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Data fitting in Monod-type models with nonlinear growth rates

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Abstract

We consider the classical Monod model and modify it by introducing generic growth rate for the biomass that is monotonically increasing with respect to the functional response. We show that this modification does not lead to qualitatively new possibilities for the model solutions. On the other hand, however, it allows better flexibility in terms of fitting experimental data. In particular, we use experimental data for microbial growth under inhibitory conditions, make parametric identification and show that the modified model performs far better than the classical one.

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1 Introduction

The basic model, describing bacterial growth processes in continuous bioreactors is the Monod model [9]:

$$\begin{aligned}\frac{ds}{dt} &= \left(s^{(0)} - s\right) D - \frac{\alpha V_{max} s x}{s + K_s},\\ \frac{dx}{dt} &= \left(\frac{V_{max} s}{s + K_s} - D\right) x,\\ x(0) &= x_0,\ s(0) = s_0,\end{aligned} \tag{1}$$

where $s(t)$ and $x(t)$ denote, respectively, concentrations of substrate and biomass at time t, $s^{(0)}$ is the inlet substrate concentration and D is the wash-out rate. The Monod function $V_{max}s/(s+K_s)$ describes consumption, and α characterizes the effectiveness of the biotechnological process, i.e. "transformation" of substrate into biomass.

It is well-known, however, that the classical models perform well in describing experimental data, when microorganisms are in optimal conditions, but not in suboptimal or inhibiting conditions [1, 11]. That is, there might be certain conditions of the environment like temperature, pH, substances (other than the limiting substrate) that make the conditions not optimal. The main purpose of this paper is to study whether using generic growth rate for the biomass could give better results when describing experimental data in this case. In the present article, we shall consider an example of the inhibiting effect of high sugar concentrations on two wine-making strains.

Considering predator-prey type systems, A.J. Terry [10] suggested that in some cases the growth rate of organisms is not adequately modelled by a linear, with respect to the consumption, function. The reasoning behind this statement is the following. It needs a certain level of energy intake before an organism can reproduce. Thus, until a certain threshold is reached the reproduction should be zero, rather than a linearly increasing function. Also, if the functional response is sufficiently large, the reproduction rate reaches a plateau level, since no organism can reproduce infinitely fast.

Using the latter idea, in Section 2, we modify the classical Monod model (1) by using generic growth rate for the biomass. In Section 3, the dynamical behavior of the model's solutions is studied. Then, in Section 5, we use experimental data from two wine-making strains (described in Section 4) to compare the ability of the classical and the modified model to fit real data.

2 Mathematical model

Using the idea from [10], we consider the following model:

$$
\begin{aligned}
\frac{ds}{dt} &= \left(s^{(0)} - s\right) D - \frac{\alpha V_{max} s x}{s + K_s}, \\
\frac{dx}{dt} &= \left[\mathcal{B}\left(\frac{V_{max} s}{s + K_s}\right) - D\right] x, \\
x(0) &= x_0, \ s(0) = s_0.
\end{aligned}
\tag{2}
$$

We denote $\mathcal{F}(s) = V_{max} s/(s+K_s)$ and let the function $\mathcal{B}(\mathcal{F})$ be defined in the following manner:

(i) $\mathcal{B} = \mathcal{B}(\mathcal{F}) = \mathcal{B}(\mathcal{F}(s))$ is continuously differentiable w.r.t. $\mathcal{F} \geq 0$ and $s \geq 0$;

(ii) $\mathcal{B}(0) = 0$ and $0 \leq \mathcal{B}(\mathcal{F}) \leq C\,\mathcal{F}$ for some constant $C > 0$;

(iii) there exist non-negative constants $A_1 < A_2$ (A_2 possibly equal to $+\infty$) such that, for $s \geq 0$, $\mathcal{B}' = d\mathcal{B}/d\mathcal{F} = 0$ if $\mathcal{F} \in [0, A_1] \cup [A_2, +\infty)$, and $\mathcal{B}' > 0$ if $\mathcal{F} \in (A_1, A_2)$.

In our numerical experiments, we shall use the following possible form of $\mathcal{B}(\mathcal{F})$, satisfying the aforementioned conditions [10]:

$$
\mathcal{B}(\mathcal{F}) = \begin{cases} 0, & 0 \leq \mathcal{F} \leq A_1, \\ \beta \cos^2 \left\{ \left(\frac{\pi}{2}\right) \left(1 + \left[\frac{\mathcal{F} - A_1}{A_2 - A_1}\right]\right) \right\}, & A_1 \leq \mathcal{F} \leq A_2, \\ \beta, & \mathcal{F} \geq A_2. \end{cases}
$$

Remark 1. We shall neglect the death rate of the microorganisms, assuming that it is much smaller than the wash-out rate or, for a batch process, the time scale is such that it affects the process little.

3 Dynamical behavior of the model solutions

As we shall show in the present section, the results for the classical Monod model (see e.g., [9]) can be easily extended for the model with generic growth function for the biomass.

Nondimensionalizing the model (2) with $\bar{s} = s/s^{(0)}$, $\bar{t} = Dt$, $\bar{x} = \alpha x/(Ds^{(0)})$, $\bar{\mathcal{B}} = \mathcal{B}/D$, $\bar{K}_s = K_s/s^{(0)}$ and skipping the bars for notational simplicity, we consider the system

$$\begin{aligned} \frac{ds}{dt} &= 1 - s - \frac{V_{max} s x}{s + K_s}, \\ \frac{dx}{dt} &= \left[\mathcal{B}\left(\frac{V_{max} s}{s + K_s} \right) - 1 \right] x. \end{aligned} \tag{3}$$

Let s^* denote the unique positive value for s, such that

$$\mathcal{B}\left(\frac{V_{max} s}{s + K_s} \right) = 1.$$

If such a value does not exist, we define $s^* := +\infty$. Let us further define

$$x^* := \frac{(s^* + K_s)(1 - s^*)}{V_{max} s^*}.$$

Then, obviously, the system (2) can have up to two equilibrium points—$E_1 = (1, 0)$, which always exists, and $E^* = (s^*, x^*)$, which exists only when the inequalities $0 < s^* < 1$ hold true.

Lemma 1. *When the internal equilibrium* $E^* = (s^*, x^*)$ *exists, i.e. when* $0 < s^* < 1$, *then there exists time* $T > 0$ *such that* $s(t) < 1$ *for each* $t \geq T$.

Proof. Assume that $s(t) \geq 1$ for each $t > 0$. Then we have

$$\frac{ds}{dt} = 1 - s - \frac{V_{max} s}{s + K_s} x < 0.$$

Barbălat's Lemma (cf. [4]) implies that $\lim_{t \to +\infty} s(t) = 1$ and $\lim_{t \to +\infty} x(t) = 0$ are fulfilled. Using the monotonicity of $\mathcal{B}$ we obtain that

$$\mathcal{B}(\mathcal{F}(s)) - 1 \geq \mathcal{B}(\mathcal{F}(1)) - \mathcal{B}(\mathcal{F}(s^*)) > 0,$$

and thus $\dfrac{dx}{dt} = (\mathcal{B}(\mathcal{F}(s)) - 1)x > 0$ for all sufficiently large t, which contradicts the boundedness of $x(t)$. This contradiction shows that there exists a sufficiently large $T > 0$ such that $s(T) \leq 1$ is satisfied. Moreover, if the equality $s(\tilde{t}) = 1$ holds true for some $\tilde{t} \geq T$, then

$$\frac{ds}{dt}(\tilde{t}) = 1 - s(\tilde{t}) - \frac{V_{max} s(\tilde{t})}{s(\tilde{t}) + K_s} x(\tilde{t}) = -\frac{V_{max} s(\tilde{t})}{s(\tilde{t}) + K_s} x(\tilde{t}) < 0.$$

The last inequality shows that $s(t) < 1$ for each $t > T$. □

Remark 2. Let us note that in order to study the dynamics of (3), it is sufficient to only consider the positively invariant set $\Delta = \{(s, x) \in \mathbb{R}^2 : 0 < s < 1, x > 0\}$, since all trajectories with positive initial conditions enter it for finite time.

The dynamics of (2) is completely characterized by the following two theorems.

Theorem 1. *In the case, when the system* (2) *has no internal equilibria, i.e. when* $s^* > 1$ *holds, then the boundary equilibrium* $E_1 = (1, 0)$ *is globally asymptotically stable.*

Proof. Consider the function $V(s, x) = x$ on the positively invariant set Δ.

For the directional derivative over the trajectories of (2) the following is satisfied:

$$\dot{V} = \left[\mathcal{B}\left(\frac{V_{max} s}{s + K_s}\right) - 1\right] x.$$

The latter is always non-positive in Δ, taking into account that

$$\mathcal{B}\left(\frac{V_{max}s}{s+K_s}\right) < \mathcal{B}\left(\frac{V_{max}}{1+K_s}\right) < \mathcal{B}\left(\frac{V_{max}s^*}{s^*+K_s}\right) = 1.$$

Further, $\dot{V} = 0$ exactly when $x = 0$.

Now, from the LaSalle Invariance Principle it holds that the ω-limit set is contained in the set $\{(x,s) \in cl\Delta : x = 0\}$. However, all trajectories in it are attracted by the equilibrium point E_1, which concludes the proof of the theorem. □

Before we consider the case, when the internal equilibrium point E^* exists, we shall prove the following lemma, necessary for the proof of E^* being globally asymptotically stable.

Lemma 2. *In the case, when the internal equilibrium* $E^* = (s^*, x^*)$ *exists, i.e. when* $0 < s^* < 1$ *hold, the equilibrium point* E_1 *in an unstable equilibrium.*

Proof. The variational matrix of the system (2), computed at the point E_1, has the following form:

$$\begin{bmatrix} -1 & -\frac{V_{max}}{1+K_s} \\ 0 & \mathcal{B}\left(\frac{V_{max}}{1+K_s}\right) - 1 \end{bmatrix}.$$

Taking into account that from the monotonicity of $\mathcal{B}$ and the condition $0 < s^* < 1$ it follows that

$$\mathcal{B}\left(\frac{V_{max}}{1+K_s}\right) > \mathcal{B}\left(\frac{V_{max}s^*}{s^*+K_s}\right) = 1,$$

it can be easily checked that E_1 is a saddle point, i.e. it is unstable. □

Theorem 2. *In the case, when the internal equilibrium* $E^* = (s^*, x^*)$ *exists, i.e. when* $0 < s^* < 1$, *it is globally asymptotically stable.*

Proof. Choose and fix an arbitrary initial point $(s(0), x(0)) \in \Delta$.

We consider the function

$$V(s,x) = \int_{s^*}^{s} \frac{\mathcal{B}(\mathcal{F}(\xi))-1}{1-\xi} d\xi + \frac{1}{x^*}\int_{x^*}^{x} \frac{\eta - x^*}{\eta} d\eta$$

on the set Δ, where $\mathcal{F} = V_{max}s/(s+K_s)$ is the Monod function, modifying an idea from [12]. Its directional derivative over the trajectories of (2) is

$$\begin{aligned}\dot{V} &= \nabla V \cdot \left(\frac{ds}{dt}, \frac{dx}{dt}\right)\\ &= \frac{\mathcal{B}(\mathcal{F}(s))-1}{1-s}\left(1 - s - \frac{V_{max}sx}{s+K_s}\right) + \frac{1}{x^*}\cdot\frac{x-x^*}{x}\cdot\left(\mathcal{B}(\mathcal{F}(s))-1\right)x\\ &= x\left(\mathcal{B}(\mathcal{F}(s))-1\right)\left[-\frac{V_{max}s}{(s+K_s)(1-s)} + \frac{V_{max}s^*}{(s^*+K_s)(1-s^*)}\right].\end{aligned}$$

The latter is obviously non-positive on Δ, since either the second term is positive, and the third one is negative, or vice versa, depending on whether $s > s^*$, or $s < s^*$.

Denote by $L^+(s(0), x(0))$ the ω-limit set of the solution $(s(t), x(t))$ of (3) starting with $(s(0), x(0)) \in \Delta$. It is known that $L^+(s(0), x(0))$ is invariant with respect to the trajectories of (3). Also (because each trajectory of the model is contained in a compact set), $L^+(s(0), x(0)$ is nonempty, compact and connected, and is contained in the closure $cl\Delta$, i.e. $L^+(s(0), x(0)) \subset \{(s,x) : 0 \le s \le 1, x \ge 0\}$.

According to the LaSalle invariance principle every solution of the model starting from a point of Δ is defined in the interval $[0, +\infty)$ and approaches the largest invariant set which is contained in the closure of the set Z, where the derivative $\dot{V}$ of V with respect to the trajectories of the model is equal to zero, i.e. $Z := \{(s,x) \in \Delta : \dot{V}(s,x) = 0\}$.

Let $(\tilde{s}, \tilde{x})$ be an arbitrary point from $L^+(s(0), x(0))$. Then the following two cases are possible: (i) $\tilde{s} = 1$ and $\tilde{x} = 0$; (ii) $\tilde{s} = s^\star$ and $\tilde{x} = x^\star$.

Assume that case (i) holds true. The continuity of $\mathcal{B}(\cdot)$ implies the existence of a neighborhood U of $\tilde{s}$ so that for each point $s \in U$ the inequality $\mathcal{B}(\mathcal{F}(s)) > \mathcal{B}(\mathcal{F}(s^\star))$ holds true. Hence, if a trajectory

$(s(t), x(t))$ enters the set U, then the right-hand side of the second equation in the model is strongly positive (because $x(t) > 0$). This means that $x(t)$ will strictly increase and the trajectory could not tend to the point $(\tilde{s}, \tilde{x})$ for which $\tilde{x} = 0$. So we obtain that case (*i*) is impossible. Hence the case (*ii*) is fulfilled. From here it follows that $Z = \{E^\star\}$, and therefore $L^+(s(0), x(0)) = \{(s^*, x^*)\}$. This completes the proof of Theorem 2. $\square$

4 Physical experiments

Data from two different wine-making strains, namely, *Saccharomyces cerevisiae* MB and *Saccharomyces cerevisiae* FR were used [2].

Yeast are unicellular fungi, used as a model organism in molecular biology and with a particularly important role in genetic engineering. In general, these microscopic fungi have diverse natural habitats—plant leaves and flowers, soil, and salt water, but also they can be found on the surfaces and in the intestinal tracts of warm-blooded animals, as symbionts or as parasites. Fermented beverages and foods have been significant for people for millennia. The main fermenting agent, *Saccharomyces cerevisiae*, is used in the production of beer, wine, and bread, as well as fermented dairy products worldwide. Also, *Saccharomyces cerevisiae* is often taken as a vitamin supplement, because it is 50 percent protein and a rich source of B vitamins, niacin, and folic acid [8]. Amid the typical characteristics of *Saccharomyces cerevisiae* are:

- they metabolise sugars with concentrations $170 - 220 g/l$ [7], where concentrations above $250 g/l$ may cause inhibition effect on the fermentation [6];
- in general, their species can survive up to 15 % alcoholic content [3]; there are still variations.

Data is obtained from a batch process. *Saccharomyces cerevisiae* strains were cultivated on grape juice from *Vitis vinifera cv. Merlot* which possess important qualities in the biotechnology of wine making. The species were put in inhibitory conditions because of the high concentration of sugars in the initial cultures.

5 Data analysis

We compare models (1) and (2) in terms of their ability to fit the experimental data.

5.1 Parametric identification procedure

We use the following procedure to obtain the best fit in the least squares sense. Let us be given n measurements,

$$\{(t_i, x_i, s_i) : i = \overline{1, n}\},$$

where x_i and s_i are the biomass and substrate concentration at time t_i, respectively.

We define the following objective function:

$$\varepsilon(\mathbf{p}) = \sum_{i=1}^{n} \left[\left(\frac{x(t_i; \mathbf{p}) - x_i}{x_i} \right)^2 + \left(\frac{s(t_i; \mathbf{p}) - s_i}{s_i} \right)^2 \right],$$

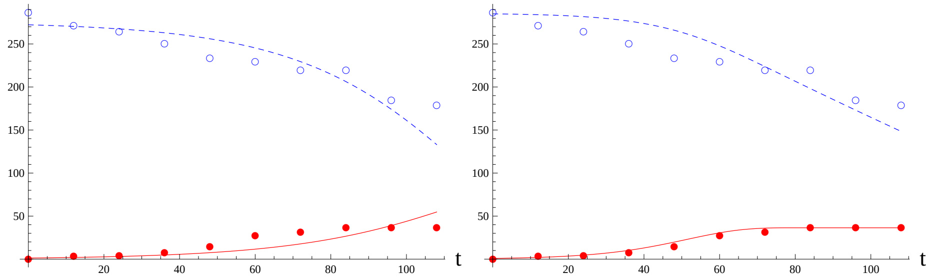
where $\mathbf{p} = (\alpha, V_{max}, K_s, s_0, x_0)$ for the Monod model (1) or $\mathbf{p} = (\alpha, V_{max}, K_s,$
$\beta, A_1, A_2, s_0, x_0)$ for the model (2) and $x(t; \mathbf{p})$ and $s(t; \mathbf{p})$ are the solutions of the respective model for given parameter values $\mathbf{p}$.

Minimizing the latter with respect to $\mathbf{p}$, we obtain the optimal parameter values. We solve this minimization problem, by using the Wolfram Mathematica implementation of the Nelder–Mead method.

5.2 Numerical experiments

We consider the first phase of the growth process, until the population reaches its quasi-stationary state.

FR Strain. We obtain the best fit with the Monod model (1) for the following values of the model parameters—$\alpha = 0.4099686$, $V_{max} = 909.6993014$, and $K_s = 5.61387864 \times 10^6$. As can be seen from Fig. 1, this model fails to adequately describe the growth dynamics. Obviously, the main reason lies in the fact that the growth of the bacteria is supposed to be linear w.r.t. the consumption. The behaviour of the experimental data, however, rejects this suggestion. The organism's growth stops, even though there is sufficient substrate left in the culture.



(a) Model (1).

(b) Model (2).

Figure 1: Growth dynamics of *Saccharomyces cerevisiae* FR. The dashed line and the solid line represent solution of the mathematical model for the substrate and biomass, respectively. The empty and filled-in circles correspond to substrate and biomass experimental data.

A much better fit is obtained using model (2) for the following values of the model parameters—$\alpha = 10.0155702$, $V_{max} = 0.287346$, $K_s = 2.03163$, $\beta = 0.498609$, $A_1 = 0.284397$, $A_2 = 0.288714$ (see Fig. 1b).

One might argue that the failure of model (1) lies in the fact that we have neglected the death rate. Even adding this term, however, the model is still unable to describe well the quasi-stationary phase of the process. Considering a constant per-capita death rate d, we obtain the results, depicted in Fig. 2, for parameter values $\alpha = 0.0407204$, $V_{max} = 1465.12$, $K_s = 2.78048 \times 10^6$, and $d = 0.0985754$.

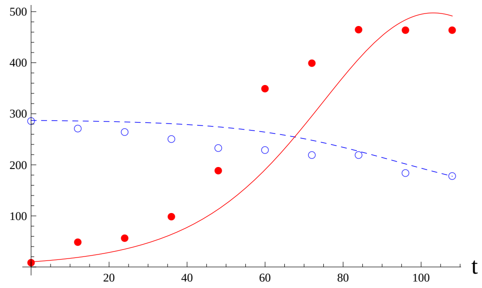
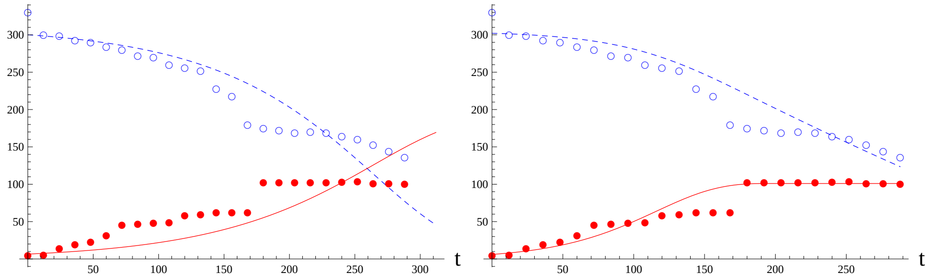


Figure 2: Growth dynamics of *Saccharomyces cerevisiae* FR, modelled by (2). The dashed line and the solid line represent solution of the mathematical model for the substrate and biomass, respectively. The empty and filled-in circles correspond to substrate and biomass experimental data.

Similar observations, as in the case of the FR strain, can be made about the best fit obtained for the set of experimental data for the MB strain, see Fig. 3a for the results, obtained with the Monod model and Fig. 3b for the ones, obtained with the model (2).



(a) Model (1)

(b) Model (2)

Figure 3: Growth dynamics of *Saccharomyces cerevisiae* MB. The dashed line and the solid line represent solution of the mathematical model for the substrate and biomass, respectively. The empty and filled-in circles correspond to substrate and biomass experimental data.

6 Conclusions and discussion

We have showed that introducing nonlinear growth rate for the biomass in the classical Monod model does not change its asymptotic behavior and can, thus, model two principal situation—when the organisms get washed out from the bioreactor and when a stable stationary state is reached. Those correspond to what is observed in real-life one substrate–one biomass systems.

On the other hand, however, our experiments show that the modified model is more flexible in terms of fitting real data. While the only possibility for the classical model to reach a quasy-stationary state for the biomass in a batch process is when all substrate is exhausted from the bioreactor, the modified model allows to describe more complex behaviour, resulting from the interplay between all the conditions in the medium. Those might be incorporated in the parameters, defining the thresholds in the growth rate. Thus, further studies could focus on defining specific forms of the growth rate that are most plausible from biological point of view. Furthermore, various scenarios, where classical models fail, could be studied, so that acceptable growth functions are derived.

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