

## ARTICLE; BIOINFORMATICS

### Time lag model for batch bioreactor simulation accounting the effect of micro-organism mortality

Andrey Zahariev<sup>a\*</sup>, Hristo Kiskinov<sup>a</sup>, Angel Angelov<sup>b</sup> and Stoyan Zlatev<sup>a</sup>

<sup>a</sup>Faculty of Mathematics and Informatics, University of Plovdiv, Plovdiv, Bulgaria; <sup>b</sup>Department of Biotechnologies, University of Food Technologies, Plovdiv, Bulgaria

(Received 28 May 2014; accepted 28 July 2014)

In the present work, a generalization of the classical model of Monod accounting the influence of both delayed and instant mortalities on the dynamics of the micro-organism population is proposed. The model was analysed and compared with respect to its quality and applicability for simulation of the cultivation process of micro-organisms. Existence of a unique global positive solution of the Cauchy problem for the proposed model is proved and explicit relations between the decay parameters and the nutrition substrate concentration are obtained. These mathematical results allow us to calculate the nutrient substrate concentration which guarantees that the biomass concentration is maximal for every specific type of taxonomic groups of micro-organisms (bacteria, yeasts).

**Keywords:** Monod model; micro-organism cultivation; dynamics of micro-organism populations; batch bioreactor; delay equations

#### Introduction

It is well known that the Monod-type microbial growth models describe adequately bioprocesses appearing in bioreactors and this explains why the Monod-type models are still actual from the theoretical as well as practical point of view.[1] The classical model of Monod [2] of aerobic periodic cultivation of micro-organisms (bacteria, yeasts)

$$\begin{aligned} s'(t) &= -\alpha\mu(s(t))x(t) \\ x'(t) &= \mu(s(t))x(t) \end{aligned} \quad (1)$$

where  $t \in R_+ = [0, \infty)$ , and  $x(\cdot)$  and  $s(\cdot) : R \rightarrow R_+$  are the concentrations of micro-organisms and the substrate, respectively, has been studied in details by many authors. [1–6] The function  $\mu(s)$  is the intrinsic specific rate of micro-organism population growth and the parameter  $\alpha > 1$  is called economic coefficient (rate of yield). Note that  $\mu(s)$  is designed to reflect the limiting influence of the substrate on the microbial growth. It is practically established that the model of microbial growth (1) with monotonously increasing functions of Monod type  $\mu_M(s) = \mu_{\max} \frac{s}{k+s}$  or of Webb type  $\mu_{W1}(s) = \mu_{\max} \frac{s(1+sk_I^{-1})}{k+s+s^2k_I^{-1}}$ , where  $\mu_{\max}$  (maximal specifically possible producing rate),  $k$  (constant of half saturation) and  $k_I$  (inhibition constant) are positive constants, adequately describes the dynamics of this process at certain

favourable conditions permitting the micro-organisms actively to produce specific enzymes, which are necessary for assimilation and dissimilation of the nutrient substrates. Thus the micro-organisms reproduce themselves at the maximal possible rate  $\mu_{\max}$ . However, that small or large amount of substrate may have an inhibiting (decreasing) effect on the specific rate of microbial growth. In order to reflect this phenomenon in model (1), Haldane [7] and Andrews [3] have suggested the unimodal functions  $\mu_H(s) = \mu_{\max} \frac{s}{(k+s)(1+sk_I^{-1})}$  and  $\mu_A(s) = \mu_{\max} \frac{s}{k+s+s^2k_I^{-1}}$ , respectively. These functions are also similar and special cases of the Webb [8] function  $\mu_W(s) = \mu_{\max} \frac{s(1+\beta sk_I^{-1})}{k+s+s^2k_I^{-1}}$  which is unimodal too, when  $\beta \in [0, 1)$ . Besides,  $\mu_W(s) \approx \mu_M(s)$  if  $0 \leq s \ll \sqrt{k_I}$ . It means that the parameter  $k_I$  determines in a way the inhibitory phase of the population growth.[6] The basic properties and the graphs of all the four functions are given in [9] where the system

$$\begin{aligned} s'(t) &= -\alpha\mu(s(t))x(t) \\ x'(t) &= \mu(s(t))x(t) - k_d x(t) \end{aligned} \quad (2)$$

introduced in [10] is under consideration. Here,  $k_d > 0$  is the specific rate of decay of the micro-organism population. The necessity of models of the kind (2) arises due to unfavourable conditions in the bioreactor. Theoretical and computational analysis of model (2) is fulfilled in [10].

\*Corresponding author. Email: [zandrey@uni-plovdiv.bg](mailto:zandrey@uni-plovdiv.bg)

This includes establishing of explicit dependencies between  $x$  and  $s$  for all four above-mentioned functions as well as between  $t$  and  $s$  for the first three of them when  $k_d = 0$ . In a previous work,[11] we study the delay analogue of (2), namely

$$\begin{aligned} s'(t) &= -\alpha\mu(s(t))x(t) \\ x'(t) &= \mu(s(t))x(t) - k_mx(t-\tau) \end{aligned} \quad (3)$$

with an initial condition

$$x(t) = \varphi(t), s(t) = \psi(t) \text{ for } t \in [-\tau, 0] \quad (4)$$

where  $t \in R_+$ ,  $\varphi, \psi: [-\tau, 0] \rightarrow R_+$ ,  $\mu: R \rightarrow R$  and  $k_m > 0$  is the specific rate of decay of the micro-organism population. The model (3) was proposed under the reasonable assumption that the individuals of every kind of population have their specific average lifetime  $\tau$  in the bioreactor, which implies that the population decay at the moment  $t$  is directly proportional to the micro-organism quantity at the moment  $t - \tau$ .

## Materials and methods

### Materials

Our mathematical model is applicable for all types of bioreactors for aerobic periodic cultivation of micro-organisms (bacteria, yeasts).

### Methods

Since the time of V. Volterra, functional-differential equations (FDEs) are widely used to model biological processes. The transmission of control signals in biological systems is related to such long processes as birth, growth (development) and death. Because of this, the evolution of biological systems depends in an essential way on the whole previous history, and can be modelled in general only by FDEs. Moreover, using FDEs allows us to take into account various insecure factors such as finite lifetime and interaction time; inhomogeneity of the populations lifetime; finite acceptance time for external signals and finite time for elaborating counteractions; pollution effects, resulting in additional mortality with time delay; and spatial environmental heterogeneity. The importance of the aftereffects in population dynamics, and the new effects stipulated by it, determines the practical reason to create delay models which are used to control processes of microbiological growth of cells and production of a useful product. We consider one of them, describing the periodical aerobic reproduction of micro-organisms.

## Results and discussion

### Statement of the problem

From the biotechnological point of view, we presume that the micro-organism mortality is one of the most significant factors influencing successful micro-organism cultivation. Therefore, it is very important to create models which take into account more precisely the micro-organism mortality impact on the population dynamics. In the present paper, we follow this direction and study a combination of models (2) and (3) of the kind

$$\begin{aligned} s'(t) &= -\alpha\mu(s(t))x(t) \\ x'(t) &= \mu(s(t))x(t) - l_1x(t) - l_2x(t-\tau) \end{aligned} \quad (5)$$

$$x(t) = \varphi(t), s(t) = \psi(t) \text{ for } t \in [-\tau, 0] \quad (6)$$

where  $t \in R_+$ ,  $\varphi, \psi: [-\tau, 0] \rightarrow R_+$ ,  $\tau > 0$ ,  $\mu: R \rightarrow R$ ,  $l_1, l_2 > 0$  are the specific rates of decay of the micro-organism population. The model (5) describes more precisely the impact of the microorganism mortality for different kinds of micro-organism populations in comparison with the models (2) and (3) taking into account not only the micro-organism mortality in the same moment, but also that in a previous moment. It means that the individuals of every kind of population have their own specific average lifetime  $\tau$  in the bioreactor. It is clear from the biological point of view that the micro-organism mortality in the moment  $t$  is caused in general by natural reasons, i.e. it is proportional to the quantity of those micro-organisms that have begun their lives in the moment  $t - \tau$  described by the term  $l_2x(t - \tau)$  included in the model (5). Thus, we take into account the influence of both (instant and delayed) micro-organism mortalities on the population dynamics. Under biological reasons, one may suppose  $l_1 < l_2$  (a majority of the micro-organism population will die after the expiry of its average lifetime  $\tau$ ), but in our exposition below we will not do that.

In our consideration, we make the following assumption: the material composition is uniform in the reactor and intracellular, while nonuniform space distribution is ignored.

Our basic purpose in this work is to give an explicit answer of the following two practical questions which play an important role in the aerobic periodic cultivation of micro-organisms:

- (1) For every choice of the function  $\mu(s)$ , how to calculate practically the minimal concentration of the nutrient substrate  $s_0$  which is necessary to start an increasing micro-organisms reproduction for some period.
- (2) How to establish practically that the biomass concentration is maximal, i.e. the cultivation process enters in the stationary phase.

Let  $J \subseteq R$  be an arbitrary interval. For every function  $f : J \rightarrow R$ , we set by definition  $\|f\| := \sup_{t \in J} |f(t)|$  if  $\sup_{t \in J} |f(t)| < \infty$ . We shall denote by  $\|F\|_2$  the Euclidean norm of  $F \in R^2$ , by  $C(J, R^2)$  the set of all continuous vector functions  $F : J \rightarrow R^2$  and by  $AC(J, R^2)$  the set of all vector functions  $F(t)$  which are absolutely continuous on every closed subinterval of  $J$ . For the vector function  $F \in C(J, R^2) F(t) = (x(t), s(t))^T$ , we set by definition  $\|F\|_2 := (\|x\|^2 + \|s\|^2)^{\frac{1}{2}}$  if  $\sup_{t \in J} |x(t)| < \infty$  and  $\sup_{t \in J} |s(t)| < \infty$ . Further, we shall use the following definitions:

**Definition 2.1.** Vector function  $F(t) = (x(t), s(t))^T$ ,  $F \in AC([0, t_F], R^2)$ ,  $t_F > 0$  is said to be a solution of the initial value problem (IVP) (5)–(6) in the interval  $[0, t_F]$  if it satisfies Equation (5) for almost all  $t \in [0, t_F]$  and the initial condition (6) for  $t \in [-\tau, 0]$ .

**Definition 2.2.** A solution  $F(t) = (x(t), s(t))^T$  of the IVP (5)–(6) in the interval  $[0, t_F]$  is said to be positive (non-negative) in the interval  $[0, t_F^+)$ ,  $0 < t_F^+ < t_F$  if  $x(t) > 0, s(t) > 0 (x(t) \geq 0, s(t) \geq 0)$  for all  $t \in [0, t_F^+)$ .

**Definition 2.3.** We will say that the property  $P$  is ultimately fulfilled for some function  $f : [-\tau, \infty) \rightarrow R$  if there exists a point  $t_P \geq 0$ , such that for the function  $f$  the property  $P$  holds for each  $t \geq t_P$ .

The basic tasks concerning the model (5) to be solved in the present paper are the existence and uniqueness of a solution of the IVP (5)–(6), analysis of the dynamics of  $x(t)$  when the nutrient substrate diminishes on a finite or infinite period and studying the influence of the correlation between the parameter  $l_1, l_2$  and the values of the function  $\mu(s(t))$  on the dynamics of  $x(t)$ .

**Main results**

We denote by (H) the following conditions:

- (H1).  $\varphi(t) = 0$  for  $t \in [-\tau, 0)$  and  $\varphi(0) = x_0 > 0$ .
- (H2).  $\psi(t) = s_0 > 0$  for  $t \in [-\tau, 0]$ .
- (H3). There exists  $h > 0$  such that the function  $\mu : R \rightarrow R$  is nondecreasing, continuous and bounded for  $s \in [-h, \infty)$  and  $\mu(0) = 0$ .

**Lemma 1.** Let the conditions (H) be fulfilled.

Then there exists a function  $F(t) \in AC(R_+, R^2)$  which is the unique solution of the IVP (5)–(6) in  $R_+$ .

**Proof.** Denote by  $\phi(t) = (\varphi(t), \psi(t))^T$  the initial vector function and define for each  $t \in R_+$  the function

$$\Phi(t, F_t) = \begin{pmatrix} \mu(s_t(0))x_t(0) - l_1x_t(0) - l_2x_t(-\tau) \\ -\alpha\mu(s_t(0))x_t(0) \end{pmatrix} \quad (7)$$

where  $F_t(\theta) = \begin{pmatrix} x_t(\theta) \\ s_t(\theta) \end{pmatrix} = \begin{pmatrix} x(t+\theta) \\ s(t+\theta) \end{pmatrix}$ ,  $F_0(\theta) = \phi(\theta)$  for  $-\tau \leq \theta \leq 0$ .

Let us denote by  $M_\phi$  the set of all vector functions  $F : [-\tau, \infty) \rightarrow R^2$ , such that  $F_0(\theta) = \phi(\theta)$ ,  $\theta \in [-\tau, 0]$  and the restriction  $F|_{R_+}$  is a continuous vector function. We will denote by  $D_\phi$  the set  $D_\phi = \{(t, F_t) | t \in R_+, F \in M_\phi\}$  equipped with the metric function  $d((t_1, \varsigma_1), (t_2, \varsigma_2)) = |t_1 - t_2| + \|\varsigma_1^* - \varsigma_2^*\|_2$ ,  $(t_1, \varsigma_1), (t_2, \varsigma_2) \in D_\phi$  (see [12] Chapter 3, Subs. 2.4), where  $\varsigma_i^*(t) = \varsigma_i(t)$  for all  $t_i \geq \tau$  and if  $0 \leq t_i < \tau$ , then  $\|\Phi(t, \varsigma(t))\|_2 \leq L\|\varsigma\|_2$ . We set  $\varsigma_i^*(t) = \varsigma_i(t)$  for  $-t_i \leq t \leq 0$  and  $\varsigma_i^*(s) = \varsigma_i(-t_i)$  for  $-\tau \leq s \leq -t_i, i = 1, 2$ .

From the conditions (H), it follows that if the function  $\Phi : D_\phi \rightarrow R^2$  is defined by (7), then the map  $t \rightarrow \Phi(t, F_t)$  is continuous for any  $F \in M_\phi$ . Moreover, for every fixed  $t \in R_+$ , the function  $\Phi(t, \varsigma)$  is continuous in every function  $\varsigma : C([-\tau, 0], R^2)$ . Let  $(t_0, \varsigma_0) \in D_\phi$  be an arbitrary point,  $0 < r < t_0$  and consider the neighbourhood  $B_r = \{(t, \varsigma) \in D_\phi | d((t, \varsigma), (t_0, \varsigma_0)) < r\}$ . Then, there exists a constant  $L(r, t_0, \varsigma_0) > 0$  such that the inequality  $\|\Phi(t, \varsigma_1) - \Phi(t, \varsigma_2)\|_2 \leq L\|\varsigma_1 - \varsigma_2\|_2$  holds for every two points  $(t, \varsigma_1), (t, \varsigma_2) \in B_r$ . Since  $\Phi(t, 0) = 0$ ,  $\varphi(t)$  is uniformly continuous in  $[-\tau, 0)$ ,  $\varphi(0^-) = 0 \neq \varphi(0)$  and  $\psi(t)$  is uniformly continuous in  $[-\tau, 0]$ , then there exists a point  $t_F > 0$  such that the IVP (5)–(6) has a unique solution on the interval  $[0, t_F]$ . The function  $\Phi(t, \varsigma)$  defined by equality (7) is sublinear: that is, for each point  $(t, \varsigma) \in D_\phi$ , the inequality holds. Therefore,  $t_F = \infty$  (see [12] Chapter 3, Subs. 2.2–2.4).

**Theorem 2.** Let the conditions (H) be fulfilled.

Then for every solution  $F(t) = (x(t), s(t))^T$  of the IVP (5)–(6) for which there exists a point  $t_0^s > 0$  such that  $x(t_0^s) = 0$  and  $x(t) > 0$  for  $t \in [0, t_0^s)$ , then  $s(t) > 0$  for each  $t \in [0, t_0^s)$ .

**Proof.** Assume there exists a point  $t_0^s \in [0, t_0^x)$  such that  $s(t_0^s) = 0$  and  $s(t) > 0$  for  $t \in [0, t_0^s)$ . Then, it follows from (5) that  $s'(t_0^s) = 0$  and consequently  $t_0^s$  is either an inflection point or a point of local minimum for  $s(t)$ . Since  $x(t_0^s) > 0$ , then there exists  $\delta > 0$  such that  $x(t) > 0$  for  $t \in (t_0^s - \delta, t_0^s + \delta)$ . From the first equation of (5) it follows that  $t_0^s$  cannot be neither an inflection point nor a point of local minimum for  $s(t)$ .

**Corollary 3.** Let the conditions (H) be fulfilled.

Then for every solution  $F(t) = (x(t), s(t))^T$  of the IVP (5)–(6) for which there exists a point  $t_0^s > 0$  such that  $s(t_0^s) = 0$  and  $s(t) > 0$  for  $t \in [0, t_0^s)$  there exists a point  $t_0^x$  such that  $t_0^x \leq t_0^s, x(t_0^x) = 0$  and  $x(t) > 0$  for  $t \in [0, t_0^x)$ .

**Proof.** Assume there does not exist a point  $t_0^x$  such that  $t_0^x \leq t_0^s$  and  $x(t_0^x) = 0$ . Then, we obtain that  $x(t) > 0$  for  $t \in [0, t_0^s)$ , which contradicts the conclusion of Theorem 2.

**Theorem 4.** Let the conditions (H) be fulfilled.

Then for every solution of the IVP (5)–(6) there exists a point  $t_F^+ > \tau$  such that the solution  $F(t)$  is positive and  $s(t)$  is decreasing for  $t \in [0, t_F^+)$ .

**Proof.** Let the function  $F(t) \in AC(R_+, R^2)$  be a solution, existing according to Lemma 1, in the interval  $R_+$ . From conditions (H) it follows that there exists a point  $t_F > 0$  such that  $F(t) = (x(t), s(t))^T$  is a positive solution in the interval  $[0, t_F]$ . It is easy to see that from (5) it follows  $x(t) > 0$  for  $t \in [0, \tau]$ . Now assume there exists a point  $t_0^s \in (0, \tau]$  such that  $s(t_0^s) = 0$  and  $s(t) > 0$  for  $t \in [0, t_0^s)$ . From (5) it follows that  $s'(t_0^s) = 0$  and from condition (H3) it follows that  $t_0^s$  is either an inflection point for  $s(t)$  or  $s(t)$  has minimum in the point  $t_0^s$  which is impossible because from (5) it follows that  $t_0^s$  can be neither an inflection point, nor a point of local minimum for  $s(t)$ . Therefore,  $s(t) > 0$  for  $t \in [0, \tau]$ . Then, there exists a point  $t_F^+$  ( $t_F^+ > \tau$ ) such that  $x(t) > 0$  for  $t \in [0, t_F^+)$  and Theorem 2 implies  $s(t) > 0$  for  $t \in [0, t_F^+)$ . From conditions (H) and (5), it follows that  $s(t)$  is decreasing for  $t \in [0, t_F^+)$ .

Let us denote by  $s_{\min} = \inf_{t \in R_+} s(t)$ .

**Theorem 5.** Let the conditions (H) be fulfilled.

Then, for every solution  $F(t) = (x(t), s(t))^T$  of the IVP (5)–(6) we have  $\mu(s_{\min}) < l_1 + l_2$ .

**Proof.** Assume that  $\mu(s_{\min}) \geq l_1 + l_2$ . Then, from condition (H3) it follows that  $s_{\min} > 0$ . Since  $s_{\min} > 0$  and  $\mu(s(t)) - l_1 \geq l_2 > 0$  for  $t \in R_+$ , then from Theorem 4 it follows that the inequalities  $x'(t) \geq (\mu(s(t)) - l_1)x(t) > 0$  are obviously true for  $t \in [0, \tau]$  and therefore,  $x'(\tau - 0) > 0$  and  $x(t)$  is strictly increasing in the same interval. From (1.5) and Theorem 4 it follows that the inequalities

$$\begin{aligned} x'(\tau + 0) &= \mu(s(\tau))x(\tau) - l_1x(\tau) - l_2x(0) \geq \\ &\geq (\mu(s(\tau)) - l_1)x(\tau) - l_2\varphi(0) \geq l_2(x(\tau) - \varphi(0)) > 0 \end{aligned}$$

hold. Assume there exists a point  $t^* > \tau$  such that  $x'(t^*) \leq 0$  and denote  $t_0 = \inf_{\tau \leq t \leq t^*} \{t | x'(t) \leq 0\}$ . Then we have  $x'(t_0) = 0$  and  $x(t_0) > 0$ . Condition (H3), Theorem 2 and the assumption  $\mu(s_{\min}) \geq l_1 + l_2 > 0$  imply the inequalities  $\mu(s(t)) \geq \mu(s(t_0)) \geq \mu(s_{\min}) \geq l_1 + l_2$  and therefore the following inequality

$$\begin{aligned} x'(t) &\geq (\mu(s(t_0)) - l_1)x(t) - l_2x(t - \tau) \geq \\ &\geq l_2(x(t) - x(t - \tau)) > 0 \end{aligned} \tag{8}$$

holds for  $t \in [\tau, t_0]$ . Considering that  $x(t_0) > 0$  and  $x \in C^1((\tau, \infty), R)$ , from inequalities (8) it follows  $x'(t_0) > 0$  which contradicts  $x'(t_0) = 0$ . Therefore,  $x'(t) > 0$  for each  $t \in R_+$  and the first equation of (5) implies that  $s'(t) \leq -\alpha\mu(s_{\min})x_0 < 0$ . Consequently, the function  $s(t)$  is negative and decreasing for all sufficiently large  $t$  which contradicts the assumption that  $s_{\min} > 0$ .

**Theorem 6.** Let the following conditions be fulfilled:

- (1) Conditions (H) are fulfilled.
- (2)  $\mu(s_0) > l_1 + l_2$ .

Then for any positive solution  $F(t) = (x(t), s(t))^T$  of the IVP (5)–(6) in  $R_+$ , the following equalities  $s_{\min} = \lim_{t \rightarrow \infty} s(t)$  and  $\lim_{t \rightarrow \infty} x(t) = 0$  are valid.

**Proof.** Let  $F(t) = (x(t), s(t))^T$  be a positive solution of the IVP (5)–(6). Then, Theorem 5 implies the inequalities  $0 \leq \mu(s_{\min}) < l_1 + l_2$ . From (5) it follows that  $s(t)$  is a positive decreasing function for each  $t \in R_+$  and consequently  $s_{\min} = \lim_{t \rightarrow \infty} s(t) \geq 0$ . Since in virtue of Theorem 5 we have that  $0 \leq \mu(s_{\min}) < l_1 + l_2$ , then there exists a unique point  $t_0 > \tau$  such that  $\mu(s(t_0)) = l_1 + l_2$  (apparently  $x'(t_0) \geq 0$ ). Therefore, either  $x'(t) \geq 0$  for each  $t \in R_+$  which is impossible, or there exists a point  $t^x > t_0$  such that  $x'(t^x) < 0$ . If we denote by  $t_0^x = \inf_{t_0 \leq t < t^x} \{t | x'(t) < 0\}$ , then we have  $x'(t_0^x) = 0$ . Suppose that there exists a point  $t_+^x > t^x$  such that  $x'(t_+^x) > 0$  and denote  $t_0^x = \inf_{t_0^x \leq t < t_+^x} \{t | x'(t) \geq 0\}$ . Then, evidently,  $x'(t_0^x) = 0$  and  $x'(t) < 0$  for  $t \in (t_0^x, t_+^x)$ .

Consider the case  $t_1^x - t_0^x > \tau$ . Then, for each  $t \in (t_0^x + \tau, t_1^x)$  for which  $\mu(s(t)) - l_1 \leq 0$  it follows from (5) that  $x'(t) \leq -l_2x(t - \tau) < 0$  and since  $x(t_1^x - \tau) > 0$ , therefore we have  $x'(t_1^x) < 0$  which is a contradiction. In the case when  $t \in (t_0^x + \tau, t_1^x)$  and  $\mu(s(t)) - l_1 > 0$ , the following inequalities

$$\begin{aligned} x'(t) &= \mu(s(t))x(t) - l_1x(t) - l_2x(t - \tau) \leq \\ &\leq (\mu(s(t)) - l_1)x(t) - l_2x(t - \tau) \leq \\ &\leq l_2(x(t) - x(t - \tau)) < 0 \end{aligned} \tag{9}$$

hold. From (9) it follows that  $x'(t_1^x) < 0$  which is impossible.

Consequently,  $t_1^x - t_0^x \leq \tau$  and let  $t \in (t_0^x, t_1^x)$ . Then,  $t - \tau \leq t_0^x$  and from (5) for which  $\mu(s(t)) - l_1 > 0$ , we obtain

$$\begin{aligned} x'(t) &= (\mu(s(t)) - l_1)x(t) - l_2x(t - \tau) \leq \\ &\leq (\mu(s(t)) - l_1 - l_2)x(t) < 0. \end{aligned}$$

In the case when  $\mu(s(t)) - l_1 \leq 0$ , the inequalities  $x'(t) \leq -l_2x(t - \tau) < 0$  hold and therefore  $x'(t_1^x) < 0$  which is impossible. Then, we ultimately have that  $x'(t) < 0$  and therefore  $\lim_{t \rightarrow \infty} x(t) = c \geq 0$ . Let us assume that  $c > 0$ . Since  $x'(t) < 0$ , ultimately then it follows from (5) that  $s'(t) \leq -\alpha\mu(s_{\min})\min\{c, x_0\} < 0$  and consequently, we have  $\lim_{t \rightarrow \infty} s(t) = -\infty$  which is a contradiction. Therefore,  $c = 0$ .

### Discussion

The proposed model (5) is analysed and compared from mathematical point of view with respect to its quality and applicability for simulation of the cultivation process of micro-organisms. This analysis includes proof of existence of a unique global positive solution of the Cauchy problem (5)–(6) (Lemma 1). The biological sense of Theorem 2 and Corollary 3 is that if the concentration of the

nutrient substrate in some finite moment becomes equal to zero, then it is impossible to have live micro-organisms after this moment. Moreover, Theorem 6 implies that if the nutrition substrate vanishes ( $\lim_{t \rightarrow \infty} s(t) = 0$ ), then the concentration of the live micro-organisms vanishes too ( $\lim_{t \rightarrow \infty} x(t) = 0$ ). Thus, the model (5) describes the real process more adequately in comparison to the classical model (2) where the nutrition substrate can vanish ( $\lim_{t \rightarrow \infty} s(t) = 0$ ), but the concentration of the live micro-organisms stay positive ( $\lim_{t \rightarrow \infty} x(t) = x_0 + \alpha s_0 > 0$ ) and even increases, which is impossible from the biological point of view.

The biological sense of Theorem 5 and the second equation from the model (5) is that the inequality

$$\mu(s_0) > l_1 + l_2 \quad (10)$$

implies increasing reproductive rate of the micro-organism population (at least for period  $\tau$ ) and there exists a moment  $t_{\max}$  such that the biomass concentration is maximal and

$$\mu(s(t_{\max})) = l_1 + l_2. \quad (11)$$

The importance of these relations for the practice is that they give explicit (computational) answers to the questions (1) and (2).

The inequality (10) allows for every specific choice of the function  $\mu(s)$  practically to calculate the minimal concentration of the nutrient substrate  $s_0$  which is necessary for an increasing bacterial concentration at least for period  $\tau$ .

From the equality (11), we can calculate the critical concentration of the nutrition substrate  $s_c = s(t_{\max})$  which guarantees that the biomass concentration is maximal. It is enough to measure periodically the nutrient substrate concentration until it reaches the critical level  $s_c = s(t_{\max})$ . Solve Equation (11) with respect to  $s$ :  $s_c = \mu^{-1}(l_1 + l_2)$ .

Let us explain this when, for example  $\mu_M(s) = \mu_{\max} \frac{s}{k+s}$ . It follows from (10) that if  $s_0 > \frac{k(l_1+l_2)}{\mu_{\max}-l_1-l_2}$ , then we will have increasing bacterial concentration at least for period  $\tau$  and when the nutrient substrate concentration  $s$  reaches, according to (11), the critical level  $s_c = \frac{k(l_1+l_2)}{\mu_{\max}-l_1-l_2}$ , then the biomass concentration is maximal.

## Conclusion

In this work, a model of aerobic periodic cultivation of micro-organisms (bacteria, yeasts) is introduced and studied. It is more precise comparing to previous models.

Our model allows us to calculate practically the minimal initial concentration of the nutrient substrate, which is necessary to start an increasing micro-organism reproduction for a given period, for every specific rate of micro-organism population growth. Moreover, it allows with a simple measuring of the concentration of the nutrient substrate to establish when the biomass concentration is maximal, i.e. the cultivation process enters in the stationary phase.

## Disclosure statement

No potential conflict of interest was reported by the author(s).

## Funding

This work was partially supported by Plovdiv University NPD [grant number N113 FMI-002].

## References

- [1] Gochev V, Montero G, Kostov G, Toscano L, Stoycheva M, Krastanov A, Georgieva A. Nutritive medium engineering enhanced production of extracellular lipase by trichoderma longibrachiatum. *Biotechnol Biotechnol Equip.* 2012;26(2):2875–2882.
- [2] Monod J. *Recherches sur la croissance des cultures bacteriennes* [Research on the growth of bacterial cultures]. Paris: Hermann; 1942.
- [3] Andrews J. A mathematical model for the continuous culture of microorganisms utilizing inhibitory substrates. *Biotechnol Bioeng.* 1968;10:707–723.
- [4] Monod J. The growth of bacterial cultures. *Ann Rev Microbiol.* 1949;3:371–394.
- [5] Murray J. *Lectures on nonlinear-differential-equation models in biology*. Oxford: Clarendon Press; 1977.
- [6] Pirt S. *Principles of microbe and cell cultivation*. Oxford: Blackwell Scientific Publications; 1975.
- [7] Haldane J. *Enzymes*. London: Longmans; 1930.
- [8] Webb J. *Enzyme and metabolic inhibitors*. New York (NY): Academic Press; 1963.
- [9] Alt R, Markov S. Theoretical and computational studies of some bioreactor models. *Comp Math Appl.* 2012;64:350–360.
- [10] Markov S. On the mathematical modelling of microbial growth: some computational aspects. *Serdica J Comput.* 2011;5(2):153–168.
- [11] Kiskinov H, Zahariev A, Zlatev S. A generalized model of Monod including a delayed decay in bacterial populations. *C R Acad Bulg Sci.* 2014;67(2):173–180.
- [12] Kolmanovskii V, Myshkis A. *Introduction to the theory and applications of functional differential equations, mathematics and its applications*. Dordrecht: Kluwer Academic Publishers; 1999.