Simulation of Mammalian Cell Population Dynamics

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Abstract. *Applied is a systems view to modelling of mammalian cell cultivation in a bioreactor for biotechnological applications. Proposed is a model with a hierarchical structure with three levels: the macroscopic level of a reactor, microscopic level of a cell population, and molecular level of protein interactions with cyclin dependent kineases. The macroscopic model provides basis for production process control by optimal feeding of nutrients and growth factors during cultivation. The microscopic model simplifies a cell population into three pools of cells: P proliferating, Q quiescent, and D dead cells. Dynamics of cell population is determined by the least square estimation of the specific rates of the pool transitions. The molecular model enables theoretical basis for prediction of G_1/S transition and the rate of transition from proliferating cells into quiescent state. Conceptual application of the molecular model in conjunction with the mammalian cell production system is discussed.*

Keywords. mammalian cell, cell cycle, bioreactor control

1. Introduction

Integration and automation of experimental methods of molecular biology has produced massive data basis, which led to development of bioinformatics and functional understanding of biological systems. Development of mathematical models is aimed to synthesis of biological knowledge into principal functional units, which can be studied from the view of mathematical systems theory. Present knowledge and models of biological production systems at macroscopic level (bioreactors) are highly developed, but fundamental biological phenomena are incorporated in reactor interactions as regression models. Such models of biological phenomena are usually applicable under similar experimental and essentially steady state conditions, and understanding of dynamic transients is not well understood or modelled. However, a systems view and bioinformatics, supported with dynamic experiments on molecular level by microarray techniques, provides potential integration of knowledge and understanding of complexity of biological systems under dynamically varying conditions [1-4].

2. Mass balances of a cultivation process

Considered is a general model of a biorector production system for mammalian cell cultivation, such as for suspended BHK cells (Fig. 1).

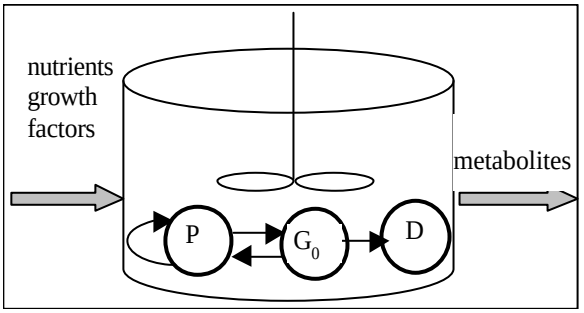


Figure 1. Schematic diagram of a perfusion (flow through) reactor system for the cell cultivation. Cell population is segregated into the pools of: P- proliferating, G_0- arrested, D- nonviable cells.

In a general case, cultivation media is continuously or intermittently fed to the reactor, and likewise, toxic metabolites and nonviable cells are withdrawn from the reactor. The macroscopic

balances are derived from the assumption of homogeneous distribution of cells and cultivation components. Cultivation media contains chemically defined components, oxygen, and complex serum with growth factors. The balances of nutrients account for inlet and outlet flows, consumption by cells, and the effect of dilution by volume change. The balances have the following general form:

$$\frac{dc_s}{dt} = q_{in} \cdot c_{sf} - q_{out} \cdot c_s - Y_{s/P} \cdot m_p \cdot c_P - \\ - Y_{s/Q} \cdot c_Q - \frac{1}{V} \cdot \frac{dV}{dt} \cdot c_s \quad (1)$$

The given form applies for the main nutrients present in liquid phase, such as glucose, glutamine, and amino acids. The consumption rate is divided into the rate of consumption by proliferating cells and non-growing cells (quiescent cells). The balance of gaseous nutrient (oxygen) and metabolite (CO_2) has the same form, but the exchange rate with gaseous phase is modelled by the volumetric coefficient of mass transfer:

$$\frac{dc_o}{dt} = k_l a \cdot \left(c_o^* - c_o\right) - Y_{o/P} \cdot m_p \cdot c_P - \\ - Y_{o/Q} \cdot c_Q - \frac{1}{V} \cdot \frac{dV}{dt} \cdot c_o \quad (2)$$

The balances of products of cell metabolism account for metabolite production by growing and nongrowing cells (but metabolically active), and concentration decrease due to outflows and volume dilution:

$$\frac{dc_m}{dt} = -q_{out} \cdot c_m + Y_{m/P} \cdot m_p \cdot c_P + \\ + Y_{m/Q} \cdot c_Q - \frac{1}{V} \cdot \frac{dV}{dt} \cdot c_m \quad (3)$$

The balance (3) is applied to the main metabolite, such as lactate produced in carbon source metabolism in glycolysis, ammonia produced in interaction of glutamine with intermediates of TCA, and CO_2 produced in respiration. Yields factors are the main model parameters, which are usually determined by least square fitting of the model to experimental data. The fitting procedure does not lead to predictive models, and is seldom applicable under different experimental conditions. Biological information represented by interconnected network of intracellular reactions with superimposed principles of homeostasis of free energy and reductive power enables metabolic flux analysis and prediction of yield factors for microbial populations under very broad conditions. However, when the metabollic flux analysis is applied to cell cultures the main modelling problem is nonuniformity of metabolic states in population. Various modelling attempts could be applied, such as population segregation with deterministic transitions between cell pools, or a stochastic modelling of transitions [5-6].

3. Cell cycle dynamics

A simple concept of deterministic modelling of cell population dynamics is based on segregation of cells into the pools of viable and nonviable cells. Viable cells can be further divided into growing cells, and cells which failed to enter mitosis and are present in an metabollically arrested states (Fig. 2). The transition between proliferating (P) and quiescent (Q) cells is modelled as a reversible process, which leads to the following balance:

$$\frac{dc_P}{dt} = m_p(t) \cdot c_P - m_Q(t) \cdot c_P + m_{-Q}(t) \cdot c_Q \\ - \frac{1}{V} \cdot \frac{dV}{dt} \cdot c_P \quad (4)$$

It accounts for cell proliferation, the transition rates, and the dilution by reactor volume change.

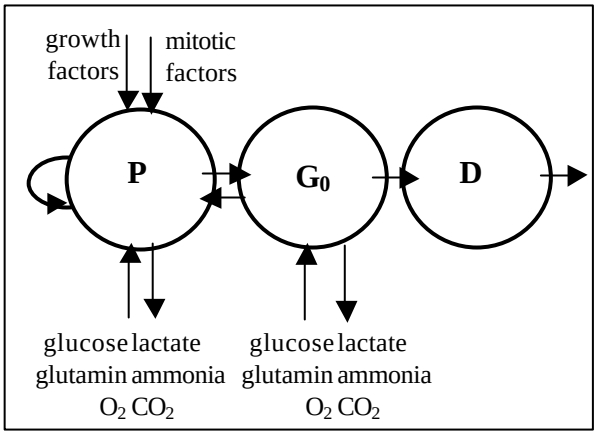


Figure 2. Schematic diagram of the reaction rates (denoted as lines with arrows) between the pools of proliferating, quiescent and dead cells, and exchange rates of key nutrients and metabolites with cell environment.

Analogously, the balance for arrested cells accounts for the same effects but with the addition of specific death rate:

$$\frac{dc_Q}{dt} = m_Q(t)\cdot c_P - m_{-Q}(t)\cdot c_Q - m_d(t)\cdot c_Q - \frac{1}{V}\cdot\frac{dV}{dt}\cdot c_Q \quad (5)$$

The balance of nonviable cells includes loss of dead cells by lysis and disintegration of apoptotic cells: :

$$\frac{dc_D}{dt} = m_D(t)\cdot c_Q - m_L\cdot c_Q - \frac{1}{V}\cdot\frac{dV}{dt}\cdot c_D \quad (6)$$

Parameters of the cell dynamics, i.e. the specific rates in the balances (4-6) can be estimated by regression to experimental data. However, least square estimates of the specific rates are only useful in reactor analysis under similar physical and biochemical conditions. The predictive modelling of the specific mitotic rate and cell dying by apoptosis and/or necrosis is the central objective. The simple conceptual model of phases in a cell cycle is presented in Fig. 3.

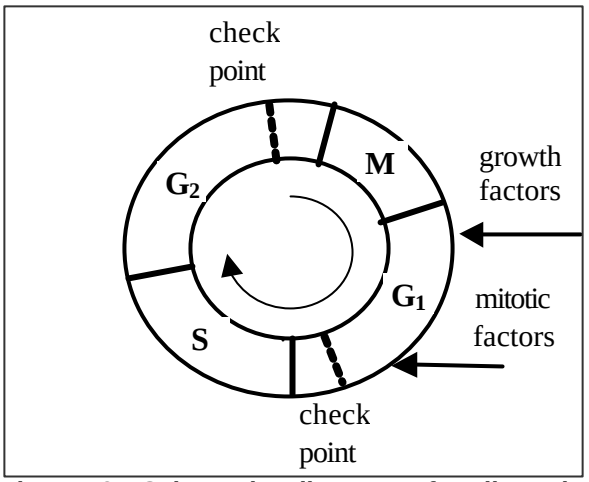


Figure 3. Schematic diagram of cell cycle phases: M mitotic, G_1 gap phase, S synthesis phase, G_2 gap phase. The restriction (check) points are depicted by the broken lines, and arrows represent intake of the extracellular growth and mitotic factors.

The checking points are "information nodes" of responses of complex protein interactions. A failure to pass the checking point from the first gap phase G_1 to DNA synthesis phase S results in transition of a cell from growing metabolism to arrested state. Complexity of interactions is driven (controlled) by hierarchy of protein modifications of cyclin depended kineases (2-3,7-8). Intrinsic rates of interactions of proteins at molecular level require highly sophisticated experimental systems. Nevertheless, the modelling concept is applicable in sense of prediction of qualitative features under dynamic conditions. From the systems view, the interactions can be analysed as reactions resulting into three classes: signal progressing reactions, signal amplification, and signal attenuation reactions. Modifications of chemical signals occur by catalytic and inhibitory effects.

Model of a single step progression reaction is given by:

$$A_1 \rightarrow A_2 \qquad r = k\cdot c_{A1} \quad (7)$$

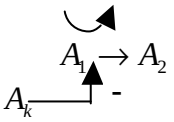
It is the first order rate, which also includes enzymatic reactions at substrate concentration lower than the corresponding enzyme saturation constant.

Numerous reactions are effected by interaction with proteins. A simple single step model for the amplification (gain) effect is represented by:

$$A_1 + A_k \rightarrow A_2 + A_k \qquad r = k\cdot c_A\cdot c_k \quad (8)$$

Rate of consumption of substrate A_1 is activated by interaction of A_k. The reaction does not change mass balance of k-th molecule.

A simple model for the competitive enzyme inhibition is applied for attenuation of chemical signal:



$$r = k\cdot\frac{c_{A1}}{K_1 + c_{A1} + K_{IK}\cdot c_{Ak}} \quad (9)$$

System of reactions for a model of G_1/S transition is schematically presented in Fig. 4. From systems view, it is a functional unit, which exchange mass, energy, and information with the rest of a cell. Exchange of mass and free energy occurs through simultaneous reactions denoted as consumption rates from sources and their consequent degradation reactions outside the unit (sinks). The goal is modelled is process of production of the mitotic response activated by mitotic stimulus. When mitotic response is bellow the critical level metabolism is arrested.

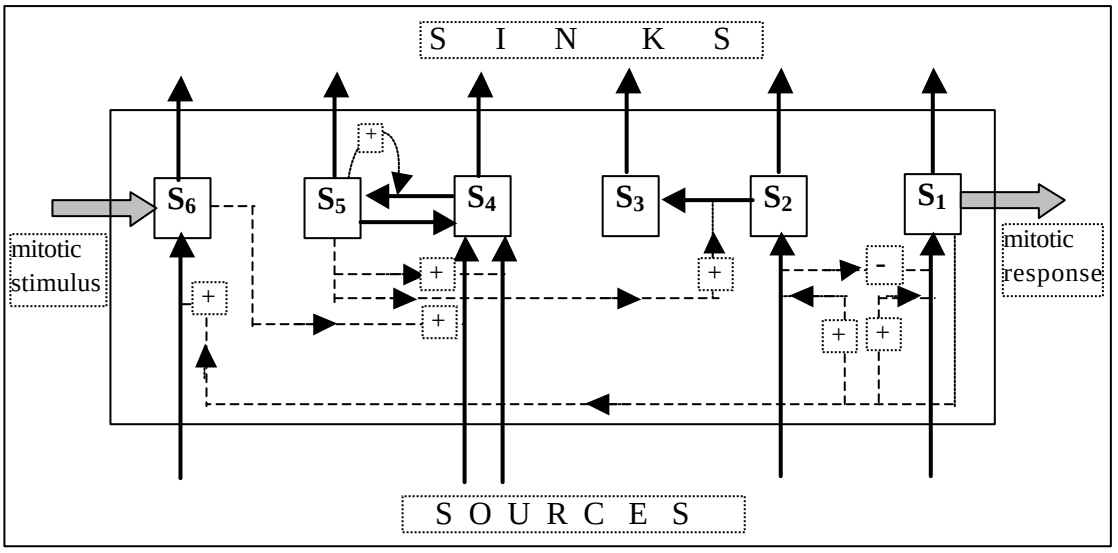


Figure 4. Schematic of protein interactions that control activities of cyclin-dependent kineases during the mitotic transition G_1/ S. Arrows with full lines denote reaction rates, dotted lines with arrows denote (+) positive and (-) negative catalytic interactions. Labels are: S_1:E2F, S_2: PRB, S_3: PRB-p, S_4: cycE/cdk2(I), S_5: cycE/cdk2(A), S_6: AP-1.

The network model includes short range and long range, positive and negative, feedforward and feedback loops. Hierarchy of the control loops provide synchronism of the whole network. Qualitative predictions of the model can be disclosed by a bifurcation analysis in a parametric space [4]. Here is applied numerical simulation of the model with aim to relate the simulation results to estimated specific mitotic rate (generation time) of BHK cells in a production reactor. Applied is software *Mathematica* [9] and NDSolve algorithm for numerical integration of stiff differential equations.

4. Results and discussion

Simulation results are presented in Fig. 5-8. Simulation experiments are motivated by analysis of effects of unsteady conditions in a cell production reactor on specific rate of mitosis. Unsteady conditions are result of liquid weaves in a specific type of reactor, or by periodic viscous stress on cells in an impeller agitated reactor. The unsteady conditions are modelled as a periodic variation of the mitogenic stimuli (factor). For periodic perturbations used is a sine signal with a constant frequency and amplitude. For simulation of perturbations due to intermittent addition of serum with growth factors applied is simple square impulse. Simulation results are compared by account of total content of mitogenic factor evaluated by time integration of the model input function.

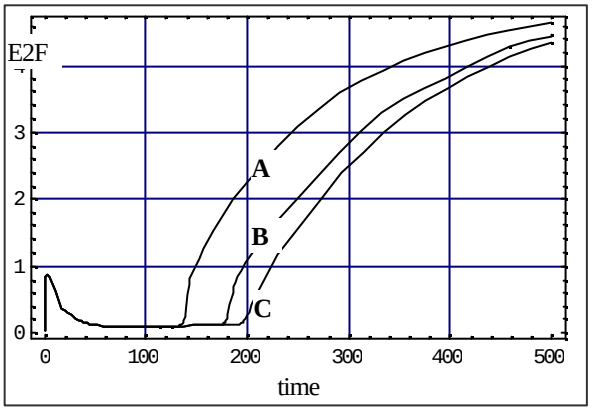


Figure 5. Simulation of the transcription factor E2F at the level of the mitogenic factor m=10 and periodic variations with frequencies: A) = 1; B) = 0.05; C) = 0.01.

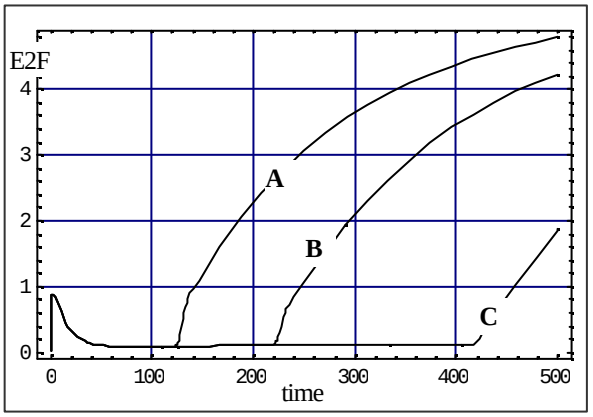


Figure 6. Simulation of the transcription factor E2F at the periodic variation with the frequency = 1 at the level of the mitogenic factors A) m = 25; B) m = 10; C) m = 7.5.

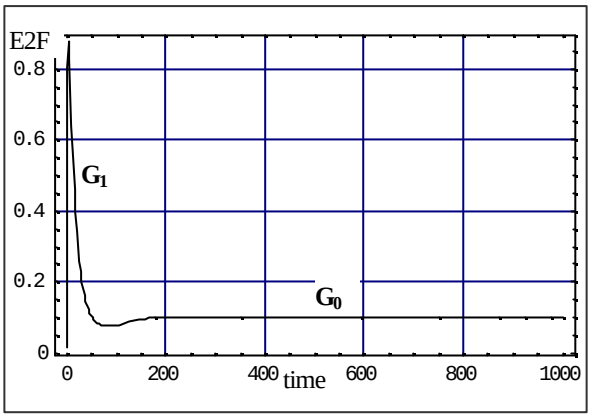


Figure 7. Simulation of the transcription factor E2F at the level of mitotic factor m = 2.5 and transition from G_1 to quiescent state G_0.

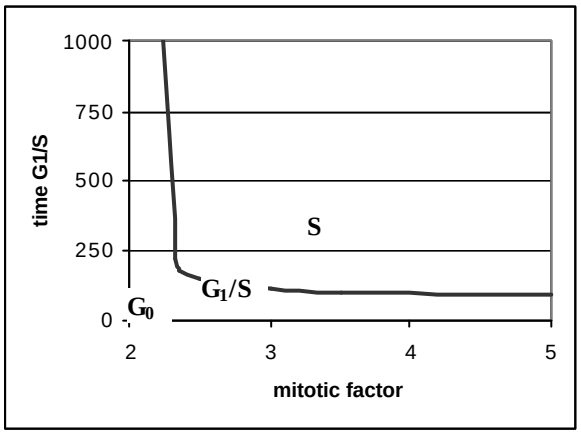


Figure 8. Simulation of the time period for transition from G_1 to S phase at the constant impulse of the mitotic factor in the range of m= 2 - 5.

In Fig. 5. are the results of simulation of the mitotic response (level of E2F) for frequencies of input stimuli from $w = 1$ to $w = 0.01$ time units (approximately in minutes). Time average of the mitogenic stimuli is the same m = 10. Predicted are durations of G_1/S transitions from 150 to 200 time units (min.). Increase of the frequency results in decraese of the transition time, but the effect is relatively minor. It could be interpreted as an effect of a better synchronisation between the input stimuli and dynamics of the protein interactions. More profound effect is produced when the time average of the input is changed, Fig. 6. The results are obtained at periodic variation of frequency $w = 1$ and average values of m =25, 10 and 7.5. Results indicate almost linear proportionality between specific mitotic rate and the average level *m*. When the level of mitogenic stimuli is bellow a critical value a response fail, and transition to an arrested state occurs (Fig. 7). Besides simulations of the periodic perturbations, a result for a constant level impulse of stimuli is given in Fig. 8. Here is plotted G_1/S transition time as function of *m*. Determined is the critical value needed for the transition (m = 2.3). Above the critical level time transition decreases dramatically (a bifurcation point), and on further increase remains almost constant.

5. Conclusions

Presented is a hierarchical model on macroscopic (reactor), microscopic (cell), and molecular levels of mammalian cell cultivation.

The model integrates essential macroscopic balances with cell population and protein interactions of cyclin dependednt kineases for prediction of specific mitotic rate (proportional to duration of G_1/S phase).

The model is aimed for improvement of bioreactor process control for biotechnological production with mammalian cell cultures.

6. References

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