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PERFORMANCE OF INHIBITION FUNCTIONS WITH TOTAL INHIBITION CONCENTRATION

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Abstract. Several biokinetic models which include a total inhibition concentration term (modified Aiba model, modified Haldane model (I) and (II)) are considered to account for substrate inhibitory effects. Their performance is compared on the basis of different experimental data in the literature and of parameter sensitivity analysis.

Keywords :microbial growth modeling, substrate inhibition, parameter sensitivity analysis.

1 Introduction

For a long time, the modeling of the dependence of the specific growth rate in microbial growth systems with respect to the process components and the physico-chemical conditions has been an active research area. Substrate inhibition has been in particular the subject of modeling efforts. In 1968, Andrews [2] suggested that the inhibitory effect of substrate concentration on the microbial specific growth rate can be treated by the first equation in Table 1 which was initially derived by Haldane [4] to describe the inhibition phenomena in enzymatic reactions. Several authors, however, have pointed out that the analysis of growth curves according to the Haldane (or Aiba) equation has some drawbacks because these models imply that the cells remain able to grow even when the substrate concentration is infinitely large, in contradiction with many practical situations. Indeed, a number of experimental results - especially when alcohols (i.e. methanol, ethanol, butanol, ...) are used as a limiting nutrient - have been reported where there is clearly a finite upper value of the substrate concentration above which the microbial growth stops [7], [8], [10]. Consequently, new models for substrate inhibition kinetics have been proposed by Wayman and Tseng [11], by Luong [7], and Han and Levenspiel [5]. These are listed in Table 1. In these models, the parameters $\mu^*, K_s, K_i, S_c, S^*$ and n are positive constants. In particular, S_c, in the model of Wayman and Tseng [11] represents a threshold substrate concentration below which the cells grow without inhibition, and S^*, a *total substrate inhibition* concentration above which the microbial growth stops.

Model	Ref.
Models without total inhibition	
1. Hyperbolic type	
$\mu(S) = \frac{\mu^*}{1+\frac{K_s}{S}+K_i S}$	[4]
2. Exponential type	
$\mu(S) = \frac{\mu^*}{1+\frac{K_s}{S}} e^{-K_i S}$	[1]
$\mu(S) = \mu^* \left[e^{-\frac{S}{K_i}} - e^{-\frac{S}{K_s}}\right]$	[3]
Models with total inhibition	
3. Linear type	
$\mu(S) = \frac{\mu^* S}{K_s+S}$ when $S < S_c$	
$= \frac{\mu^* S}{K_s+S} - K_i(S - S_c)$ otherwise	[11]
4. Power functional type	
$\mu(S) = \frac{\mu^*}{1+\frac{K_s}{S}} \left(1 - \frac{S}{S^*}\right)^n$	[6], [7]
$\mu(S) = \mu^* \left(1 - \frac{S}{S^*}\right)^n \frac{S}{S+K_s\left(1-\frac{S}{S^*}\right)^m}$	[5]

Table 1. Models for the substrate inhibition from the literature

The concept of a total inhibition concentration was initially proposed by Levenspiel [6] to model product inhibition phenomena. In this paper, we show how the original models of Haldane and Aiba can also be modified to account for the existence of a total inhibition concentration. Three new kinetic models are presented in Section 2. They combine the two following key features. They are flexible enough to fit all possible forms of substrate inhibition with only four kinetic constants (instead of five for the Han & Levenspiel model). And the three models can be viewed as extended Monod kinetic models : if there is no inhibition, they reduce to simple Monod kinetics; and even in presence of inhibition, at low substrate concentrations they increase in accordance with the Monod equation.

We have carried out in section 3 a systematic comparison of these three new kinetic models with three previous models of Table 1 (Haldane model, Levenspiel-Luong model, and Han & Levenspiel model) on basis of various experimental data patterns taken from the literature. The comparison is pursued in Section 4 via a parameter sensitivity analysis.

2 Extension of Haldane and Aiba models

The concept of a total substrate inhibition concentration ($= S^*$) is introduced as follows. Let the term $K_i S$ be replaced by $\frac{K_i S}{f(S^*,S)}$ in the Haldane and Aiba model equations, with $f(S^*,S)$ such that : 1) $\mu(S)$ must be positive in the range $0 \leq S \leq S^*$ and 2) S^* is a total inhibition concentration of substrate, i.e. $\mu(S^*) = 0$. It is easy to verify that a linear function of the form : $f(S^*,S) = (S^* - S)$ satisfies both conditions. Hence we obtain the following models :

$$\begin{aligned} &Modified \qquad Haldane\ model\ (I) : \\ &\mu(S) = \frac{\mu^*}{1 + \frac{K_s}{S} + K_i \frac{S}{S^*-S}} \qquad (1) \\ &Modified \qquad Aiba\ model : \\ &\mu(S) = \frac{\mu^*}{1 + \frac{K_s}{S}} e^{-K_i \frac{S}{S^*-S}} \qquad (2) \end{aligned}$$

If K_i is larger than 1, the growth inhibition phase can be described as a decreasing curve with a concave pattern. When K_i is below 1, a decreasing curve with a convex pattern is also possible for the description of the growth inhibition phase (Fig. 1(I)).

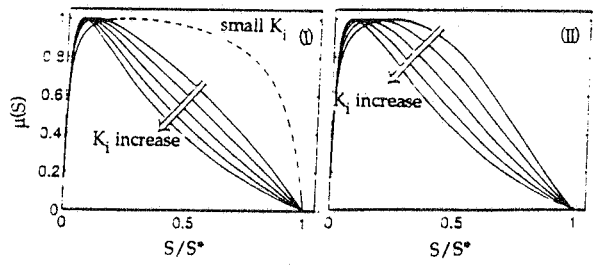

Fig.1. : Influence of K_i in the modified Haldane models (I) and (II)

2.1 Modified Haldane model (II)

The specific growth rate $\mu(S)$ of some microorganisms has been reported to exhibit three distinct growth phases [8], [9] : a growth stimulation phase, a growth saturation phase, and a growth inhibition phase 2. The above two models do not appear to be flexible enough to account for such a growth saturation phase. That's why we propose to modify the Haldane model as follows :

$$\begin{aligned} &Modified \qquad Haldane\ model\ (II) : \\ &\mu(S) = \frac{\mu^*}{1 + \frac{K_s}{S} - \frac{S}{S^*} + K_i \frac{S}{S^*-S}} \qquad (3) \end{aligned}$$

as in equations (1) and (2), the parameter S^* of equation (3) still represents the total substrate inhibition concentration above which cells do not grow i.e., $\mu(S^*) = 0$, and the value of K_i has an important influence on the curve shape limited in the growth inhibition phase. More specifically, in this case, the major variation effects of the parameter K_i seem to be located at the beginning of the growth inhibition phase (See Figure 1(II)). Finally, we note that all three new models can be viewed as extended Monod type models because in the extreme case where $S \ll S^*$ and/or S^* tends to infinity we have:

$$\mu(S)_{|S \ll S^* or S^* \to \infty} \simeq \frac{\mu^*}{1 + \frac{K_s}{S}}.$$

3 Results and discussion

Three kinds of substrate-inhibited growth data drawn from the literature have been used to compare the performance of six canditate models, and more specifically their flexibilities to fit the different experimental data patterns. Here we shall present only some of the modelling results : detailed results for the 6 models for a set of convex pattern inhibition data, graphical results of the modified Haldane model (II) for two data sets (linear and concave patterns).

3.1 Example 1 : Convex inhibition pattern

We start our model comparisons with experimental data drawn from Pilat and Prokop [8], where the tolerance of *C. boidinii* 11Bh to methanol was investigated. Figure 2(a)-(f) show the best fitting results of the experimental data for the six candidate models. The evaluated parameters of each model and fitting qualities are listed in Table 2.

#	μ^* (h^{-1})	K_s $\frac{v}{v}\%$	$K_i^\dagger$	n	S^* $\frac{v}{v}\%$	m	σ^*, 10^{-3}
1	0.2	0.2	3.7	-	-	-	5.3
2	0.15	0.1	0.28	-	8.37	-	1
3	0.16	0.12	-	0.5	7.54	-	1.7
4	0.15	0.11	0.29	-	7.7	-	1.1
5	0.16	0.12	-	0.5	7.54	0.4	1.8
6	0.13	0.08	0.64	-	8.23	-	0.8

† : for the Haldane model K_i $\frac{v}{v}\%$

Table 2. Parameters for Pilat & Prokop data (1:Haldane, 2:M. Aiba, 3:Levenspiel-Luong, 4:M. Haldane (I), 5:Han & Levenspiel, 6:M. Haldane (II)).

First of all , all models involving a total inhibitions S^* turns out to have a small value of $\sigma^*(< 0.0018)$. Only the original Haldane model gives a large value of σ^*, at least three time larger than the worst case of the other candidate models.

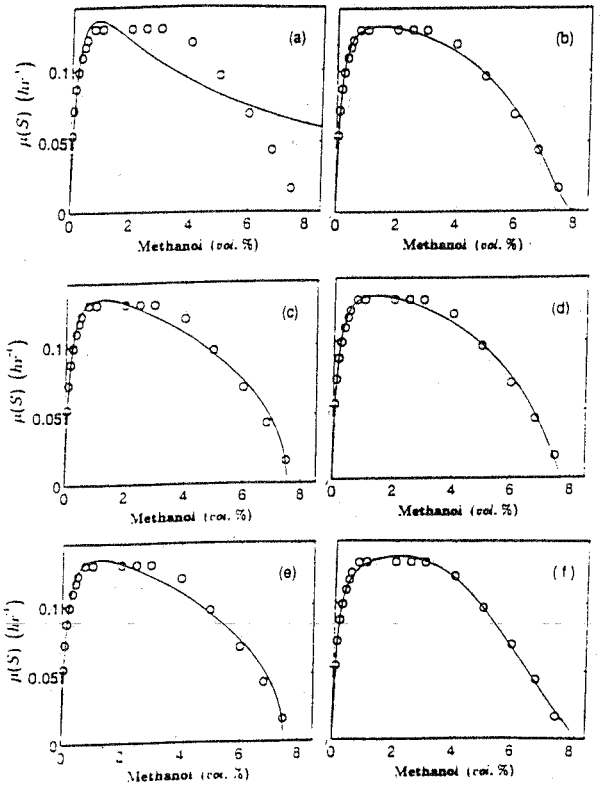

Fig. 2. : Model fitting for Pilat and Prokop data

Figure 2(a) shows that the original Haldane model is not suited for this type of substrate-inhibited growth data. Not only the growth saturation phase but also the growth inhibition phase are not fitted well. Figure 2(c) shows that the Levenspiel-Luong model is not able to describe the linear decrease of the specific growth rate and presents a sudden drop in the very near of S^*. In spite of having five parameters, the Han & Levenspiel model is not better here than the Levenspiel-Luong model. In addition, both models do not turn out to be able to predict a large growth saturation phase (See Figure 2(e)). Note also that all evaluated parameters of the Levenspiel-Luong model are quite similar to those of the Han & Levenspiel model. The contribution of the term $\left(1 - \frac{S}{7.54}\right)^{0.4}$ in Han & Levenspiel model to data fitting is negligible.

This situation is also similar for both modified Haldane (I) and modified Aiba models. As shown in Figure 2(b) and (d), descriptions of the growth inhibition phase of both models are quite good but here again both models are not able to exhibit a large growth saturation phase.

The modified Haldane model (II) turns out to give good fitting results. As shown in Figure 2(f), the best fitting curve exhibits a good matching with the whole data set of Pilat and Prokop. The introduction of the term $\left(-\frac{S}{S^*}\right)$ in the model allows to have a broader region with a rather flat profile in the growth saturation phase. Consequently, the modified Haldane model (II) gives the lowest value of $\sigma^*(= 0.0008)$ among all candidate models.

3.2 Examples 2 and 3 : Linear and concave patterns

In order to illustrate the flexibility of the modified Haldane model (II), figure 3 shows the fitting of the model to two different set of experimental data : one with a linear decrease pattern and one with a concave inhibition pattern (both drawn from [10]).

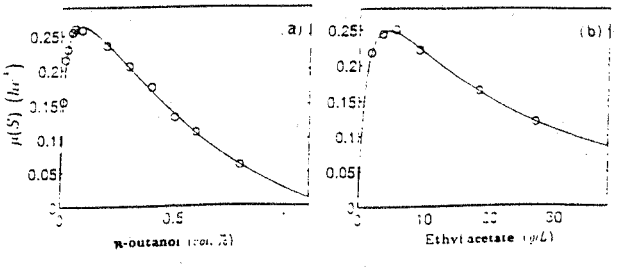

Fig.3. : Modified Haldane model for two data sets (linear (a), concave (b)).

4 Parameter sensitivity analysis

The relative parameter sensitivity functions of the six candidate models have been derived and evaluated using the estimated parameters listed in Table 2 (Figure 4(a)-(e)). First of all, the sensitivity peaks of K_s parameter of all candidate models concentrate in low substrate concentrations and splits from other sensitivity curves. Thus, the role of K_s parameter in all candidate models is the same : to determine the curve behaviour at low substrate concentrations. However, the sensitivity curves of K_i, n and S^*, which seem to determine the curve behaviour in growth inhibition phase, is quite different from each other.

In the Haldane model case (Figure 4(a)), the sensitivity curve of parameter K_i does not concentrate at high substrate concentrations. Furthermore, the sensitivity is not larger than that of μ^* at high substrate concentrations. Therefore the parameter K_i does not significantly affect the curve shape in the growth inhibition phase.

For the modified Aiba model (Figure 4(b)), the parameter sensitivities of K_i and S^* are closely located together between 6 and $8v/v\%$ of n-butanol. The sensitivity curve of S^* shows the sudden drop close to the point of $S = S^*$. On the other hand, the S* sensitivity curve of the Levenspiel-Luong model is sharply increasing close to $S = S^*$ (See Figure

4(c)). In both cases, there is no available parameter to affect the curve behaviour in the intermediate substrate concentrations. The comments about the modified Haldane model (I) (Figure 4(d)) are similar to those of the Levenspiel-Luong model case, yet with a small shift to the left of the peak of K_i sensitivity curve.

The results of the parameter sensitivity analysis of the Han & Levenspiel model are shown in Figure 4(e). In spite of having quite different model structure, all curves, except that of m, are the same to those of the Levenspiel-Luong model. Furthermore, the sensitivity of parameter m turns out to be almost negligible comparing with the other curves in this case.

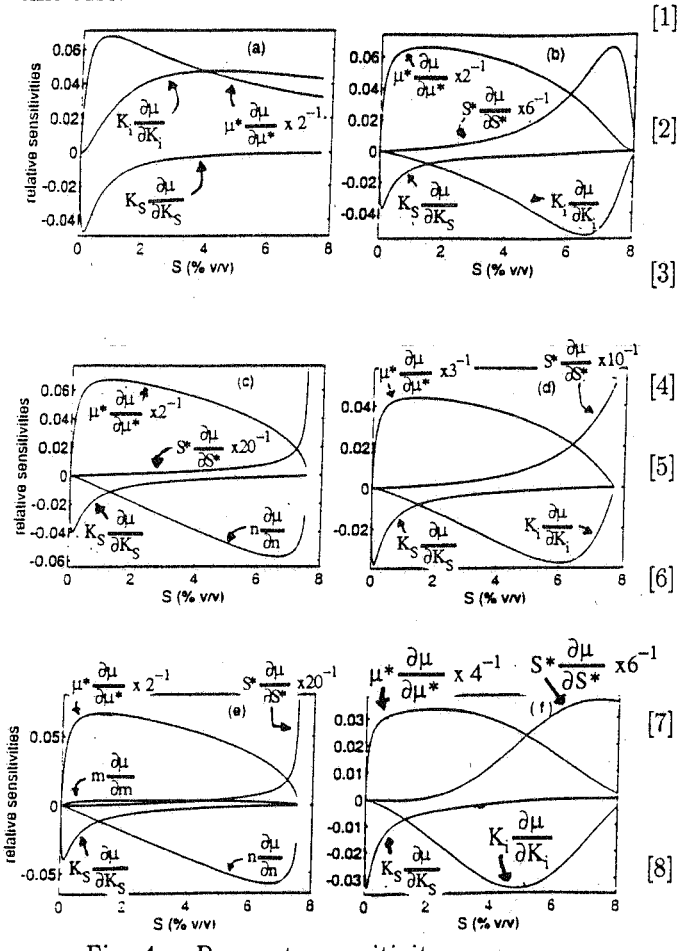

Fig. 4. : Parameter sensitivity curves.

Figure 4(f) shows the parameter sensitivity analysis of the modified Haldane model (II). In comparison with the above models, its parameters are consistent because the most intensive points of each sensitivity curve are clearly separated and independent to each other. In the growth stimulation phase, the parameter K_s has a major effect on the curve shape change. Beyond this level, i.e. in the growth saturation phase, the parameter μ^* plays a key role to determine the maximum growth rate. And in the growth inhibition region, the growth curve is influenced by the parameter K_i and S^* successively.

5 Conclusions

Comparative modeling studies on substrate inhibition in microbial growth are carried out based on three kinds of inhibition patterns. The modified Haldane model (II) gives a good fit for experimental data with a large growth saturation phase between the growth stimulation and inhibition phases. And the parameter sensitivity analysis emphasizes that this model has a simple model structure, yet flexible enough for representing the microbial growth kinetics with a limited number of parameters.

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