

An Alternative Approach for Oscillatory Behaviour Control in a Nonlinear Bioprocess

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Abstract: The nonlinear nature of bioprocesses often leads to the occurrence of the self-sustained oscillations of the biomass concentration in continuous flow bioreactors. For some practical reasons, sometimes it is necessary to control the oscillatory behaviour, which is usually achieved by changing the dilution rate, but this is not always the best option. Hence, the main idea of this paper is to introduce an additional substrate, which has been previously diluted. Based on the numerical analysis of an unstructured mathematical model, it is shown that by mixing two various substrates (the main and the diluted one), it is possible to induce or eliminate the sustained oscillations. As a result, the contribution of both substrates to the mixture and the degree of dilution of the additional substrate can be treated as new control variables.

Keywords: Limit cycle, Self-sustained oscillations, Double substrate, Bioreactor, Nonlinear process.

1. INTRODUCTION

Many previous studies, both theoretical and experimental, have dealt with analysis of the dynamic properties of bioreactors, where the main role is played by microorganisms. At the same time, it is emphasized that the growth of microorganisms is a very complex process, especially in the presence of several available substrates. Moreover, microorganisms can change their metabolic pathway and, as a result, they consume only one of the available substrates on which microorganisms can grow faster (Egli, 1995). Because of the nonlinear nature of these processes, various nonlinear phenomena such as multiplicity of steady states, limit cycles or even chaos (Graham et al., 2007) are often observed. Particular attention is drawn to the self-sustained oscillations (SSO) of biomass concentration, which take place in continuous flow bioreactors for some range of dilution rates at constant substrate concentration in the feed (Sohn et al., 2000). Many papers also describe the application of the oscillatory behaviour in improvement of the bioreactor performance. For instance, in (Balakrishnan and Yang, 2002; Nelson and Sidhu, 2005) it has been shown that the operation of bioreactor in the range of SSO may result in higher average biomass concentrations (or biomass productivity) in comparison to the results obtained at steady states. On the other hand, the oscillatory behaviour is usually avoided in practice due to the necessity of using more expensive equipment (Silveston et al., 1995) and system of surge tanks (Bai et al., 2009). Moreover, the oscillatory mode of operation not always leads to an increase in bioreactor improvement (Bruce et al., 1991). Hence, the choice of the desired mode of operation of the bioreactor is equally important and interesting problem. In practice, it is usually done by changing the flow rate, which is equivalent to changing the dilution rate. As a result, most bioreactors

operate at steady states for small dilution rates. This, in turn, may lead to lower biomass productivities, in comparison to the values obtained in the range of SSO and there is no possibility for arbitrary selection of the dilution rate. Hence, the present paper describes an alternative approach involving a mixture of two various substrates of similar properties, but with different inlet concentrations. The main idea is to introduce an additional substrate, which is diluted and fed into the bioreactor at the expense of the main substrate. It means that the contribution of the individual substrates to the mixture and the degree of dilution can be treated as new control variables. This will be discussed based on the numerical analysis of an unstructured mathematical model of the bioreactor system. However, it should be noted that the growth of microorganisms on the basis of two various substrates is much more complex process, than the growth limited by a single substrate. This is because there are various patterns of substrate consumption and varying degrees of interaction between substrates (Egli, 1995). Hence, the choice of an appropriate mathematical model must first be preceded by a discussion of the case where the bioreactor is fed with a single limiting substrate. The main results of this paper are obtained by numerical simulations of the model equations including detection of the SSO according to the algorithm described in (Skupin, 2010) and by numerical bifurcation analysis in XPPAut (Ermentrout, 2002).

2. GROWTH LIMITED BY A SINGLE SUBSTRATE

Because, the processes involving living microorganisms are very complex (there are hundreds of intermediate intracellular reactions), hence, we will focus our attention on the application of unstructured mathematical models to describe dynamics of the continuous flow bioreactor. In such models there is only one variable (X) used to represent the

concentration of microorganisms (biomass). One of the well-known unstructured mathematical models describing the growth of biomass in continuous flow bioreactor was proposed by Monod (1950) and by Novick and Szilard (1950):

$$\frac{dS}{dt} = D(S_{in} - S) - \frac{\mu(S)}{Y} X \quad (1)$$

$$\frac{dX}{dt} = (\mu(S) - D)X \quad (2)$$

$$\mu(S) = \frac{\mu_m S}{S + K_s} \quad (3)$$

where: S_{in} , S – inlet and outlet substrate concentrations, respectively [g/L], X – biomass concentration [g/L], D – dilution rate [1/h], Y – yield coefficient [g/g], $\mu(S)$ – specific growth rate [1/h], which is described by the Monod equation (3), K_s – half saturation constant [g/L], μ_m – maximum specific growth rate [1/h].

In (Crooke et al., 1980; Crooke and Tanner, 1982) it has been shown that for the model described by (1)-(3) with constant yield coefficient ($Y=\text{const}$) there is no SSO. However, a slight modification in the model may result in qualitatively different behaviour of the system (Ivanitskaya et al., 1989). Hence, to describe the oscillatory behaviour, it is often assumed that the yield coefficient is dependent on substrate concentration S in the following fashion (Essajee and Tanner, 1979; Huang, 1990):

$$Y(S) = \alpha + \beta S \quad (4)$$

where: α [g/g], β [L/g] – are constant and positive coefficients. A hypothesis concerning the dependence of the yield coefficient on the substrate concentration is well-known in the literature and results from the fact that some fraction of substrate is used to support basic life functions of microorganisms (Pirt, 1965). This fact has been proved in many experimental studies (see e.g. Hempfling and Mainzer, 1975; Panikov, 1995).

In turn, in (Huang, 1990) it has been proved that if the yield coefficient $Y(S)$ is an increasing function of S for $S > 0$ and $\alpha, \beta > 0$, then the system (1)-(3) exhibits the oscillatory behaviour (SSO of biomass concentration) for some range of dilution rates D . It has been confirmed in (Pilyugin and Waltman, 2003) by formulating necessary conditions for the existence of periodic solutions of the system (1)-(3) with variable yield coefficient (4).

In the next paragraphs of this section, we will recall the basic properties of the system (1)-(3) with variable yield coefficient (4) that can be found in (Nelson and Sidhu, 2005; Pilyugin and Waltman, 2003). These properties will be necessary in further analysis of the bioreactor fed with a mixture of two substrates. The main difference with respect to the cited papers is that we do not introduce dimensionless variables in order to have a physical interpretation of the results.

By setting the derivatives in (1)-(2) equal to zero we can easily find two equilibrium points corresponding to washout and no washout states, respectively:

$$S_1^* = S_{in}, X_1^* = 0 \quad (5)$$

$$S_2^* = \frac{DK_s}{\mu_m - D}, X_2^* = (\alpha + \beta S_2^*)(S_{in} - S_2^*) \quad (6)$$

The Jacobian matrix for the system (1)-(4) at the washout equilibrium (5) has a form:

$$J(S_1^*, X_1^*) = \begin{bmatrix} -D & -\frac{\mu_m S_{in}}{S_{in} + K_s} \cdot \frac{1}{\alpha + \beta S_{in}} \\ 0 & -D + \frac{\mu_m S_{in}}{S_{in} + K_s} \end{bmatrix} \quad (7)$$

Based on the eigenvalues of (7), the conditions for the local stability of (5) are:

$$D > 0 \quad \text{and} \quad \frac{\mu_m S_{in}}{S_{in} + K_s} < D \quad (8)$$

It means that for $D > \mu_m S_{in} / (S_{in} + K_s) =: D_c$ all the biomass is washed out of the reactor and the biomass concentration drops to zero. The parameter D_c is called the critical dilution rate. For $0 < D < D_c$ the equilibrium point (5) is a saddle point which means that for $X(0) = 0$ [g/L] (no microorganism at $t = 0$) and for $S(0) \geq 0$ each trajectory will tend to $(S_1^*, X_1^*) = (S_{in}, 0)$ as time tends to infinity (Pilyugin and Waltman, 2003).

Likewise, the Jacobian matrix calculated at the equilibrium point (6):

$$J(S_2^*, X_2^*) = \begin{bmatrix} -D - \frac{\mu'(S_2^*)(\alpha + \beta S_2^*) - D\beta}{(\alpha + \beta S_2^*)^2} X_2^* & -\frac{D}{\alpha + \beta S_2^*} \\ \mu'(S_2^*) X_2^* & 0 \end{bmatrix} \quad (9)$$

In turn, based on the eigenvalues of (9), the conditions for the local stability of (6) are:

$$D + \frac{\mu'(S_2^*)(\alpha + \beta S_2^*) - D\beta}{(\alpha + \beta S_2^*)^2} X_2^* > 0 \quad \text{and} \quad \frac{\mu'(S_2^*) X_2^* D}{\alpha + \beta S_2^*} > 0 \quad (10)$$

The latter inequality (10) is always satisfied for $S_2^* \in [0, S_{in})$ and $D > 0$, whereas the former inequality (10) is satisfied only for some positive values of D . This can be shown by plotting a bifurcation diagram in XPPAUT for system (1)-(4). Treating the dilution rate D as a bifurcation parameter, Figure 1 presents steady state values of biomass concentration versus D . For this purpose, the parameter values were taken from (Balakrishnan and Yang, 2002). The thin continuous lines represent stable branches and the broken lines are unstable ones. The white circle represents a point for which $D = D_c$ and the grey shaded area represents maximum and minimum values of biomass concentration in the range of SSO. It can be clearly seen that for $D > D_c$ the system (1)-(4) at the equilibrium point (5) is stable and the biomass concentration is equal to zero. The unstable branch between black filled in

squares (representing Hopf points) corresponds to the occurrence of the stable limit cycle for some range of dilution rates. In this range, the black dots represent the average values of biomass concentration.

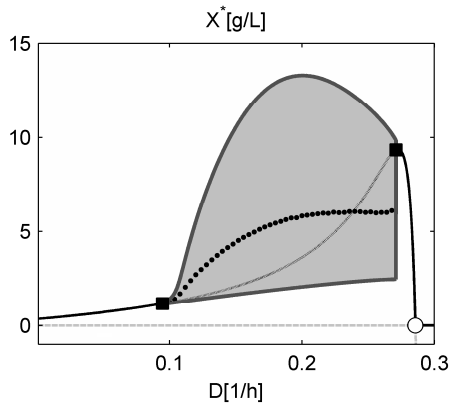


Fig. 1. Steady state diagram showing biomass concentration versus dilution rate D for system (1)-(3) with variable yield coefficient $Y=\alpha+\beta S$.

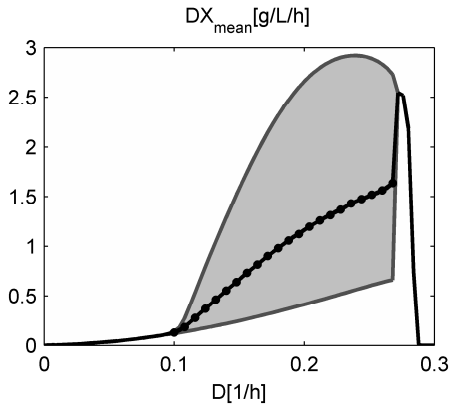


Fig. 2. Average biomass productivity DX_{mean} versus dilution rate D . The grey shaded area represents maximum and minimum values of biomass productivity.

The next important parameter that should be taken into account is a biomass productivity DX [g/L/h], which is equal to the mass of biomass produced per unit time, per unit volume of the medium in the bioreactor. By making several simulation runs of the system (1)-(4), it is possible to show the dependency of the average biomass productivity on the dilution rate D . The obtained results were double-checked in XPPAut. The maximum biomass productivity is obtained at steady state in a narrow range of dilution rates. However, due to the high risk of washout, it is recommended to avoid this range. Moreover, due to the reasons mentioned in the introduction section, the oscillatory mode of operation of the bioreactor is usually avoided in practice. Therefore, most of bioreactors operate at steady states for smaller values of dilution rates and for smaller values of biomass productivities in comparison to the results that can be obtained in the range of SSO (Balakrishnan and Yang, 2002). This is clearly seen in Figure 2. Hence, it is proposed a method involving a mixture of two various substrates of similar properties, which means that the growth of microorganisms can be limited by each of the two substrates separately. In other words, we

assume that these two substrates are substitutable (Egli, 1995). Furthermore, it is assumed that the SSO are observed for some range of dilution rates for each single limiting substrate. Examples of such substrates are glucose and galactose (Beuse et al., 1999).

3. GROWTH LIMITED BY DOUBLE SUBSTRATES

Figure 3 presents the general scheme of the continuous flow bioreactor fed with the mixture of two substrates. The contribution of the individual substrates to the mixture is set by the three-port valve, which will be represented in the mathematical model by the parameter $r \in [0,1]$, which can be treated as a new control variable. It means that the substrate of inlet concentration S_{in2} is fed into the reactor chamber at the expense of the main substrate of inlet concentration S_{in1} . Because, it is assumed that the SSO can be observed for each single limiting substrate, hence, in order to choose the desired mode of operation of the bioreactor, the second substrate of inlet concentration S_{in2} is diluted with distilled water. The degree of dilution will be represented in the mathematical model by the parameter $\gamma \in [0,1]$ ($\gamma=1$ means totally diluted substrate). The degree of dilution can also be treated as a new control variable.

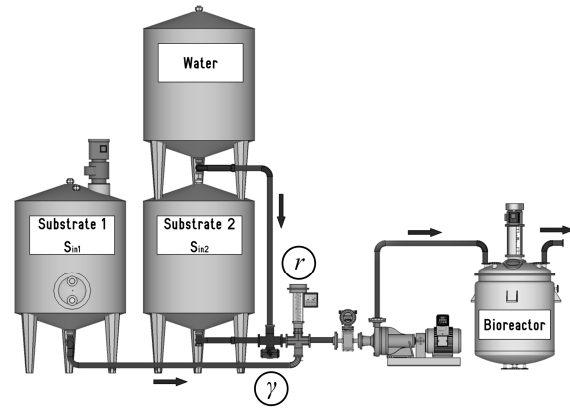


Fig. 3. The general scheme of the bioreactor system, with marking of the new control variables, i.e. r and γ parameters.

Based on the mass balance of substrates and microorganisms, the general form of the mathematical model of the continuous flow bioreactor is as follows:

$$\frac{dS_1}{dt} = D((1-r) \cdot S_{in1} - S_1) - \frac{\mu_1(S_1, S_2)}{Y_1(S_1)} X \quad (11)$$

$$\frac{dS_2}{dt} = D(r \cdot (1-\gamma) \cdot S_{in2} - S_2) - \frac{\mu_2(S_1, S_2)}{Y_2(S_2)} X \quad (12)$$

$$\frac{dX}{dt} = -DX + \mu(\mu_1, \mu_2) X \quad (13)$$

where: S_1, S_2 – are outlet concentrations of substrates (the bioreactor is well-mixed) [g/L], S_{in1}, S_{in2} – are inlet concentrations of the main and additional substrate [g/L], respectively, X – biomass concentration [g/L], $\mu_i(S_1, S_2)$ – i -th specific growth rate [1/h] ($i=1,2$), which can be simultaneously dependent on both substrate concentrations,

$Y_i(S_i)=\alpha_i+\beta_i S_i$ – i -th variable yield coefficient [g/g] (where $\alpha_i, \beta_i > 0$ are constant parameters, $i=1,2$) and μ is the overall specific growth rate [1/h], which, in general, can be dependent on the individual specific growth rates μ_i ($i=1,2$).

Of course, the presented model is incomplete and it is necessary to discuss and choose an appropriate equation describing the overall specific growth rate μ . However, this requires a good understanding of the consumption mechanisms, since the growth limited by two or more substrates is much more complex than the growth limited by a single substrate. Egli (1995) presented a thorough study of the possible consumption patterns in the presence of several available substrates. Among these patterns, the most frequently observed are: simultaneous utilisation of both substrates in the whole range of dilution rates (until washout occurs) and the diauxic growth (Egli, 1995; Narang et al., 1997a, 1997b; Jones and Kompala, 1999). In the continuous flow bioreactors, the diauxic growth pattern is observed when there is a simultaneous utilisation of both limiting substrates for small dilution rates ($D \leq D_{trans}$) and for $D > D_{trans}$ the only utilised substrate is a substrate on which microorganisms can grow faster (Egli, 1995). The parameter D_{trans} is called a transition dilution rate. On the other hand, another frequently observed phenomenon is a substrate or product inhibition, e.g. too high concentrations of one substrate can inhibit the growth of microorganisms or the utilisation of another substrate. However, due to the limited length of this paper, it is not possible to discuss all cases. Therefore, in the next section, our attention will be focused on the frequent case of simultaneous utilisation of two substitutable substrates. In such the case, the overall specific growth rate can be described by the equation proposed in (Yoon et al., 1977):

$$\mu(S_1, S_2) = \mu_1(S_1, S_2) + \mu_2(S_1, S_2) \quad (14)$$

$$\mu_1(S_1, S_2) = \frac{\mu_{m1} S_1}{K_{s1} + S_1 + a_2 S_2} \quad (15)$$

$$\mu_2(S_1, S_2) = \frac{\mu_{m2} S_2}{K_{s2} + S_2 + a_1 S_1} \quad (16)$$

where: $a_1 = K_{s2}/K_{s1}$, $a_2 = K_{s1}/K_{s2}$ are dimensionless coefficients representing the inhibition effects of the individual substrates on the growth of microorganisms.

From the model equations (11)-(13) and (14)-(16), it is easy to notice that for $r=0$ the bioreactor is fed only by the main substrate of inlet concentration S_{in1} and for $r=1$ – by the diluted substrate of inlet concentration $(1-\gamma) \cdot S_{in2}$. The next step is to find an appropriate degree of dilution (γ) of the additional substrate. As we shall see, the degree of dilution should be sufficiently high to have a possibility to attenuate the sustained oscillations, but sufficiently small to not cause a significant reduction in the biomass productivity (Skupin and Metzger, 2012). It is obvious that the smaller the inlet concentration $(1-\gamma) \cdot S_{in2}$, the smaller is the biomass concentration (and the biomass productivity as well), what has been shown in (Nelson and Sidhu, 2005) for a single limiting substrate. Hence, the degree of dilution is assumed to be equal $\gamma=0.35$.

Although, the model (11)-(13) consists of only three differential equations, it is a very laborious task to find all the equilibrium points. Therefore, our analysis will be limited to the numerical integration of the equation and bifurcation analysis in XPPAut. For simplicity, but without loss of generality, we assume the same parameter values for half saturation constants $K_{s1}=K_{s2}=1.75$ [g/L] and for maximum specific growth rates $\mu_{m1}=\mu_{m2}=0.3$ [1/h]. These parameter values are taken from (Balakrishnan and Yang, 2002). In turn, the parameters $\alpha_1=\alpha_2=0.2$ [-] and $\beta_1=\beta_2=0.06$ [L/g] in variable yield coefficients $Y_1(S_1)$ and $Y_2(S_2)$ are chosen so that the system (11)-(16) exhibits the sustained oscillations for some range of dilution rates for each limiting substrate separately. Moreover, the inlet substrate concentrations are $S_{in1}=S_{in2}=35.0$ [g/L].

4. RESULTS

The model equations were numerically integrated for several values of r and D changing in steps of size $\Delta r=0.02$ and $\Delta D=0.002$ [1/h], respectively. The initial conditions $X_0=0.1$ [g/L] and $S_{10}=S_{20}=0.0$ [g/L] were kept constant in each simulation run, and the obtained results were double-checked in XPPAut. Figure 4 presents the average biomass concentration obtained for the case when two substrates are fed into the bioreactor. The influence of both parameters (r and D) on the existence of the SSO is presented in Figure 5. It can be clearly seen that the assumed degree of dilution allows for induction or elimination of the SSO of biomass concentration. Otherwise, e.g. for too low degrees of dilution, the attenuation of oscillations in the whole range of dilution rates would be impossible (Skupin and Metzger, 2012). Moreover, irrespective of the value of the r parameter, the washout always occurs for some critical dilution rate and the biomass concentration drops to zero.

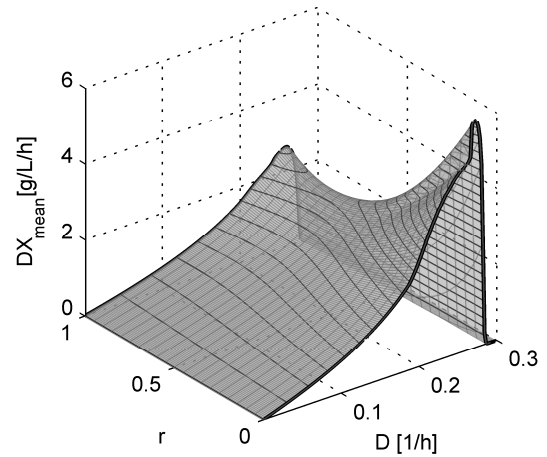


Fig. 4. Average biomass productivity DX_{mean} versus dilution rate D and r parameter for a mixture of two substrates.

For a more in-depth analysis of the system, the obtained results were complemented by the numerical bifurcation analysis in XPPAut. Treating the dilution rate D as a bifurcation parameter, Figures 6, 7 and 8 show steady state diagrams of the biomass concentration for fixed values of the r parameter.

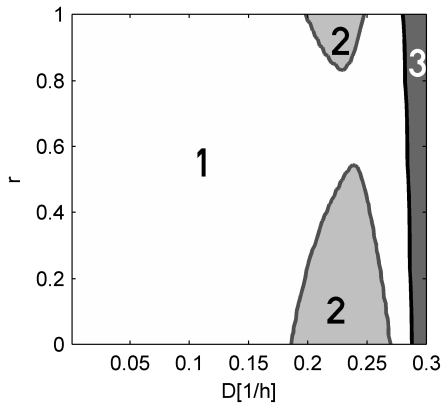


Fig. 5. The parameter plane (D, r) with marking of the regions of: 1 – steady state, 2 – SSO, 3 – washout.

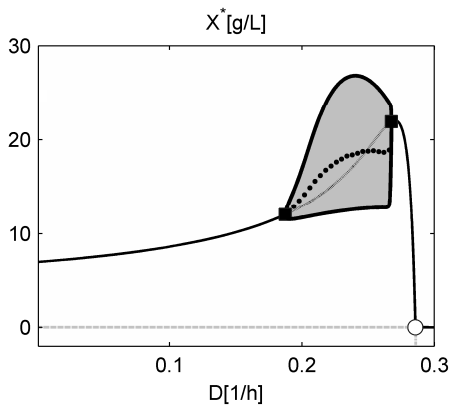


Fig. 6. Steady-state diagram for $r=0$ (the main substrate)

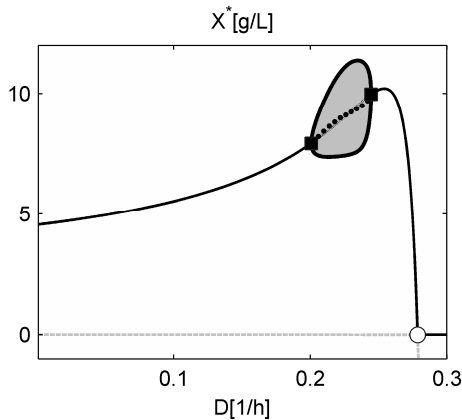


Fig. 7. Steady-state diagram for $r=1$ (the diluted substrate)

The two extreme cases, in which the bioreactor is fed either with the main substrate ($r=0$) of inlet concentration S_{in1} or with the diluted substrate ($r=1$) of inlet concentration $(1-\gamma) \cdot S_{in2}$ are illustrated in Figures 6 and 7. The notations representing Hopf points, stable and unstable branches or maximum and minimum biomass concentrations are the same as in Figure 1. The case of intermediate value of the r parameter (the mixture of substrates) is depicted in Figure 8. In turn, Figure 9 shows the average biomass productivity obtained for two cases, when bioreactor is fed only with the main substrate ($r=0$) or with the mixture of substrates ($r=0.6$).

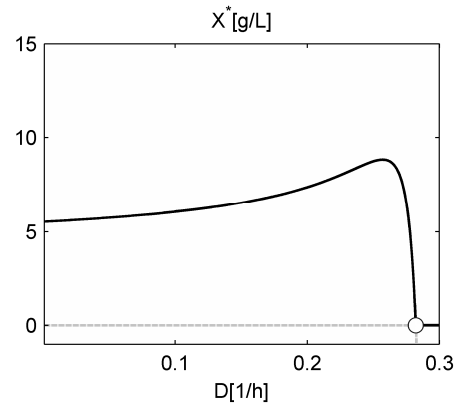


Fig. 8. Steady-state diagram for $r=0.6$ (the mixture of substrates)

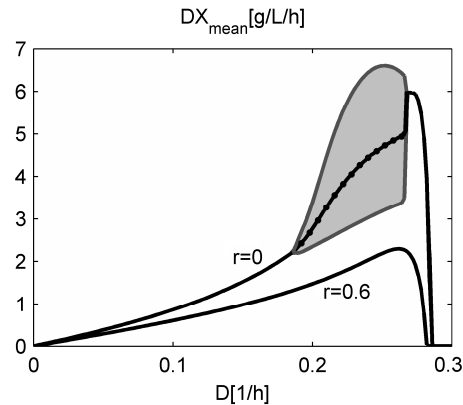


Fig. 9. The comparison of average biomass productivities for the original substrate ($r=0$) and for the mixture of substrates ($r=0.6$)

It is therefore clear that for the appropriate contribution of both substrates to the mixture, which is indicated by the r parameter, it is possible to induce or eliminate the SSO. In other words, the additional control variable r allows us to control the oscillatory behaviour. At the same time, it is still possible to maintain sufficiently high values of the biomass productivity, which are comparable to the ones obtained in the range of steady states for a single limiting substrate. It means that the approach involving a mixture of two various substrates of similar properties allow us to choose the desired mode of operation of the bioreactor with no significant reduction in the biomass productivity.

5. CONCLUSIONS

The occurrence of the SSO of biomass concentration for some range of dilution rates for a single limiting substrate has been described in the previous papers by other authors. They have concluded that the elimination (or induction) of these oscillations is possible by changing the operating conditions (e.g. dilution rate, pH, temperature, dissolved oxygen concentration). However, in most cases, these parameters have to be kept constant; therefore most bioreactors operate in the range of steady states for small dilution rates (Balakrishnan and Yang, 2002). In the presented paper, based on the numerical analysis of the mathematical model, it has been shown that by setting an appropriate contribution of two

various substrates of similar properties, it is possible to induce or eliminate the SSO of biomass concentration in the whole range of dilution rates. Moreover, by setting an appropriate degree of dilution of the additional substrate, it is possible to maintain the biomass productivity at the acceptable level. As a result, the r and γ parameters can be treated as control variables, which introduce two additional degrees of freedom that can be employed to develop new control strategies.

ACKNOWLEDGEMENTS

This work was supported by the National Science Centre under grant No. 2012/05/B/ST7/00096 and by the Ministry of Science and Higher Education under grant BK-UiUA.

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