Bioprocess Engineering 11 (1994) 185–188 © Springer-Verlag 1994

Extended monod kinetics for substrate inhibited systems

A. Sivakumar, T. Srinivasaraghavan, T. Swaminathan, A. Baradarajan

Abstract The biochemical route is identified to be one of the simplest and cheapest means by which valuable chemicals are being synthesized. Microorganisms play a vital role in carrying out these processes. However, the kinetic studies relevant to this process is scarce. Most often inhibition effects due to either cells or substrates or products affect the performance of such processes. This paper deals with the study of various model equations for substrate inhibition kinetics. An attempt has been made to study the applicability of various model equations for substrate inhibited systems by fitting their experimental data and evaluating various parameters including the standard deviations in each case. Finally, a new model has been brought out which gives the best fit for almost all the systems.

List of symbols

C_s	g/l	substrate concentration
C_{s0}	g/l	threshold substrate concentration
K_I	g/l	inhibition constant
K_M, K_s	g/l	half saturation constant of growth kinetics
m, n	–	constants
μ	1/h	specific growth rate
μ_{max}	1/h	maximal specific growth rate

1 Introduction

The production of various industrially important compounds are being carried out using microbes. The fermentative processes offer a great deal of advantage in terms of reducing the process cost and the raw material utility [1]. However, these processes are governed by the specificity of the microorganisms and the metabolic regulations are, in turn, dependent on the process parameters. Hence, monitoring the growth of microorganisms, their behaviour towards various levels of substrates and their role in the overall productivity of the process needs careful kinetic studies. In most of the biotechnological processes, high concentrations of substrates or products often lead to inhibitory effects, which results in poor utilisation of the substrates [2]. This also decreases both the product yields and fermentation rates. The metabolic processes of the microorganisms are inhibited by certain nutrients at sufficiently higher levels [3]. This paper deals with the approach towards identification and formulation of a suitable mathematical model for regulation and application of certain microbial processes with relevance to substrate inhibition kinetics.

2 Development of the model equations

The growth of the microorganisms is a very complex phenomenon. One of the simplest and the most widely used model for growth kinetics was proposed by Monod.

$$\mu = \mu_{max} C_s/(K_M + C_s). \quad (1)$$

However it has also failed in some cases, where unusual or extraordinary K_M values can be obtained due to multi-S-limitation, insufficient mixing in the liquid phase, external transport limitation, internal transport limitation, ionic strength, high cell concentration, endogenous metabolism, non-stationary processing, product inhibition and biosorption [3].

The value of μ according to Monod kinetics approaches it's asymptote too slowly to be a proper approximation of the experimental data even in simple cases.

In most of the cases, inhibition effects due to excess of one of the nutrients affects the growth rate. The substrate inhibition in particular occurs at higher levels. The high osmotic shock is induced at these levels of substrates resulting in a decrease in the water activity. The model equations for substrate inhibition are like Monod's relationship, being derived from theories on the inhibition of a single enzyme [4]. The inhibition kinetics for a single inhibitor [5] is given in Table 1.

The effect of substrate and product inhibition using Saccharomyces cerevisiae for ethanol production was studied by Thatipamala et al. [6]. A general model for microbial growth under any form of dual limitation was proposed by Mankad et al. [7].

3 Results and discussion

In our attempt to study the substrate inhibition phenomenon, we have chosen the systems as given in the Table 3. Among these the data for the production of 2,3-butanediol by K. oxytoca were obtained by performing batch culture studies [8].

The model equations given in the Table 2 were fitted by graphical method into the various systems as given in the Table 3.

Received 25 August 1993

A. Sivakumar, T. Srinivasaraghavan, T. Swaminathan, A. Baradarajan
Centre for Biosciences and Biotechnology, Indian Institute of Technology, Madras-600 036, India

Correspondence to: A Baradarajan

The authors wish to thank Mr. S. Venkatesan for the excellent typing of the manuscript.

It is seen that Han and Levenspiel model gives the best fit for all the systems. Among the other three models the Wayman and Tseng model fits reasonably well but marginally better than Haldane and Andrews and Noack models (Figs. 1–5).

Haldane and Andrews and Noack models gave almost similar values as the equations are similar (recall Eqs. (2) and (3) in Table 2). Since $K_s C_s/K_I$, the extra factor in the denominator of Eq. (3) is not large compared to the other factors, the two models yield similar results. The Han and Levenspiel model, Eq. (5), gives a better fit especially at higher levels of substrate due to the following reasons.

At low substrate concentrations, the second term in the denominator of Eq. (5), i.e. $K_M(1-C_s/K_I)^m$, has a significant effect as the factor $(1-C_s/K_I)$ is appreciable. But as the substrate concentration increases, this factor keeps decreasing and at high substrate concentrations $C_s/[C_s+K_s(1-C_s/K_I)^m]$ tends to 1.

Table 1. Inhibition kinetics for a single inhibitor

Model	Year	Reference
Haldane	1930	13
Webb	1963	3
Ierusalimsky	1965	3
Andrews and Noack	1968	3
Yano et al.	1969	3
Edwards	1970	13
Yano and Koya	1973	14
Wayman and Tseng	1976	12
Ghose and Tyagi		
Dagley and Hinshelwood	1952	3
Chen et al.	1976	15
Bazua and Wilke	1977	3
Levenspiel	1980	3
Han and Levenspiel	1988	2
Present model	1993	This work

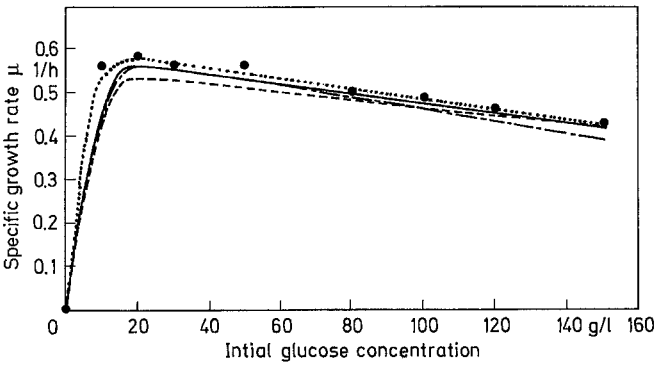

Fig. 1. Effect of initial glucose on the specific growth rate of K. oxytoca. ● experimental values of μ, predicted values of μ using Han and Levenspiel model (——), Haldane model (– – – –), Wayman and Tseng model (— – — –), Andrews and Noack model (— . . — . .), Present model (.....)

Table 2. Models representing substrate inhibition

Model	Form of normalized kinetics	Equation
Haldane (1965)	$\dfrac{C_s}{K_M+C_s+C_s^2/K_I}$	(2)
Andrews and Noack (1968)	$\dfrac{C_s}{(C_s+K_s)(1+C_s/K_I)}$	(3)
Wayman and Tseng (1976)	$\dfrac{C_s}{(K_M+C_s)}-K_I\min(C_s-C_{s0})$	(4)
Han and Levenspiel (1988)	$(1-C_s/K_I)^n\dfrac{C_s}{C_s+K_M(1-C_s/K_I)^m}$	(5)
Present model (1993)	$(1-C_s/K_I)^n\dfrac{C_s}{C_s+K_M(1-C_s/C_{s0})^m}$	(6)

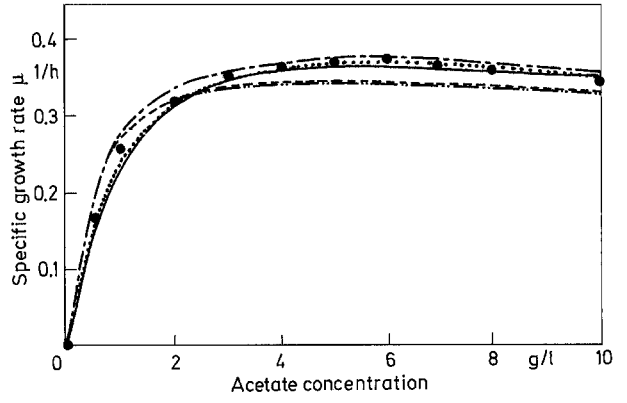

Fig. 2. Effect of sodium acetate on the specific growth rate of C. utilis. ● experimental values of μ, predicted values of μ using Han and Levenspiel model (——), Haldane model (– – – –), Wayman and Tseng model (— – — –), Andrews and Noack model (— . . — . .), Present model (.....)

Table 3. Systems exhibiting substrate inhibition and their calculated kinetic constants

System	Microorg.	Substrate	Conc. (g/l)	μ_m (h^{-1})	K_M (g/l)	K_I (g/l)	n	m	Fig.	Ref.
1	K. oxytoca	D-Glucose	0–150	0.586	0.75	400	0.708	3.813	1	8
2	C. utilis	CH_3COONa	0–10	0.406	0.43	50	0.746	0.691	2	9
3	C. utilis	CH_3COONa	0–3	0.399	0.17	10	1.492	1.360	3	10
4	Mixed culture	Sucrose	0–4	0.745	0.36	8	0.749	1.397	4	11
5	P. methanica	Methanol	0–50	0.220	1.28	57	0.860	1.765	5	12

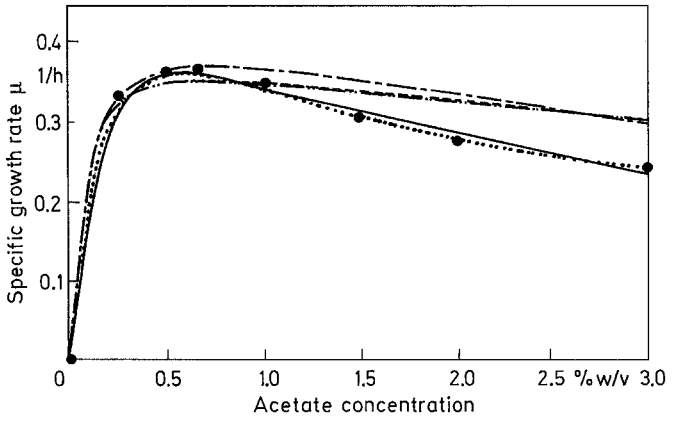

Fig. 3. Effect of sodium acetate on the specific growth rate of C. utilis. ● experimental values of μ, predicted values of μ using Han and Levenspiel model (——), Haldane model (– – – –), Wayman and Tseng model (— — — —), Andrews and Noack model (— . . — . .), Present model (.....)

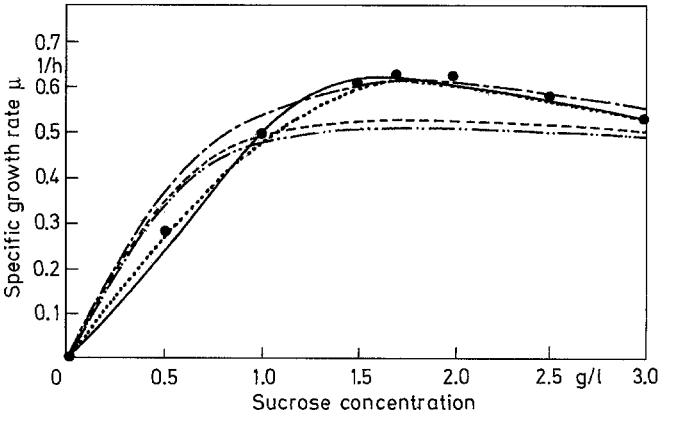

Fig. 4. Effect of sucrose on the specific growth rate of a mixed culture. ● experimental values of μ, predicted values of μ using Han and Levenspiel model (——), Haldane model (– – – –), Wayman and Tseng model (— — — —), Andrews and Noack model (— . . — . .), Present model (.....)

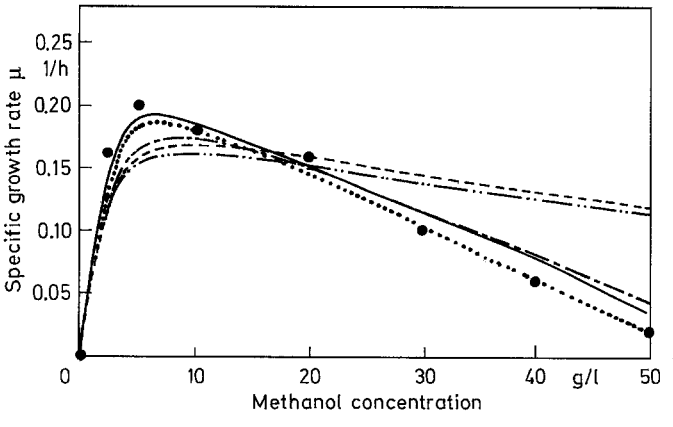

Fig. 5. Effect of methanol on the specific growth rate of P. methanica. ● experimental values of μ, predicted values of μ using Han and Levenspiel model (——), Haldane model (– – – –), Wayman and Tseng model (— — — —), Andrews and Noack model (— . . — . .), Present model (.....)

Table 4. Standard deviation values

	Han and Levenspiel	Haldane	Wayman and Tseng	Andrews and Noack	Present model
System 1	0.0135	0.0255	0.0242	0.0289	0.0125
System 2	0.0053	0.0244	0.0221	0.0254	0.0045
System 3	0.0150	0.0340	0.0365	0.0325	0.0054
System 4	0.0321	0.0797	0.0606	0.0880	0.0052
System 5	0.0121	0.0518	0.0188	0.0497	0.0126

Hence it declines as a function of $(1-C_s/K_I)^m$. In the Haldane model, Eq. (2),

$$\mu=\frac{\mu_{\max} C_s}{1+(K_m/C_s)+(C_s/K_I)} \tag{7}$$

at low substrate values $K_m/C_s \gg C_s/K_I$, hence it increases as a function of C_s. Later C_s/K_I becomes significant as C_s increases, however the magnitude is not large enough for the model values to drop accurately. Hence, the Han and Levenspiel model gives a better fit. In the Wayman and Tseng model, Eqn (4) in Table 2, a correction factor has been added to the Monod model to fit for the inhibition phase, which is not as accurate as the Han and Levenspiel model.

From the comparison of the various standard deviation values, it was clear that the Han and Levenspiel model fitted the systems best. Further, it was also noticed that it was in the initial stages that the model showed maximum deviation from the experimental points and in most cases, it was below the experimental curve. Hence, an attempt was made to increase the value of μ as found by the model. After trying various combinations, we found that changing the K_I in the denominator to C_{s0}, i.e. threshold substrate concentration, reduced the denominator, thereby increasing the value of μ appropriately to fit the experimental curve better. The corresponding standard deviation values of the present model have also been listed out in Table 4. The Table 4 and Figs. 1–5 clearly shows that the new model fits most systems better than the Han and Levenspiel model. The model equation has been divided into two parts – one for substrate concentrations less than C_{s0} and other for those greater than C_{s0}. Among the model equations represented in Table 2, the following Table 4 gives the standard deviation values of the systems for the best fitting models.

4 Concluding remarks

Among the model equations, the Han and Levenspiel model and the Haldane model seem to give a reasonable fit for all the systems studied. In most of the cases, the Han and Levenspiel model gives a better fit. However, the present model which is a modification of the above model gives the best fit for all the systems.

References

1. **Underkoefler, L. A.; Hickey, R. J.:** Industrial fermentations, Vol 1, pp. 1–13. New York: Chemical Publishing Co. 1954
2. **Han, K.; Levenspiel, O.:** Biotechnol. Bioeng. 32 (1988) 430–437
3. **Moser, A:** Biotechnology, Rehm, H. -J. and Reed, G. (Eds), vol. 2. Verlagsgesellschaft mBH, Weinheim(FRG) 1985
4. **Dixon, M.; Webb, E. C.:** Enzymes, pp. 62–181. London: Longmans, Green 1958

5. **Bellgardt, K. H.:** Cell models. In: Biotechnology, vol. 4, Verlagsgesellschaft mBH, Weinheim (FRG) (2nd completely revised edition) 1991
6. **Thatipamala, R.; Rohani, S.; Hill, G. A.:** Biotechnol. Bioeng. 40 (1992) 289
7. **Mankad, T.; Naumann, E. B.:** Chem. Eng. J. 48 (1992) B9
8. **Sivakumar, A.; Srinivasaraghavan, T.; Swaminathan, T.; Baradarajan, A.:** 45th Ann. session of the IIChE, Manipal 1992
9. **Ko, R. Y. C.; Edwards, V. H.:** Biotechnol. Bioeng. 17 (1975) 965
10. **Jackson, J. V., Edwards, V. H.:** Biotechnol. Bioeng. 17 (1975) 943
11. **Udayakumar, M. R.:** M. S. Thesis, IIT, Madras, 1991
12. **Wayman, M.; Tseng, M. C.:** Biotechnol. Bioeng. 18 (1976) 383
13. **Edwards, V. H.:** Biotechnol. Bioeng. 12 (1970) 679
14. **Yano, T.; Koya, S.:** J. Gen. Appl. Microbiol. 19 (1973) 97
15. **Chen, B. J.; Lim, H. C.; Tsao, G. T.:** Biotechnol. Bioeng. 18 (1976) 1629