

*Dedicated to the memories of my biology teachers
Dr. Kalcho Markov and Dr. Roumen Tsanev*

ON THE MATHEMATICAL MODELLING OF MICROBIAL GROWTH: SOME COMPUTATIONAL ASPECTS*

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ABSTRACT. We propose a new approach to the mathematical modelling of microbial growth. Our approach differs from familiar Monod type models by considering two phases in the physiological states of the microorganisms and makes use of basic relations from enzyme kinetics. Such an approach may be useful in the modelling and control of biotechnological processes, where microorganisms are used for various biodegradation purposes and are often put under extreme inhibitory conditions. Some computational experiments are performed in support of our modelling approach.

1. Introduction. In this work we present a new approach to the mathematical modelling of microbial growth. Our approach takes dynamically into account inhibition on microbial growth caused by shortage or excess of nutrient

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substrate. It is hoped that such an approach can be useful in the modelling of biotechnological processes, where microorganisms are used for various biodegradation purposes.

It has been recognized since long that classical microbial growth models using Monod function describe adequately bio-processes under favorable conditions, when microorganisms actively produce specific enzymes for the degradation and consumption of nutrient substrates and thus divide and grow at the maximal possible rate. However, the environment in bio-reactors sometimes becomes unfavorable due to depletion of nutrient, or due to super-abundance of nutrient substrate. In such cases microbial growth may be inhibited.

Under such extreme conditions Monod type models may fail to reflect adequately the inhibition on microbial growth and thus the dynamics of the corresponding bio-processes. Modifications of these models in various directions have been proposed in order to suitably reflect inhibition on microbial growth. One possible direction is to allow some of the parameters in the model to depend on the nutrient substrate and/or on other quantities. Within this direction Monod function is replaced by other specific expressions [6]. Another modelling direction is based on the assumption that microorganisms of the same species behave rather differently under favorable and unfavorable conditions actually changing their physiological states. Hence microorganisms can be classified into two or more groups corresponding to their physiological states/phases. Mathematically this means assigning different variables to the bio-masses of microorganisms of different states and considering the corresponding microbial populations as different species.

Our proposed approach can be viewed as a hybrid of the above-mentioned two modelling directions. Microbial growth is tightly related to enzyme production, so it is natural to use ideas from enzyme kinetics in the modelling of bio-processes involving microbial populations. Recall that the Michaelis-Menten differential equation describing the uptake of substrate by enzymes is integrated in the Monod type models. Our idea is to use instead the Henri-Michaelis-Menten system of ODE (under the Briggs-Haldane interpretation), which involves not only the substrate dynamics, but also the dynamics of the enzyme concentration (free and bounded). This idea allows us to introduce special variables corresponding to microbial population in two different states/phases. In order to explain and motivate our modelling approach to microbial growth we recall next some known facts from enzyme kinetics in relation to Monod type models.

2. Enzyme kinetics and microbial growth. Microorganisms produce enzymes so it is natural to look for analogies between models of microbial growth and those of enzyme kinetics. Enzyme kinetics models in their simplest form (when the enzymes possess just one active site) are usually met in the literature in two variants. In the first variant the dynamics of the substrate uptake is presented by a single ODE for the substrate concentration, which we shall further refer to as Michaelis-Menten ODE, or Michaelis-Menten law, MM-law for short. The MM-law concentrates on the substrate dynamics and says nothing about the dynamics of the remaining three counterparts: the two forms of the enzyme (free and bounded) and the product. The second variant is presented by four ODEs describing the dynamics of all four components: the substrate, the two forms of the enzyme, and the product. This variant is further referred to as Henri-Michaelis-Menten system of ODE's, HMM-law for short. We wish to emphasize that the MM-law for the substrate uptake is an approximation to the HMM-law, see e.g. [11]. We shall next discuss the relation between the Monod model and the MM-law, resp. the HMM-law.

2.1. Microbial growth models and MM-law. Consider a classical model of microbial growth involving a single microbial species in a batch mode chemostat/bioreactor of the form

$$\begin{aligned} (1) \quad & ds/dt = -\alpha\mu(s)x, \\ (2) \quad & dx/dt = \mu(s)x - k_d x, \end{aligned}$$

with initial conditions $s(0) = s_0 > 0$, $x(0) = x_0 > 0$. Here $x = x(t)$ is the biomass, $s = s(t)$ is the concentration of the substrate in the chemostat, k_d is a decay (death rate) constant and $\mu(s)$ is a function depending on the substrate s , see e.g. [1], [2], [5], [14]. A commonly used function $\mu(s)$ is the Monod function:

$$(3) \quad \mu(s) = \mu_{\max} \frac{s}{K_s + s},$$

where K_s is a positive constant.

The solutions for x, s of (1)–(2) using Monod function (3) are visualized in Figure 1. One can see that the biomass x initially increases, then reaches its maximum and after that the biomass x starts to decrease due to depletion of nutrient substrate. Note that $\mu(s) \rightarrow 0$ with $s \rightarrow 0$ so that equation (2) becomes approximately $dx/dt \approx -k_d x$ for small s . Hence $x \rightarrow 0$ with $t \rightarrow \infty$. This shows that model (1)–(3) does reflect the inhibition on microbial growth due to shortage of nutrient substrate. Nevertheless, it has been pointed out in the literature that Monod type models of the form (1)–(2) do not model sufficiently

realistically inhibition on microbial growth even when other specific functions $\mu(s)$ are used, see e.g. [6].

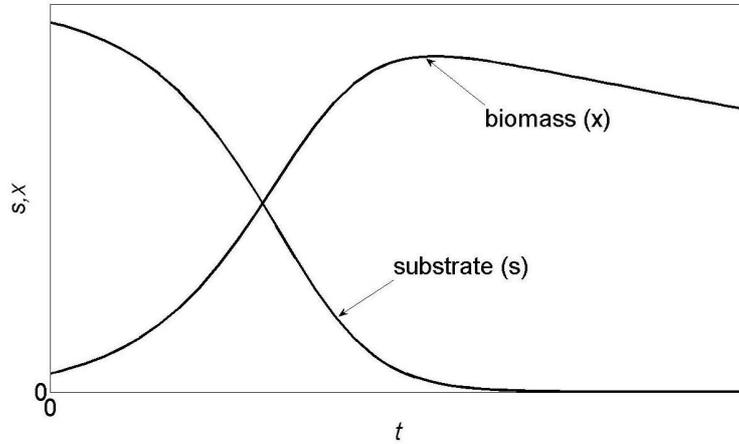


Fig. 1. Solutions for x, s of Monod model (1), (2), (3)

Let us consider the substrate uptake (1) in the Monod model using Monod function (3) in a batch mode chemostat/bioreactor:

$$(4) \quad \frac{ds}{dt} = -\alpha\mu_{\max} \frac{s}{K_s + s} x.$$

During the initial (lag) phase the biomass x is nearly constant, $x \approx \text{const} = c$, so that we have approximately

$$(5) \quad \frac{ds}{dt} \approx -\alpha c \mu_{\max} \frac{s}{K_s + s},$$

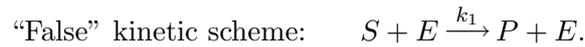
This shows that for time intervals when the biomass is nearly constant, $x \approx \text{const}$, the Monod model (5) describes the nutrient uptake similarly to the MM-law; recall that the latter reads [11]:

$$(6) \quad \frac{ds}{dt} = -V_{\max} \frac{s}{K_m + s}.$$

The recognition of the close relation between Monod growth models and those of enzyme kinetics allows us to make a step further in this direction. Next we intend to explore some relations between microbial growth models and the HMM-law instead of the MM-law. This will give us a possibility to introduce additional

inhibition factors. We shall thus look in the sequel for analogies between enzyme-substrate dynamics described by the HMM-law and microbial growth dynamics.

2.2. Basic enzyme kinetics: MM-law and HMM-law. We can speculate that before the invention of enzyme kinetics some hundred years ago, cf.[13], the transition of substrate S into product P under a catalyst enzyme E was thought to happen according to the following “false” kinetic scheme:



Applying the mass action law, the above kinetic scheme leads to a simple differential equation for the substrate concentration s :

$$(7) \quad \frac{ds}{dt} = -k_1 e s, \quad s(0) = s_0,$$

where $e = \text{const} = e_0$. The solution of (7) is an exponential decay function, which is visualized in Figure 2 (see lower graphic). The exponential decay contradicts the experimentally observed uptake of s , with almost constant rate in a certain time interval (see upper graphic). The experimentally observed discrepancy between the empirical data and expected theoretical solution based on the “false” kinetic scheme has led to the discovery of the Henri-Michaelis-Menten kinetic equation.

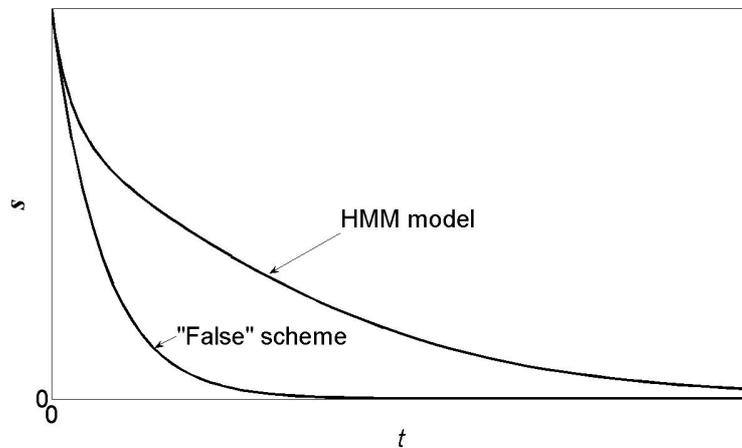
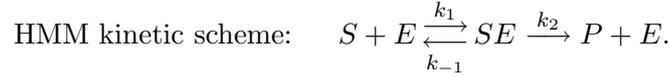


Fig. 2. Substrate dynamics according to the “false” scheme (below) and the HMM-scheme (above)

Remark. The “false” kinetic scheme is applicable to large classes of catalytic chemical reactions, so it has been believed that the same scheme applies to enzymatic reactions.

We next briefly recall the HMM-law of enzyme kinetics. The HMM-law is presented by the following kinetic scheme:



The HMM-law says that during the transition of the substrate S into product P the enzyme E bounds the substrate into a complex SE .

The basic enzyme kinetics HMM-law is mathematically presented by a system of ODEs for the concentrations: $s = [S]$, $e = [E]$, $c = [SE]$, $p = [P]$. Applying the Law of Mass Action we formulate the HMM-law in the form of the following system of ODEs:

$$(8) \quad \begin{aligned} \frac{ds}{dt} &= -k_1 es + k_{-1}c, & \frac{de}{dt} &= -k_1 es + (k_{-1} + k_2)c, \\ \frac{dc}{dt} &= k_1 es - (k_{-1} + k_2)c, & \frac{dp}{dt} &= k_2c. \end{aligned}$$

with initial conditions $s(0) = s_0$, $e(0) = e_0$, $c(0) = 0$, $p(0) = 0$.

The upper graphics in Figure 2 presents the solution for the substrate s to system (8) and demonstrates the basic properties of the HMM-scheme discovered by V. Henri (1901–1902) [7]–[9] and A. J. Brown (1902) [4], and experimentally confirmed by L. Michaelis, M. Menten (1913) [10]. The solutions for s, e, c, p to system (8) are visualized in Figure 3. Note that the graphics for s in Figure 2 (HMM model) and in Figure 3 are the same.

To explain the relation between the HMM-law (8) and the MM-law (6) as far as the substrate dynamics is considered we next recall the Briggs-Haldane derivation of MM-uptake [3] following [11].

From (8) using that $de/dt + dc/dt = 0$ implies $e(t) + c(t) = e_0$ and thus $e = e_0 - c$, system (8) reduces to two equations for s and c :

$$\begin{aligned} ds/dt &= -k_1 e_0 s + (k_1 s + k_{-1})c, \\ dc/dt &= k_1 e_0 s - (k_1 s + k_{-1} + k_2)c, \end{aligned}$$

with initial conditions $s(0) = s_0$, $c(0) = 0$.

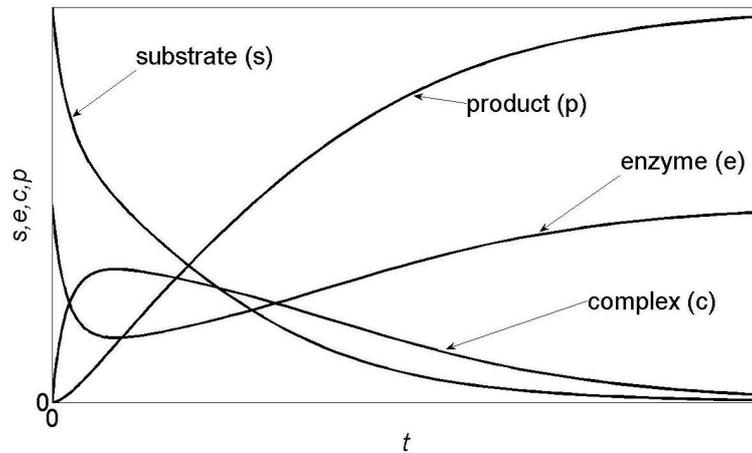


Fig. 3. Graphics of the solutions of system (8)

Assuming $s_0 \gg e_0$ and $dc/dt \approx 0$ (concentration c is at equilibrium), we have approximately:

$$c(t) \approx \frac{e_0 s(t)}{s(t) + K_m}, \quad K_m = \frac{k_{-1} + k_2}{k_1},$$

which on substituting into the first equation (for s) gives the MM-law (6):

$$\frac{ds}{dt} \approx -\frac{k_2 e_0 s}{s + K_m} = -\frac{V_{max} s}{K_m + s}.$$

The graphics of the substrate dynamics according to the MM-law (upper graphic) and to the HMM-law (lower graphic) presented in Figure 4 show that the two models produce different solutions. The upper graphic corresponding to the MM-law is an approximation of the lower graphic which originates from the true kinetic HMM-law. It can be proved that the difference between the two solutions for s does not exceed e_0 .

It is to be noted that the substrate variable s participates in the denominator in (6), whereas there are no terms with denominators in system (8). This suggests that the equations modelling microbial growth that involve s in the denominators of similar terms, may be considered as approximations of systems of larger dimension with no denominators involving s .

We emphasize that the model (3) using Monod function involves the (approximate) MM-law. Our idea developed in the next section is to involve the (true) HMM-law instead.

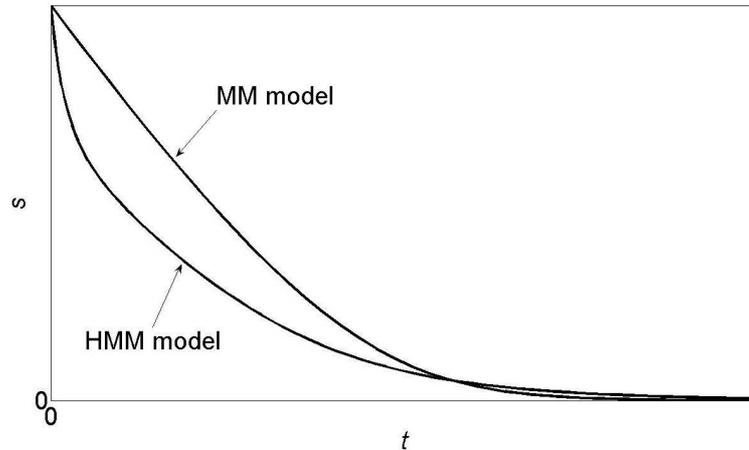


Fig. 4. Graphics of the substrate dynamics according to the MM-law and HMM-law

3. Microbial growth models and the HMM-law. As demonstrated in Figure 4, the MM-law (6) for the substrate uptake is an approximation of the HMM-law. This approximation is good when the ratio e_0/s_0 is small [12]. Note that a small ratio e_0/s_0 is typical for laboratory experiments (in vitro), but may be rather big in vivo [12].

Our idea is, instead of relating the microbial growth model to the (approximate) MM-law, to relate it to the original HMM-law which is the exact mathematical description of the Henri-Michaelis-Menten kinetic equation and should not depend on the ratio e_0/s_0 . Besides, this will give us the freedom to classify microorganisms into two sub-populations, and consequently, to introduce a more effective inhibitory mechanism.

We next discuss the necessity of introducing phases in the modelling of microbial growth.

3.1. Phases in microbial growth. Bacterial growth in batch culture can be modelled using four different phases, cf. e. g. [6], [15]:

(A) **lag phase:** During the lag phase, bacteria adapt themselves to growth conditions. It is the period where the individual bacteria are maturing and not yet able to divide. During the lag phase of the bacterial growth cycle, synthesis of RNA, enzymes and other molecules occurs.

(B) **log phase:** Exponential or log phase is a period characterized by cell doubling. The number of new bacteria appearing per unit time is proportional to the present population. If growth is not limited, doubling will continue at a constant growth rate so both the number of cells and the rate of population

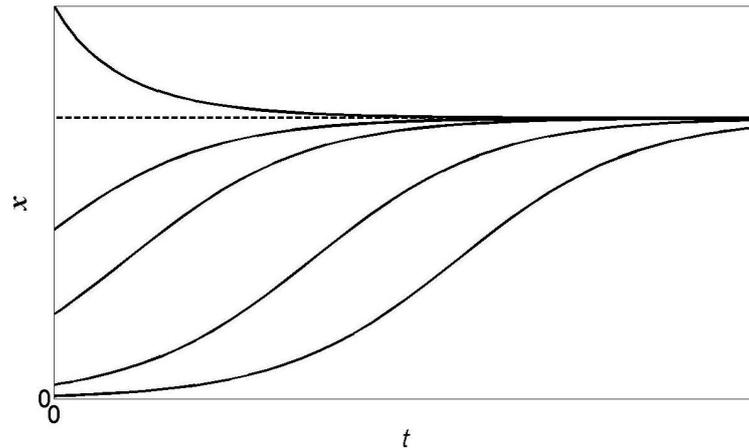


Fig. 5. Solutions to Verhulst-Pearl DE with five different initial values

increase doubles with each consecutive time period.

(C) **stationary phase**: During the stationary phase, the growth rate slows down as a result of nutrient depletion and accumulation of toxic products. This phase is reached as the bacteria begin to exhaust the resources that are available to them. This phase is a constant value as the rate of bacterial growth is equal to the rate of bacterial death.

(D) **death phase**: At the death phase, bacteria run out of nutrient substrate and die.

The first three of the above four phases of microbial growth are modelled by the logistic curves which solve the well-known Verhulst-Pearl ODE: $dx/dt = ax(1 - x/k)$, see the two lower graphics in Figure 5. (The upper three curves show the behavior of microbial growth when the initial state $x(0)$ is close to the carrying capacity k .)

Note that Verhulst-Pearl ODE describes the variation of the biomass x , but does not involve the dynamics of the substrate nutrient. Monod type models involve both the substrate and the biomass, but do not make use of microbial phases.

3.2. Introducing phases in microbial growth model. Our idea is to introduce phases in the microbial growth model suitably interpreting and slightly modifying the HMM-law. Aiming at as simple a model as possible we subdivide the microbial population into two subgroups:

- microorganisms in phases (A) and (C) are grouped into one subclass

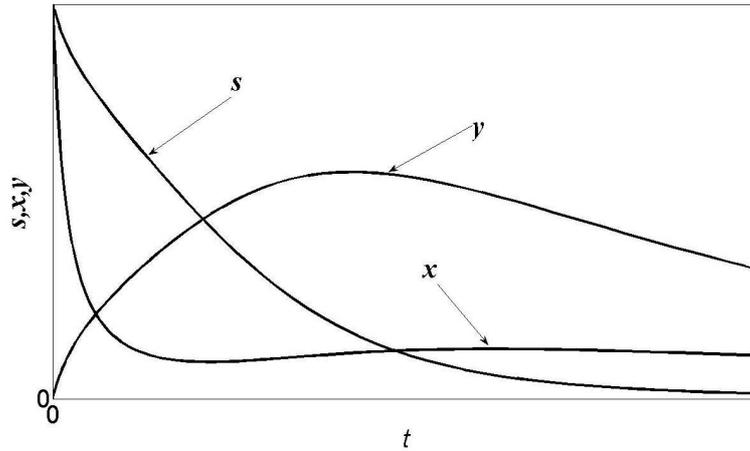


Fig. 6. Solutions to system (9)–(11)

with biomass denoted x . All microorganisms in that class experience extreme conditions (fasting or overfed), they are not able to immediately produce enzymes;

— active (viable) microorganisms in phase (B), which possess a complete active set of enzymes, denoted by y .

In this work we shall not assign a special subgroup to bacteria in dying state (D).

Under these assumptions it seems useful to look for certain analogies between a microbial–nutrient system and an enzyme–substrate system. We shall relate the subpopulation of microorganisms under stress x to free enzymes e and the subpopulation of active microorganisms y to bounded enzymes c . Thus a possibly plausible dynamic model of a batch mode bio-reactor constructed in analogy to the HMM-law of enzyme kinetics (8), after ignoring the reverse reaction with rate constant k_{-1} in the HMM-scheme, that is assuming $k_{-1} = 0$ in system (8), looks as follows:

$$\begin{aligned}\frac{ds}{dt} &= -k_1xs, \\ \frac{dx}{dt} &= -k_1xs + k_2y, \\ \frac{dy}{dt} &= k_1xs - k_2y,\end{aligned}$$

with initial conditions $s(0) = s_0$, $x(0) = x_0$, $y(0) = y_0$. Adding appropriate additional terms we obtain a variety of models. Below we present two such models.

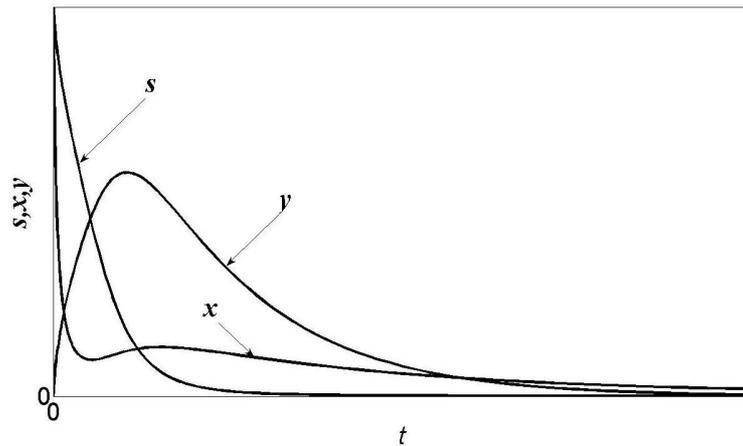


Fig. 7. Solutions to system (9)–(11) in a longer time interval

3.3. Two microbial growth models using phases.

Model 1. Consider the following system of ODE's as a model of a microbial-nutrient batch mode bio-reactor:

$$(9) \quad \frac{ds}{dt} = -k_1xs - \beta ys,$$

$$(10) \quad \frac{dx}{dt} = -k_1xs + k_2y - k_d x^2,$$

$$(11) \quad \frac{dy}{dt} = k_1xs - k_2y + \beta ys,$$

with the initial conditions $s(0) = s_0$, $x(0) = x_0$, $y(0) = y_0$. One may compare systems (8) and (9)–(11) to see that some terms reflect direct analogies, while others reflect specific characteristics of the particular system. The terms participating in system (9)–(11) have the following meaning:

k_1xs — models the consume of s by bacteria x and the transition of (fasting) bacteria x into (viable, active) bacteria y ;

βys — models the consume of s by bacteria y and the increase of bacteria biomass y due to nutrition and reproduction;

k_2y — models the transition of bacteria y into x due to depletion of s ;

$k_d x^2$ — models the competition and decay of (starving) bacteria x .

The meaning of the coefficients k_1 and k_2 is similar to that in the enzymatic HMM system (8). k_d is a decay rate, whereas β is reproduction rate; assumed is $\beta < k_1$.

In our numerical experiments we have assumed y_0 is a small quantity (or zero), meaning that at $t = 0$ most (all) of the microorganisms are in starving phase.

The solutions are visualized in Figures 6, 7. The parameters in system (9)–(11) are chosen as follows: $k_1 = 0.05$; $k_2 = 0.1$; $k_d = 0.3$; $\beta = 0.05$; and the initial conditions are: $s_0 = 10$; $x_0 = 10$; $y_0 = 0$. Observed is a fast growth of the population of active bacteria during a sufficient supply of substrate nutrient s . The decay in the total amount of biomass $x + y$ due to substrate depletion is also clearly seen in Figure 7.

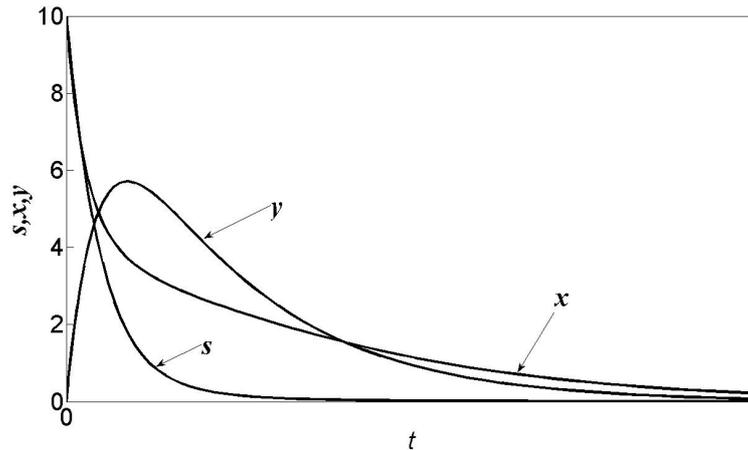


Fig. 8. Solutions to system (12)–(14) with $\alpha = 0.01$, $\beta = 0.04$ and $k_d = 0.1$

Model 2. Consider next the following system of ODE's:

$$(12) \quad \frac{ds}{dt} = -k_1xs - (\alpha + \beta)ys,$$

$$(13) \quad \frac{dx}{dt} = -k_1xs + k_2y + \alpha ys - k_dx,$$

$$(14) \quad \frac{dy}{dt} = k_1xs - k_2y + (\beta - \alpha)ys - k_dy,$$

with the initial conditions $s(0) = s_0$, $x(0) = x_0$, $y(0) = y_0 = 0$.

The new terms involved in system (12)–(14) have the following meaning:

k_dx — models the decay of bacteria x ;

k_dy — models the decay of bacteria y .

The meaning of the remaining terms and coefficients is similar to that in the system (9)–(11) with one difference: in equation (13) the term αys with $\alpha \leq \beta$

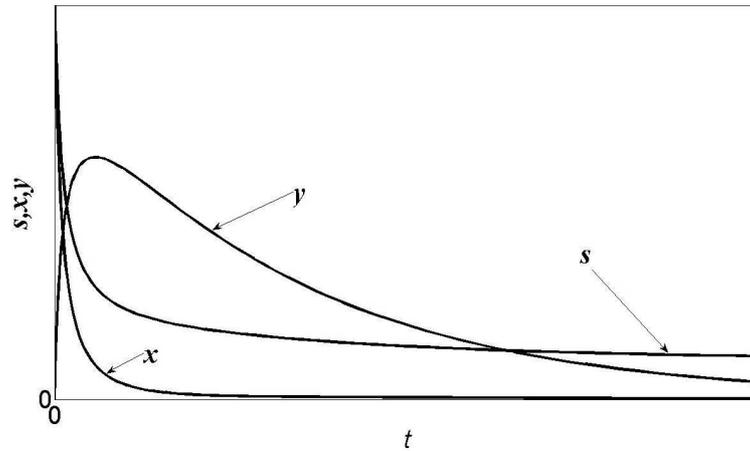


Fig. 9. Solutions to system (12)–(14) with $\alpha = 0.01$, $\beta = 0.04$ and $k_d = 0.3$

models a part of (overfed, poisoned) bacteria y which pass from compartment y to compartment x . In what follows we assume $\beta \leq k_1$.

In Fig. 8 the solutions of model (12)–(14) are visualized for values of the coefficients: $k_1 = 0.05$, $k_2 = 0.03$, $\alpha = 0.01$, $\beta = 0.04$ and $k_d = 0.1$.

In Fig. 9 all coefficients are same as in Fig. 8 except that the decay rate is bigger ($k_d = 0.3$ against $k_d = 0.1$). It can be observed that when the decay rate is bigger, the bacteria may die before the substrate has been utilized.

In our next numerical experiment we exchange the values for the coefficients α and β . The parameters in system (12)–(14) are chosen as follows: $k_1 = 0.05$; $k_2 = 0.03$; $k_d = 0.1$; $\alpha = 0.04$; $\beta = 0.01$ and the initial conditions are: $s_0 = 10$; $x_0 = 2$; $y_0 = 0.0$. The solutions are visualized in Figure 10. The decrease in the total amount of biomass $x + y$ due to substrate depletion is again clearly observed.

Our numerical simulations show that the two models (9)–(11) and (12)–(14) adequately reflect the inhibition on microbial growth due to nutrient depletion. The presence of more parameters and terms allows us the freedom to tune the model better to particular realistic situations. The behavior of the computed solutions is close to the experimentally observed behavior of microbial growth.

3.4. Relation to Monod type models. Here we analyze the relation between the proposed microbial growth models and the Monod models of the type (1)–(2). To this end consider the simplified system (9)–(11) where the mortality

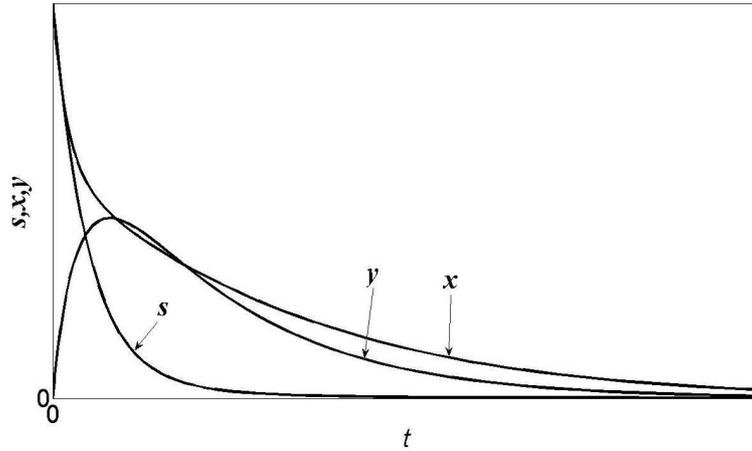


Fig. 10. Solutions to (12)–(14) with $\alpha = 0.04$, $\beta = 0.01$ and $k_d = 0.1$

rate of bacteria x is taken to be $k_d = 0$, that is:

$$(15) \quad \frac{ds}{dt} = -k_1xs - \beta ys,$$

$$(16) \quad \frac{dx}{dt} = -k_1xs + k_2y,$$

$$(17) \quad \frac{dy}{dt} = k_1xs - k_2y + \beta ys,$$

with the initial conditions $s(0) = s_0$, $x(0) = x_0$, $y(0) = y_0 = 0$.

Adding equations (16)–(17) we obtain $x' + y' = \beta ys$, which after integration gives: $x(t) = x_0 - y + \phi$, with $\phi = \beta \int_0^t ysd\tau$.

We shall next do some simple calculations based on the approximation $y' \approx 0$, which is often a realistic assumption at least for the time period when y is close to its maximum. From $y' = 0$ and (17) we calculate y in terms of s from $k_1(x_0 - y + \phi)s - k_2y + \beta ys = 0$, obtaining thus:

$$y = \frac{k_1(x_0 + \phi)s}{k_2 + (k_1 - \beta)s}.$$

Substituting this into (15) (which due to $y' = 0$ can be written as $s' = -k_2y$) gives:

$$s' = -k_1xs - \beta ys = -k_2y = -\frac{k_1k_2(x_0 + \phi)s}{k_2 + (k_1 - \beta)s},$$

which can be written as:

$$s' = -\frac{k_2(x_0 + \phi)s}{k_2/k_1 + (1 - \beta/k_1)s}.$$

Noticing that $x_0 + \phi = x + y$ is the total biomass we see that the above DE coincides (up to some parameters) with the MM-uptake which is characteristic for Monod type models (1)–(3).

4. Conclusions. It has been noted [6] that Monod type models are most adequate when microorganisms are in active states, which explains why once disturbed bio-reactors often go out of control. Our proposed approach to bacterial growth modelling provides various possibilities to flexibly account for specific microbial competence under various types of environmental conditions and thus may be suitable for the reliable modelling and control of certain bio-reactors. We formulate two particular models involving microbial phases and show that the computed solutions adequately reflect practically observed inhibition effects. For the construction of our models we make substantial use of ideas inspired by enzyme kinetics mechanisms.

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REFERENCES

- [1] BURHAN N., TS. SAPUNDZHIEV, V. BESCHKOV. Mathematical modelling of cyclodextrin-glucano-transferase production by batch cultivation. *Biochemical Engineering J.*, **24** (2005), 73–77.
- [2] BURHAN N., TS. SAPUNDZHIEV, V. BESCHKOV. Mathematical modelling of cyclodextrin-glucano-transferase production by immobilised cells of *Bacillus circulans* ATCC21783 at batch cultivation. *Biochemical Engineering J.*, **35** (2007), 114–119.
- [3] BRIGGS, G. E., J. B. S. HALDANE. A note on the kinetic of enzyme action. *Biochem. J.*, **19** (1925), 338–339.
- [4] BROWN A. J. Enzyme action. *J. Chem. Soc.*, **81** (1902), 373–386.

- [5] DIMITROVA N. Local Bifurcations in a Nonlinear Model of a Bioreactor. *Serdica Journal of Computing*, **3** (2009), No 2,107–132.
- [6] GERBER M., R. SPAN. An Analysis of Available Mathematical Models for Anaerobic Digestion of Organic Substances for Production of Biogas. proc. IGRC, Paris, 2008.
- [7] HENRI V. Recherches sur la loi de l'action de la sucrase. *C. R. Hebd. Acad. Sci.*, **133** (1901), 891–899.
- [8] HENRI V. Ueber das Gesetz der Wirkung des Invertins. *Z. Phys. Chem.*, **39** (1901), 194–216.
- [9] HENRI V. Theorie generale de l'action de quelques diastases. *C. R. Hebd. Acad. Sci.*, **135** (1902), 916–919.
- [10] MICHAELIS L., M. L. MENTEN. Die Kinetik der Invertinwirkung. *Biochem. Z.*, **49** (1913), 333–369.
- [11] MURRAY J. D. *Mathematical Biology: I. An Introduction*, Third Edition, Springer, 2002.
- [12] SCHNELL S., P. K. MAINI. Enzyme kinetics at high enzyme concentration. *Bull. Math. Biol.*, **62** (2000), 483–499.
- [13] SCHNELL S., P. K. MAINI. A century of enzyme kinetics: Reliability of the K_M and v_{\max} estimates. *Comments on Theoretical Biology*, **8** (2003), 169–187.
- [14] SMITH H. L., P. WALTMAN. *The theory of the chemostat. Dynamics of microbial competition*. Cambridge University Press, 1995.
- [15] http://en.wikipedia.org/wiki/Bacterial_growth

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