MODELING AND SIMULATION OF THE BIOPROCESS WITH RECIRCULATION

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The bioprocess models with recirculation present an integration of the model of continuous bioreaction system and the model of separation system. The reaction bioprocess is integrated with separation the biomass, formed product, no consumed substrate or inhibitory substance. In this paper the simulation model of recirculation bioprocess was developed, which may be applied for increasing the biomass productivity and product biosynthesis, increasing the conversion of a substrate to product, mixing efficiency and secondary CO_2 separation.
The goal of the work is optimal bioprocess configuration, which is determined by simulation optimization. The optimal cchemostat state as referent was used. Step-by-step method simulation is necessary because the initial bioprocess state is changing with recirculation in the each step. The simulation experiment confirms that at the recirculation ratio $\alpha = 0.275$ and the concentration factor C=4 the maximum glucose conversion to the ethanol and at ten times larger dilution rate.
Key words: Bioprocess with recirculation, modeling and simulation, simulation optimization.

INTRODUCTION

The recirculatio technique with separation is primarily used for bioprocess productivity increase for biomass synthesis, but it can also be applied for bioprocess efficiency increase for product synthesis as well as for the increase of the total substrate conversion into the product. Most frequently, the bioprocess is combined with the separator S for obtained biomass, product formed and non-consumed substrate.

At biomass production, the permanent inoculation of the fresh sterile medium, the maintenance of exponential growth phase or the achievement of biomass more that critical are provided by biomass recirculation. The application of the cell biomass recirculation at cchemostat enables bioprocess progress in the conditions when the dilution rate D is considerably higher than the specific microbial growth rate μ [1].

Considering the fact that biosystem functions with the dilution rate higher than the specific growth rate, the volumetric bioprocess productivity is increased. Furthermore, the increase of the cell concentration enables proportional increase of substrate consumption rate. The instability border of classical cchemostat, which is because of cell washing from bioreactor connected to the condition $D \approx \mu$, during which the maximal bioprocess productivity, is changed towards higher values [2,3].

In this paper, the possibility of application of simulation optimization technique for improvement of configuration of bioprocess with recirculation is researched, with the aim to increase productivity and outlet ethanol concentration [4,5]. Simulation optimization aims at determining the best values of input parameters, given an output criterion. This approach allows a large variety of new types of problems to be solved, such as design of complex dynamic biosystems [6].

PROCESS MODELLING

The complex bioprocess was decomposed to the reaction-transformation and separation subsystem by using subsystem analysis. The bioprocess scheme is shown in Figure 1.
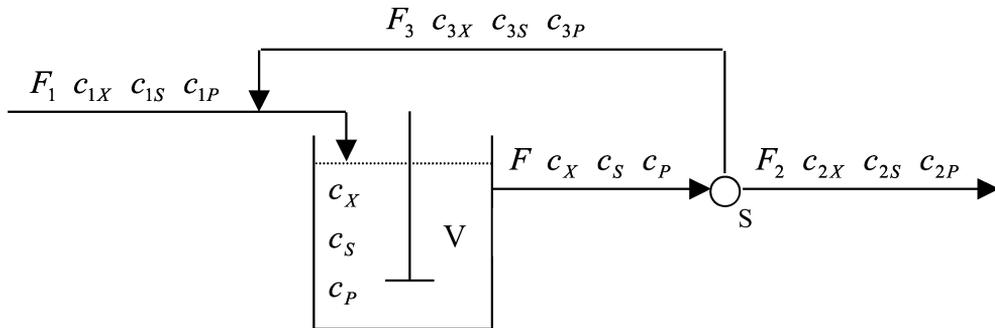

Figure 1. Scheme of the bioprocess with recirculation

Transformation process balance:

$$\frac{d(V c_X)}{dt} = F_1 c_{1X} + F_3 c_{3X} + r_X V - F c_X \tag{1}$$

$$\frac{d(V c_S)}{dt} = F_1 c_{1S} + F_3 c_{3S} - r_S V - F c_S \tag{2}$$

$$\frac{d(V c_P)}{dt} = F_1 c_{1P} + F_3 c_{3P} + r_P V - F c_P \tag{3}$$

$$\frac{dV}{dt} = F_1 + F_3 - F \tag{4}$$

Separation process balance:

$$F c_X - F_2 c_{2X} - F_3 c_{3X} = 0 \tag{5}$$

$$F c_S - F_2 c_{2S} - F_3 c_{3S} = 0 \tag{6}$$

$$F c_P - F_2 c_{2P} - F_3 c_{3P} = 0 \tag{7}$$

$$F - F_2 - F_3 = 0 \tag{8}$$

Initial conditions: $t = 0$, $c_X(0) = c_{X0}$, $c_S(0) = c_{S0}$, $c_P(0) = 0$, $V = V_0$ \quad (9)

If in sterile inlet flow F_1, only the limiting substrate with concentration c_{1S} ($c_{1X} = c_{1P} = 0$) is present, and if the recirculation flow F_3 is presented by recirculation ratio $\alpha = F_3 / F_1$ based on volumetric flow rates, and the concentration in recirculation flow c_{3X} is expressed by concentration factor $C_X^{konc} = c_{3X}/c_X$, the model of reaction subsystem at constant volume, is reduced in the following equation system:

$$V\frac{dc_X}{dt} = \alpha\, F_1 \cdot c_X C_X^{konc} + r_X V - (1+\alpha)F_1 \cdot c_X \tag{10}$$

$$V\frac{dc_S}{dt} = F_1\, c_{1S} + \alpha\, F_1 \cdot c_S - r_S V - (1+\alpha)F_1 \cdot c_S \tag{11}$$

$$V\frac{dc_P}{dt} = \alpha\, F_1 \cdot c_P + r_P V - (1+\alpha)F_1 \cdot c_P \tag{12}$$

$$\frac{dV}{dt} = 0 \tag{13}$$

Initial conditions: $t = 0$, $c_X(0) = c_{X0} = 0$, $c_S(0) = c_{S0}$, $c_P(0) = 0$, $V = V_0$ (14)

The border bioprocess models with recirculation are generated for the biomass production process with complete biomass separation and recirculation (Model 1), for the process with complete substrate separation and recirculation with the goal of getting the total substrate conversion (Model 2) and for the process with complete separation of the inhibitory product, with the goal of the maximum substrate conversion and process productivity (Model 3).

Model 1. The biomass is completely separated in the separator and returns in the bioprocess by the flow F_3 (condition: $F_3 c_{3X} = F c_X$, $c_{3S} = 0$, $c_{3P} = 0$), and outlet reaction mixture (reactant and product) leaves the separator by the flow F_2 (condition: $c_{2X} = 0$, $c_{2S} = c_S$, $c_{2P} = c_P$),

$$\frac{d(V\, c_X)}{dt} = r_X V$$

$$\frac{d(V\, c_S)}{dt} = F_1\, c_{1S} - r_S V - F c_S$$

$$\frac{d(V\, c_P)}{dt} = r_P V - F c_P$$

$$dV/dt = F_1 + F_3 - F$$

Initial conditions: $t = 0$, $c_X(0) = c_{X0} = 0$, $c_S(0) = c_{S0}$, $c_P(0) = 0$, $V = V_0$

Model 2. The substrate that has not reacted is separated in the separator and returned in the bioprocess by the flow F_3 (condition: $F_3 c_{3S} = F c_S$, $c_{3X} = 0$, $c_{3P} = 0$), and outlet reaction mixture (biomass and product) leaves the separator by the flow F_2 (condition: $c_{2X} = c_X$, $c_{2S} = 0$, $c_{2P} = c_P$),

$$\frac{d(V c_X)}{dt} = r_X V - F c_X$$

$$\frac{d(V c_S)}{dt} = F_1 c_{1S} - r_S V$$

$$\frac{d(V c_P)}{dt} = r_P V - F c_P$$

$$dV/dt = F_1 + F_3 - F$$

Initial conditions: $t = 0$, $c_X(0) = c_{X0} = 0$, $c_S(0) = c_{S0}$, $c_P(0) = 0$, $V = V_0$

Model 3. The formed product is separated in the separator and is taken away by the flow F_2 (condition: $F_2 c_{2P} = F c_P$, $c_{2X}=0$, $c_{2S}=0$), and outlet reaction mixture (biomass and substrate) is returned in the bioprocess by the flow F_3 (condition: $F_3 c_{3X}=F c_X$, $F_3 c_{3S}=F c_S$, $c_{3P}=0$)

$$\frac{d(V c_X)}{dt} = r_X V$$

$$\frac{d(V c_S)}{dt} = F_1 c_{1S} - r_S V$$

$$\frac{d(V c_P)}{dt} = r_P V - F c_P$$

$$dV/dt = F_1 + F_3 - F$$

Initial conditions: $t = 0$, $c_X(0) = c_{X0} = 0$, $c_S(0) = c_{S0}$, $c_P(0) = 0$, $V = V_0$

By including the relations for recirculation ratio α and concentrating factor C_X^{conc}, and by expressing of the volumetric reaction rates (r_X, r_S, r_P) with suitable specific reaction rates (μ, v_S, v_P), the models of reaction subsystem Model 1-3 are transformed in the following form:

Model 1.1 The biomass is completely separated in the separator and is returned in the bioprocess, and outlet reaction mixture (reactant and product) leaves the separator by the flow F_2 (condition: $F_3 c_{3X}=F c_X$)

$$V \frac{dc_X}{dt} = r_X V = c_X \mu\, V$$

$$V \frac{d c_S}{dt} = F_1 c_{1S} - r_S V - (1+\alpha)F_1 \cdot c_S = F_1 c_{1S} - c_X v_S V - (1+\alpha)F_1 \cdot c_S$$

$$V \frac{dc_P}{dt} = r_P V - (1+\alpha)F_1 \cdot c_P = c_X v_P V - (1+\alpha)F_1 \cdot c_P$$

$$dV/dt = 0$$

Initial conditions: $t = 0$, $c_X(0) = c_{X0} = 0$, $c_S(0) = c_{S0}$, $c_P(0) = 0$, $V = V_0$

Model 2.1 The substrate which has not reacted is separated in the separator and is returned in the bioprocess, and the outlet reaction mixture (biomass and product) leaves the separator by the flow F_2 (condition: $F_3 c_{3S} = F c_S$)

$$V\frac{dc_X}{dt} = r_X\,V - (1+\alpha)F_1 \cdot c_X = c_X \mu\;V - (1+\alpha)F_1 \cdot c_X$$

$$V\frac{dc_S}{dt} = F_1\,c_{1S} - r_S\,V = F_1\,c_{1S} - c_X v_S\,V$$

$$V\frac{dc_P}{dt} = r_P\,V - (1+\alpha)F_1 \cdot c_P = c_X v_P\,V - (1+\alpha)F_1 \cdot c_P$$

$$dV/dt = 0$$

Initial conditions: $t = 0$, $c_X(0) = c_{X0} = 0$, $c_S(0) = c_{S0}$, $c_P(0) = 0$, $V = V_0$

Model 3.1 The formed product is separated in the separator and is taken away by the flow F_2, and the outlet reaction mixture (biomass and substrate) is back in the bioprocess (condition: $F_2 c_{P2} = F c_P$),

$$V\frac{dc_X}{dt} = r_X\,V = c_X \mu\;V$$

$$V\frac{dc_S}{dt} = F_1\,c_{1S} - r_S\,V = F_1\,c_{1S} - c_X v_S\,V$$

$$V\frac{dc_P}{dt} = r_P\,V - (1+\alpha)F_1 \cdot c_P = c_X v_P\,V - (1+\alpha)F_1 \cdot c_P$$

$$dV/dt = 0$$

Initial conditions: $t = 0$, $c_X(0) = c_{X0} = 0$, $c_S(0) = c_{S0}$, $c_P(0) = 0$, $V = V_0$

KINETIC MODEL

The kinetic model is semi-empirical, non-structured with aspect of cell composition and distributed with aspect of biomass quantification according as total concentration of biomass, which is uniform distribution at volume of fluid culture.
The kinetic model is generated form mixed semi-empirical biomass growth model, which includes modified non-competitive substrate inhibition and linear product inhibition.

$$r_X = \frac{dc_X}{dt} = c_X \cdot \frac{\mu_m \cdot c_S}{K_M + c_S + \dfrac{c_S^2}{K_{IS}}} \cdot \left(1 - \frac{c_P}{P_m}\right) - m \cdot c_X \tag{15}$$

$$-r_S = -\frac{dc_S}{dt} = c_X \cdot \frac{v_{ms} \cdot c_S}{K_M + c_S + \dfrac{c_S^2}{K_{IS}}} \cdot \left(1 - \frac{c_P}{P_{mm}}\right) \tag{16}$$

$$r_P = \frac{dc_P}{dt} = c_X \cdot \frac{v_{mp} \cdot c_S}{K_M + c_S + \dfrac{c_S^2}{K_{IS}}} \cdot \left(1 - \frac{c_P}{P_{mm}}\right) \qquad (17)$$

The empirical model parameters P_m and P_{mm} are determined in the independent experiment. They represent the corresponding ethanol concentrations, which growth cells and ethanol synthesis stopped respectively. With biochemical aspect these parameters represent the biological switching effect, which is specific characteristic of the cell culture *Saccharomyces cerevisiae*.

The experimental data from kinetic experiments were used for kinetic parameter estimation. The model parameters were calculated based on ordinary least squared method. The Levenberg-Marquardt iterative fitting method is used at parameter estimation [7,8]. The model validity is statistically estimated by Fischer's test and preliminary by correlation coefficient. The results of the statistical test present in Table 1, show good comparison of the experimental and simulation data.

Table 1. The kinetic model Eqs. (15-17) parameter estimation, for different initial substrate concentration (c_{S0}=50-250 g/dm^3, c_{X0}=0.75 g/dm^3, P_m=84, P_{mm}=95)

c_{S0}	μ_m	v_{ms}	v_{mp}	K_M	K_{IS}	F_{calc}^{tot}	F_{tab}	r_{cor}
50	0.272	2.603	1.224	1.393	401.800	0.105	2.484	1.000
100	0.229	2.380	1.104	0.688	422.300	0.043	2.084	1.000
150	0.195	2.434	1.069	1.764	470.800	0.472	1.841	0.999
200	0.181	1.972	0.815	4.560	239.900	0.277	1.592	0.999
250	0.116	1.376	0.543	8.917	361.700	0.335	1.278	1.000

RESULTS AND DISCUSSION

Analysis of the bioprocess with recirculation

The simulation model of bioprocess with recirculation flow obtained from Eqs. (1-8), is analyzed for different conditions of separation subsystem. The bioprocess with recirculation flow represents the integration of continual flow reaction system and separation system. The recirculation techniques represents permanent inoculation of reaction subsystem, that excludes the need to produce the starter culture all trough the process.

The shortage of this bioprocess configuration is in time accumulation of metabolites in reaction subsystem and in engineering problems in realization of the setting separation degree in the separation subsystem.

Analyzing the reaction-separation model, the main contribution of bioprocess with recirculation flow can be confirmed. The model is derived under the presumption that the inlet flow is sterile, and that the bioprocess is initialized by impulse injection of biomass at the start of the process, which is realized in the model by specifying the zero inlet biomass concentration $c_{1X} = 0$ in inlet flow F_1, i.e. $c_X(0) = c_{X0}$ as the initial condition that is mathematical formalized the impulse biomass injection.

$$V \frac{dc_X}{dt} = \alpha \, F_1 \cdot c_X C_X^{konc} + r_X V - (1+\alpha) F_1 \cdot c_X \qquad (18)$$

$$V \frac{dc_S}{dt} = F_1 \, c_{1S} + \alpha \, F_1 \cdot c_S - r_S V - (1+\alpha) F_1 \cdot c_S \qquad (19)$$

$$V \frac{dc_P}{dt} = \alpha \, F_1 \cdot c_P + r_P V - (1+\alpha) F_1 \cdot c_P \qquad (20)$$

$$dV / dt = 0 \qquad (21)$$

Initial conditions: $t = 0$, $c_X(0) = c_{X0} = 0$, $c_S(0) = c_{S0}$, $c_P(0) = 0$, $V = V_0$ $\qquad (22)$

In steady state conditions, the model of biomass forming is transformed in the following form:

$$\frac{1}{V} \left(\alpha \, F_1 \cdot c_X C_X^{konc} + r_X V - (1+\alpha) F_1 \cdot c_X \right) = 0 \qquad (23)$$

By introduction of the specific growth rate $r_X = \mu \, c_X$ in Eq. (23) it follows,

$$D = \frac{F_1}{V} = \frac{\mu}{1 + \alpha - \alpha \, C_X^{konc}} \qquad (24)$$

that represents the function of dilution rate D from the specific growth rate μ, recirculation ratio α and concentrating factor C_X^{konc}.

The Eq. (24) show that the dilution rate is higher that specific growth rate in the process. The concentrating factor is much higher than one $C_X^{konc} >> 1$, and the recirculation ratio is lower than the one $\alpha < 1$, so that the denominator of the equation is less than one. In the chemostat with cell recirculation, the cell concentration is higher for $(1 / 1 + \alpha - \alpha \, C_X^{konc})$ times in comparison with the standard chemostat.

With the research of the bioprocess with recirculation, the control values, such as inlet flows and their corresponding concentrations are changing. The values of the recirculation flow, main flow and concentrations are also changed, depending on the concentration factor and recirculation ratio. These changes lead to changes of initial

values of the reaction subsystem state and that leads to change of the kinetic parameter values.

In each time interval (t_i, t_{i+1}), which is determined by simulation step Δt, the simulated process state variables in time t_i, will be initial condition for simulation in time t_{i+1}. Therefore, in each simulation step, the values of kinetic parameters with values of changeable states will change, especially with the actual concentration of limiting substrate.

The module for specification of control values (input variable), components with kinetic model, corresponding simulation algorithm and components for simulated response show constitute the basic structure of the process simulation model. Besides, the component with the model for the determining of the values of the kinetic parameter models in the function of the initial concentration of limiting substrate is included.

Simulation of the bioprocess with recirculation

In development of the software applications object oriented transformation method was used. The objects of complex bioprocess are modeled by heterogeneous program packages. This development dynamics of the engineering program packages are directly incorporated in software applications. Program components were generated with MathCAD program package [9]. The new generating program components were integrated in Mathconnex environment. Matconnex is a stable explorer for visual integration of heterogeneous program packages for creation of the continual simulation [10]. The process is simulated on the process simulation model, with kinetic model Eqs. (15-17) and with confirmed parameters of kinetic model from the Table 1. The structure of the simulation software is shown in Figure 6.

The simulation software of the process with recirculation, consist of three basic components: "TRANSFORMATION PROCESS" and "SEPARATION PROCESS" and component "VARIABILITY KINETIC PARAMETERS" for estimation of variable values of the kinetic parameters in the function of the reaction subsystem state change.
The input simulator system is consisted of a module for specification of the starting conditions in reaction subsystem "INITIAL CONDITIONS", the module for volumetric flow specification "FLOW" and special modules for component concentration specification in input flow F_1 and recirculation flow F_3.
The output simulator subsystem is especially separated for reaction subsystem and show by the graph "REACTOR DYNAMICS", and the graphs "VOLUME" and "BIOMASS" are also shown because of the clarity.

The time changes of the concentration of biomass, glucose, ethanol and reaction subsystem values in the reactor i.e. output flow F are shown in the corresponding graphs.

The graphs "SEPARATOR DYNAMICS" and 'BIOMASS DYNAMICS' show concentration changes of biomass, glucose and ethanol at the exit of the separator S in flow F_2. These changes in flow F_2 are at the same time total response time changes of the state of the bioprocess with recirculation.

In the component "SEPARATION PROCESS" the predefined value of the concentrating factor (CF) is set, for example biomass on Figure 2, from which the biomass concentration is defined and the models for separation of substrate and product are introduced, Eqs. (5-8). The dilution rate is defined from the input flow F_1 and the effective reaction volume and according to the Eq. (24) the values of the possible dilution rate above the values of the specific biomass growth rate is estimated, and this is comparatively used in relation to the standard chemostat.

The simulation software is transformed in the simulator of steady state chemostat in the testing phase by the appropriate flow choice ($F_3=0$, $F_1=F_2=0.2$ dm^3/h) and their content and by additional defining of the separation ratio ($c_{3X}=CX$, $c_{3S}=CS$ i $c_{3P}=CP$). Considering the effect of cell washing by continual flow trough chemostat, it can be supposed that the culture is permanently in the phase of exponential growth, so, in the kinetic model, the maintenance coefficient of biomass m can be approximation neglected $m=0$. During the simulation, the maximum dilution rate limited by the washing effect and corresponding bioprocess state are defined.

At dilution rate D=0.02 h^{-1} and by excluding of the separation subsystem from the simulation, by the condition $c_{3X}=CX$, $c_{3S}=CS$ i $c_{3P}=CP$, after 40 h of non-steady state, the process gets into kvazi-steady state when the maximum biomass concentration CX=10.327 g/dm^3, ethanol CP=69.979 g/dm^3 and maximum conversion, i.e. minimum glucose output CS=1.587 g/dm^3. The simulation chemostat response got on the simulation software for bioprocess with recirculation is shown on Figure 2. At dilution rate D=0.2 h^{-1} chemostat washing occurs.
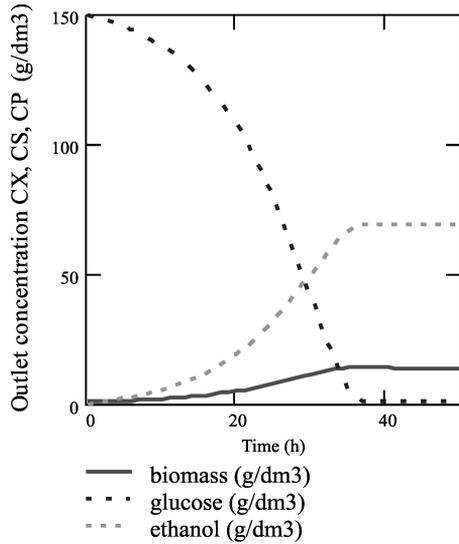
Figure 2. The simulation of the chemostat on the bioprocess simulation model with separation and biomass recirculation (D=0.02 h^{-1}, $\alpha = 0$, $C_X^{konc} = 1$).

In Figure 3, the biomass dynamics in reaction and separation subsystem, is comparatively presented for chemostat and the process with recirculation. The simulation response obtained at the dilution rate D=0.02 h^{-1}. The process with recirculation is simulated at the dilution rate D=0.10 h^{-1}, at recirculation rate α=0.275 and concentration factors CF=2 i CF=4.
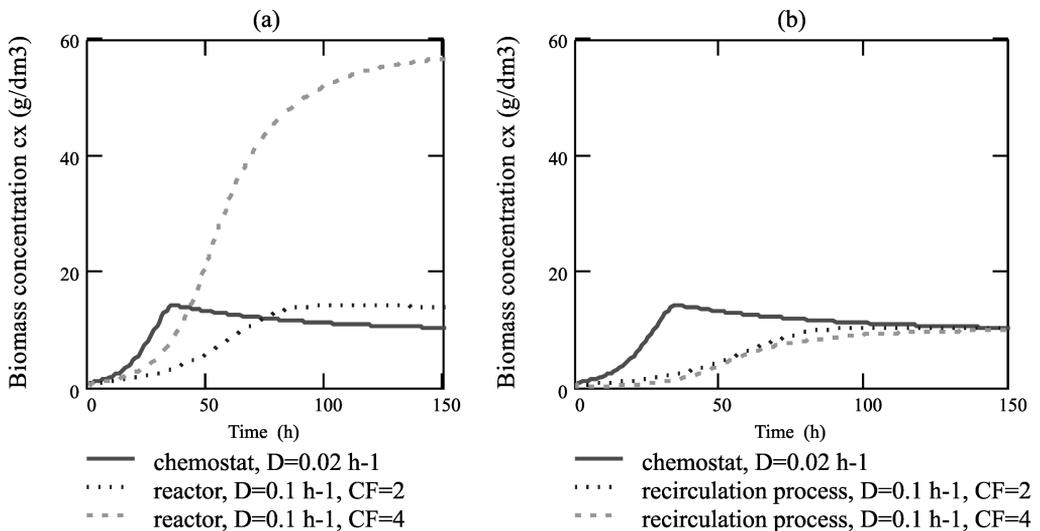
Figure 3. Biomass dynamics at the outlet of the reactor (a) and the separator (b)

The corresponding changes of the concentrations of glucose and ethanol are shown in Figure 4.
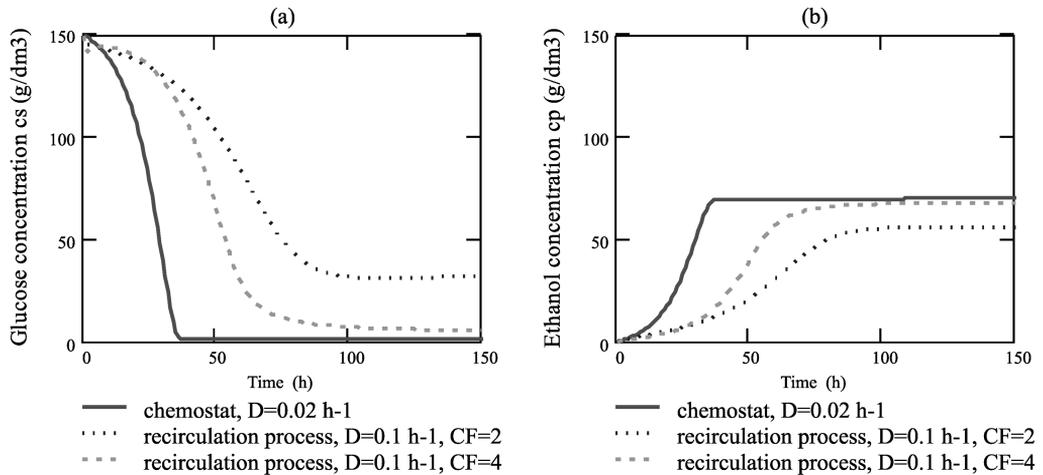
Figure 4. Dynamics of glucose (a) and ethanol (b) in recirculation bioprocess

The main aim of the recirculation, the productivity increase of the glucose consumption and ethanol forming is comparatively illustrated in Figure 5 for chemostat and recirculation bioprocess with different concentration factor.
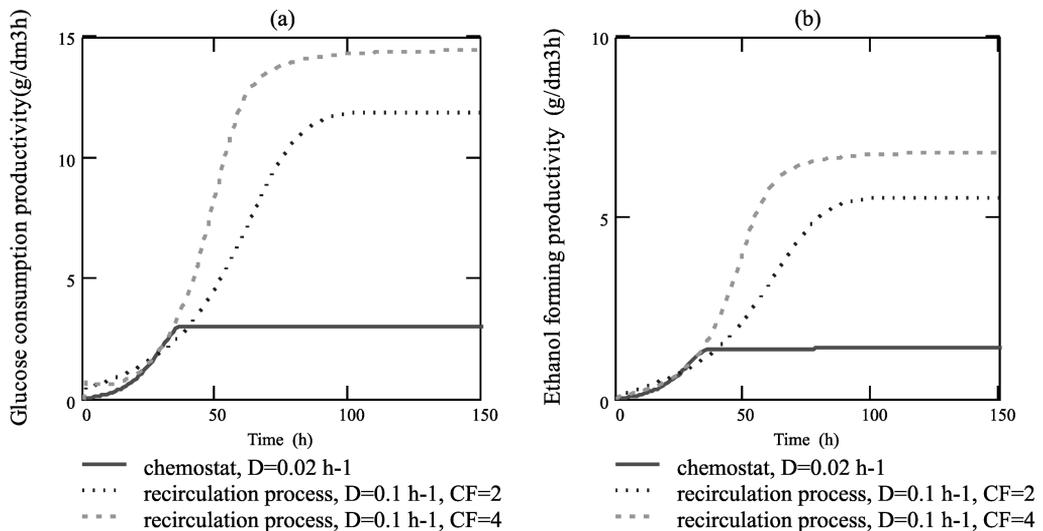

Figure 5. The productivity of glucose consumption (a) and ethanol forming (b)

On Figure 3, the bioprocess simulation with recirculation at recirculation ratio $\alpha = 0.275$ and concentrating factor $C_X^{konc} = 4$ is shown. At dilution rate of D=0.2 hr^1 at the output from the reaction subsystem of kvazi-steady state values are: CX=56.686 g/dm³, CS=5.585 g/dm³ and CP=68.164 g/dm³ and they are obtained after 70 h of non-steady state process. At the same time, the corresponding values at the separator output or with inlet flow F_2 from the total process are: CX=9.926 g/dm³, CS=5.576 g/dm³ and CP=68.169 g/dm³. Almost theoretical glucose conversion and maximum ethanol concentration are obtained at ten times higher dilution rate.

The initial conditions specified by the input module "INITIAL CONDITIONS" (V=10 dm³, CX=0.75 g/dm³ i CS=150 g/dm³, CP=0 g/dm³) are used for both the chemostat and the process with recirculation of separated biomass simulation.

Kinetic and inhibitory model parameters in the form of specific values, at different initial biomass concentration and initial substrate concentration c_{S0}=150 g/dm³ are obtained with the method of initial rates. Fischer's test confirms the good agreement with experimental data (Table 2). The parameter values from Table 2 show the relative stability and small variations with the biomass concentration change. However, the changes of parameter values with the change of substrate concentration (Table 1) are important. Therefore, the component "VARIABILITY KINETIC PARAMETERS" is integrated in the process simulator, to predict the kinetic parameter values with the change of process state in each step of the simulation.

Table 2. The kinetic model Eqs. (15-17) parameter estimation, for different initial biomass concentration (c_{S0}=150 g/dm³, P_m=84, P_{mm}=95)

c_{X0}	μ_m	v_{ms}	v_{mp}	K_M	K_{IS}	F_{rac}^{tot}	F_{tab}	r_{cor}
0.75	0.196	2.433	1.069	1.756	471.2	0.518	1.861	0.999
1.50	0.185	2.519	1.100	1.882	395.2	0.057	1.984	1.000
3.00	0.192	2.423	1.058	1.088	450.2	0.069	2.168	1.000
9.00	0.181	2.407	1.057	1.855	529.6	0.033	2.818	1.000
15.00	0.173	2.275	0.998	1.238	814.0	0.029	3.179	1.000

At recirculation steady state process (Figure 5) the high productivity and conversion values are achieved. At outlet ethanol concentration c_P=57.719 g/dm³ the coefficient of ethanol yield is $Y_{P/S}$=0.387. At the same time the degree of glucose conversion into biomass and ethanol is 0.936. The theoretical ethanol yield in the absence of cell growth is $Y_{P/S}$ =0.511.

The separation and biomass recirculation techniques increases biomass concentration in reaction subsystem, that enables bioprocess progress with almost theoretical yield and ethanol concentration and total glucose conversion, at dilution rates that are ten times higher in comparison with chemostat.
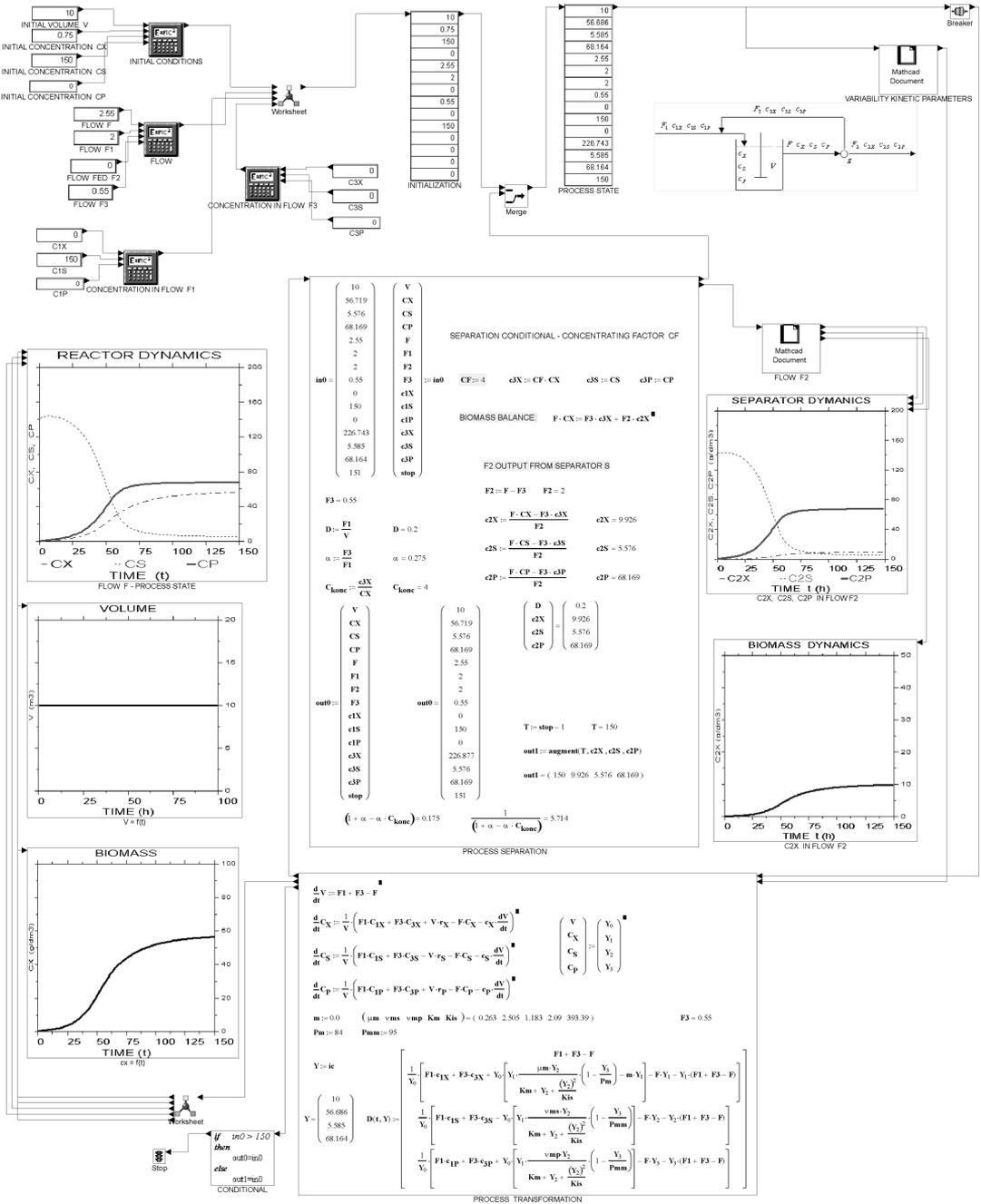
Figure 6. The simulation of flow bioprocess with separation and biomass recirculation, at dilution rate $D=0.2$ h^{-1}, recirculation ratio $\alpha = 0.275$ and concentrating factor $C_X^{konc} = 4$.

CONCLUSION

The computer-aided modeling and bioprocess simulation enables optimal bioprocess configuration with recirculation flow. The subsystem analysis and compartment approach at modeling enable independent development and testing of simulation software for reaction and separation process. The process simulator is formed by integration of operational simulators. The function of the classical chemostat can be simulated by appropriate choice of control values, e.g. by excluding separation process. The obtained values of the steady state chemostat quantities are used as referential values.

The optimal values of chemostat productivity are limited by the cell-washing phenomenon. This limitation leads to relatively low steady state cell concentration in chemostat, which results in low output ethanol concentration. The optimal values cannot be increased above the values obtained by optimization of the nutrient medium and process parameters, e.g. temperature, pH values and mixing. The optimal values of chemostat state are c_X=10.327 g/dm^{-3}, c_S=1.587 gdm^{-3} and c_P=69.979 g/dm^{-3}, and they are obtained at dilution rate D=0.02 h^{-1}.

The separation subsystem, which is realized by centrifuge, increases the cell concentration that continually returns into the reaction subsystem by partial recirculation flow. The concentrating factor is also limited by centrifugal efficiency and recirculation flow techniques.

At concentrating degree C_X^{conc} and recirculation ratio α=0.275, the dilution rate that enables the complete substrate conversion is determined (D=0.2) by simulation optimization. The kvazi-steady state of reaction subsystem (c_X=56.686 gdm^{-3} c_S=5.585 gdm^{-3} c_P=68.164 gdm^{-3}) shows considerably higher biomass concentration at the same time. The total recirculation flow effect is show by simulation output from the separator (c_X=9.926 g/dm^{-3}, c_S=5.576 gdm^{-3} i c_P= 68.169 g/dm^{-3}). With almost total conversion of glucose, the dilution rate in the process with recirculation flow is almost ten times higher. The bioprocess productivity proportionally increases at the same time.

The ratio of chemostat productivity DP_1=1.363 gdm^{-3}h^{-1} and bioprocess with recirculation DP_2=13.634 gdm^{-3}h^{-1}, confirms the effectively of the applied method. The application of simulation optimization is efficient approach that enables the configuration of complex bioprocess with the aim to increase productivity. The high productivity and output ethanol concentration considerably decreases ethanol separation expenses in the rectification phase.

Nomenclature

c_X, c_S, c_P	$g_X\,dm^{-3}$	cell, substrate and product concentration in reaction subsystem
c_X, c_S, c_P	$g_X\,dm^{-3}$	cell, substrate and product concentration in reaction subsystem
c_{1X}, c_{1S}, c_{1P}	$g_X\,dm^{-3}$	cell, substrate and product concentration in inlet flow F_1
c_{2X}, c_{2S}, c_{2P}	$g_X\,dm^{-3}$	cell, substrate and product concentration in outlet flow F_2
c_{3X}, c_{3S}, c_{3P}	$g_X\,dm^{-3}$	cell, substrate and product concentration in flow F_3
c_{3X}, c_{3S}, c_{3P}	$g_X\,dm^{-3}$	cell, substrate and product concentration in recirculation flow F_3
C_X^{konc}	-	concentrating factor (CF)
D	h^{-1}	dilution rate
F_1, F_2, F_3	$dm^{-3}h^{-1}$	inlet, outlet and recirculation flow
F_{calc}^{tot}	-	total Fisher's calculated values for validation test
K_M	$g_S\,dm^{-3}$	Monod's constant of cells growth rate
K_{IS}	$g_S\,dm^{-3}$	inhibition constant for noncompetitive inhibition by substrate
K_{IP}	$g_P\,dm^{-3}$	inhibition constant for competitive inhibition by product
m	$g_X\,g_X^{-1}\,h^{-1}$	maintenance coefficient for cells growth
P_m	$g_S\,dm^{-3}$	ethanol concentration that stops cell growth
P_{mm}	$g_S\,dm^{-3}$	ethanol concentration that stops ethanol biosynthesis
r_X	$g_X\,dm^{-3}\,h^{-1}$	rate of growth (production) of cells related to glucose
r_S	$g_S\,dm^{-3}\,h^{-1}$	rate of substrate consumption
r_P	$g_P\,dm^{-3}\,h^{-1}$	rate of product formation
r_N	$g_X\,dm^{-3}\,h^{-1}$	rate of growth (production) of cells related to nitrogen
r_{cor}	-	correlation coefficient
V	dm^3	reaction subsystem volume

Greek symbols

α	-	recirculation ratio
μ_m	$g_X\,g_X^{-1}\,h^{-1}$	maximum specific growth rate
v_S	$g_S\,g_X^{-1}\,h^{-1}$	specific rate of substrate consumption
v_{ms}	$g_S\,g_X^{-1}\,h^{-1}$	maximum specific rate of substrate consumption
v_P	$g_P\,g_X^{-1}\,h^{-1}$	specific rate of product formation
v_{mp}	$g_P\,g_X^{-1}\,h^{-1}$	maximum specific rate of product formation

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Izvod

MODELOVANJE I SIMUALCIJA BIOPROCESA S POVRATNIM TOKOM
Naučni rad

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Modeli bioprocesa s povratnim tokom pretstavljaju integraciju modela kontinualnog bioreakcionog sistema i modela separacionog sistema. Reakcioni bioproces se integriše sa separacijom biomase, nastalog proizvoda, neproreagovanog supstrata ili inhibitorne supstance. U radu je razvijen simulacioni model recirkulacionog bioprocesa, koji se može primeniti za povećanje produktivnosti biomase i biosinteze proizvoda, povećanje konverzije supstrata u proizvod, efikasnosti mešanja i izdvajanja sporednog CO_2.
Cilj rada je optimalna konfiguracija bioprocesa s recirkulacijom koja se nalazi simulacionom optimizacijom. Optimalno stanje chemostata korišćeno je kao referentno. Simulacija korak-po-korak je neophodna metoda jer se početno stanje bioprocesa s recirkulacijom menja u svakom koraku simulacije. Simulacioni eksperiment potvrđuje da se pri recirkulacionom odnosu $\alpha = 0.275$ i faktoru koncentrovanja C=4 postiže maksimalna konverzija glukoze u etanol i pri 10 puta većoj brzini razeđenja.

Ključne reči: Bioproces sa recirkulacijom, modelovanje i simulacija, optimizacija simulacijom.