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ON PARAMETER ESTIMATION OF MONOD'S BACTERIAL GROWTH MODEL FROM BATCH CULTURE DATA

A. CORMAN[1] AND A. PAVE*

Laboratoire de Biologie des Sols (E.R.A. 848), Université Lyon I, Bâtiment 741, 43, boulevard du 11 Novembre 1918, 69622 VILLEURBANNE cedex, FRANCE
**Laboratoire de Biométrie (L.A. 243), Université Lyon I, Bâtiment 741, 43, boulevard du 11 Novembre 1918, 69622 VILLEURBANNE cedex, FRANCE*

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This is a report of a method to estimate the three parameters of Monod's bacterial growth model, μ_0, K_s and Y, by linearization and integration of data from batch culture experiments. This method can be used with either substrate or biomass data. The technique is tested on three sets of data, from the literature or from our laboratory. The accuracy of the values obtained for the parameters is appreciated by using them as initial estimates of a non linear fitting procedure. It is shown that in some cases the method can provide fairly precise estimates for the three parameters.

MONOD's equations (*1*) have been widely used to describe bacterial-substrate relationships in various situations: batch and continuous culture, and for different environmental conditions: pure culture, soil or activated sludge.

Though many of these uses are theoretically subject to criticism (in the case of non steady state conditions, for instance) (*2*, *3*), the original model or extensions made to take into account maintenance metabolism, limiting effects of high substrate concentration (*4*), temperature, dissolved oxygen or pH effects (*5–7*), still provide an important conceptual basis for bacterial kinetics studies, because of the biological significance of the parameters involved (*8*).

However, estimation of these parameters is not easy. Experimentally, if maximum growth rate can be obtained from batch studies, determination of the saturation constant is often tedious, requiring chemostat cultures over long time periods especially for slow growing organisms (*9*), although this parameter has been

[1] To whom correspondence should be sent. Present address: Laboratoire de Biométrie, Université Lyon I, Bâtiment 741, 43, boulevard du 11 Novembre 1918, 69622 VILLEURBANNE cedex, FRANCE.

shown to account for an issue of competition between species (*10*) or diffusion limiting effects (*11*).

Mathematically, a major drawback of this model is the fact that its sole analytical expression yields time as the predicted variable, which hinders its flexibility and complicates parameter estimation.

Thus to avoid this disadvantage, one must use other models providing analytical expression of biomass as a function of time. For instance, the generalized logistic equation offers this characteristic (and some other advantages (*12*)), but its use involves many more parameters, some of which have no direct biological meaning. Therefore, the effect of limiting substrate is not explicitly considered.

Because of non-linearity, Monod parameter estimation requires an iterative method for sum of squares minimization. The problem is to get accurate initial estimates, to ensure that the algorithm will converge to values corresponding to a global minimum.

The two basic Monod assumptions are:

1-Growth yield is constant over all substrate consumption:

$$x-x_0=-Y(s-s_0) \tag{1}$$

where x is the biomass at time t ($x=x_0$ at $t=0$); s is the concentration of limiting substrate at time t ($s=s_0$ at $t=0$); Y is the growth yield (unit of biomass·unit of of substrate^{-1}).

Derivating (1) gives:

$$\frac{dx}{ds}=-Y \tag{2}$$

2-Growth rate is a hyperbolic function of substrate concentration:

$$\frac{dx}{dt}=\mu x=\frac{\mu_0 s}{K_s+s}x \tag{3}$$

with μ : specific growth rate, having maximum value μ_0 (unit of time^{-1})
K_s: substrate concentration ensuring $\mu=(\mu_0/2)$ (unit of substrate)

This paper reports a method to get suitable initial estimates for the three parameters of eq. (1) and eq. (3), from batch culture data. The principle of the method is to find a linear expression of the model by combination of experimental data. An analog method was proposed previously for other models which are written in terms of differential equations (i. e. multilinear differential systems of chemical kinetics (*13*)). The technique is applied to three sets of data from published studies or from our laboratory.

Because our method does not take into account the classical model of error (i. e. normality, zero-mean, additivity (*14*)), the accuracy of the estimations obtained has to be tested by using them as initial values of a non linear fitting pro-

cedure. Statistical properties of estimates will then be studied by applying estimation procedures on a hundred randomized data sets.

THEORY

From eq. (1), substrate can be written as a function of biomass:

$$s=s_0-(x-x_0)/Y \tag{4}$$

Combining eq. (3) and eq. (4) leads to:

$$\frac{dx}{dt}=\frac{\mu_0(s_0-(x-x_0)/Y)x}{K_s+s_0-(x-x_0)/Y} \tag{5}$$

Multiplying each side of eq. (5) by $K_s+s_0-(x-x_0)/Y$ and rearranging gives:

$$\left(K_s+s_0+\frac{x_0}{Y}\right)\frac{dx}{dt}-\frac{x}{Y}\frac{dx}{dt}=-\frac{\mu_0}{Y}x^2+\mu_0\left(s_0+\frac{x_0}{Y}\right)x \tag{6}$$

Integrating eq. (6) and rearranging, we finally get:

$$\frac{1}{2}(x^2-x_0^2)=(Y(K_s+s_0)+x_0)(x-x_0)-\mu_0(Ys_0+x_0)\int_0^t x\,dt+\mu_0\int_0^t x^2\,dt \tag{7}$$

Experimental results can be summarized by $n+1$ couples (t_0, x_0), (t_1, x_1), . . . , (t_i, x_i), . . . , (t_n, x_n), when t_i is the time at which biomass x_i has been determined. Then, if we denote:

$$R_i=\frac{1}{2}(x_i^2-x_0^2)$$

$$U_i=(x_i-x_0)$$

$$V_i=\int_0^{t_i} x\,dt$$

$$W_i=\int_0^{t_i} x^2\,dt$$

and

$$A=Y(K_s+s_0)+x_0,\quad B=-\mu_0(Ys_0+x_0),\quad C=\mu_0,\quad \text{eq. (7) becomes}$$

$$R_i=AU_i+BV_i+CW_i \tag{8}$$

Equation (8) is a linear expression where R_i, U_i, V_i and W_i can be calculated from experimental data. Calculation of V_i and W_i requires numerical integration of the data. This has been done by using a parabolic approximation on three successive points (Appendix I).

Then A, B and C can be estimated by classical multilinear regression which consists of minimizing the following sum of squares:

$$S=\sum_{i=1}^{n}[R_i-(AU_i+BV_i+CW_i)]^2$$

The well-known solution is

$$\hat{\Theta}=(X^tX)^{-1}XY$$

where X is the $n\times 3$ matrix $X=\begin{pmatrix} U_1 & V_1 & W_1 \\ U_2 & V_2 & W_2 \\ \vdots & \vdots & \vdots \\ U_n & V_n & W_n \end{pmatrix}$

Y is the $n\times 1$ matrix $Y=\begin{pmatrix} R_1 \\ R_2 \\ \vdots \\ R_n \end{pmatrix}$

and

$\hat{\Theta}$ the vector of parameters estimates $\hat{\Theta}=\begin{pmatrix} \hat{A} \\ \hat{B} \\ \hat{C} \end{pmatrix}$

Then estimates of parameters μ_0, K_s and Y can be obtained easily:

$$\mu_0=C$$

$$Y=-\frac{1}{s_0}\left(x_0+\frac{B}{C}\right)$$

$$K_s=\frac{1}{Y}(A-x_0)-s_0$$

REMARK

From eq. (1), the same approach can be taken using substrate instead of biomass as predicted variable.

Then the terms of eq. (8) become:

$$R_i=\frac{1}{2}(s_i^2-s_0^2)$$

$$U_i=(s_i-s_0)$$

$$V_i=\int_0^{t_i} s\,dt$$

$$W_i=\int_0^{t_i} s^2\,dt$$

and for the constants:

$$A=-K_s,\quad B=-\mu_0\left(s_0+\frac{x_0}{Y}\right),\quad C=\mu_0.$$

METHODS

The program of initial estimation was written in BASIC, including the following procedures:

1) integration of time dependent variable and its square by parabolic approximation (Appendix I).

2) parameter estimation by multiple linear regression.

3) simulation of the process, with estimates obtained, by numerical integration of differential equation (a classical RK4 method was used). Goodness of fit was appreciated by calculating the following ratio:

$$T=\frac{\sum_{i=1}^{n}\left|\frac{\text{(calculated value)}-\text{(observed value)}}{\text{(observed value)}}\right|}{n}\times 100$$

T is the average relative error (*12*), n is the number of data estimated. The first value is not estimated but used as initial condition for integration. Then for final parameter estimation, an adaptation of the Gauss Marquardt procedure proposed by FLETCHER (*14*) (Harwell Library) was used to minimize the following sum of squares:

$$S=\sum_{i=1}^{n}\{x_i-f(t_i, \mu_0, K_s, Y)\}^2$$

This procedure requires calculation of the sensitivity functions T (i.e. partial differential equations with respect to parameters μ_0, K_s and Y) (Appendix II).

Calculations were done on an S 140 Data General Computer under Advanced Operating System.

RESULTS

Three sets of data were used. The first, obtained by MONOD (*1*), concerns *Escherichia coli* growth on glucose. As shown in Table 1 (col. 2, 3 and 4) and Fig. 1, there is good agreement between experimental and calculated data. Estimates obtained with the linearization method are rather good. Parameter values are not greatly modified by the use of the non linear fitting method and the average relative error is not significantly lowered (Table 5).

By interpolation of his own data, Monod estimated μ_0 ($=1.35$ div$\cdot$hr$^{-1}=0.935$ hr^{-1}) and K_s ($=4$ mg), Y ($=0.295$ unit O.D. mg^{-1}). These values are close to our estimations except for K_s, which can be explained, as shown below, by the great variability of this parameter.

A statistical study of the 3 parameters of the model has been made using this set of data, by considering each calculated cell concentration as the mean of a Gaussian distribution whose variance was estimated from the residual sum of squares $\{\sigma^2=\text{Res.S.S.}/(n-3)\}$, because 3 parameters have been estimated. So 100 randomized data sets have been obtained on which the two estimation methods are

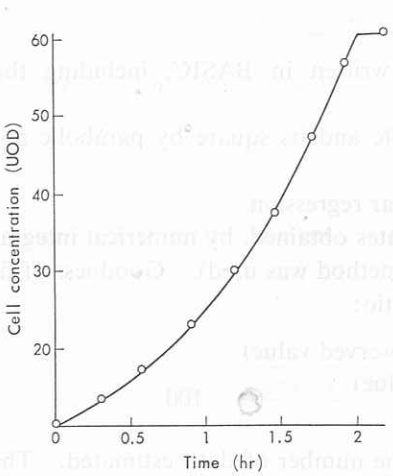

Fig. 1.

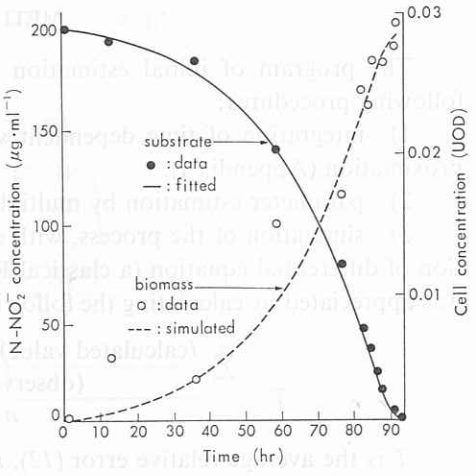

Fig. 2.

Fig. 1. Growth kinetics of *Escherichia coli* on glucose. Experimental data are from MONOD (*1*) and curve is calculated with final estimates (see Table 5).
○, Data values; —, fitted values.

Fig. 2. Growth kinetics of *Nitrobacter winogradskyi* and $N\text{-}NO_2^-$ consumption (Full line: calculated $N\text{-}NO_2^-$ concentration with non linear fitting procedure (see Table 5)–Dotted line: calculated optical density with parameter values obtained on substrate).

Table 1. Comparison of observed values with calculated values. Experimental data are issued from the 1 st run of MONOD (*1*) with *E. coli* grown on glucose.

Time (hr)	Observed cell conc. (U.O.D.)	Calculated cell conc. (U.O.D.)[a]	Calculated cell conc. (U.O.D.)[b]
0	10.2	—	—
0.3	13.2	13.3	13.2
0.57	16.8	16.8	16.8
0.90	22.5	22.4	22.4
1.2	29.4	29.2	29.2
1.47	36.5	36.9	36.9
1.72	46.0	45.8	45.8
1.95	55.5	55.5	55.5
2.21	59.5	59.3	59.5

[a] Estimation made with initial guesses.
[b] Estimation made with final guesses.

applied. The statistical properties of the estimates can be derived (Table 2).

At first, the 9 data points of Monod's experiment were randomized, but this lead to too large a variance for K_s, so that almost 50% of estimates obtained for this parameter were negative. To avoid this, two points have been added between the

Table 2. Statistics on Monod parameters obtained on 100 randomized data sets with two estimation method (values obtained with initial estimation procedure).

	μ_0	K_s	Y
Mean	0.892 (0.894)	1.71 (2.24)	0.310 (0.310)
Population ±confidence limits $P=0.95$	0.034 (0.040)	1.66 (2.80)	0.003 (0.003)
Correlation coefficients	1	0.93 (0.96) 1	0.81 (0.81) 0.70 (0.74) 1

Table 3. Observed and predicted $N\text{-}NO_2^-$ concentrations during the course of a batch culture of *Nitrobacter winogradskyi* (data from our laboratory).

Time (hr)	Observed $N\text{-}NO_2^-$ conc. ($\mu g \cdot ml^{-1}$)	Calculated $N\text{-}NO_2^-$ conc. (initial guess)	Calculated $N\text{-}NO_2^-$ conc. (final guess)
0	199.9	—	—
12	192.2	195.8	195.5
36	181.35	179.8	178.1
59	136.4	143.4	138.7
77	77.5	84.6	75.7
83	44.95	55.9	45.4
85	34.1	45.2	34.5
87	22.78	34.1	23.5
89	14.1	22.8	13.2
92	3.04	7.5	2.5
93	0	3.8	1.0

last two, at time 2.0 and 2.1. Experimentally this emphasizes the importance of an intensified sampling at the end of the kinetics. Even so the dispersion of K_s remains large. Comparing the two estimation methods, it appears that means and variances are close in the two cases. We only noted that the non-linear fitting procedure tends to normalize parameter distributions. However the high correlation between parameters must be kept in mind when comparing estimations made on different experiments.

Another test was made with data obtained during the growth of *Nitrobacter winogradskyi* on a medium containing 200 $\mu g \cdot ml^{-1}$ N as $NaNO_2$ (unpublished data from our laboratory; methods and medium are in (*15*); temperature is 28°). Parameter estimation was made on substrate measurements because they had a somewhat better precision than those made on biomass by optical density. Comparison between observed and calculated values is shown in Table 3 and in Fig. 2. Here the use of the non-linear method has significantly increased the precision of the fitting, as shown by the decrease of the relative error (Table 5). (In this case relative error does not take into account the last point because the experimental value

equals 0). Use of the parameters obtained on substrate leads to a good simulation of the course of the biomass growth (Fig. 2).

The last set of data used in the program is that of Luedecking, cited by EDWARDS

Table 4. Observed and predicted cell concentrations during the growth of *Lactobacillus delbruckii* (data of LUEDECKING, cited by EDWARDS and WILKE (*12*)).

Time (hr)	Observed cell conc. (U.O.D/ml)	Calculated cell conc. U.O.D./ml (initial guess)	Calculated cell conc. U.O.D./ml (final guess)
1.00	0.123	—	—
2.00	0.139	0.192	0.191
3.00	0.283	0.299	0.296
4.00	0.442	0.465	0.458
5.50	0.82	0.894	0.875
6.25	1.12	1.23	1.20
7.17	1.77	1.81	1.76
7.70	2.24	2.25	2.18
8.25	2.73	2.81	2.71
9.00	3.77	3.74	3.60
9.53	4.43	4.51	4.35
10.00	5.00	5.26	5.08
10.50	5.72	6.11	5.90
11.00	6.78	6.95	6.74
11.50	7.51	7.73	7.52
12.00	8.23	8.37	8.21
12.33	8.63	8.71	8.58
12.67	8.78	8.97	8.89
13.00	9.27	9.17	9.13
13.50	9.49	9.36	9.38
14.00	9.38	9.47	9.52

Table 5. Monod parameters estimates and average relative error obtained with two estimation methods on three sets of experimental batch culture data: (1) method of linearization after integration of data. (2) non-linear least square minimization (Gauss-Mararquardt method).

		Escherichia coli on glucose	*Nitrobacter winogradskyi* on $NaNO_2$	*Lactobacillus delbruckii*
Estimations made on		(biomass)	(substrate)	(biomass)
μ_0	(1)	0.886	0.0382	0.680
	(2)	0.886	0.0387	0.692
K_s	(1)	1.54	6	52.7
	(2)	1.51	7.5	57.0
Y	(1)	0.307	0.139×10^{-3}	0.0948
	(2)	0.309	0.130×10^{-3}	0.0960
Average relative error	(1)	0.47%	37%	5.1%
	(2)	0.33%	4.2%	4.1%

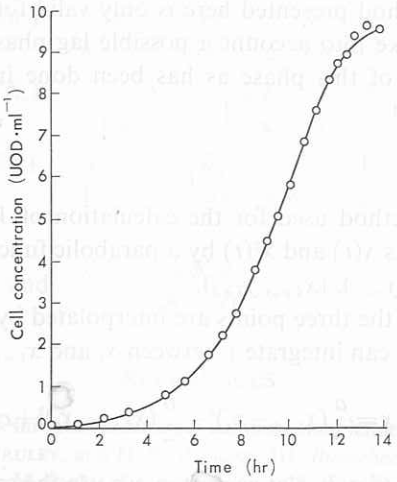

Fig. 3. Growth kinetics of *Lactobacillus delbruckii*. Experimental data are from LUEDECKING, cited by EDWARDS and WILKE (*12*) and the curve is calculated with final estimates (see Table 5).

○, Data values; —, fitted values.

and WILKE (*14*). The bacterium involved in this batch kinetic study is *Lactobacillus delbruckii*. As no substrate data was available, initial substrate concentration was arbitrarily chosen as 100, and the yield was assumed to be constant.

Agreement between observed and calculated data is still rather good (Table 4, Fig. 3). As in the first case, estimates obtained by our method are near those given by the non-linear method (Table 5). The use of a generalized logistic model to represent this set of data leads to an average error of 4. 1 % (*12*), but requires the use of 7 parameters.

CONCLUSION

The method developed here provides fairly good initial estimates for a non-linear procedure of fitting to experimental data from batch cultures. In some cases parameter values obtained in this way are close to final estimates obtained by sum of squares minimization. This method can be easily implemented on micro or mini computers, so that many experimenters can use it. But, as with most linearization methods, its use theoretically introduces a bias, because the assumption of additive errors on the linearized model is not verified; but the smaller the experimental errors the lower will be the bias. Clearly the way chosen to solve the problem depends on the objectives; in particular, if identification is made for the command of a process, it will be necessary to get more accurate estimates for parameters (by successive use of the initial estimation method, by of non-linear least squares procedure and possibly by further experimentation after analysis of sensitivity functions).

Moreover, the method presented here is only valid for data from the growth phase, and does not take into account a possible lag phase. It can be modified easily to fit the length of this phase as has been done in some cases for other models (*16*).

Appendix I.

The integration method used for the calculation of V_i and W_i (eq. (8)) approximates the functions $x(t)$ and $x^2(t)$ by a parabolic function on three successive points: (x_i, y_i), (x_{i+1}, y_{i+1}), (x_{i+2}, y_{i+2}).

If we consider that the three points are interpolated by a function of the type: $y=ax^2+bx+c$, then we can integrate y between x_i and x_{i+1}, obtaining:

$$\int_{x_i}^{x_{i+1}} y(x)\mathrm{d}x=\frac{a}{3}(x_{i+1}-x_i)^3+\frac{b}{2}(x_{i+1}-x_i)^2+c(x_{i+1}-x_i)$$

For determination of a, b, c at each step, we use the set of the three following equations, yielding three unknowns

$$ax_i^2+bx_i+c=y_i$$
$$ax_{i+1}^2+bx_{i+1}+c=y_{i+1}$$
$$ax_{i+2}^2+bx_{i+2}+c=y_{i+2}$$

This system admits the following solutions:

$$a=\frac{(y_{i+2}-y_i)(x_{i+1}-x_i)-(y_{i+1}-y_i)(x_{i+2}-x_i)}{(x_{i+2}^2-x_i^2)(x_{i+1}-x_i)-(x_{i+2}-x_i)(x_{i+1}^2-x_i^2)}$$

$$b=\frac{y_{i+1}-y_i-a(x_{i+1}-x_i^2)}{(x_{i+1}-x_i)}$$

$$c=y_i-bx_i-ax_i^2$$

With this integration method, there is no need to consider constant and even number of time intervals as in, for example, Simpson's rule.

Appendix II.

Non-linear fitting procedure requires calculation of the partial differential equations with respect to parameters (sensitivity functions).

So if we consider the model:

$$f(x)=\frac{\mathrm{d}x}{\mathrm{d}t}=\mu_0\frac{xs}{K_s+s}$$

with

$$s=s_0-\frac{1}{Y}(x-x_0)\,.$$

The three sensitivity functions are given by:

$$\frac{d(\partial x/\partial \mu_0)}{dt} = \frac{xs}{K_s+s} + \left(\frac{\partial f}{\partial x} - \frac{1}{Y}\frac{\partial f}{\partial s}\right)\frac{\partial x}{\partial \mu_0}$$

$$\frac{d(\partial x/\partial K_s)}{dt} = -\frac{\mu_0 xs}{(K_s+s)^2} + \left(\frac{\partial f}{\partial x} - \frac{1}{Y}\frac{\partial f}{\partial s}\right)\frac{\partial x}{\partial K_s}$$

$$\frac{d(\partial x/\partial Y)}{dt} = \frac{1}{Y^2}(x-x_0)\frac{\partial f}{\partial s} + \left(\frac{\partial f}{\partial x} - \frac{1}{Y}\frac{\partial f}{\partial s}\right)\frac{\partial x}{\partial Y}$$

with

$$\frac{\partial f}{\partial x} = \frac{\mu_0 s}{K_s+s}, \quad \text{and} \quad \frac{\partial f}{\partial s} = \frac{\mu_0 K_s x}{(K_s+s)^2}.$$

REFERENCES

1) J. Monod, Recherches sur la croissance des cultures bactériennes, Herman ed., Paris (1942).
2) T. B. Young, D. F. Bruley, and H. R. Bungay, III, *Biotechnol. Bioeng.*, **12**, 747 (1970).
3) C. P. Jeffreson and J. M. Smith, *Chem. Eng. Sci.*, **28**, 629 (1973).
4) V. H. Edwards, *Biotechnol. Bioeng.*, **12**, 679 (1970).
5) H. Laudelour, R. Lambert, J. L. Fripiat, and M. L. Pham, *Ann. Microbiol.* (*Inst. Pasteur*), **125–B**, 75 (1974).
6) H. Laudelout, R. Lambert, and M. L. Pham, *Ann. Microbiol.* (*Inst. Pasteur*), **127-A**, 367 (1976).
7) D. N. Ryder and C. G. Sinclair, *Biotechnol. Bioeng.*, **14**, 787 (1972).
8) M. J. Bazin, P. T. Saunders, and J. I. Prosser, CRC critical reviews in microbiology, 463 (1976).
9) K. J. Williamson and P. L. McCarty, *Biotechnol. Bioeng.*, **17**, 915 (1975).
10) H. Veldkamp and H. W. Jannasch, *J. Appl. Chem. Biotechnol.*, **22**, 105 (1972).
11) M. K. Stenstrom and R. A. Poduska, *Water Res.*, **14**, 643 (1980).
12) V. H. Edwards and C. R. Wilke, *Biotechnol. Bioeng.*, **10**, 205 (1968).
13) A. Pave, Contribution à la théorie et à la pratique des modèles mathématiques pour l'analyse dynamique des systémes biologiques. Etudes de quelques cas typiques en biologie cellulaire et moléculaire. Thèse Doct. ès Sciences, Université Lyon I, France (1980).
14) J. V. Beck and K. J. Arnold, Parameters Estimation in Engineering and Science, J. Wiley and Sons, New York (1977).
15) A. Josserand, G. Gay, and G. Faurie, *Microb. Ecol.*, **7**, 275 (1981).
16) N. C. Chiang, Etude thermodynamique et cinétique de la croissance de *Nitrobacter* sp., Thèse Doct. ès Sci., Univ. Catholique LOUVAIN, Belgium (1969).