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Stability characteristics of a biochemical reactor with predator–prey relationship
A substrate inhibition case

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Abstract

The present work characterizes static behaviour of a CSTBR with existing food chain relationship, for a case when base carbon and energy source exhibits toxic influence on enzymatic pathways of all microorganisms. Some bifurcation diagrams for representative process parameters are presented, together with characteristic phase plane plots. Conditions leading to induction of persistent oscillations of substrate and cell concentration are derived, and ways to avoid them are also given herein.

Keywords: Food chain; Predator–prey relationship; Substrate inhibition

1. Introduction

In biochemical reactor systems that are open to environmental influences, microbial composition often differs from that of inoculum. Organisms, e.g., bacteria, that are directly responsible for converting supplied substrate into (as in wastewater plants) non-toxic products, become a basis of a food chain. These bacteria serve as a food source for holozoic organisms, e.g., protozoa. This means, that microbial reactor is a suitable place for creation of a classical predator–prey system. Such a system, through a number of mechanisms, deeply influences a sequence of biochemical processes.

Chemical engineers and biotechnologists early had to tackle with problems that arise from a presence of a protozoa in a bioreactor. These were noticed soon after first runs of activated sludge plants. Effects of protozoan activity in inner environment of these plants were also noted and early works discussed possible advantageous influences that protozoa could exert on a process. An opinion on the role that protozoa, especially of *Ciliata* type, play in biocenosis of a bioreactor, was changing over a span of several decades. Protozoa were thought of as an indispensable component of properly operated aerated-sludge plant [1], but also as an obstacle in overall biochemical reactions, that take place in a reactor [2]. At present, existence of protozoa in activated sludge is treated positively as one of the conditions for effective biochemical purification, as pointed out in work of Ratsak et al. [3]. The following factors are quoted to justify beneficial effects of protozoan predation on bacteria in biochemical units.

(a) Flocculation of bacteria into biological flocs: what prevents wash-out, broadens a range of allowable flowrates, and increases subsequent sedimentation velocity [4]. This flocculation may be caused by adhesion of bacteria to a mucus excreted by protozoa during digestion, as suggested early by Watson [5]. This hypothesis suggests, that process of protozoa feeding contains a feedback loop in itself. Namely, if predators (protozoa) absorb some quantity of free-swimming preys (bacteria), it results in occlusion of the rest of bacteria in secreted mucus, and that in turn creates a shortage of food for protozoa. Actually, in correctly operated processes with a diversified biocenosis, a microbial flocs may contain up to 90% of all bacteria [6]. Other researchers, e.g., Güde [7], explain a process of microbial flocs creation in activated sludge as an selective adaptation of bacteria to predator activity. True reasons are probably a combination of above-mentioned effects.

(b) Removal of free-swimming bacteria, i.e., those open to influence of predacious protozoa, gives significant improvement in product quality (e.g., purified water in case of wastewater treatment plant) [4].

(c) Reduction of biomass produced, that would have to be utilised otherwise [8]. In aerated sludge plants, excessive biomass is often a troublesome waste, requiring dewatering and subsequent treatment.

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(d) Some species of protozoa, strongly adapted to feeding on fibrilous forms of bacteria, prevent from activated sludge bulking [9].

(e) There are hints to believe that swimming protozoa intensify a flow of nutrients and dissolved oxygen to bacterial aggregates, thus decreasing diffusional resistances [10]. This occurs due to intensive movement of some parts of protozoa cell.

(f) In microscopic investigations of activated sludge, protozoa are convenient indicators of its physiological state [11].

From the above-mentioned data, it may be concluded that the effects of presence of protozoa in biochemical systems is beyond doubt a phenomenon that should not be ignored. Consequently, that explains numerous attempts to establish a mathematical model of bioreactor biocenosis, that would allow to anticipate, a priori, effects of protozoa activity on a process efficiency. Since number of possible substrates, microbial species that inhabit the reactor, and resulting food-chain interconnections is obviously vast, at present it seems impossible to take all of them into account during model formulation. Even existing environmental and laboratory experimental data often contradict each other. Facing this, it sounds reasonable to utilise unstructured kinetic models in a study of steady-state and dynamic behaviour of a biochemical system. A simplicity of such an approach, together with generality, have an advantage over sophisticated models, that include some effects, while still neglecting another.

Relatively simple mathematical model for predator–prey system was first derived in a book by Lotka and Volterra [12]. More precise formulation was given by Kolmogorov [13], who formulated, basing on an intuitive knowledge of dynamical features of such a system, general principles that model equations should obey. Basically, all attempts to predator–prey system modelling can be divided into formulation of continuous models [14] or discrete ones [15]. As far as chemical engineering is concerned, continuous models are preferred, due to both ease of mathematical manipulation, and life-time of organisms under consideration. One may also mention about stochastic approach [16], that is less popular, although offers great perspectives.

Beginning from the early 1970s, one can notice steadily increasing number of publications on biochemical reactor modelling, either theoretical or experimental, that take into account an existence of a predator–prey relationships. A skeleton of almost every mathematical model consists of three differential (or difference) equations. These describe mass balance of a limiting substrate, bacteria (prey) and protozoa (predator). Besides these equations, additional balance equations are usually formulated, their number and character depending on assumptions related to the reactor biocenosis. For instance, Canale et al. [17], took into account fact that substrate could appear in two forms, one that is readily degradable by bacteria and the other, more resistant to bioconversion. Curds [18] considered appearance of more complex food chain, that included two kinds of both substrate, bacteria and protozoa. Toyoda and Kanki [19] in their investigation of a three-phase bioreactor, divided predators into two groups, namely free-swimming and 'creeping' ones, with different feeding habits. Sudo et al. [20] formulated model that regards flocs as undergoing cyclical processes of disintegration and restoration, what in turn causes periodical shortages of food supply for predators, since only bacteria on an outer surface of a floc are available for protozoa. Ratnam et al. [21] took into account adhesion of bacteria to reactor walls and biofilm production. Pavlou [22] developed a model with assumption, that some part of bacteria in a reactor is hidden from predation. A numerical analysis of coupled biochemical reactors with prey and predators was carried out by Taylor et al. [23], who demonstrated effects of overlapping oscillations, which each of the reactors generated independently.

The above-mentioned works deal mainly with dynamics of a bioreactor. They prove the existence of stationary attractors, like stable node and stable focus, and non-stationary, such as a limit cycle. Apart from these, appearance of unstable focus was shown. So far, literature lacks steady-state multiplicity and stability analysis, for case when non-Monod, or substrate-inhibition kinetic models are concerned. This problem is therefore dealt with in the present work.

2. Model formulation

Let us consider a continuously stirred biochemical tank reactor with two types of microorganisms. Its mathematical model is formulated based on the following assumptions.

(a) Two averaged 'pseudospecies' inhabit the bioreactor: bacteria and protozoa, the latter preying on the former. In fact, this is a simplification of a real situation, when biocenosis consists of many species.

(b) Specific rate of substrate consumption by bacteria is expressed by Haldane equation. A growth of protozoa may be approximated using the quasi-Monod kinetic, as justified by the work of Proper and Garner [24]. Here, protozoan growth rate expression is modified by insertion of term that takes into account susceptibility of eukaryotic organisms to a toxic substrate.

(c) A reaction broth is assumed to be homogeneous.

(d) Growth of bacteria is limited by one substrate. In case of an aerobic process this assumption means that liquid is saturated in dissolved oxygen. Protozoa are unable to feed on dissolved substrate.

(e) A part of a reactants' stream that leaves the reactor is recycled back to it, after some dewatering (by sedimentation process). In practice, sedimentation module can be a settler or a hydrocyclone with a low shear stress. Dynamic of that module is not considered here.

(f) There are no time delays, that could result from recycling part of an outlet stream, dynamics of flocs formation, or feeding and growth mechanisms.

(g) Death and lysis of cells are neglected, and entire biomass is postulated to exhibit a metabolic activity at the same level.

(h) Yield coefficients for bacteria and protozoa are taken as constants.

The considered reactor, together with characteristic flowrates and concentrations is depicted in Fig. 1. Dimensionless concentrations of substrate, bacteria and protozoa are defined with respect to substrate concentration in the feed stream as follows:

$$\alpha=\frac{c_A}{c_{Af}},\ \beta=\frac{c_B}{c_{Af}},\ \gamma=\frac{c_P}{c_{Af}} \qquad \alpha,\beta,\gamma\in[0;1] \tag{1}$$

The recycle stream, after passing through sedimentation module, possesses higher concentration of biomass than that in the reactor. A degree of biomass densification is defined by η coefficient:

$$\eta=\frac{c_B-c_{BE}}{c_B}=\frac{c_P-c_{PE}}{c_P} \qquad \eta\in[0;1) \tag{2}$$

where c_{BE} and c_{PE} are concentrations of bacteria and protozoa, respectively in a stream that leaves a separator. Equations describing reactor dynamics are given below.

$$\frac{d\alpha}{dt}=\frac{1}{\tau}(1-\alpha)-r_A(\alpha,\beta) \tag{3a}$$

$$\frac{d\beta}{dt}=\frac{(\eta-1)}{\tau}\beta+r_B(\alpha,\beta,\gamma) \tag{3b}$$

$$\frac{d\gamma}{dt}=\frac{(\eta-1)}{\tau}\gamma+r_P(\alpha,\beta,\gamma) \tag{3c}$$

The overall residence time τ is computed with respect to feed stream F_{V0}. This formulation allows to omit a recirculation coefficient in model Eqs. (3a), (3b) and (3c). Terms r_A, r_B and r_P that appear in Eqs. (3a), (3b) and (3c) are kinetic functions defining, respectively the rate of uptake of a limiting substrate, growth rate of bacteria and protozoa. Some elementary conditions these functions should fulfil were defined by Kolmogorov [13]. It may be proved that expressions given below are consistent with these conditions:

$$r_A(\alpha,\beta)=\frac{1}{Y_{BA}}\mu(\alpha)\beta \tag{4a}$$

$$r_B(\alpha,\beta,\gamma)=\mu(\alpha)\beta-\frac{1}{Y_{PB}}g(\alpha,\beta)\gamma \tag{4b}$$

$$r_P(\alpha,\beta,\gamma)=g(\alpha,\beta)\gamma \tag{4c}$$

Functions $\mu(\alpha)$ and $g(\alpha,\beta)$ are the specific uptake rates of substrate and bacteria

$$\mu(\alpha)=\frac{k\alpha}{K_S+\alpha+\dfrac{\alpha^2}{K_I}} \tag{5a}$$

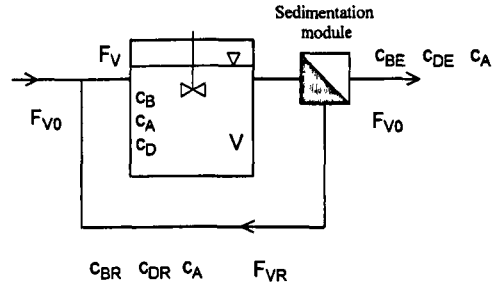

Fig. 1. A continuous stirred tank bioreactor with recycle and biomass sedimentation.

$$g(\alpha,\beta)=\frac{k_P\beta}{K_{SP}+\beta+\dfrac{\alpha^2}{K_{IP}}} \tag{5b}$$

In a vector notation, Eqs. (3a), (3b) and (3c) may be rewritten as follows:

$$\frac{dx}{dt}=f(x,\lambda,\xi), \qquad x=(\alpha,\beta,\gamma) \tag{6}$$

At the steady states $dx/dt=0$, thus Eq. (6) take the form:

$$0=f(x,\lambda,\xi) \tag{7}$$

where x is a state variable vector, whereas λ and ξ are vectors of parameters, divided into process parameters and kinetic parameters:

$$\lambda=[\tau,\eta,c_{Af}],\ \xi=[Y_{BA},Y_{PB},k,k_P,K_S,K_{SP},K_I,K_{IP}]$$

Values of kinetic parameters, both for protozoa and for bacteria, may be estimated quantitatively only in a relative manner. Exact values, obtained from pure cultures, so far, give only a general overview of growth kinetics when processes with mixed cultures are concerned. The fact that data from pure cultures are non-additive, might be ascribed, e.g., to the fact that protozoa can excrete compounds that stimulate bacterial growth (Mallory et al. [25]). A solution to the problem in question is to assume values of kinetic constants, that may be thought of as 'generic' for bacteria and coexisting protozoa. Basing on this reasoning, data given by Pawlowsky and Howell [26] ($k=0.26\ h^{-1}$, $K_s=0.0254\ kg\ m^{-3}$, $K_I=0.173\ kg\ m^{-3}$, $Y_{BA}=0.616$), were taken for an analysis of the model. These data were obtained from experiments with phenol biodegradation, which is a troublesome waste component, e.g., in oil refining industry. Specific bacteria uptake rate, defined by Eq. (5b), is an analogue of a Haldane equation. Yield coefficient of protozoa from bacteria is assumed to be constant, what is justified by experimental works by Curds and Cockburn [27]. Kinetic coefficients for protozoa should comply with a condition, that organisms on a higher trophic level have longer characteristic times (e.g., of cell fission or biomass doubling), than those occupying preceding position in a food chain. This constraint is, apart from specific examples, universally obeyed. In accordance with that rule, values of kinetic coefficients for protozoa are taken as $k_P=1/2\cdot k$ and $K_{SP}=2\cdot K_S$. A coefficient that reflects

inhibitory influence of substrate on protozoa metabolism $K_{IP}=2/3\cdot K_I$, takes into account their higher sensitivity to dissolved substrate. Yield coefficient of protozoa biomass from unit biomass of bacteria, obtained empirically, varies from 0.35 to 0.7. In the present work value $Y_{PB}=0.5$ was used.

Admissible values of parameters in vector λ should be subject to some constraints. Concentration of limiting, toxic substrate in a feed stream will be bounded by value of 1.5 kg/m^3. When phenol is used as the substrate, there exists a possibility of its biodegradation by bacteria even in a case of higher concentration. However, viability of eukaryotic organisms (that protozoa represent) is rather doubtful with such an environment toxicity level. Since death of microbial cells in kinetic terms (Eqs. (5a) and (5b)) is omitted, it seems rational to assume rather short residence time of all microorganisms in the bioreactor. Implicitly, this means that ecosystem does not reach an age which could be considered as advanced. Here, a value $\tau_{max}=20$ h is accepted, what in most cases is a short residence time, when microbial reactors with toxic substrate are in focus. Although it is possible to reach a high densification ratio η, even up to 0.9, concentration of a biomass in a recycled stream would in that case contradict some assumption made during model formulation. Firstly, it would denote substantial difference between residence time of liquid and biomass. Secondly, if one assumes the time that reactants spend in a sedimentation module is roughly proportional to densification coefficient, for high values of η time lag of state variables would be significant, in relation to their values in reactor. That, in turn, would influence the reactor dynamics. In the light of this reasoning, a value of η in computations will not exceed 0.4.

3. Quantitative analysis of the model

In system of Eq. (7) variable γ appears linear. It is therefore possible to eliminate it from the system, what would result in a decrease of dimensionality of the model by one. Although such operation offers easier determination of attractors in a modified state space (α, β), it would on the other hand complicate interpretation of the results. This is because there are two possible types of solution, with $\gamma=0$ and $\gamma>0$, both being numerically correct, but each denoting different physical state of the reactor.

Structure of the stationary states for nonlinear objects can be represented in a form of a parametric dependence $x=f(\lambda)$. Inlet substrate concentration c_{Af}, in a case of the specific example of a reactor used in waste treatment industry, is a parameter that cannot be freely adjusted. Similar situation in case of a densification coefficient η can be ascribed to a fact that sedimentative properties are highly dependent on biomass separator used. On the other hand, residence time τ is relatively easily adjustable to a required value. Consequently, it is beneficial to create and analyse parametric dependence diagrams, setting τ as a bifurcation parameter.

For subsequent comparison, introductory analysis of a reactor without protozoa ($\gamma=0$) should be done first. In that case (Fig. 2) diagrams of parametric dependence $\alpha=f_1(\tau)$, $\beta=f_2(\tau)$ consist of trivial solution branches (washout steady state) and of a nonzero steady-state solutions branch, that forks out from the previous in a point of static-trivial bifurcation. A limit point singularity is also encountered on the non-zero branch. The τ coordinate for this point denotes the lowest value of the residence time, that may be attained in a properly operated reactor. Increase of substrate concentration in an inlet stream makes the static bifurcation point occurs at higher values of τ. With $K_I\to\infty$ a middle, nonzero and unstable stationary state vanishes, and Eq. (5b) transforms into Monod equation of growth. It may be shown, that for the case without a predator and with kinetics considered, only stationary attractors occur, i.e., stable and unstable nodes, and saddle points, the latter denoting middle stationary state.

When a predator is introduced into the system, its static characteristics undergoes a qualitative change. The second transcritical bifurcation appears in a point where solutions with nonzero protozoa concentration branch out. The highest concentration of microorganisms and the greatest attainable substrate conversion, which may be obtained in a reactor with an existing predator, are characterized by points on stationary state branch, that are located in vicinity of the new static bifurcation point τ_{BS1}. Locus of a steady state conversion, given by parametric dependence on τ, is definitely different in case with $\gamma>0$, in comparison with situation when $\gamma=0$. If the predator is absent from the bioreactor, decrease of values of τ toward τ_{LP}, (i.e., approaching the point where productivity is lost) results in slow reduction of a substrate conversion and bacteria concentration in an outlet stream.

When a predator appears, static characteristics changes. Substrate conversion and bacteria concentration increase, together with residence time reduction, until a singularity point of dependence $\alpha=f_1(\tau)$, $\beta=f_2(\tau)$, $\gamma>0$ occurs.

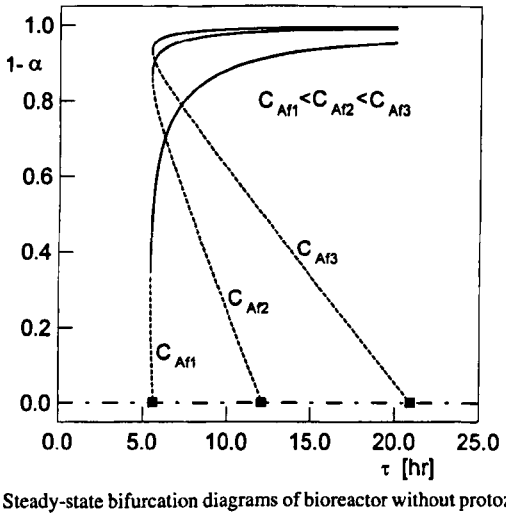

Fig. 2. Steady-state bifurcation diagrams of bioreactor without protozoa, for several values of c_{Af}. ($\eta=0.3$) (———) Stable states; (- - -) unstable states.

Increase of values of these state variables may be monotonical, or they may attain a local minimum. Values of concentrations α and β are at the same time lower than those in a case without a predator. Further decrease of τ results in washout of protozoa from the reactor, i.e., values of γ are equal 0. Since then, values of state variables α and β are identical to those in a case without protozoa. The value of a bifurcation point mentioned above has an abscissa τ that is placed in a neighbourhood of value for τ_{LP}. Since a situation, when τ in the reactor falls below τ_{LP} corresponds to settling on a lower, stable and trivial steady state, then protozoa cell concentration is a representative indicator for a danger of a complete loss of reactor productivity. The protozoa cell concentration decreases monotonically when $\tau \rightarrow \tau_{BS1}$ from a right hand side.

The fact, that signs of first derivatives $d\beta/d\tau$ and $d\gamma/d\tau$ are mutually opposite, can be ascribed to differences in kinetic coefficients of bacteria and protozoa populations. That in turn results in uniqueness of nontrivial stable state, and in further consequence the lack of limit point on branches $\alpha = f_1(\tau)$, $\beta = f_2(\tau)$, for the process carried out in presence of the predator. Moreover, stationary attractors' characteristics of a considered system changes. Since part of eigenvalues $\mu = \kappa + i\nu$ of a Jacobian matrix, obtained from linearization of the model equations, have nonzero imaginary parts, resulting solutions are expected to be periodic in time.

From bifurcation diagrams depicted in Fig. 3, one may conclude that three types of solutions may be achieved in a bioreactor: coexistence of both bacteria an protozoa, presence of bacteria alone, or washout of all microorganisms. When a value of $\tau < \tau_{BS1}$ is fixed in a reactor, introduction of the protozoa to the system, resulting with its successful inoculation, is impossible. On the other hand, for values of residence time greater than τ_{BS1}, successful inoculation of the predator depends on initial values of the state vector; this results from reactor dynamics.

When one scans a trivial solution branch, starting from $\tau = 0$ and moving toward high values of residence time, a static bifurcation point is encountered (Fig. 4). In this point, previous stable and trivial solution becomes unstable, and new branch emanates, with initial derivative $d\gamma/d\tau > 0$. The new branch denotes appearance of a stable node solution type in a state space. Moving further right on a nontrivial solution branch, a point τ_{OS} is met, where one pair of eigenvalues changes from real to complex conjugate. It implies that the system, when perturbed locally, returns to the previous state through dumped oscillations. With further increase of values of τ, one approaches a point, where product $\kappa_{1,2}^{(i)} \cdot \kappa_{1,2}^{(i+1)} < 0$, $\nu_{1,2} \neq 0$. This is a Hopf bifurcation point (center), where a 'soft' transition to sustained oscillations takes place. Upon passing the point, previous stable nodes becomes unstable, and values of α, β, γ in the state space tends toward dynamic attractor. The attractor is a limit cycle, that surrounds unstable nodes of steady state solutions; amplitudes of the concentrations grow steadily with increasing values of τ. These oscillations are undesirable as far as efficiency and safety of the process is considered. If phenolic compound is a limiting substrate (as in the case under study), its large fluctuations can cause transient concentrations in outlet stream that are above admissible level (even with acceptable mean concentration), and result in environmental pollution.

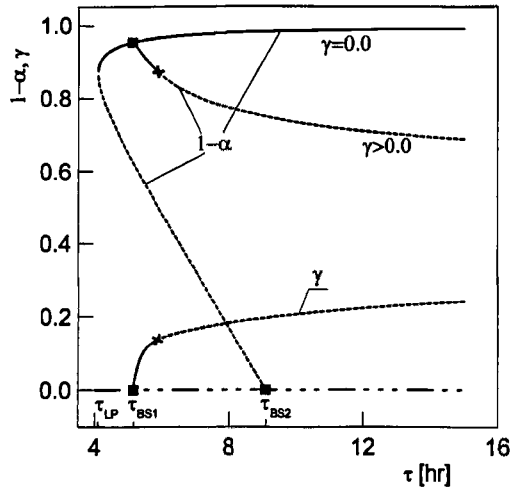

Fig. 3. Steady-states bifurcation diagram at the presence of bacteria and protozoa ($\eta = 0.3$, $c_{Af} = 1.0\ kg\ m^{-3}$). (———) Stable states; (- - -) unstable states; (■) static bifurcation point; (x) Hopf bifurcation point.

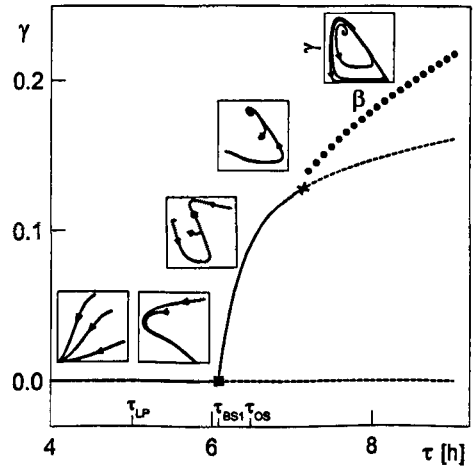

Fig. 4. Dependence of protozoa cell concentration γ on τ and phase portraits of bioreactor in (β, γ) space, corresponding to different values of residence time τ. (τ_{LP}, τ_{OS1}, τ_{OS}—singular points).

Change of value of the η coefficient, which indicates degree of densification of a biomass in a recycled stream has got an effect different from that in a case without a predator. Increase of values of the parameter causes densification not only of bacteria, that convert substrate, but also predators, that prey on these bacteria. Generally, increase of densification coefficient moves static bifurcation point (and also the point with the lowest possible τ in the working bioreactor), toward lower values of residence time. Simultaneously, this increase can cause changes in steady state structure and the reactor dynamics. This is exemplified by Fig. 5a–e, which

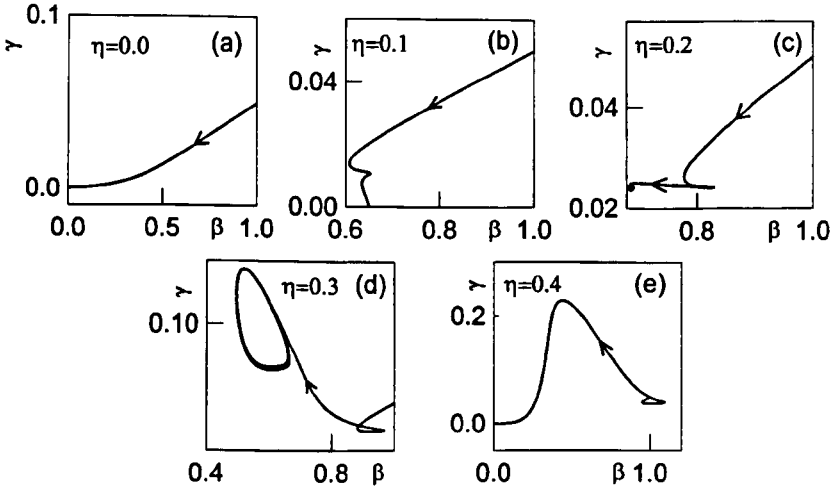

Fig. 5. Phase portraits of bioreactor in (β, γ) space for several values of densification coefficient η (c_{Af} = 1.0 kg m^{-3}).

are plotted for several values of η. When $\eta = 0$, a washout and complete loss of productivity occur. With increase of η to 0.1 the system reaches a stationary state; this is connected with complete predator extinction. Fixing value of η at 0.2 results in nonzero steady state values of both β and γ. On setting $\eta = 0.3$, sustained oscillations of concentration arise in the bioreactor. With $\eta = 0.4$ washout and biological 'death' of the bioreactor occurs again. The last phenomenon takes place in a virtue of change of attractor basin and is therefore of global type.

From the preceding analysis one may infer that there are three possible types of attractors of model Eq. (6) with nonzero protozoa concentration: a stable node, a stable focus and a limit cycle. Residence time domain for each of the attractor types depends on values of the two others process parameters, namely c_{Af} and η. Fig. 6a, b present dependence of range of τ for which a specific attractor is expected, on c_{Af}, for two arbitrarily chosen values of η, i.e., $\eta = 0.1$ and $\eta = 0.3$.

In the region of low c_{Af}, values of τ corresponding to the existence of a limit cycle, are rather high. Moreover, washout of the protozoa occurs with relatively high values of residence time. The last observation results from a lower inflow of substrate, that can be utilised by bacteria, which are an intermediate component of a food chain sequence phenol-bacteria-protozoa. Stable focus solutions are encountered for a wide range of τ, whereas the τ range of stable nodes is substantially smaller. Range of values of τ for stationary solution becomes considerably narrower along with increasing values of c_{Af}. For high inlet substrate concentration, domain of τ, that guarantees existence of stable focus, is very small. A domain of τ for stable node solutions at the same time is somewhat wider; domains relation between the two mentioned types of solution is reversed with change form low to high c_{Af}.

Apart from the stationary solutions, there exists the limit cycle for almost all values of τ. This dynamic attractor accompanies majority of nonzero solutions. In a case of higher densification coefficient (Fig. 6b), relationships discussed above are topologically identical, but changes of types of solution occurs for lower values of τ.

As far as industrial bioreactors are concerned, there are several circumstances that can cause unavailability of some

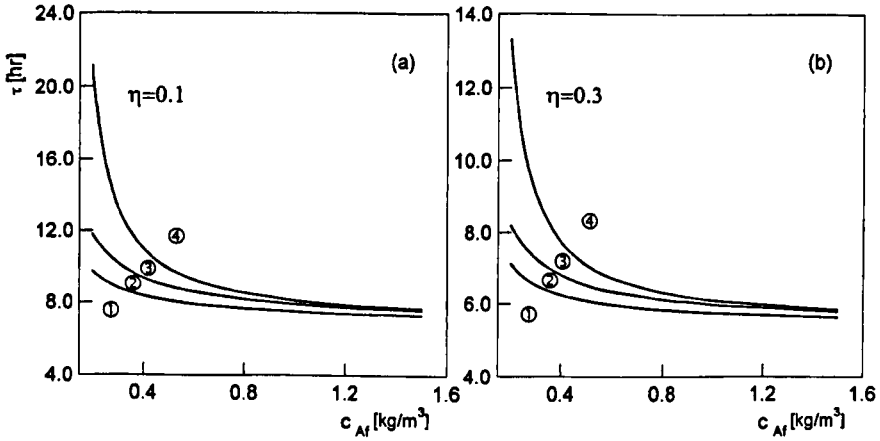

Fig. 6. Plot of dependence $\tau = f(c_{Af})$ expressing change of steady-state solutions type for model Eq. (7). (a—$\eta = 0.1$; b—$\eta = 0.3$) (1) wash-out, (2) stable node, (3) stable focus, (4) limit cycle.

part of bacteria for protozoa. For example, bacteria inside activated sludge flocs or those living inside a biofilm developed on the reactor wall, are hardly available as a food source for free-swimming protozoa. One can take this fact into account through introduction of some minor changes to functions describing growth of both bacteria and predators. To this end we utilise a 'hiding place for prey' concept, that came from the field of population ecology (see for instance, Maynard Smith [15]). With assumption that some constant part of bacteria, expressed by the dimensionless concentration β^* (for simplicity, relative to the whole reacting volume), is inaccessible for protozoa, the kinetic equations (Eqs. (4b) and (4c)) can be rewritten as follows:

$$r_B(\alpha,\beta,\gamma)=\mu(\alpha)\beta-\frac{1}{Y_{PB}}g(\alpha,\beta_s)\gamma \quad (8a)$$

$$r_P(\alpha,\beta,\gamma)=g(\alpha,\beta_s)\gamma \quad (8b)$$

A quantity β_s is concentration of bacteria exposed to predation by protozoa, defined as:

$$\beta_s=\begin{cases}\beta-\beta^* & \text{for } \beta>\beta^* \\ 0 & \text{for } \beta\le\beta^*\end{cases} \quad (9)$$

With assumption of negligible diffusional resistance, the modification introduced is not contradictory to homogeneity of reaction mixture, postulated during formulation of the model. There are also other possibilities of definition of bacteria reservoir unusable for protozoa, e.g., as proportional to concentration of all bacteria suspended in activated sludge liquor.

After introducing of the modifications quoted above, solution characteristic of Eqs. (6) and (7) undergoes apparent change. A domain of values of τ for which solutions of unstable focus type exist, together with encircling limit cycle, is bounded on both ends. Change of attractors characteristics from stationary to dynamic ones, and in reverse direction, occurs at either of the two points of Hopf bifurcation (Fig. 7). For values of τ lower than value for the first (leftmost) bifurcation, the solutions can be of stable node or stable focus type; that is, similarly to the situation when all bacteria are open to influence of protozoa. With values of τ increased over value of the rightmost Hopf bifurcation, only stable focus are found. Both points of dynamic catastrophe are characterized by 'soft' transition from oscillations to stable states (i.e., there is no unstable limit cycle for given kinetic parameters). This behaviour can be visualized by plotting values of τ domains for each type of solution, vs. c_{Af}, as it is done in Fig. 8. For values c_{Af} below 0.445 kg/m^3 a limit cycle does not appear at all, and majority of range of τ values correspond to solutions of stable focus type. Beyond value $c_{Af}=0.445$ there exists a domain of τ corresponding to oscillatory solution. The domain substantially broadens along with increasing c_{Af}, but even at high inlet substrate concentration there is the second point of Hopf bifurcation. After passing this point toward higher c_{Af}, sustained oscillations change to dumped oscillations.

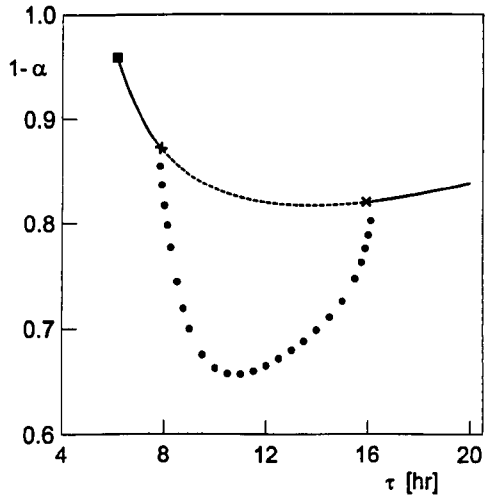

Fig. 7. Dependence of degree of conversion $(1-\alpha)$ on τ for $\gamma>0$, when hiding places for bacteria exist. ($\beta^*=0.1$). (———) Stable states; (- - -) unstable states; (■) static bifurcation point; (x) Hopf bifurcation point; (••••••••) minimal values from limit cycle.

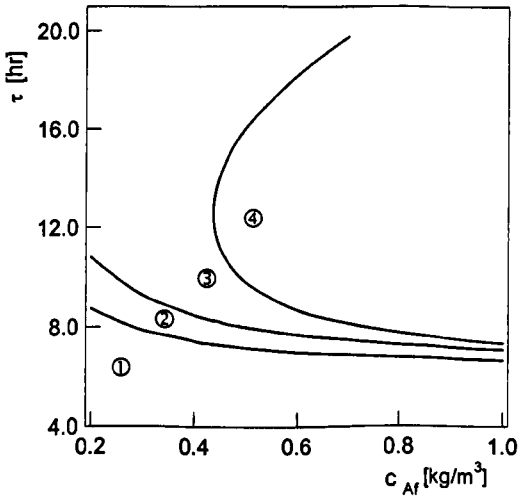

Fig. 8. Plot of dependence $\tau=f(c_{Af})$ expressing change in steady-state solutions type of model Eq. (7), for the case with hiding places for bacteria. ($\beta^*=0.1$) (1) wash-out, (2) stable node, (3) stable focus, (4) limit cycle.

The comparison of steady state structure, for cases with and without hiding places for bacteria, is done in Fig. 9a,b,c. From Fig. 9a, it may be concluded that evident change of the structure takes place. Dependence of substrate conversion degree $(1-\alpha)$ on τ is no longer monotonical, since this variable then passes through a minimum. Although the minimum exists for τ domain where steady state solutions are unstable, after passing the rightmost Hopf bifurcation point the substrate conversion rises steadily in a region of dumped oscillations. This means the substrate conversion approaches monotonically, when τ values are increased, a highest possible value, that is the one in a case without a predator. Stationary values of bacteria concentrations β for a case with hiding places (Fig. 9b) are only slightly higher in comparison to case without hiding places. On the other hand, protozoa

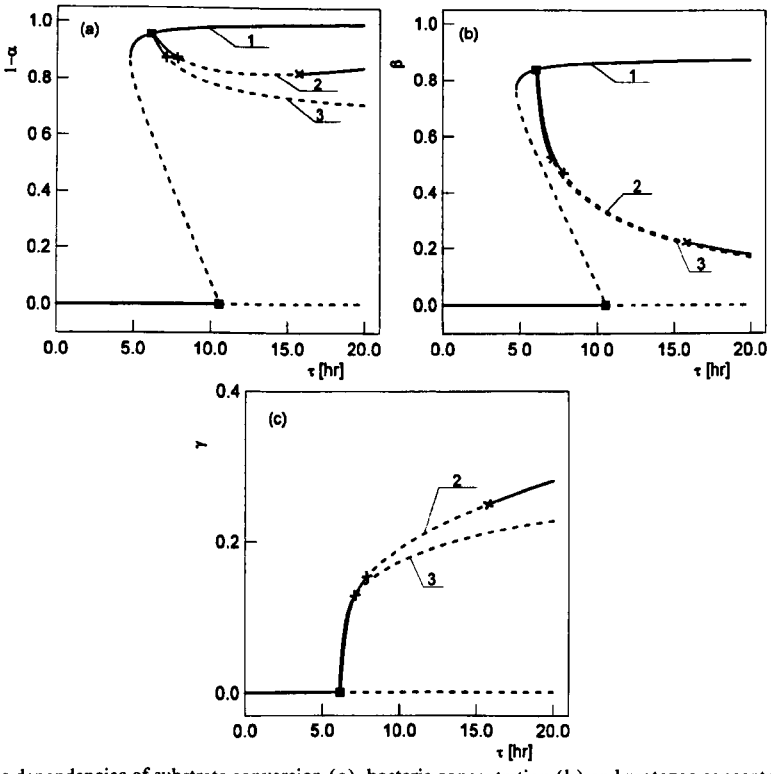

Fig. 9. Comparison of parametric dependencies of substrate conversion (a), bacteria concentration (b) and protozoa concentration (c) on residence time; (1) $\gamma=0$; (2) presence of hiding places for bacteria; (3) all bacteria available as a food source for protozoa ($\eta=0.2$, $c_{Af}=0.7$ kg m^{-3}) (———) stable states; (- - -) unstable states.

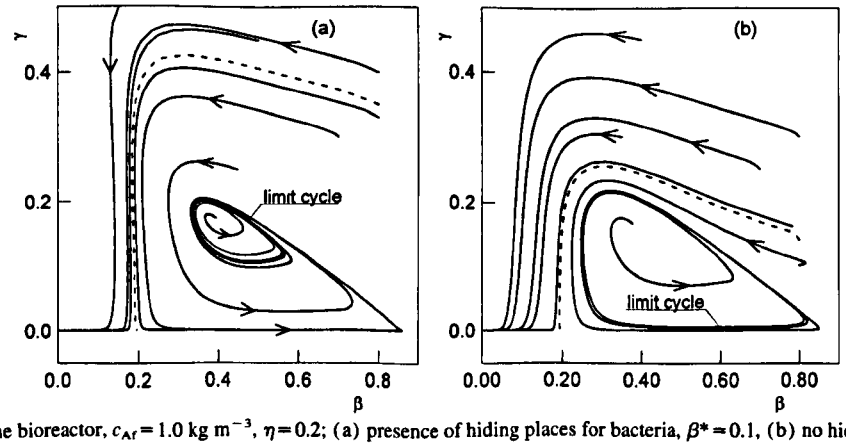

Fig. 10. Phase portraits of the bioreactor, $c_{Af}=1.0$ kg m^{-3}, $\eta=0.2$; (a) presence of hiding places for bacteria, $\beta^*=0.1$, (b) no hiding places; (- - -) separatrix.

concentration is apparently higher when part of bacteria is unavailable. This unexpected behaviour may be interpreted through a posteriori inspection of necessary conditions, that have to be accomplished so as to steady state Eq. (3c) for $d\gamma/d\tau=0$ could describe nontrivial solution. Since growth rate of protozoa $r_P(\alpha,\beta,\gamma)$ is lower when part of the bacteria is hidden from predation, then operation at a nonzero steady state (with $\gamma>0$) requires higher protozoa concentration. If not, at a given fixed fresh stream inflow F_{V0}, protozoa are flushed out of the bioreactor. This requirement for maintaining a nontrivial steady state greatly influences the reactor dynamics, and extents of basins of attractors in particular, both trivial and nonzero. Two phase portraits in Fig. 10a,b represent the situation. From these figures, one may draw a conclusion, that domain of attraction of a limit cycle enlarges in the instance of existence of hiding places for bacteria. Apart from that, amplitude of oscillation of cell and substrate concentration decreases. For values of process parameters chosen herein, in both cases (Fig. 10a and b) the limit cycle is the only possible nonzero attractor. Comparing Fig. 10a and b, a decrease of amplitude of the limit cycle is apparent. Similarly, values of the variable α are subject to change within narrower

range. From the above analysis it follows that occurrence of the hiding places in a multispecies bioreactor can in fact improve process course.

4. Conclusion

The biochemical reactor, described by sets of Eqs. (6) and (7) reveals various static characteristics, depending on presence of predator cells. In some ranges of a residence time, there exist three steady states, including the one with a nonzero protozoa cell concentration. When the reactor reaches a stable state with zero concentrations of all microorganisms, its retrieval to a previous state is possible by inoculation only, while maintaining τ at sufficiently high values. Also, occurrence of the two transcritical bifurcation points implies that, for some values of τ, a successful, stable introduction of the predator to a system (or its further existence) is impossible.

When protozoa cells are present in the reactor, a high-amplitude oscillations of a reactant concentrations may appear. A range of values of τ they cover, may be limited by assuring a proper development of microbial flocs. When these flocs are present, a second point of Hopf bifurcation emerges on a branch of parametric dependence $x=x(\tau)$ for high values of residence time. This means a decrease of oscillation amplitude takes place, what is connected with confining of range of τ for oscillatory solutions on both sides. As far as dynamics is concerned, one can eliminate occurrence of dumped oscillations through change of a biomass densification coefficient. In a case with hiding places for bacteria, a higher substrate conversion is reached, what is crucial when environmental pollution preventing is concerned. Another benefit is an increase of domain of attraction of the nonzero states, either stable or oscillatory. Because a limit point τ_{LP} in a branch of parametric dependence lies in vicinity of static bifurcation point τ_{BS1}, (by mean of τ value), so washout of a predator signify that the lowest possible residence time to be attained in an operating reactor is approached. This observation can partly explain fact that protozoa in an activated sludge may be utilised as an indicator of its current state.

5. Nomenclature

c_A, c_B, c_P	Concentrations of a substrate, bacteria and protozoa, respectively [kg m^{-3}]
c_{Af}	Substrate concentration in a feed stream [kg m^{-3}]
c_{BE}, c_{PE}	Concentrations of microorganisms in a stream leaving an installation, respectively for bacteria and protozoa [kg m^{-3}]
F_{V0}	Volumetric flow of a raw stream [m^3 h^{-1}]
F_V, F_{VR}	Volumetric flows through a reactor and recycle loop, respectively [m^3 h^{-1}]
k, k_P, K_S, K_{SP}, K_I, K_{IP}	Kinetic constants
V	Volume of the bioreactor [m^3]
Y_{BA}, Y_{PB}	Yield coefficients of bacteria and protozoa
α, β, γ	Dimensionless concentrations of substrate, bacteria and protozoa, respectively
β^*, β_s	Dimensionless concentrations of bacteria unavailable and available for protozoa
η	Biomass densification coefficient
$\mu=\kappa+i\nu$	Complex eigenvalue
τ	Residence time in a bioreactor [h]
τ_{LP}, τ_{BS1}, τ_{BS2}, τ_{OS}	Abscissa coordinates of some singular points in bifurcation diagrams [h]

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