{X}{X_0} - C_2 \ln \frac{S}{S_0} = \mu_{max} (t - t_0) \tag{9}$$

with $C_1 = (K_s Y + S_0 Y + X_0)/(Y S_0 + X_0)$ and $C_2 = K_s Y/(Y S_0 + X_0)$ because differentiating Eq. (9) with respect to t leads to

$$\frac{K_s Y + (Y S_0 + X_0)}{(Y S_0 + X_0)} \frac{1}{X} \frac{dX}{dt} - \frac{K_s Y}{(Y S_0 + X_0)} \frac{1}{S} \frac{dS}{dt} = \mu_{max}. \tag{10}$$

This is equivalent to Eq. (6) if $(Y S_0 + X_0)$ and $\frac{dX}{dt}$ are eliminated by means of Eq. (8).

2.2 Experimental setup

A chloropropham degrading biofilm culture was grown on plastic carriers (Biofilm-Chip M, anoxkaldnes Sweden) using minimal incubation medium which contained 1419.6 mg Na_2HPO_4, 1360.9 mg KH_2PO_4, 300 mg $(NH_4)2SO_4$, 98.5 mg $MgSO_4\,7H_2O$, 5.88 mg $CaCl_2\,H_2O$, 2.78 mg $FeSO_4\,7H_2O$, 1.69 mg $MnSO_4\,H_2O$,

1.15 mg $ZnSO_4\,7H_2O$, 0.38 mg $CuSO_4\,5H_2O$, 0.24 mg $CoCl_2\,6H_2O$, 0.12 mg $(NH_4)6Mo_{24}\,4H_2O$, 3.2 mg Na_2EDTA and 50 mg of chlororpropham per liter of distilled water [16, 17]. When all chloropropham was mineralized, 200 mg/l chloroproham was added to the medium until a biofilm became clearly visible on the added carriers . The biomass concentration was determined and expressed as the volatile suspended solid concentration. A batch test was set up in 250 ml autoclaved glass erlenmeyers containing 100 ml minimal medium with a final concentration of 60 mg/l chloropropham and 50 mg VSS/l culture (VSS=Volatile Suspended Solids) [14] to examine the chloropropham removal efficiency of the culture. Liquid samples for HPLC analysis were taken at 30 minutes intervals. Supernatants of the samples were analyzed by reverse-phase HPLC after the cells were removed by centrifugation at 5000 g for 10 minutes.

2.3 Analytical methods

Chloropropham and 3-chloroaniline were analysed using a high-performance liquid chromatography (HPLC) system (HP Agilent 1100 series) equipped with a G1322A degasser, a G1311A quaternary pump, a G1313A autosampler, a G1314A variable wavelength detector, a G1316A column compartment and HP Chemstation software. A Gracesmart RP-18 column (250- by 4.6-mm inner diameter, 5-m particle size; Grace, USA) was used. The mobile phase consisted of CH_3OH/0.1% H_3PO_4 (60/40) with a flow rate of 1.0 ml 1/min and the UV detector was set to 240 nm. Quantitative determination of chloropropham and 3-chloroaniline was done using an external standard ranging from 0.1 to 60 mg/l. The detection limit was $\pm$ 0.1 mg/l.

2.4 Curve fitting method

To model the evolution in time of the concentration S, it is necessary to find methods that can mathematically express their relation based on the measured concentration values at different time periods. The initial concentrations are $S_0 = 59.69\,\mathrm{mg/l}$ and $X_0 = 10\,\mathrm{mg/l}$.

Interpolation of the data points by means of polynomials (e.g. Lagrange interpolation), performs badly too much oscillatory. With the knowledge of the integrated forms Eq. (3) and Eq. (9), better results can be expected from curve fitting methods. To find the best estimations of the parameters in Eq. (3) and Eq. (9), the sum of squares of the differences $(t_i - t_i^{[pred]})$ can be minimized, with $(S_i,\, t_i)$ the i-th data point in the (concentration, time) field. $t_i^{[pred]}$ is the predicted value of time associated with the measured concentration S_i following the suggested model. The Levenberg-Marquardt algorithm is an iterative technique that locates the minimum of a function that is expressed as the sum of squares of nonlinear functions. It uses a combination of steepest descent and the Gauss-Newton method to handle nonlinear least-squares problems with a vectorial approach. An alternative method is the quasi-Newton method that works with an approximation for the Hessian which is built up from changes in the gradient.

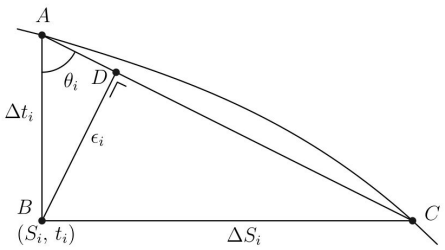

Figure 1: Orthogonal error measurement

The classical error measurement

$$E_{vert} = \sqrt{\sum_{i=1}^{n} (\Delta t_i)^2}, \quad \text{with } \Delta t_i = t_i - t_i^{[pred]}, \tag{11}$$

expresses the vertical error in the (S, t) plane with n the number of data points. A better error measurement tool is the orthogonal error

$$E_{orth} = \sqrt{\sum_{i=1}^{n} \epsilon_i^2} \tag{12}$$

$$\text{with} \qquad \Delta t_i = t_i - t_i^{[pred]} \text{ and } \Delta S_i = S_i - S_i^{[pred]} \tag{13}$$

and ϵ_i the orthogonal distance between (S_i, t_i) and the regression curve. From Figure 2 it can be derived that

$$\sin\theta_i = \frac{\Delta S_i}{\sqrt{(\Delta t_i)^2 + (\Delta S_i)^2}} \qquad \text{and} \qquad \sin\theta_i = \frac{\epsilon_i}{\Delta t_i} \tag{14}$$

as θ_i is a sharp angle in different rectangular triangles: ΔABC and ΔADB. This justifies that

$$\epsilon_i = \frac{\Delta S_i \, \Delta t_i}{\sqrt{(\Delta t_i)^2 + (\Delta S_i)^2}}. \tag{15}$$

3 Results and discussion

3.1 Initial parameter estimation

As the Levenberg-Marquardt method is an iterative method, an accurate initial estimation of K_s and $\mu_{max}$is recommendatory. Initial estimations of the parameters can be obtained from the discretized form Eq. (16) of the no growth differential equation Eq. (2).

$$\frac{\Delta t}{\Delta S} = -\frac{1}{S}\frac{K_s}{X_0\,\mu_{max}} - \frac{1}{X_0\,\mu_{max}}. \tag{16}$$

Estimations of the intercept and the slope when expressing $\frac{\Delta t}{\Delta S}$ as a function of $1/S$, lead to estimations of K_s and μ_{max} to start the iteration process based on the method of Levenberg-Marquardt. In the same way Eq. (17) can be used in case of growth based on Eq. (6).

$$X\,\frac{\Delta t}{\Delta S} = -\frac{1}{S}\,\frac{K_s\,Y}{\mu_{max}} - \frac{Y}{\mu_{max}}. \tag{17}$$

3.2 Feasibility

As for the no growth model the sensitivity coefficients $-dS/d\mu_{max}$ and dS/dK_s are linear dependent, there is no guarantee for a unique minimum. However, the two sensitivity coefficients are not proportional, so unique estimations of the parameters of the model can be expected [2].

In case of the Monod growth model, the sensitivity equations coefficients are almost proportional. Consequently the iteration process of the nonlinear regression will be hindered by the similar modes [2].

3.3 Regression analysis

Table 1: Parameter results after iteration process of curve fitting process for the growth model.

Parameter	Initial value	Value after convergence
μ_{max}(1/h)	0.0645	0.0386
K_s(mg/l)	5.79	11.22
Y	0.2	0.04

Table 2: Parameter results after iteration process of curve fitting process for the no growth model.

Parameter	Initial value	Value after convergence
μ_{max}(1/h)	0.290	0.998
K_s(mg/l)	5.203	8.852

A nonlinear regression analysis based on the Levenberg-Marquardt method for the no growth model and the growth model is performed by means of the software MATLAB [8]. The best fitted curves are plotted together with the data points in Figure 4 (no growth model and growth model) and do almost coincide.

Accurate initial parameter values were obtained from slope estimations of the linear regression model from Figure 3, also descriped in Eq. (16) and Eq. (17). The values of K_s and μ_{max} are estimated with results as in Table 1 and Table 2

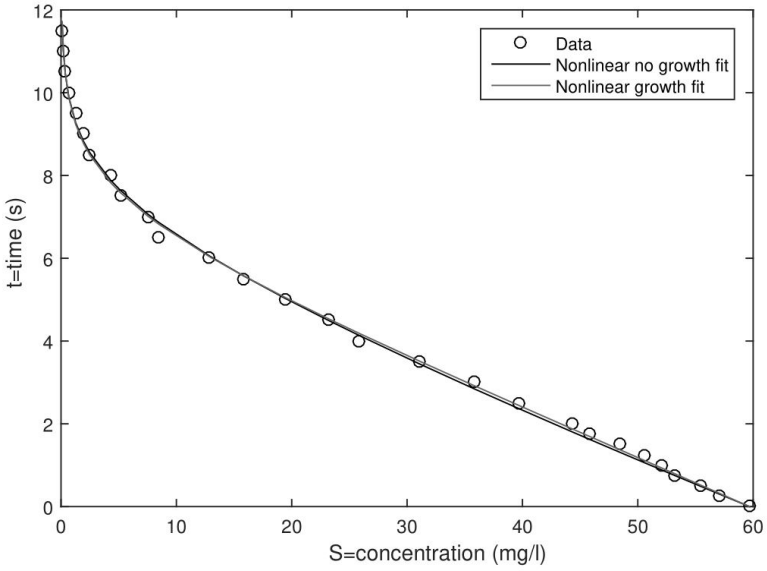

Figure 2: Orthogonal error measurement

for respectively the growth and the no growth model. Here Y is a dimensionless quantity as is expresses mg of biomass produced/mg of substrate consumed.

The no growth model requires less iterations and less function evaluations, while the growth model results in a smaller difference between the measured values and the predicted values of the model. The use of the R^2-value ($R^2 = \frac{\text{SSM}}{\text{SST}}$) to evaluate the regression models is typical for an analysis of variance. However here it is avoided as the model is non-linear [15] so the partitioning SST=SSE+SSM [5] of the total sum of squares into the sum of squares of the model SSM and the sum of squares of the error SSE does no longer hold. Table 3 also shows that the Levenberg-Marquardt method is superior to other iteration methods in iteration efficiency to reach the same model. Experiments with several more arbitrary initial parameter values lead to convergence difficulties in case of the growth model. This was predicted by the almost proportional sensitivity coefficients.

4 Conclusion

The Levenberg-Marquardt method performed as a valuable and efficient method to construct a nonlinear regression curve in this case of bacterial degradation kinetics. This is confirmed by several evaluation criteria: the number of function evaluations to calculate the cost, supplemented by an orthogonal error measurement to calculate the accuracy. This error measure takes into account

Table 3: Performance results after iteration process of curve fitting process

Model	Method	Number of iterations	Number of function evaluations	E_{orth} (seconds)
No growth	Levenberg-Marquardt	6	21	0.6775
	quasi-Newton	10	60	0.6775
	Simplex	62	117	0.6775
	Genetic Algorithm	116	5850	0.9084
Growth	quasi-Newton	32	152	0.5851
	Levenberg-Marquardt	26	121	0.5851
	Simplex	127	228	0.6775
	Genetic Algorithm	138	6950	1.264

inaccuracy for the independent time measurement as well as for the dependent concentration measurement. The initial values for the iteration process required to obtain the regression parameters, are estimated from the discrete form of the underlying differential equation. The feasibility of the iteration process is examined by considering the sensitivity equations, expressing the change in response of the concentration with a change in the parameters.

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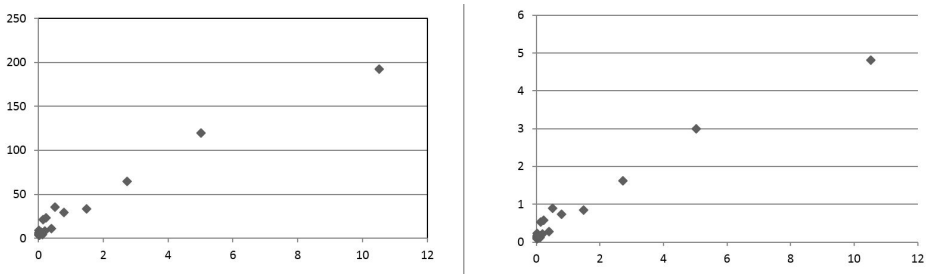

Figure 3: Data in the $(1/S, -X(\Delta t)/(\Delta S))$ diagram (left) and the $(1/S, -(\Delta t)/(\Delta S))$ diagram (right) enables the estimate of initial values of K_s and μ_{max}.

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