MODELLING AND OPTIMIZATION OF ACETONE-BUTANOL-ETHANOL FED-BATCH BIOSYNTHESIS

Brînduşa SANDU (OHREAC)[1], Tănase DOBRE[2], Oana C. PÂRVULESCU[3]

The biosynthesis of solvents with Clostridium acetobutylicum occurs due to its growth in various sweet substrates. In many cases, especially for glucose as substrate, the process is conducted in fed-batch mode, in a perfectly mixed bioreactor. The mathematical model of this process reveals the dynamics of biomass, intracellular marker, substrate, solvents (acetone-butanol-ethanol), and intermediate compounds (butyric and acetic acids) in the reaction medium. The model was used for process optimization for a scenario where the solvents production was maximized. The substrate concentration in the reactor feed, the inoculum volume, and the feed flow rate were selected as process variables.

Keywords: biosynthesis, ABE solvents mixture, *Clostridium acetobutylicum*, modelling, process optimization

1. Introduction

The energy derived from renewable materials presents a number of advantages over fossil derived energy. These include the renewability of primary sources, the environmentally friendly and beneficial of the used technologies. Because the renewable materials are agriculture products, the production of this kind of energy provides new markets for all agriculture sectors and turns agricultural wastes into more valuable products. Thus, it is worthwhile replacing fossil fuels by bioenergy carriers [1-3].

Near to ethanol and biodiesel, which are currently commonly used, butanol is one of a number of interesting energy species for future. With reference to the ethanol, the butanol is more advantageous because it has higher energy content, presents a good temperature dependency of vapour pressure, has a low higroscopicity and requires no modification for use in combustion engines [4-5]. Butanol is biologically produced along with small amounts of acetone and ethanol

[1] Eng., Dept. of Chemical and Biochemical Engineering, University POLITEHNICA of Bucharest, Romania
[2] Prof., Dept. of Chemical and Biochemical Engineering, University POLITEHNICA of Bucharest, Romania
[3] Lecturer, Dept. of Chemical and Biochemical Engineering, University POLITEHNICA of Bucharest, Romania, e-mail: oana.parvulescu@yahoo.com

by *Clostridium acetobutylicum* bacterial systems from renewable materials, under strictly anaerobic conditions. This process is called the acetone-butanol-ethanol (ABE) fermentation process. Glucose is the basic substrate in this biosynthesis. It may be derived from starch by hydrolysis thereof in the presence of amylolytic enzymes. Any other natural source of glucose (molasses, sweet wastes *etc*.) or which can be converted into glucose (*e.g.* cellulose, lignin, hemicelluloses) may be a valuable raw material for ABE biosynthesis. Glucose is used for cell growth, for acid (acetic and butyric) production during the acidogenesis fermentation phase, and for conversion of these acids into ABE products during the solventogenesis fermentation phase [6-8]. At this time China is in the forefront of efforts to resale ABE fermentation process, including plants which operate in an apparently continue system, where the fermentation is organized in an interconnected batch fermenters battery with up to 8 fermenters at 300-400 m^3 per unit [9]. The gases resulted from this anaerobic digestion can be used to generate heat or electricity. However, the bio-butanol is not marketed due to its high production cost, the raw material accounts being of about 63 % from the cost of the fermentation process [10].

In ABE biosynthesis the mathematical modelling has been used in three main areas: (i) quantitative characterization of intricate biochemical process describing the dynamics of glucose transformation in ABE, CO_2, and H_2; (ii) design, modelling, and optimization of ABE synthesis reactor as well as of ABE synthesis reactor coupled with ABE separation system; (iii) analysis of final reaction mass processing. The present paper aim is to show how can be used the fed-batch reactor for ABE biosynthesis in order to obtain a maximum transformation of substrate in solvents.

2. ABE fermentation features

The ABE anaerobic fermentation presents non-growth-associated end-products formation, which proceeds through a growth-associated intermediate products formation. It is a difficult task to study, elucidate, model, and optimize a fermentation system with such a great number of process variables and with a metabolic pathway yet not fully understood.

All kinetics used in mathematical modelling of ABE fermentation start from the representation of the metabolic pathways characterizing the culture of *Clostridium acetobutylicum* in media with glucose. For this process, there are various complex or simplified representations [11-14]. One of them, proposed in the related literature, is given in Fig. 1 [15]. All reactions mentioned in Fig. 1, except those characterizing the cell death, are enzymatic reactions controlled by enzymes produced by *Clostridium acetobutylicum*. The kinetic of each reaction is expressed by specific Monod relationships described by the equations (1)-(4). The

relations (2) and (3) highlight the participation of two substrates in the biochemical process. The relation (4) emphasizes the inhibition effect of butanol on the cell growth dynamics. On the other hand, the metabolic pathways from Fig. 1 show that there are two distinct important phases, *i.e.* acidogenesis (pathways containing acetaldehyde and butyraldehide synthesis) and solventogenesis (pathways containing acetone, ethanol, and butanol synthesis). They can be manipulated in order to produce higher amounts of butanol.

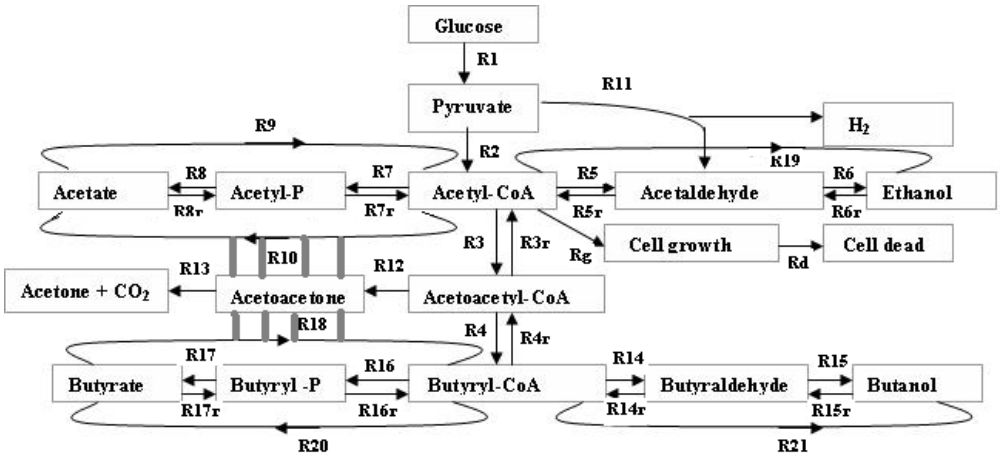

Fig. 1. Metabolic pathways at *Clostridium acetobutylicum* culture in media with glucose

$$R1 = \frac{V_{m1}[glucose][biomass]}{k_{m1} + [glucose]} \tag{1}$$

$$R10 = \frac{V_{m10}[acetate][acetoacetyl - CoA]}{(k_{m10A} + [acetate])(k_{m10B} + [acetyl - CoA])}[biomass] \tag{2}$$

$$R18 = \frac{V_{m18}[butyrate][acetoacetyl - CoA]}{(k_{m18A} + [butyrate])(k_{m18B} + [acetyl - CoA])}[biomass] \tag{3}$$

$$Rg = \frac{V_{mg}[acetoacetyl - CoA][biomass]}{k_{mg}(1 + \frac{[butanol]}{kg}) + [acetyl - CoA](1 + \frac{[butanol]}{kg})} \tag{4}$$

Generally, in the acidogenesis microorganisms have an exponential growth and acetic and butyric acids are produced along with ATP formation,

whereas in the solventogenesis the cells growth occurs in a stationary phase. The organic acids are reintroduced in the metabolic cycle and the final products, *i.e.* butanol, acetone, and ethanol, are synthesized. Additions of acetic, pyruvic or acetoacetic acids in the broth results in increased quantities of acetone formed, with no effect on butanol, while the addition of butyric acid increases the amount of butanol obtained. The yields of solvents based on the glucose substrate range around 30-34 %.

Aiming at modelling a batch reactor, where the ABE synthesis takes place, using the kinetic expressions of the reactions described in Fig. 1, 17 unsteady state differential equations can be written, each equation expressing the balance of a single species. This system of differential equations, containing 77 parameters ($V_{mi}, i = 1..34; k_{mi}, i = 1..34; 2\times4$ reactions with double substrates; 1 for cell death kinetics), gives the model of ABE solvents batch synthesis. The relation (5) of mass balance of acetyl-CoA species reveals the model complexity.

$$\frac{d[Acetyl-CoA]}{d\tau} = R2 + R3r + R5r + R7r + R9 - R3 - R5 - R7 - R10 - R19 \quad (5)$$

The most used kinetic models for ABE synthesis take into account the chemical reactions from the metabolic pathways, but with simplifications concerning the process dynamics of major products [16,17]. A model based on the intracellular marker concept is applied in this paper.

3. Model and optimization of a fed-batch reactor

The structured growth model developed by Volesky and Votruba [18] was used with some modifications. The kinetic expressions for the metabolic pathway presented in Fig. 1 form the basis of this kinetic model. To express the biomass concentration, the method of the principal indicator of the metabolic activity $Q(\tau)$ was used. As shown in relation (6), it depends on the history of cellular culture ($f(\varsigma)$) and on the state of momentary substrate concentration ($c_s(t-\varsigma)$). The relation (7), where Y_{SX} is the yield of substrate (S) conversion in biomass (X), shows how this indicator determines the cell growth rate (μ).

$$Q(\tau) = \int_0^{\tau} f(\varsigma) q[c_s(t-\varsigma)] d\varsigma \quad (6)$$

$$\mu = Y_{SX} R_S Q(\tau) \quad (7)$$

For the case of ABE synthesis via cultivation of *Clostridium acetobutylicum*, relation (7) turns into relation (8), where y is the momentary dimensionless RNA concentration in the reaction media ($y = c_{RNA} / c_{RNA_{\min}}$ with $c_{RNA_{\min}}$ as RNA concentration in the reaction media needed to start the reaction).

$$\mu = 0.56(y-1) \tag{8}$$

With these general considerations, the mathematical model of the fed-batch bioreactor (equation (9)) is given by relations (10)-(22). In fact, these relations express the balance of species considered in the ABE biosynthesis, *i.e.* the marker of RNA (y dimensionless concentration), the biomass (c_x concentration), the glucose substrate (c_S concentration), the butyric acid (c_{BA} concentration), the butanol (c_B concentration), the acetic acid (c_{AA} concentration), the acetone (c_A concentration), the ethanol (c_E concentration), the carbon dioxide (c_{CO_2} concentration), and the hydrogen (c_{H_2} concentration).

$$\frac{d(Vc_i)}{d\tau} = VR_i + Fc_{i0},\ \mathrm{i} = 1,...\mathrm{N} \tag{9}$$

$$\frac{dy}{d\tau} = \left[k_1 \frac{k_I c_s}{k_I + c_B} - 0.56(y-1) \right] y \tag{10}$$

$$\frac{dc_X}{d\tau} = 0.5(y-1)c_X - k_2 c_B c_X \tag{11}$$

$$\frac{dc_S}{d\tau} = k_3 c_S c_X - k_4 \frac{c_S c_X}{c_S + k_S} + \frac{1}{V}\frac{dV}{d\tau}(c_{SF} - c_S) \tag{12}$$

$$\frac{dc_{BA}}{d\tau} = k_5 \frac{k_I c_S}{k_I + c_B} c_X - k_6 \frac{c_{BA} c_S}{c_{BA} + k_{BA}} c_X - \frac{1}{V}\frac{dV}{d\tau} c_{BA} \tag{13}$$

$$\frac{dc_B}{d\tau} = k_7 c_S c_X - 0.831\left(\frac{dc_{BA}}{d\tau} + \frac{1}{V}\frac{dV}{d\tau} c_{BA} \right) - \frac{1}{V}\frac{dV}{d\tau} c_B \tag{14}$$

$$\frac{dc_{AA}}{d\tau} = k_8 \frac{c_S}{c_s + k_S} \frac{k_I}{k_I + c_B} c_X - k_9 \frac{c_{AA}}{c_{AA} + k_{AA}} c_X - \frac{1}{V}\frac{dV}{d\tau} c_{AA} \tag{15}$$

$$\frac{dc_A}{d\tau} = k_{10}\frac{c_S}{c_S + k_S}c_X - 0.5\left(\frac{dc_{AA}}{d\tau} + \frac{1}{V}\frac{dV}{d\tau}c_{AA}\right) - \frac{1}{V}\frac{dV}{d\tau}c_A \tag{16}$$

$$\frac{dc_E}{d\tau} = k_{11}\frac{c_S}{c_S + k_S}c_X - \frac{1}{V}\frac{dV}{d\tau}c_E \tag{17}$$

$$\frac{dc_{CO_2}}{d\tau} = k_{12}\frac{c_S}{c_S + k_S}c_X - \frac{1}{V}\frac{dV}{d\tau}c_{CO_2} \tag{18}$$

$$\frac{dc_{H_2}}{d\tau} = k_{13}\frac{c_S}{c_S + k_S}c_X - \frac{1}{V}\frac{dV}{d\tau}c_{H_2} \tag{19}$$

The relations (20) and (21), describing the initial condition for the differential equations (10)-(20), complete the mathematical model of the fed-batch bioreactor for ABE synthesis.

$$\frac{dV}{d\tau} = F \tag{20}$$

$$\begin{aligned}\tau = 0, V = V_0, y = y_0, c_X = c_{X0}, c_S = c_{S0}, c_{BA} = c_{BA0}, c_B = c_{B0}, c_{AA} = c_{AA0},\\ c_A = c_{A0}, c_E = c_{E0}, c_{CO_2} = c_{CO_{20}}, c_{H_2} = c_{H_{20}}\end{aligned} \tag{21}$$

Table 1 contains the usual values of the kinetic and Monod constants from the fed-batch ABE biosynthesis model. In real operation conditions of an ABE synthesis bioreactor there are two stages. In the first stage the bioreactor operates in batch mode.

Table 1

Values for kinetic constants (k_1.....k_{14}) and Monod constants (k_I, k_S, k_{AA}, k_{BA}) for ABE biosynthesis [18]

Parameter	Unit	Value	Parameter	Unit	Value
k_1	L/(gh)	0.0090	k_{10}	h^{-1}	0.1558
k_2	L/(gh)	0.0008	k_{11}	h^{-1}	0.0238
k_3	L/(gh)	0.0255	k_{12}	h^{-1}	0.6139
k_4	h^{-1}	0.6764	k_{13}	h^{-1}	0.0185
k_5	L/(gh)	0.0136	k_{14}	h^{-1}	0.00013
k_6	h^{-1}	0.1170	k_I	g L	0.83300
k_7	L/(gh)	0.0113	k_S	g/L	2.0
k_8	h^{-1}	0.7150	k_{AA}	g/L	0.9
k_9	h^{-1}	0.1350	k_{BA}	g/L	0.5

The second stage corresponds with fed-batch operation mode. The time when occurs the switch from the first stage to the second stage is an important parameter for a bioreactor exploitation. The model characterizing the first stage operation of the bioreactor derives from the fed-batch model considering $dV/d\tau \to 0$. At the switching time the final state of the first stage becomes initial state for the second operating stage of the bioreactor.

The model presented can be used to develop some optimization problems related to the operation of ABE synthesis in the fed-batch bioreactors. In this work the maximizing of the solvents production as an optimization case was considered. This problem requires to obtain the reactor feed flow rate (F), the concentration of substrate in feed flow (c_{SF}), and the time position of switching from batch reactor to fed-batch reactor (τ_{sc}) in order to obtain a maximum quantity of solvents in the final reaction media. In this purpose, the function given by relation (22), showing the sum of carbon in desirable (+) and undesirable (-) compounds, has to be maximized.

$$F_{opt}(V_0, F, c_{SF}, \tau_{sc}) = \frac{V_0}{V_f}(0.646c_{Bf} + 0.622c_{Af} + 0.527c_{Ef} - 0.4c_{Sf} - 0.4806c_{Xf} - 0.44c_{AAf} - 0.4865c_{Bf}) \tag{22}$$

4. Results and discussions

In order to obtain data for establishing the maximum of the optimization function and also to show the specificity of the investigated process, the process simulation was performed according to the model input data summarized in Table 2. Each model simulation presents the substrate utilization rate, production rate, cell growth rate, physiological state marker and allows the computation of the optimization function (Figs. 2-12).

Figs. 2-6 highlight the effect of feed flow rate on dynamics of solvents concentration and also on major participants to ABE biosynthesis (substrate, acetic acid, butyric acid, biomass, and the marker of biological activity). Results depicted in Figs. 2-6 show that a total processing time over 50 h is not recommended. Regarding the evolution of the solvents concentration a capping type growth was found. This behaviour is due to the *Clostridium acetobutylicum* bacterial system inhibition by butanol end product. The operation with low feed flow rate appears to be recommended from the point of view of the concentration level of a single solvent or of total solvents. If the interest is the solvents productivity then, with respect to total solvents, the final solvents mass ranges from $26V_0$ kg at F=$0.002V_0$ to $17.8\times6\times V_0$ kg at F=$0.1V_0$.

Table 2

Input conditions for ABE biosynthesis in batch-fed batch system

Parameter type	Parameter name	Symbol	Value	Unit
Manipulated	Switching time	τ_{sc}	5, 10, 15, 20	h
	Feed flow rate	F, $dV/d\tau$	$0.002V_0$, $0.01V_0$, $0.025V_0$, $0.05V_0$, $0.1V_0$	L/h
	Glucose concentration in the reactor feed	c_{SF}	20, 50, 80, 100, 120	g/L
Fixed	Glucose concentration	c_{S0}	50	g/L
	Biomass concentration	c_{X0}	0.6	g/L
	Butanol concentration	c_{B0}	0.5	g/L
	Acetone concentration	c_{A0}	0.81	g/L
	Ethanol concentration	c_{E0}	0.24	g/L
	Butyric acid concentration	c_{BA0}	5.88	g/L
	Acetic acid concentration	c_{AA0}	3.28	g/L
	CO_2 concentration	c_{CO20}	0	g/L
	Hydrogen concentration	c_{H20}	0	g/L
	Marker concentration	y_0	1.2	-
	Batch reactor volume	V_0	V_0	m^3

This behaviour can be observed if it changes the switching time from 5 to 20 h. With the use of the presented F dependence on V_0, the changing of V_0 keeps unchanged the above mentioned dependencies. The figures above discussed seem to indicate that the flow rate F=0.025V_0 is desirable in the operation with reactor passing from batch state to fed-batch state. Consequently, the figures showing the effect of substrate concentration from the bioreactor feed on dynamics of ABE synthesis (Figs. 7-11) has been obtained for this feed flow rate.

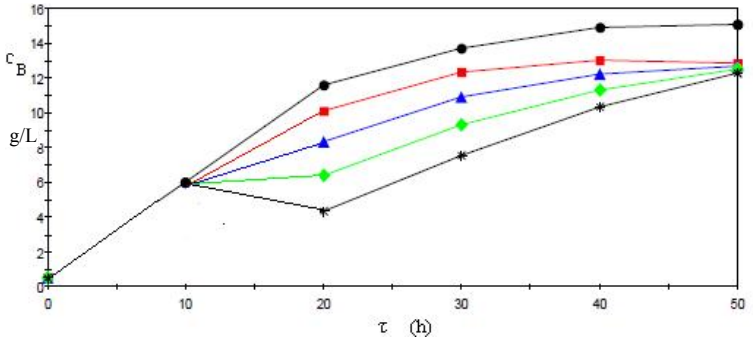

Fig. 2. Effect of feed flow rate on butanol concentration dynamics (c_{S0}=50 g/L, c_{SF}=100 g/L, t=30 °C, V_0=2 L, ● F=0.002V_0, ■ F=0.01V_0, ▲ F=0.025V_0, ♦ F=0.05V_0, *F=0.1V_0, τ_{sc}=10 h)

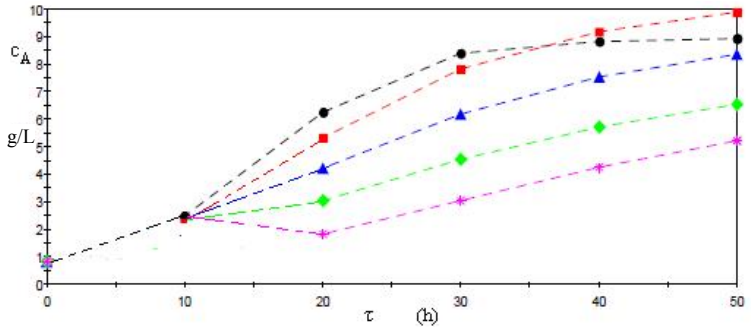

Fig. 3. Effect of feed flow rate on acetone concentration dynamics (c_{S0}=50 g/L, c_{SF}=100 g/L, t=30 °C, V_0=2 L, ● F=0.002V_0, ■ F=0.01V_0, ▲ F=0.025V_0, ♦ F=0.05V_0, *F=0.1V_0, τ_{sc}=10 h)

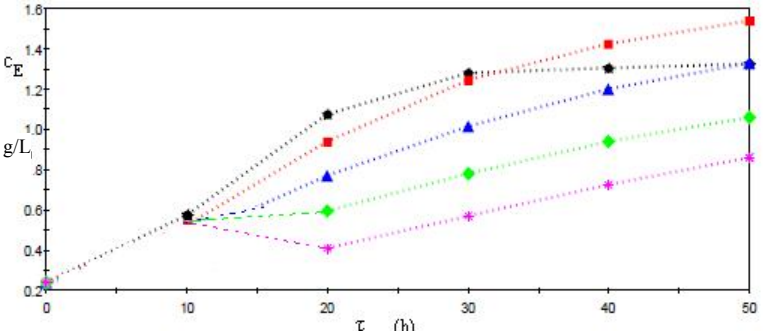

Fig. 4. Effect of feed flow rate on ethanol concentration dynamics (c_{S0}=50 g/L, c_{SF}=100 g/L, t=30 °C, V_0=2 L, ● F=0.002V_0, ■ F=0.01V_0, ▲ F=0.025V_0, ♦ F=0.05V_0, *F=0.1V_0, τ_{sc}=10 h)

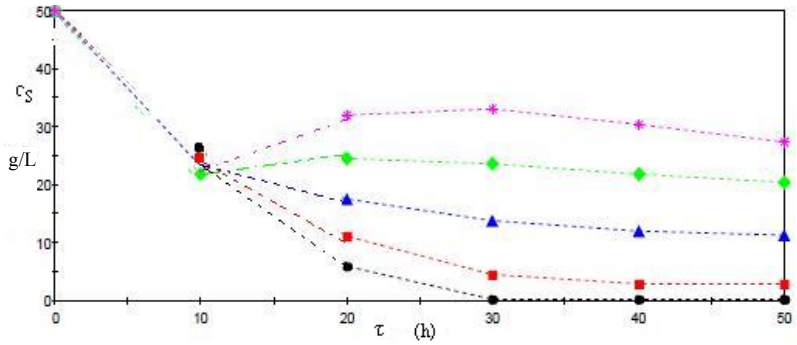

Fig. 5. Effect of feed flow rate on substrate concentration dynamics (c_{S0}=50 g/L, c_{SF}=100 g/L, t=30 °C, V_0=2 L, ● F=0.002V_0, ■ F=0.01V_0, ▲ F=0.025V_0, ♦ F=0.05V_0, *F=0.1V_0, τ_{sc}=10 h)

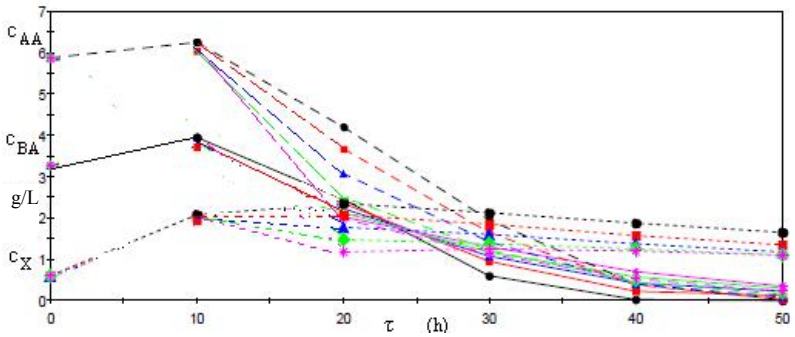

Fig. 6. Effect of feed flow rate on acetic acid, butyric acid, and biomass concentration dynamics (c_{S0}=50 g/L, c_{SF}=100 g/L, t=30 °C, V_0=2 L, ● F=0.002V_0, ■ F=0.01V_0, ▲ F=0.025V_0, ♦ F=0.05V_0, *F=0.1V_0, τ_{sc}=10 h)

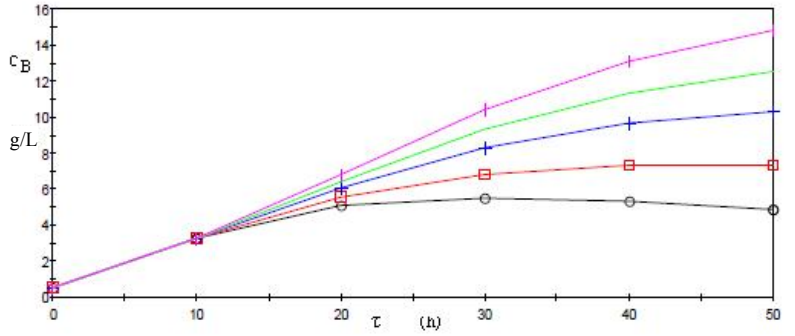

Fig. 7. Effect of substrate concentration in the bioreactor feed on butanol concentration dynamics (c_{S0}=50 g/L, F=0.025V_0 L/h, t=30 °C, V_0=2 L, ○ c_{SF}=20 g/L, □ c_{SF}=50 g/L, + c_{SF}=80 g/L, - c_{SF}=100 g/L , I c_{SF}=120 g/L, τ_{sc}=10 h)

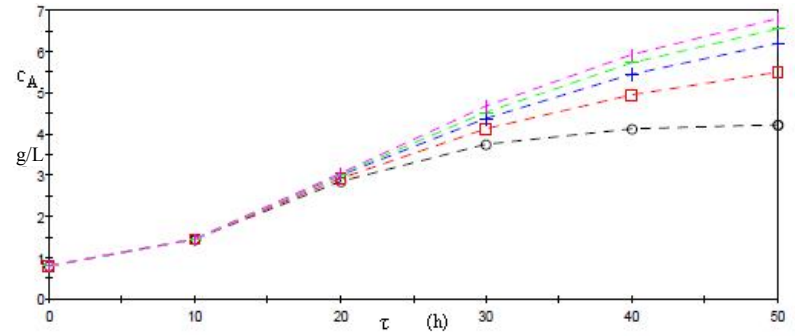

Fig. 8. Effect of substrate concentration in the bioreactor feed on acetone concentration dynamics (c_{S0}=50 g/L, F=0.025V_0 L/h, t=30 °C, V_0=2 L, ○ c_{SF}=20 g/L, □ c_{SF}=50 g/L, + c_{SF}=80 g/L, - c_{SF}=100 g/L , I c_{SF}=120 g/L, τ_{sc}=10 h)

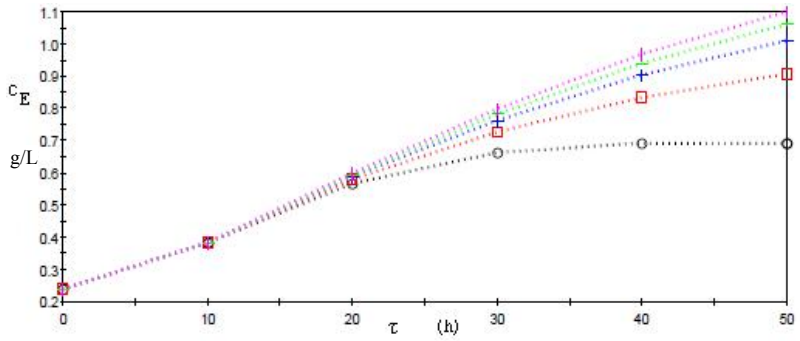

Fig. 9. Effect of substrate concentration in the bioreactor feed on ethanol concentration dynamics (c_{S0}=50 g/L, F=0.025V_0 L/h, t=30 °C, V_0=2 L, ○ c_{SF}=20 g/L, □ c_{SF}=50 g/L, + c_{SF}=80 g/L, - c_{SF}=100 g/L , I c_{SF}=120 g/L, τ_{sc}=10 h)

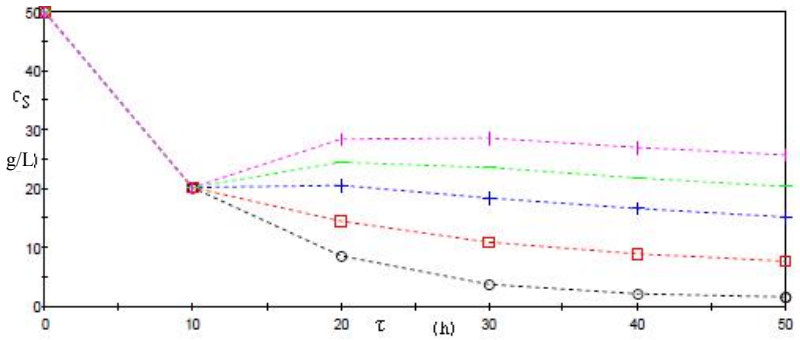

Fig. 10. Effect of substrate concentration in the bioreactor feed on substrate concentration dynamics (c_{S0}=50 g/L, F=0.025V_0 L/h, t=30 °C, V_0=2 L, ○ c_{SF}=20 g/L, □ c_{SF}=50 g/L, + c_{SF}=80 g/L, - c_{SF}=100 g/L , I c_{SF}=120 g/L, τ_{sc}=10 h)

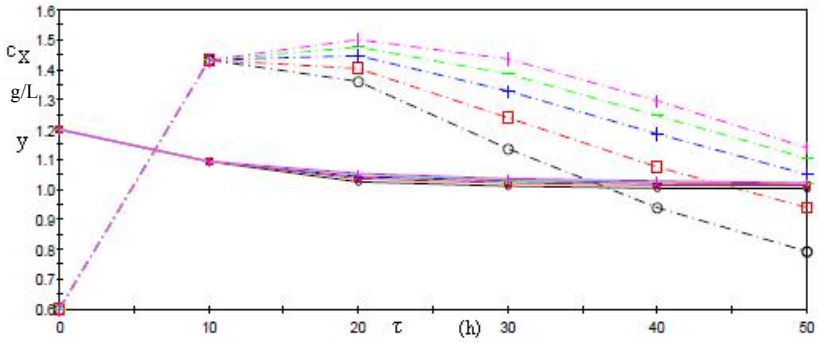

Fig. 11. Effect of substrate concentration in the bioreactor feed on biomass (c_X) and physiological marker (y) dynamics (c_{S0}=50 g/L, F=0.025V_0 L/h, t=30 °C, V_0=2 L, ○ c_{SF}=20 g/L, □ c_{SF}=50 g/L, + c_{SF}=80 g/L, - c_{SF}=100 g/L , I c_{SF}=120 g/L, τ_{sc}=10 h)

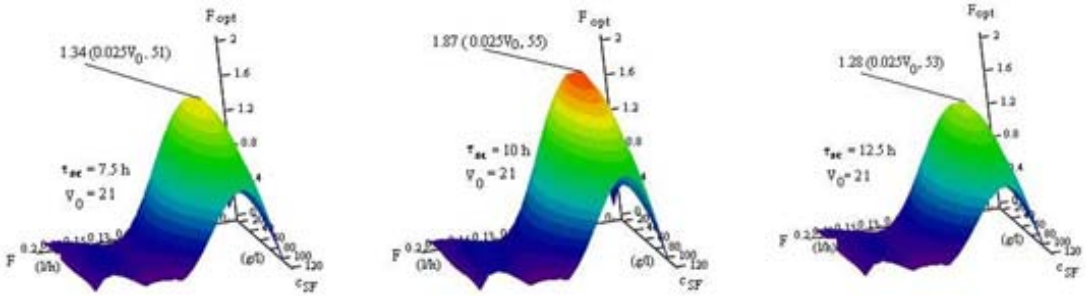

Fig. 12. Effect of feed flow rate (F), substrate concentration in the bioreactor feed (c_{SF}), and switching time (τ_{sc}) on optimization function (t=30 °C, V_0=2 L)

The physiology of the microbial culture is not fully understood. It is obvious that an increase in the glucose concentration in the feeding stream produces a higher amount of butanol in the fermentation broth. It can be seen in the model simulations depicted in Fig. 7. An increasing in the reactor feed of glucose concentration from 20 to 120 g/L determines a change of the butanol concentration in the final reaction mass from 4 to 16 g/L. For acetone or ethanol the same effect was found (Fig. 8 and Fig. 9). As shown in Fig. 10 and Fig. 5, an increase in the sugar (glucose) in the reactor feed and/or an increase in the feed flow rate over $0.025V_0$ when c_{SF}=50 g/L, lead to a high residual sugar in the broth, which makes the process economically unfeasible.

It is noteworthy that the considered optimization function penalizes the high concentration of the substrate in the synthesis broth. Fig. 11 emphasizes that the dynamics of microbial activity marker (y) is less influenced by the substrate concentration in the reactor feed.

The following calculation sequence has to be applied in order to find the maximum of the optimization function:

1. Assign a value in the field of technological interest for each control variable (*e.g.* F=$0.002V_0$, $0.01V_0$, $0.025V_0$, $0.05V_0$, $0.1V_0$, $0.2V_0$ v.u./h; c_{SF}=20, 50, 80, 100, 120 g/L; τ_{sc}=5, 7.5, 10, 12.5, 15 h);
2. Choose a value for τ_{sc};
3. Choose a value for c_{SF};
4. Simulate the process for each value of F variable and after each simulation computes the F_{opt} value;
1. Skip to next value of c_{SF} and repeat the calculation from the step 4;
2. Skip to next value of τ_{sc} and repeat the calculation from the step 3;
3. For each τ_{sc} value find the maximum of the goal function (22) by it 3D plotting versus feed flow rate (F) and substrate concentration in the bioreactor feed (c_{SF}).

Fig. 12 reveals the results of calculations performed according to the above presented algorithm. It can be concluded that the solvents production is maximized when the bioreactor operates at a switching time τ_{sc}=10 h followed by a feeding with a substrate solution having a concentration c_{SF}=55 g/L and a flow rate F=0.025V_0 v.u./h.

5. Conclusions

For solvents biosynthesis in *Clostridium acetobutylicum* culture on glucose substrate, mathematical modelling was used to develop a reliable comparison between the dynamic profiles of concentration for different products in the fermentation broth. The maximization of solvents production was chosen as an optimization case for fed-batch bioreactor operation. The switching time, the substrate concentration in the bioreactor feed, and the bioreactor feed flow were selected as control variables. As the bioreactor worked under conditions presented in Table 2, a maximum production of solvents was obtained at a switching time of 10 h, substrate concentration in the feed of 55 g/L, and the bioreactor feed flow rate of 0.025V_0 v.u/h. The simulations shown that the bioreactor operation over 50 h did not bring a significant increase in the solvents amount in the reaction medium.

R E F E R E N C E S

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