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APPARENT ELIMINATION OF INHIBITION PHENOMENON CAUSED BY PROPER DESIGN OF A BUBBLE TANK BIOREACTOR

The paper concerns an aerobic microbiological process occurring in a bubble tank bioreactor. If the process is inhibited by a carbonaceous substrate, then the steady states characteristics obtained using models without biofilm indicates a danger of irreversible washout of the biomass. It was shown that taking into account the biofilm immobilized on bioreactor's walls and proper design of the bioreactor can lead to disappearance of the region of the multiple steady-states, i.e. to disappearance of the turning point at the steady state branches. Therefore, the operation of the bioreactor is safe in a wide range of feed flow rates, even those leading to the washout of the biomass from the liquid phase. This property was called apparent elimination of inhibition phenomenon.

1. INTRODUCTION

Among bioreactors for aerobic microbiological processes, a bubble tank reactor is the simplest in construction. The widespread use of such bioreactors is justified by rather slow rates of microbiological processes. From the formal point of view, they can be considered autocatalytic processes, where the role of a catalyst play active cells of microorganisms. Bubble tank bioreactors are used both as small chemostats for laboratory kinetic experiments, and as large-scale industry apparatuses.

Prevailing number of microbiological models of tank bioreactors [1, 2] or objects with longitudinal dispersion, e.g. aeration ditches [3–5] generally, do not take into account the presence of microorganisms in the biofilm formed on walls of an apparatus. Meanwhile, according to opinions of Loosdrecht et al. [6] and Eberl et al. [7], formation of biofilms in aquatic systems is generally an unavoidable phenomenon. Therefore, in modeling of microbiological reactors, their presence should be taken into

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account and the participation in total rate of the process in the apparatus should be evaluated.

The paper presents the effects of assuming the presence of the biofilm on bioreactor's walls and interphase biomass transfer on technological properties of an aerobic microbiological process. Such properties are: yield of the apparatus determined by the conversion degree of the carbonaceous substrate, resistance to washout of microorganisms and stability of steady states. It was shown that assuming the presence of the biofilm on inner walls of the apparatus and biomass transfer to the liquid phase reveals new technological properties of the bioreactor. As an example, biodegradation of phenol by *Pseudomonas putida* bacteria was chosen due to the fact that phenol is a strongly toxic compound, causing danger for living aquatic organisms, it inhibits microbial growth, and the kinetics of aerobic biodegradation of phenol is known. Based on those experiments, the influence of the presence of the biofilm, the rate of the biomass transfer and, what is more important, additional surfaces placed inside the bioreactor on basic technological properties of the bioreactor were evaluated.

2. MODEL OF THE BIOREACTOR

Biofilm, also called biological film, is a multi-component structure, containing mainly water, cells of microorganisms and extracellular polymers (EPS), formed on a solid substratum. The biofilm is a complex substance because of its composition, morphology and processes occurring inside it.

Biofilms are effective tools in enhancing productivity of microbiological processes. Numerous investigations have been conducted on improvement of biofilm reactors technology [8, 9]. Biofilms are purposefully used especially in two- and three-phase fluidized-bed bioreactors, in which share of the biofilm is considerable because of large specific surface of solid carriers [10, 11].

Presence of biofilms and interphase biomass transfer affect stationary and dynamical properties of bioreactors. Detachment of the biofilm causes active biomass transfer to the liquid phase, so this process affects the concentration of microorganisms in the liquid phase. In turn, access of microorganisms in the liquid phase to the substrates is easier than in the biofilm, hence transfer of the active biomass from the biofilm to the liquid phase influences general rate of the process in the bioreactor.

Taking into account the presence of the biofilm immobilized on the bioreactor's walls is equivalent to the assumption, that the microbiological process proceeds in both phases, that is in the liquid and in the biofilm, and between both phases additionally mass transfer of reagents takes place. Then we are dealing with so called mixed, that is suspended and attached growth of the biomass.

In computations, perfect mixing of the liquid and piston flow of the gas phase were assumed. These assumptions are common in modeling microbiological processes

in tank bubble reactors [12]. Equations describing the process in steady state are as follows:

- liquid phase:

$$F_V^c\left(c_{Af}^c - c_A^c\right) - V\varepsilon^c r_A^c\left(c_A^c, c_B^c, c_T^c\right) - V\varepsilon^c a_s k_{sA}\left(c_A^c - c_{As}\right) = 0 \tag{1a}$$

$$-F_V^c c_B^c + V\varepsilon^c r_B^c\left(c_A^c, c_B^c, c_T^c\right) + V\varepsilon^c a_s r_{det} = 0 \tag{1b}$$

$$\begin{aligned} &F_V^c\left(c_{Tf}^c - c_T^c\right) + Vak_{cT}\int_0^1\left(\frac{c_T^g(Z)}{K} - c_T^c\right)dZ \\ &\qquad - V\varepsilon^c r_T^c\left(c_A^c, c_B^c, c_T^c\right) - V\varepsilon^c a_s k_{sT}\left(c_T^c - c_{Ts}\right) = 0 \end{aligned} \tag{1c}$$

- gas phase:

$$\frac{dc_T^g}{dZ} = -\frac{Hak_c}{\varepsilon^g u_b}\left(\frac{c_T^g(Z)}{K} - c_T^c\right),\ c_T^g(0) = c_{Tf}^g \tag{2}$$

By analytical integration of Eq. (2), the following function is obtained:

$$c_T^g(Z) = Kc_T^c + \left(c_{Tf}^g - Kc_T^c\right)\exp\left(-\frac{\tau_b ak_c}{K}Z\right) \tag{3}$$

where $Z = \dfrac{h}{H} \in [0, 1]$, whereas $\tau_b = \dfrac{H}{\varepsilon^g u_b}$.

In Equations (1), the uptake rates of the substrates and the rate of biomass growth depend on the process analyzed. According to model proposed by Seker et al. [13], the process is described by the Haldane–Monod kinetic equation. The uptake rates of the substrates and the rate of biomass growth thus have the following form:

$$r_A^c\left(c_A^c, c_B^c, c_T^c\right) = \frac{1}{w_{BA}} f_1\left(c_A^c\right) \times f_2\left(c_T^c\right) \times c_B^c \tag{4a}$$

$$r_T^c\left(c_A^c, c_B^c, c_T^c\right) = \frac{1}{w_{BT}} f_1\left(c_A^c\right) \times f_2\left(c_T^c\right) \times c_B^c \tag{4b}$$

$$r_B^c\left(c_A^c, c_B^c, c_T^c\right) = f_1\left(c_A^c\right) \times f_2\left(c_T^c\right) \times c_B^c \tag{4c}$$

where

$$f_1\left(c_A^c\right)=\frac{kc_A^c}{K_A+c_A^c+\dfrac{(c_A^c)^2}{K_{in}}},\qquad f_2\left(c_T^c\right)=\frac{c_T^c}{K_T+c_T^c} \tag{5}$$

Appropriate expressions describing solid phase of the biofilm have to be added to Eqs. (1)–(2):

$$D_{eA}\frac{d^2c_A^b}{dx^2}-r_A^b(c_A^b,c_T^b)=0 \tag{6a}$$

$$D_{eT}\frac{d^2c_T^b}{dx^2}-r_T^b(c_A^b,c_T^b)=0 \tag{6b}$$

The following boundary conditions are connected with the differential Eqs. (6):

$$\frac{dc_A^b(0)}{dx}=0 \tag{7a}$$

$$\frac{dc_T^b(0)}{dx}=0 \tag{7b}$$

$$D_{eA}\frac{dc_A^b(L_a)}{dx}=k_{sA}\left[c_A^c-c_A^b(L_a)\right] \tag{7c}$$

$$D_{eT}\frac{dc_T^b(L_a)}{dx}=k_{sT}\left[c_T^c-c_T^b(L_a)\right] \tag{7d}$$

The uptake rates of the substrates A and T in the biofilm are given as:

$$r_A^b\left(c_A^b,c_T^b\right)=\frac{1}{w_{BA}}f_1\left(c_A^b\right)\times f_2\left(c_T^b\right)\times\rho_b \tag{8a}$$

$$r_T^b\left(c_A^b,c_T^b\right)=\frac{1}{w_{BT}}f_1\left(c_A^b\right)\times f_2\left(c_T^b\right)\times\rho_b \tag{8b}$$

In the aerobic processes, not only kinetic data connected with the rate of microbiological processes is significant, but also other parameters determining the heterogeneous system, which is the environment of the process. The following quantities belong

to this group: the degree of gas hold-up ε^g and the volumetric mass transfer coefficient of oxygen from the air to the liquid phase ak_c. They depend on physicochemical properties of the liquid and gas phase and on the velocity of the air u_{0g}. Their values were calculated from experimental equations given by Shah et al. [14].

In Equations (1), the parameter a_s is a contact (specific) surface area of the biofilm with the liquid phase referred to the volume of the liquid, that is $a_s = S/V^c$. Its value depends on the geometry and design of the bioreactor. In this paper, it was calculated as:

$$a_s = (1+\xi)\zeta \frac{S}{V^c} = (1+\xi)\zeta \frac{4H+d}{Hd} \tag{9}$$

where the parameter ζ determines the biofilm external surface enlargement. This coefficient is equal to unity for a biofilm perfectly flat and totally covering the surface of solid substratum. In general, the coefficient ζ takes values both higher and lower than unity [15]. In turn, the parameter ξ depends on the internal design and equipment of the bioreactor. If no additional elements are placed inside the apparatus, $\xi = 0$. Then the biofilm settles only on the lateral surface and on the bottom of the apparatus (Fig. 1a).

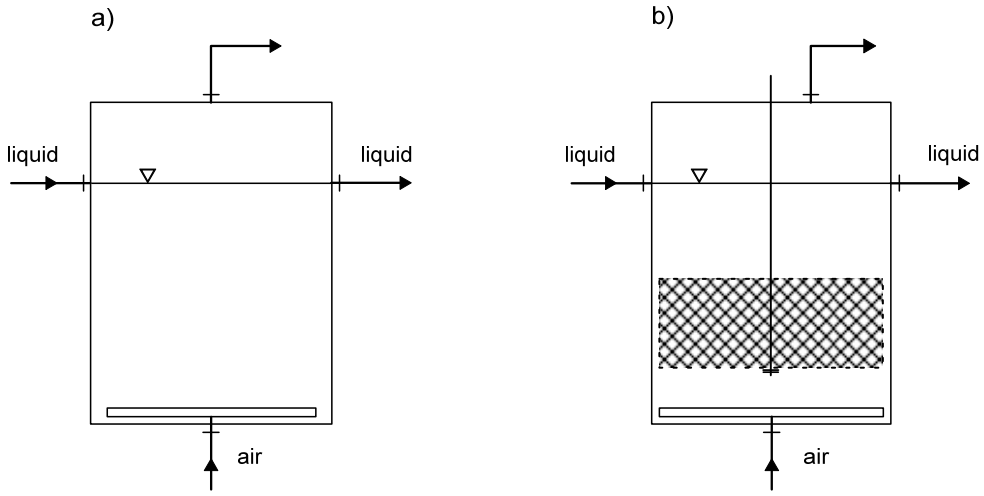

Fig. 1. Two designs of a bubble tank bioreactor: a) without packing, b) with packing

The biofilm detachment rate r_{det} and the biomass transfer to the liquid phase depend on hydrodynamic and kinetic conditions and age of the biofilm. Four mechanisms leading to the detachment of the biofilm are identified: erosion, sloughing, abrasion and grazing. No reliable equations exist determining the rate of the biofilms detachment in tank bioreactors, taking all these elements into consideration. Despite of

this, it is possible to achieve quantitative evaluation of the influence of the interphase biomass transfer assuming that some part of it formed in the biofilm is transferred to the liquid phase. This evaluation can be achieved by comparing the results with those for the process without taking into account the biofilm growth and its immobilization on the reactor's walls. It is also possible to determine the limits of the total active biomass transfer from the biofilm to the liquid phase, as:

$$0 \le r_{\text{det}} \le r_{\text{det, max}} \tag{10}$$

where

$$r_{\text{det, max}} = L_a \overline{r}_{\text{B}}^{\,b}, \qquad \text{while} \qquad \overline{r}_{\text{B}}^{\,b} = \frac{1}{L_a} \int_0^{L_a} r_{\text{B}}^{b}[c_{\text{A}}^{b}(x), c_{\text{T}}^{b}(x)]dx \tag{11}$$

The rate of the biomass transfer from the biofilm to the liquid phase can be thus expressed quantitatively as

$$r_{\text{det}} = X_{\text{B}} L_a \overline{r}_{\text{B}}^{\,b}, \qquad 0 \le X_{\text{B}} \le 1 \tag{12}$$

where X_{B} is the fraction of the active biomass transferred from the biofilm to the liquid phase. It is simultaneously convenient way of quantitative description of biomass transfer between both phases which can be used in simulations and design calculations.

The boundary value problem (6)–(7) was solved using the shooting method. By this method, both a particulate steady state and steady state branches can be determined [16].

3. RESULTS AND DISCUSSION

The results obtained for the apparatus without the biofilm are the basis for discussion about the consequences of the presence of a biofilm on the apparatus walls and interphase biomass transfer as well. A model without a biofilm is thus a particular case of the model proposed in this work. Figure 2 presents the steady state branches of the degree of conversion of the carbonaceous substrate $\alpha(\tau^c)$, the dimensionless concentration of the biomass in the liquid phase $\beta(\tau^c)$ and the dimensionless concentration of dissolved oxygen $\gamma(\tau^c)$, where:

$$\alpha = \frac{c_{\text{A}f}^{c} - c_{\text{A}}^{c}}{c_{\text{A}f}^{c}}, \qquad \beta = \frac{c_{\text{B}}^{c}}{c_{\text{A}f}^{c}}, \qquad \gamma = \frac{c_{\text{T}}^{c}}{c_{\text{A}f}^{c}}, \qquad \tau^c = \frac{V^c}{F_V^c} \tag{13}$$

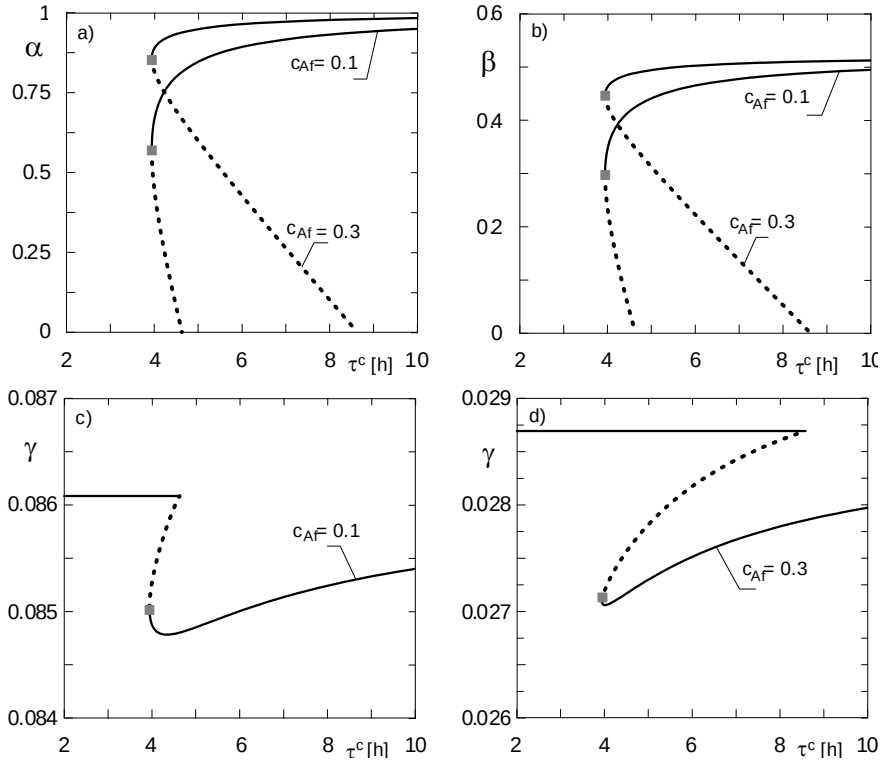

Fig. 2. Steady state branches for suspended growth model for two values of the concentration of the carbonaceous substrate feeding the bioreactor c_{Af}: a) $\alpha(\tau)$, b) $\beta(\tau)$, c) and d) $\gamma(\tau)$ (description in the text)

Results presented in Fig. 2 were obtained for two values of the concentration of the carbonaceous substrate feeding the reactor. Solid lines represent stable steady states, whereas dashed lines – unstable steady states. While decreasing the mean residence time of the liquid phase, a critical value of this parameter is achieved. Below this value the washout of the biomass occurs and loss of bioreactor productivity as well. According to models assuming lack of the biofilm on the bioreactor's walls, this loss is irreversible. Turning points corresponding to this critical value of the mean residence time of the liquid phase on the steady state branches are marked with a grey square. The presence of the turning point on the branches of the steady states and resultant consequences are caused by the inhibiting influence of the carbonaceous substrate expressed in the Haldane kinetic model. It appears that this phenomenon can be eliminated and the washout of the biomass can be avoided by a proper design of the bioreactor. The crux of this change consists in increasing of the internal specific surface area a_s available for the biofilm immobilization. There are many ways of increasing this surface.

In order to evaluate the value of the internal specific surface area required for total elimination of the inhibiting influence of the carbonaceous substrate, the model of the bioreactor presented above has to be used. The values of parameters of the model used in computations are given in Table 1. The tickness of the active biofilm L_a was determined using the method described elsewhere [17].

Table 1

Values of parameters determining process conditions

Parameter	Value	Unit	Reference
a_s	5; 50	m^{-1}	
c_{Af}	0.1–0.3	$kg \cdot m^{-3}$	
d	1	m	
D_{eA}	$3.3 \cdot 10^{-6}$	$m^2 \cdot h^{-1}$	[18,19]
D_{eT}	$8.28 \cdot 10^{-6}$	$m^2 \cdot h^{-1}$	[18,19]
H_0	1	m	
k	0.569	h^{-1}	[13]
K_A	0.01854	$kg \cdot m^{-3}$	[13]
K_{in}	0.09937	$kg \cdot m^{-3}$	[13]
K_T	$4.8 \cdot 10^{-5}$	$kg \cdot m^{-3}$	[13]
K	30	–	[13]
k_{sA}	0.144	$m \cdot h^{-1}$	[18,19]
k_{sT}	0.277	$m \cdot h^{-1}$	[18,19]
L_a	0.07	mm	[17]
u_{og}	0.1	$m \cdot s^{-1}$	
w_{BA}	0.521	kg B·[kg A]$^{-1}$	[13]
w_{BT}	0.338	kg B·[kg T]$^{-1}$	[13]
ζ	2	–	
ρ_b	100	$kg \cdot m^{-3}$	[18,19]

The results of computations are shown in Figs. 3 and 4. The steady state branches $\alpha(\tau^c)$, $\beta(\tau^c)$ and $\gamma(\tau^c)$ in the figures represent two limiting cases, $r_{det} = 0$ and $r_{det} = r_{det,\,max}$, respectively (Eq. (10)). The shape of the steady state branches in Fig. 3 and 4 completely differs from those obtained for the process without the biofilm immobilization on the bioreactor's walls. The difference, which is first of all qualitative, implicates qualitatively different stationary properties of the bioreactor. The presence of the biofilm causes that the irreversible washout of the biomass is not observed. Gradual decreasing of the mean residence time of the liquid phase in the bioreactor, that is increase of the flow rate of the liquid phase, causes admittedly, lower degree of conversion of the carbonaceous substrate, but does not lead to complete inhibition of the process. It arises from Fig. 3b that even lack of the biomass in the liquid phase for sufficiently low residence time τ^c is compensated by its presence in the biofilm.

The shape and the position of the steady state branches prove a strong influence of the specific surface area a_s which is available for the biofilm immobilization. The results presented in Figs. 3 and 4 were obtained for two values of the internal surface area, $a_s = 5$ m^{-1} and $a_s = 50$ m^{-1}, respectively. It was shown that even complete lack of the interphase transfer of the active biomass ($X_B = 0$) does not lead to the loss of the bioreactor productivity.

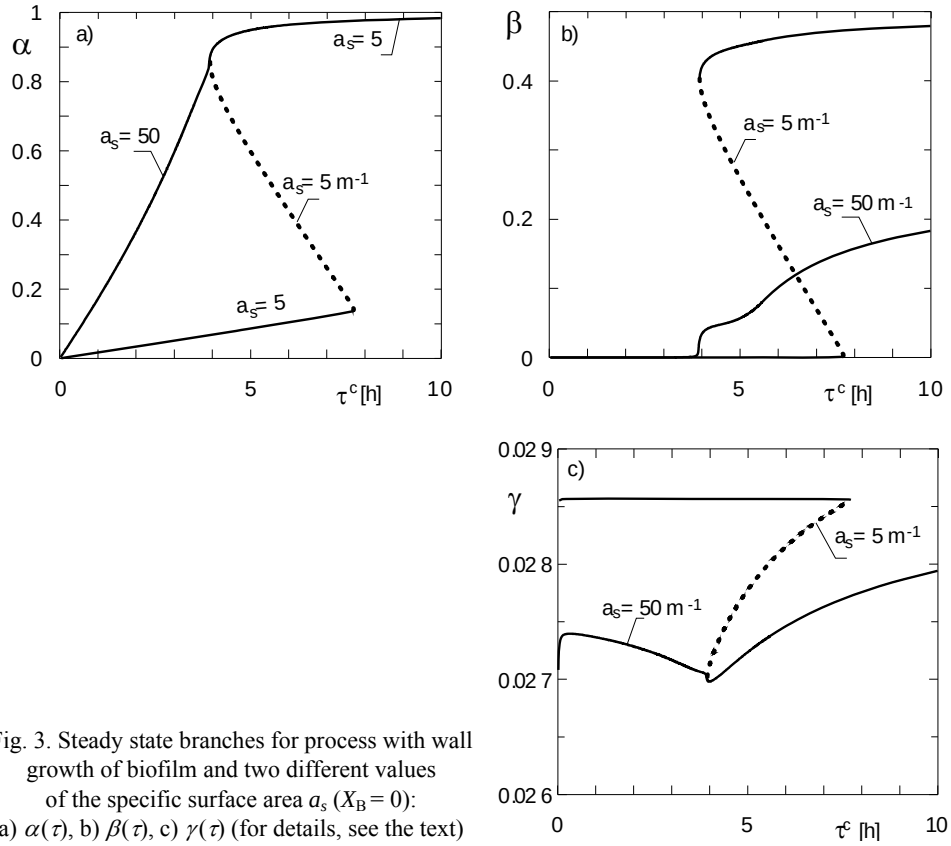

Fig. 3. Steady state branches for process with wall growth of biofilm and two different values of the specific surface area a_s ($X_B = 0$): a) $\alpha(\tau)$, b) $\beta(\tau)$, c) $\gamma(\tau)$ (for details, see the text)

For sufficiently high values of the specific surface area a_s the turning points at the steady state branches disappear. It changes diametrically the stationary character of the bioreactor. The absence of the turning point indicates an elimination of a process danger, that is a violent and considerable decrease of the conversion degree of the substrate from the upper to bottom stable steady state. This phenomenon is independent of the rate of the interphase transfer of the biomass. The value of the specific surface area a_s can be determined during designing of the bioreactor. It can be achieved by many ways. A simple manner of increasing the value of a_s is installation of a stationary packing or a static mixer (Fig. 1b).

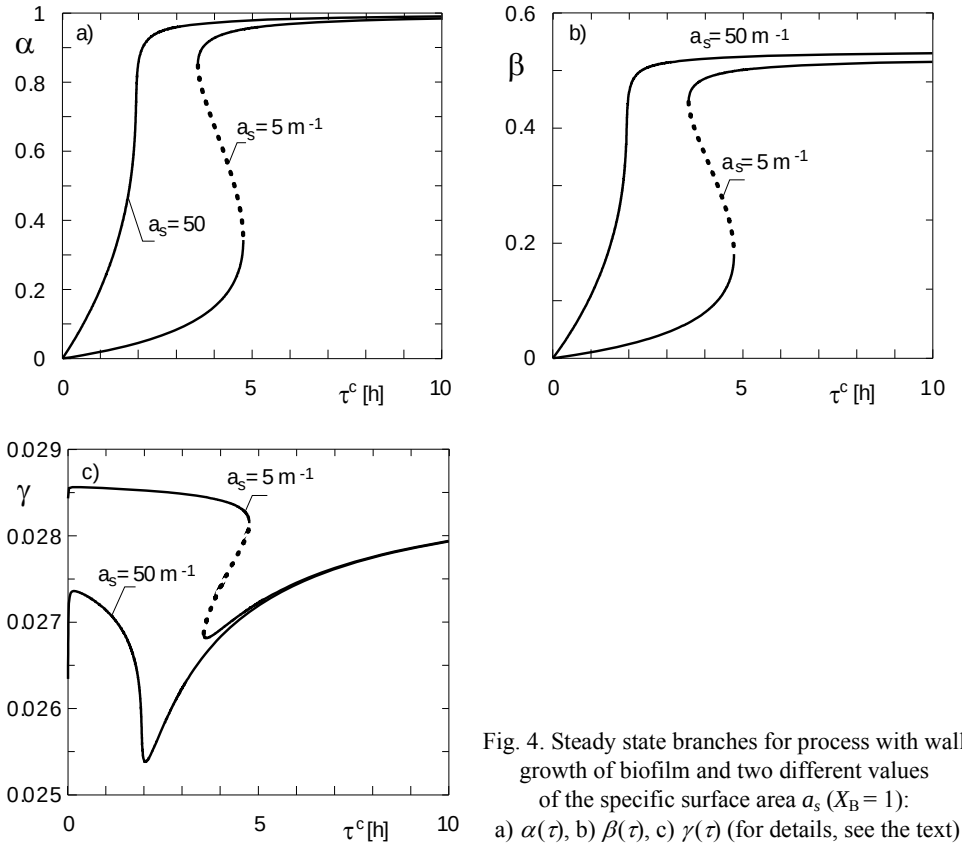

Fig. 4. Steady state branches for process with wall growth of biofilm and two different values of the specific surface area a_s ($X_B = 1$): a) $\alpha(\tau)$, b) $\beta(\tau)$, c) $\gamma(\tau)$ (for details, see the text)

Let us consider a numerical example. The dimensions of the reactor are $d = H = 1$ m. Its volume $V = 0.25\pi = 0.7854$ m^3 and the inner surface area $S = 1.25\pi = 3.927$ m^2. The specific surface area without internal development a_s is 5 m^{-1} (Fig. 1a). In order to obtain a reactor of the specific surface area $a_s = 50$ m^{-1}, an additional surface of 39.27 m^2 – 3.927 m^2 = 35.343 m^2 is required. If for this purpose Raschig rings of the nominal diameter 50 mm and the specific surface 141 m^{-1} are used [20], then 3.343/141 = 0.25 m^3 of the packing is required. Its share in the whole volume of the reactor equals 0.25/0.7854 = 0.318 that is about 32% of the volume of the reactor. A scheme of this design is shown in Fig. 1b. For structural packing with a higher specific surface, as in static mixers, proportionally lower amount is required. Placing the stationary packing in a basket or in a moving grid facilitates replacement, mechanical washout with water stream or changing the volume of the packing during stoppages in the work of the bioreactor.

Assuming the biomass transfer is the cause of the presence of the microorganisms in the liquid phase below the time of washout of the microorganisms from the reactor

(Fig. 4b). From comparison of the steady state branches $\beta(\tau^c)$ in Fig. 3b and 4b, it arises that the concentration of the biomass in the liquid phase strongly depends on the values of the specific surface a_s, and the interphase transfer the biomass. Therefore, the phenomenon can be used in experimental research on the biomass detachment rate.

4. CONCLUSIONS

It was shown that a proper design of the bubble tank bioreactor may avoid the inhibiting influence of the carbonaceous substrate. This results in change of the shape of the steady state branches and the character of the stability of these states. Disappearance of the turning point on the steady state branches also indicates a change in multiplicity of these states, therefore essential difference in the stationary characteristics of the bioreactor.

Installing of an additional surface available for immobilization of a biofilm provides therefore safe operation of the bioreactor in a wide range of feed flow rates, even for those causing the washout of the biomass from the liquid phase. Proposed design enables change of the specific surface, depending, e.g. on kinetics of the process and the concentration of the carbonaceous substrate.

The biodegradation of phenol was chosen as an example of the process, because phenol is a strongly toxic compound. Presented method and the mathematical model of the bioreactor can be extended on other toxic compounds, by choosing the proper kinetics of the microbiological process analyzed.

SYMBOLS

a_s – specific external surface area of the biofilm in the reactor, m^{-1}
A, T – carbonaceous substrate and oxygen, respectively
B – biomass
c_i – mass concentration of *i*-th species, $kg \cdot m^{-3}$
d – reactor diameter, m
D_e – effective diffusion coefficient in biofilm, $m^2 \cdot h^{-1}$
F_V – volumetric flow rate, $m^3 \cdot h^{-1}$
H – height of liquid phase in the reactor, m
k – maximum specific growth rate, h^{-1}
k_s – mass transfer coefficient, $m \cdot h^{-1}$
K – saturation constant, $kg \cdot m^{-3}$
K_{in} – inhibition constant, $kg \cdot m^{-3}$
L_a – active thickness of the biofilm, diffusional penetration depth, m
m – mass, kg
r_A – uptake rate of limiting substrate, $kg \cdot m^{-3} \cdot h^{-1}$
r_B – growth rate of biomass, $kg \cdot m^{-3} \cdot h^{-1}$
r_{det} – rate of detachment of biofilm, $kg \cdot m^{-2} \cdot h^{-1}$
t – time, h

V – working volume of the reactor, m^3
w_{BA} – growth yield coefficient, (kg B)·(kg A)$^{-1}$
x – current co-ordinate in the biofilm, m
X_B – fraction of active biomass transferred to liquid phase
Z – dimensionless co-ordinate along the bioreactor height
α – degree of conversion of carbonaceous substrate in liquid phase
β – dimensionless biomass concentration in liquid phase
γ – dimensionless concentration of dissolved oxygen in liquid phase
η – dimensionless concentration of carbonaceous substrate in the biofilm
δ – dimensionless concentration of oxygen in the biofilm
ρ_b – biomass concentration in the biofilm, kg·m^{-3}
τ^c – mean residence time of liquid phase in the reactor, h

SUBSCRIPTS AND SUPERSCRIPTS

b – biofilm phase
c – liquid phase
s – external surface of the biofilm
f – feed stream

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