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MATHEMATICAL MODELING OF INTRACELLULAR PROCESSES

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Abstract

Mathematical modeling of intracellular processes is an actively developing field of study. Different scientific groups use various approaches and principles for the modeling of all range of processes, from single biochemical reactions to cellular metabolism. Each of the approaches used has its advantages and disadvantages and requires different input. This article includes the review and analysis of the modern works in the field. The main approaches to the modeling of intracellular processes are discussed, including flux balance analysis, Petri nets, thermodynamics approaches for systems far from equilibrium, "black-box" modeling etc. Also the article involves the analysis of approaches to the structures of mathematical models, organization of links between sub-models and the possibilities of use of various methods while modeling a single metabolic process or a metabolism of a certain microorganism.

Keywords: mathematical modeling, intracellular processes, metabolism, systems far from equilibrium, Petri nets

Introduction

Representation of intracellular processes as mathematical models allows researching *in silico* and using the results as a base for planning further experimental work. Mathematical models are expected to become very helpful in detection of functions of almost every gene in cell and in the long term will make a significant contribution in general picture of processes in biological systems [1].

Nowadays the modeling of metabolic fluxes became the object of greatest interest [2, 3]. First approaches to this kind of simulation formed in early 90^s of last century [4, 5] and today are widespread. This type of simulation is known as Flux Balance Analysis (FBA).

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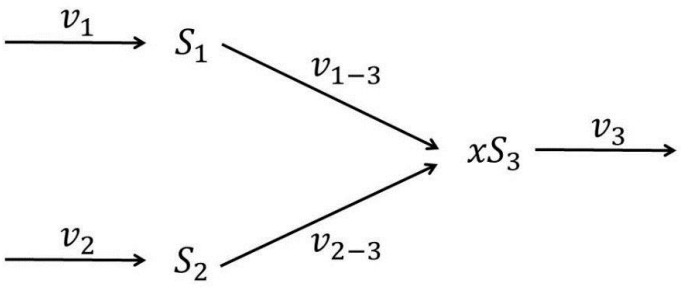
Such models give an idea of the material balance of intracellular processes in stationary conditions and in case of a complete model they allow to predict phenotype on the basis of genotype [3].

One of the most important aspects of simulation process is to respect the order of the reactions in a simulated metabolic pathway. At the same time it is necessary to count properly the changes in amount of substance in each of the reactions. Such control should help, for example, in identifying the limiting reaction in metabolic pathway. Currently there are various approaches in solving this problem: using different types of algorithmization, using mathematical apparatus, graph algorithms, in particular Petri nets [6–8].

In general today mathematical simulation of intracellular processes is actively developing branch, which can be very useful both for scientific research and for solving practical biotech problems. Below we will examine some approaches to simulation which are applied nowadays.

Analysis of Metabolic Fluxes

Today the most widespread method of modeling is a method of analysis of metabolic fluxes. It gained popularity on the grounds of its simplicity, informativity and relatively low computational cost of developed models [9]. In this type of modeling metabolic flux means the rate of transformation of substance in the metabolic pathway. So the rate of proceeding reactions is analyzed when writing the equations. Consequently, the left side of the equation will represent the speed of transformation of analyzed metabolite - dS_i/dt, and the right side will summarize the rates of reactions leading to increase or decrease in its concentration. For example, consider the following reaction scheme:



where:

S_1, S_2, S_3 – metabolites;

v_1, v_2, v_{1-3}, v_{2-3}, v_3 – reaction rate;

x – stoichiometric coefficient

For the offered reactions scheme we can write the following system of equations:

$$\frac{dS_1}{dt} = v_1 - xv_{1-3} \quad (1)$$

$$\frac{dS_2}{dt} = v_2 - xv_{2-3}$$

$$\frac{dS_3}{dt} = xv_{1-3} + xv_{2-3} - v_3$$

To sum this approach we can write down generalized equation:

$$\frac{dS_i}{dt} = \sum v_{in\_i} - \sum v_{out\_j} \quad (2)$$

where:

v_{in_i}– rate of i[th] reaction which leads to increasing the concentration of metabolite;

v_{out_j}– rate of j[th] reaction which leads to decreasing the concentration of metabolite.

A number of studies [10, 11] contains one additional summand in the equation (2) – rate of growth μ. It is noted that cell growth leads to a slight dilution of the metabolites, but the study [11] emphasizes that the dilution is not significant and does not substantially affect model accuracy. Taking into account the growth rate, the equation (2) can be written in a following way:

$$\frac{dS_i}{dt} = \sum v_{in\_i} - \sum v_{out\_j} - \mu S_i \quad (3)$$

Offered equations are solved in stationary conditions, which means, on the assumption that the system equilibrium is established [9]. In this case, the equality between fluxes leading to the formation of metabolite and fluxes leading to decrease in its concentration must be respected. Consequently, for example in the equation (3) the derivative $\frac{dS_i}{dt}$ will be equal to zero and the equation itself will look like:

$$\sum v_{in\_i} - \sum v_{out\_j} - \mu S_i = 0 \quad (4)$$

For stationary conditions solution of the system of equations is performed to maximize or minimize one or more metabolic fluxes. For example the system of equations (1) for stationary state can be written as:

$$\begin{gathered} v_1 - xv_{1-3} = 0 \\ v_2 - xv_{2-3} = 0 \\ xv_{1-3} + xv_{2-3} - v_3 = 0 \end{gathered} \quad (5)$$

The solution can be conducted to maximize the metabolic flux v_3:

$$y = v_3 \rightarrow max \quad (6)$$

where: y – the target function.

The complexity of these models depends on the number of analyzed fluxes.

Let us consider further some possible applications for the method of analysis of the balance of metabolic fluxes. Today the use of metabolic models has shown its effectiveness in the selection of potential strains, nutrient mediums, products and also in identifying ways to increase efficiency in the production of biofuels or pharmaceutical preparations [12–14].

In the study [11] the method of analysis of balance of metabolic fluxes was used to compute metabolic fluxes associated with biological carbon fixation. The authors compared six known types of fixation [11] on the criterions of maximizing the growth of biomass and energy efficiency. Three of them are present in phototrophic organisms: reductive pentose phosphate cycle (Calvin cycle), reductive citric acid cycle (Arnon cycle), 3-hydroxypropionate cycle. Their needs of light quanta (the number of photons absorbed) was calculated and compared. The most effective on this parameter is Arnon cycle (11 photons), then goes Calvin cycle (13.9 photons), and the least effective is 3-hydroxypropionate cycle (15.3 photons). The number of moles of photons for each photoautotrophic pathway of carbon fixation was converted into the total amount of energy in kilojoules spent on fixation of CO_2. Fixation of one mole of CO_2 in Calvin cycle requires 2439 kJ, in Arnon cycle – 2401 kJ, in 3-hydroxypropionate cycle – 3152 kJ. It is interesting that overwhelming majority of all primary products on the planet is made through Calvin cycle which is not the most effective. Three more pathways of carbon fixation work for chemotrophic organisms: reductive acetyl-CoA pathway (the pathway of Wood–Ljungdahl), 3-hydroxypropionate/4-hydroxybutyrate and dicarboxylate/4-hydroxybutyrate pathways. After computing the thermodynamic efficiency of all six pathways of carbon fixation it has been found that three chemotrophic pathways (the pathway of Wood–Ljungdahl, 3-hydroxypropionate/4-hydroxybutyrate and dicarboxylate/4-hydroxybutyrate pathways) are more effective than photoautotrophic pathways (less than 1000 kJ/mole CO_2). However, after inclusion to the analysis of the parameter of energy consumption for the generation of hydrogen from sunlight it turned out that energy consumption of chemotrophic pathways increases 5 times.

The overall efficiency, calculated by dividing the heat of biomass combustion by the amount of energy spent on the biomass synthesis, is highest for the Arnon cycle (25.3%). Calvin cycle is a little less effective (24.9%). Thus, from a practical point of view (taking into account the spendings for hydrogen production), the most effective of the six pathways is Arnoncycle, the Calvin cycle is not far behind. Chemotrophic pathway of carbon fixation can become profitable only with the development of cheap hydrogen generation technologies. For the synthesis of various metabolic precursors one pathways of carbon fixation may be more effective than others.

Effectiveness of researches of microorganisms' metabolism leads to the fact that today there is an opportunity to simulate the maximum possible number of metabolic pathways of any organism. In the study [3] its authors presented the results of the work on the comprehensive model of the bacterium *Mycoplasma genitalium*. The reason for choosing this bacterium was its extremely small genome, which consists of only 580 070 base pairs. Also, the genome of *M. genitalium* was completely sequenced in the period from 1993 to 1995 [15]. In 2008 the results of successful synthesis of the genome of the bacteria were published [16].

Before designing the model, the authors [3] have analyzed about 900 publications to gather information about intracellular processes of *M. genitalium*. This modeling was aimed to predict the phenotype by genotype and to try to describe the life cycle of cells, following changes in metabolic fluxes, as well as to try to assess the balance of metabolites at different periods of the cell life. In this study the metabolism was modeled by the method of balance of metabolic fluxes. The work [17] where the most complete metabolic map of *M. genitalium* was published, was taken as a basis for modeling. At the same time, degradation of RNA and proteins were modeled as a Poisson process (detailed description of the stochastic process is presented in [18]). In general, authors [3] developed 28 sub-models describing different

intracellular processes. Thus, they faced the challenge of integrating all sub-models into a single system. Today, these methods of integration are already under development [19–22]. For the integration of sub-models in this model, authors have assumed autonomy: each of the sub-models for a very short period of time (less than 1 second) is autonomous and independent from the other sub-models. During each iteration of the calculation each of the sub-models makes calculation based on the data received by all sub-models during the previous iteration.

All the variables in the model were divided into five major groups: processes associated with DNA, processes associated with RNA, processes involving proteins, processes involving various metabolites, and others which include all the processes excluded from first four groups. Testing of the model was carried out according to published reports, using more than 1,900 different parameters. For testing key parameters have been chosen, and their known values were compared with the simulation results. The point of testing itself was to simulate 128 cells of the wild strain of *M. genitalium*. The result of testing is $R^2 = 0.68$, considering analysis of cellular chemical composition, weight, and gene expression.

In general, the developed model showed a strong correlation with real data. So, the model was able to predict that the material flux through glycolysis was significantly higher than through pentose phosphate pathway or through the biosynthesis of lipids, which corresponds with experimental data [23].

One of the problems authors tried to solve by means of the developed model is prediction of behavior of DNA-binding proteins. Today active researches in the field of distribution of DNA-binding proteins [24, 25] and their diffusion dynamics [26] are being conducted. Relying on published data authors [3] included into their model 30 DNA-binding proteins, including DNA and RNA polymerases and replication initiator DNA.

Results of model calculations show that during the first 6 minutes of the cell cycle 50% of the chromosome is getting connected with at least one protein, and within 20 minutes already 90%. The basic protein is an RNA polymerase which binds to 90% of chromosome during the first 49 minutes of the cell cycle.

The model [3] was used to conduct *in silico* the research aimed to determine single genes, which significantly influence the growth of the microorganism. As a result of more than 3,000 simulations the authors were able to determine 284 genes that are essential for cell growth. The comparison with the experimental data presented in [27] showed that accuracy of the model for this task was 79%.

Global distribution of energy is one of most interesting problems solved with the help of a mathematical model of *M. genitalium* cells. During the simulations provided by means of this model authors [3] determined the dynamics of the synthesis and consumption of energy intermediates ATP, GTP, FAD(H_2), NAD(H) and NADP(H). Among the results of these simulations the most interesting was the imbalance between consumption and energy production, which amounted to 44%. It should be mentioned that the approach to determination of energy balance through the balance of energy intermediates today is actively studied by different research groups [2]. Let us consider further the results of this study in details.

In the study [2] simulation was carried out by means of method of balance of metabolic fluxes which was used by the authors to find the balance of intermediates ATP, GTP, FAD(H_2), NAD(H) and NADP(H). They looked into the processes taking place in the microorganism *Mycoplasma pneumonia*, metabolism of which described sufficiently for

modeling [23]. The developed model includes a number of subsystems, which describe processes related to power production, amino acids, nucleotides, lipids and metabolism, including cofactors, as well as the reaction of transport. Processes of biosynthesis of DNA, RNA and proteins were also included into the model. The authors analyzed the literature data and series of experiments to determine the balance of biomass in stationary conditions. As a result, they took the following equation of biomass for the model:

$$B_m = M_{DNA} + M_{RNA} + M_{Prot} + M_{lip} + M_{bs} + M_{aa} + M_{fa} + M_{cf} \quad (7)$$

where:

B_m – biomass;

M_{DNA}, M_{RNA}, M_{Prot},M_{lip}, M_{bs}, M_{aa}, M_{fa}, M_{cf} – biomass of DNA, RNA, proteins, lipids, bases, amino acids, fatty acids and cofactors respectively.

Model testing was carried out with use of own experimental data and results of published researches [28, 29].

The first task for the developed model was simulation of biomass growth. In simulation the growth was carried out within 4 days on glucose with pH value ranging from 5.5 to 8.8. Under these conditions secretion of lactic and acetic acids was observed, as well as increase of copies of lactate dehydrogenase in cell from 203 to about 1,000 for the entire simulation period. These results are consistent with the experimental data presented in [30].

Analysis of energy balance of *M. pneumonia in silico* in the study [2] established that in conditions of normal growth after 36 hours the rate of ATP synthesis is about 60,000 molecules per second. Then authors conducted a simulation of bacteria growth and compared the growth of biomass with consumption of energy in the form of ATP. The result detected significant difference between the experimental data (in particular [31]) and the model. The authors [2] concluded that, apparently, at the stage of culture growth there is a number of other reactions requiring ATP. The analysis of reactions requiring ATP *in silico* revealed that approximately 71- 88% of the available ATP is not used in processes of biomass increase. It was found out that after 36 hours of growth 9.8% of the total energy goes into protein synthesis and degradation, 8.4% into RNA synthesis and less than 0.1% into DNA synthesis. Lipid synthesis requires 0.5% of the available ATP molecules, 5.9% are consumed in the synthesis of secondary metabolites, precursor's consumption and for other processes. These calculations considered information [30] concerning half-lives of proteins (23 hours) and mRNA (1 minute). Authors decided to use this data in order to compare the energy consumption of growth-related processes and processes which are not related to growth. They found out that even during the exponential growth the input of ATP to maintain it do not exceed 7%. This distribution correlates with the data presented in [3], and perhaps its analysis in future will allow defining the calculated 44% imbalance between the production and consumption of energy intermediates.

In the forecited examples there was talk of prokaryotes, however, to date simulations using method of metabolic flux balance is also used for eukaryotes [32, 33]. Obviously, many of the models are created for the most studied microorganism – yeast *Saccharomyces cerevisiae*, genome of which has been completely sequenced by 1996 [34].

In the study [35] 234 reactions associated with the processes taking place in the mitochondria of *S. cerevisiae* were simulates. The modeling showed that during growth 138

reactions have zero rate, and stationary growth of mitochondria provided by the transport of proteins, amino acids, GDP, CDP, ions Fe_2O, protoporphyrin IX and ATP from cytoplasm. Qualitatively, the calculations are generally match with the experimental data.

The genome-scale metabolic model of *Geobactermetallireducens* consisted of 747 genes and 697 reactions including 118 unique reactions [36]. The central metabolism of *G. metallireducens* contained some energy-inefficient reactions. Benzoate up-regulated the genes for these reactions during growth on the complex electron donors for rapid energy generation. The metabolic model also shows similarities and differences to the model of a related species *G. sulfurireducens*.

The study of metabolically versatile organism *Rhodoferaxferrireducens* included iterative modeling and experimental approach [37]. Some previously unknown physiological features such as an expanded range of substrates that support growth have been discovered, as well as the stoichiometry of the electron transport chain and the ability to grow via fumarate dismutation. The genome study showed that subsurface growth is inherent to *R. ferrireducens* due to the ability to deal with various environmental insults (heavy metals, aromatic compounds, nutrient limitation and oxidative stress).

A three-dimensional reconstruction of the central metabolic network of *Thermotogamaritima* has been generated [38]. The network consisted of 478 proteins, among them 120 were determined by experiments and 358 were modeled. Among the proteins small number of basic shapes (folds) performing diverse but related functions dominated. The expansion of the essential core by nonessential proteins is gained with a few additional folds.

Ralstoniaeutropha is one of the most promising biotechnological objects. The genome-scale lithoautotrophic metabolic model of *R. eutropha* was created [39]. The stoichiometric model comprised 229 transport reactions and 1171 metabolites. The growth characteristics under lithoautotrophic conditions under varying gas mixtures were studied. Then the strategies for the production of poly-3-hydroxybutyrate under different pH values and carbon/nitrogen source uptake ratios were designed using themetabolic model. The targets for metabolic engineering essential for the production of 2-methylcitric acid in *R. eutropha* were identified using *in silico* gene knockout simulations.

In the work of Montagud et al. [40] the metabolic model of a cyanobacterium *Synechocystis sp.* PCC6803 including 882 reactions (669 genes and 790 metabolites). The detailed biomass equation contained elementary building blocks for cell growth and detailed stoichiometric representation of photosynthesis. The *in silico* metabolic engineering simulations allowed to identify and assess a set of gene knock-out candidates towards enhanced succinate production, as well as gene essentiality and hydrogen production potential. Metabolic hot-spots were also found around which gene regulation was dominant during light-shifting growth regimes.

The physiological group of purple nonsulfur bacteria consists of members possessing an extremely versatile metabolism. They can respire in the dark in the presence of oxygen, or grow by fermentation anaerobically on various organic substrates. In anaerobic conditions in the light purple nonsulfur bacteria can grow photoheterotrophically using organic substrates as carbon and electron source, or photoautotrophically with carbon dioxide as carbon source and various electron donors (elemental sulfur, thiosulfate, sulfide, hydrogen, ferrous iron) [41–43]. Bacteria can switch between different types of growth when the environmental conditions change. This ability allows them to survive and even thrive in diverse natural and anthropogenic ecosystems [44–48]. The modelling of the complex metabolic network of

purple nonsulfur bacteria is a challenge largely solved in a recent paper by Hädicke et al. [49]. The authors studied the central metabolism of three purple nonsulfur bacteria (*Rhodospirillumrubrum*, *Rhodobactersphaeroides* and *Rhodopseudomonaspalustris*) and made its stoichiometric model. Different environmental scenarios were studied then with the help of the flux variability analysis: photoautotrophic growth with hydrogen as electron donor, photoheterotrophic growth on different substrates and the role of Calvin cycle in this process, photoheterotrophic acetate metabolism (which is different in all three species studied), aerobic and anaerobic growth in darkness. The authors showed that biomass yield and CO_2 release could be calculated for a certain substrate and catabolic pathway. It correlated well with the experimental data. The role of Calvin cycle and other pathways in photoheterotrophic growth were discussed. The metabolic pathway model constructed by the authors allowed to interpret the experimental biological data and to understand better the global redox balancing mechanisms in purple nonsulfur bacteria. Moreover, the model gives the opportunity to design *in silico* new genetically engineered bacterial strains capable of biohydrogen, biopolymers, porphyrine production.

Thus, the forecited review shows that today the method of analysis of the balance of metabolic fluxes found an extensive use and allows getting adequate predictions on the results of the simulation. At the same time, this method has several drawbacks, in particular it gives opportunity to analyze only the steady metabolic flux distribution and does not take into account such factors as transportation, diffusion processes in cytoplasm, and others. And lastly, the stationarity of method prevents from effective analyze of dynamics of intracellular processes.

Models Based on Petri Nets

The method based on Petri nets is also widespread in the modeling of intracellular processes [6, 7]. Let us begin the review of models with short brief of these nets and principles they are based on.

Petri net is a set of mathematical procedures made for the simulation of dynamic systems [50–52]. In fact the net is a bipartite directed graph, where there are two types of points – positions and transitions. Positions are passive net elements, and in modeling of intracellular processes it can be certain factors, condition or chemical mixtures. Transitions respectively are active elements of the system - like, certain events and actions, such as chemical reactions. Points are connected to each other via arcs which represent interrelation between the active and passive elements. In fact the arcs describe which reactants transform into products due to a chemical reaction. It is also possible to assign to arc a multiplicand which will make allowance for stoichiometry of the modeled reaction. Shifts of material balance which become a result of reactions and transportation are modeled using tokens that move from one node to another in the direction of the process. All relocations of tokens are made in a way so their number in any given point characterizes the state of the system at a certain time. Thus, the work of Petri net in effect is relocation of tokens through the nodes. Where in following conditions are satisfied:

1. Moving of token is possible only if all the previous nodes are filled, so at least a stoichiometric amount of reactants is available for the reaction and arc factor is taken into consideration;
2. During operation all the tokens move from the basic (input) to the output points according to the multiplicands;
3. All events in net (token passing) occur automatically or in accordance with selected timeline.

Let us examine the same reaction we described for the method of analysis of metabolic fluxes balance and create a simple Petri net, that will describe it.

$$S_1 + S_2 \rightarrow xS_3 \tag{8}$$

For the sake of simplicity, let us assume that stoichiometric coefficient x is 3. Figure 1 represents the initial state of the net and the result of the reaction. In the initial state (the left part of the Figure) there are two moles of substance S_1 and one of S_2, in the Figure they are marked with black dots in the objects S_1 and S_2. The right part of the Figure shows the result of reaction: three moles of substance S_3 and one of S_1, which didn't enter the reaction as stoichiometric odd. In this example S_1, S_2 and S_3 are the positions and r is transition.

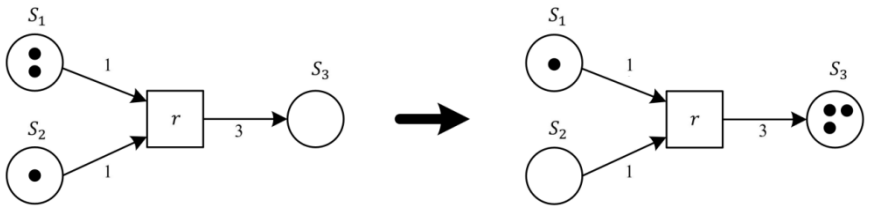


Figure 1. Petri net functioning, which describe the reaction 8.

As is seen from the description, the models based on Petri nets are not strictly formal reflection (or even approximation to it) of intercellular processes. The principles of net functioning are adaptive enough to create a rough analogue of modeled processes on the ground of already developed set of rules. As the work of this analogue is based on known set of rules, it can be successfully used for the analysis of the simulated process. That's why Petri nets are actively used nowadays for modeling of intracellular processes [6]. Next we will consider some successful examples of their application.

Let us begin with the work [53] in which the authors constructed a model based on Petri nets for modeling of such a complex phenomenon as apoptosis. This phenomenon stirs a significant scientific interest [54, 55]. Cell apoptosis is a subject of numerous studies [55–58]. In their model, authors [53] have tried to reflect two most-studied signaling pathways [56] – receptor-dependent and mitochondrial. Authors did not include inhibitors of apoptosis to the model as in their opinion, the influence of these substances is external to the simulated process and so they can be neglected not to complicate the model. Adding of inhibitors' influence is also possible by making changes in original data of the model. In developing the model, its authors took into account the intersection of two signaling pathways through a regulatory protein Bid. For purposes of accounting of enzymes involvement in reactions

authors implemented test arcs which doesn't suppose the reduction of number of enzyme molecules catalyzing the reaction. Input signals generate tokens at initial positions and they cut in the net in accordance with the set of rules. At the ending positions tokens are absorbed and removed from the net as the output signals.

Verification of model was carried out by analyzing the transitions resulting in changes in the amount of substance during the reaction [53]. In case of model inadequacy inadequate growth or reduction of the amount of substance can be shown, or the model will demonstrate the cyclical behavior. When authors introduced to the model various combinations of basic data, they received one of described in literature apoptosis signaling pathways, and so they didn't get variants with in-progress job of net, with impossible signaling pathways or endless accumulation of the substances. Thus the authors' analysis with different input signals showed that model adequately describes the given signaling pathways of apoptosis.

An important advantage of Petri nets is accountability of temporal factors. They can be accounted after extending the set rules that govern the token movements or after adding new positions, which would carry out the calculation using those or other systems of equations. An example of such a hybrid Petri net is described in a study [59]. Its authors modeled the signal circuit of dopamine and used it for analyzing delays and noise in the circuit. Another interesting example is presented in work [60], where the authors introduced the stochastic set of rules to account the temporal factor of signaling pathway of interleukin 1. This method helped to define limitative steps of this signaling pathway, which are much slower than others and so have a significant influence on the entire signal way.

As you can see from the above examples Petri nets are sufficiently flexible and interesting method of modeling of intracellular processes. However, this modeling method is a system of mathematical rules, which is not an explicit reflection of simulated processes and carries a substantial degree of conditionality. Thus, this method has lower capabilities in the matter of gaining of new information about the object in comparison with the method of metabolic fluxes balance. At the same time in modeling using Petri nets set of rules can be significantly expanded what is successfully shown in works [59, 60]. Complication of factors and positions also looks like a promising trend in application of this type of modeling. For example, the introduction of positions with inscribed formulas calculates given process with more accuracy than modeling with set of net rules. It is quite easy to integrate into such a nets degradation of proteins and RNA, model them, for example, by analogy with [3] as a Poisson process. In general we can conclude that Petri nets due to their flexibility are very interesting tool for modeling the intracellular processes.

Thermodynamics of Systems Far from Equilibrium and Some Aspects of Its Application in Modeling of Intracellular Processes

Thermodynamics of chemical reactions with fluctuations in concentrations today became a point of interest as a theoretical basis for description of various biochemical systems [61, 62]. The theoretical possibility of chemical reactions and diffusion, resulting in spontaneous evolving of the system into spatially-nonhomogeneous structures was originally demonstrated by Alan Turing [63]. The basic equation for reaction with regard for diffusion can be written as:

$$\frac{\partial u}{\partial t} = D\nabla^2 u + f(u,p) \tag{9}$$

where:

u – vector of concentrations of substances involved in the process;

D – matrix of diffusion coefficients;

p – parameters describing the kinetics of the process.

Function $f(u,p)$ characterizes the system under study. Kinetic equations of ongoing reactions serve as the description. A classic example [64] of such a description is the following system of reactions:

$$\begin{gathered} X \leftrightarrow A \\ 2X + Y \rightarrow 3X \\ B \rightarrow Y \end{gathered} \tag{10}$$

For the system (10) this type of equations (9) will look like [63]:

$$\begin{gathered} \frac{\partial u_1}{\partial t} = D_1\nabla^2 u_1 + k_2 a - k_1 u_1 + k_3 {u_1}^2 u_2 \\ \frac{\partial u_2}{\partial t} = D_2\nabla^2 u_2 + k_4 b - k_3 {u_1}^2 u_2 \end{gathered} \tag{11}$$

where:

u_1, u_2, a и b – concentrations of X, Y, A, B respectively;

$k_1 \ldots k_4$ – kinetic constant.

Obviously, if $f(u,p) = 0$, the steady state is observed in the system. Turing in his work [63] has shown that for certain values of the kinetic parameters and diffusion coefficients such a stationary state can pass into unstable, and in case of diffusion, can evolve in a spatially inhomogeneous structure. This effect is observed if one of the parameters is subjected to bifurcation.

Belousov was the first who observed a chemical reaction with auto-oscillations. His work was published in 1959 [65]. In this work Belousov tried to find an analogue of the Krebs cycle in the non-living systems. Then this reaction has been studied by Zhabotinsky. The results [66, 67] caused considerable interest, both from the point of view of studying the auto-oscillatory processes in chemistry, and also concerning the possibility to get mathematical description of autowave processes. Starting out from the Belousov-Zhabotinsky reaction, by now was discovered a big number of autowave chemical reactions. Among these systems we can mention:

- Briggs-Rauscher reaction [68];
- PA-MBO - polyacrylamide–Methylene Blue–sulfide–oxygen system [69];
- FIS - hexacyanoferrate(II)–iodate–sulfite reaction [70, 71];
- CIMA - chlorite–iodide–malonic acid–starch reaction [72].

Detailed description of theoretical questions concerning autowave chemical reactions is presented in studies [9, 73, 74].

In this article we will take a closer look only on certain issues related to the autowave processes that are more interesting from the point of view of possible application in modeling of intracellular processes.

CIMA reaction is so appealing because in a great measure it reflects the results of calculations Turing introduced in his work [63]. For this reaction a mathematical model [75–76] was developed. The fundamental principles of this model we will consider below. It is based on the following reactions:

$$MA + I_2 \rightarrow IMA + I^- + H^+ \quad (12)$$

$$ClO_2 + I^- \rightarrow ClO_2^- + \tfrac{1}{2} I_2$$

$$ClO_2^- + 4I^- + 4H^+ \rightarrow Cl^- + 2I_2 + 2H_2O$$

MA – malonic acid.

Reaction rates are determined according to the following equations:

$$r_1 = \frac{k_1[MA][I_2]}{w_1+[I_2]} \quad (13)$$
$$r_2 = k_2[ClO_2][I^-]$$
$$r_3 = k_{3a}[ClO_2^-][I^-][H^+] + k_{3b}\frac{[ClO_2^-][I_2][I^-]}{w_3+[I^-]^2}$$

where:

k_1, k_2, k_{3a}, k_{3b}, w_1, w_3 – constants.

The main difficulty in modeling of the system is to determine the diffusion coefficients. The authors [77] assumed that iodide – gel or starch reaction may be applied to reduce the rate of diffusion of the activating reaction substance. Basing on this effect we can get the diffusion coefficients which lead to formation of Turing structures as a result of CIMA reaction. In this case, the model will look like:

$$\frac{\partial u_1}{\partial t} = k_1 - u - \frac{4u_1u_2}{1+u_1^2} + \nabla^2 u_1 \quad (14)$$
$$\frac{\partial u_1}{\partial t} = k_1\left[k_3\left(u_1 - \frac{u_1u_2}{1+u_1^2}\right) + c\nabla^2 u_2\right]$$

Computational modelings showed the formation of Turing structures in the system.

The key parameter in development of such models is diffusion coefficients [61]. In study [78] presented an approach to determining of these coefficients. Let us consider it by the example of a simple reaction (description by [61, 78])

$$U + S \leftrightarrow C \quad (15)$$

After writing down the equation (9) for each of the substances we get the following system:

$$\begin{aligned} \frac{\partial u}{\partial t} &= f(u,v) - r_1 us + r_2 c + D_u \nabla^2 u \\ \frac{\partial v}{\partial t} &= g(u,v) + D_u \nabla^2 v \\ \frac{\partial c}{\partial t} &= r_1 us - r_2 c \end{aligned} \tag{16}$$

where:

u, s и c – concentrations of U, S и C respectively;

r_1– on-rate;

r_2– off-rate.

In case the reaction rates are high, the analysis of system (16) can show the S concentration is close to initial.

Addition of the first and third equations in the system (16) derives the equation for concentration of u:

$$(1+r)\frac{\partial u}{\partial t} = f(u,v) + D_u \nabla^2 u \tag{17}$$

where $r = s_0 \frac{r_1}{r_2}$, s_0 – initial concentration of the substance s.

Thus if r is much greater than unity, diffusion of substance U significantly reduced.

This approach to modeling of chemical systems has been used in work [79] to simulate the process of glycolysis. Later have appeared modifications of this model [80–82] which altogether resolve into following equations:

$$\begin{aligned} f_1(u_1,u_2) &= u_1 - u_1 u_2^2 + k_1(1-u_1) \\ f_1(u_1,u_2) &= u_2 + u_1 u_2^2 - (k_1+k_2)u_2 \end{aligned} \tag{18}$$

The results of computational simulations performed by means of this model [83], have shown a correlation with experimental data acquired subsequently [70].

Model of bifurcation has been applied for the analysis of epigenetic regulation [84]. Model developed by the authors may help to clarify the mechanisms of epigenetic regulation in the process of the cell evolvement.

In study [85], its authors dispersed the reaction mixture for the Belousov-Zhabotinsky reaction in oil droplets and thus got a kind of “chemical cages”, which demonstrated that reaction-diffusion process leads to a chemical differentiation. This differentiation in its turn led to physical morphogenesis. Authors observed five of the six structures predicted by Turing in his work [63], and as well, in the two-dimensional hexagonal arrays seven previously undescribed structures were detected. It was demonstrated that the addition into Turing theory of some factors related to the heterogeneity of proceeding processes helps to explain these new structures.

From this review we can conclude that today there are some methods of modeling of auto-oscillating and non-equilibrium processes. It is obvious that by far we don’t have enough

experimental data for a wide use of this approach for intracellular processes modeling. However, now it is already possible to rely on this method in the analysis of some processes and include received systems of equations in the general model.

Modularity in Metabolic Processes and Signaling Pathways

Analysis of dynamic characteristics of metabolic and signaling pathways is another interesting approach to modeling of intracellular processes. It also supposes division into modules, but it is carried out by means of unification of groups of metabolic reactions according to their pathways [86–88] and not on basis of additional rules. It is obvious that with such modularity significant attention should be paid to the possible impact of the next modules in metabolic pathway on the previous ones and on cycles as well. Under this approach this processes are considered as feedback, and simulation is performed in much the same way as in the electronic circuits or in control systems [88, 89]. This method gives a good mathematical tool for gene engineering projects, as it provides an opportunity to analyze the effectiveness of changes made to genome taking into account the rate of various processes [90]. For example, this approach offers possibility to analyze the combination of relatively slow processes (such as gene expression) with fast ones (as transmission of signals in the signal paths) [90].

In order to confirm the applicability of this method to the intracellular process authors of the work [90] created four recombinant strains of *S. cerevisiae*. In each of them were represented different pathways of synthesis of green fluorescent protein GFP. Some strains had implemented circuits comprising feedback, others had not. For all the strains the signal to start synthesis was the presence of doxycycline in substrate. Control of synthesis of GFP protein gave opportunity to monitor proceeding metabolic pathway and to describe it mathematically by means of analysis of the curves of varying amount of GFP [84].

Discussion

The results of studies provided by different research groups show that by far we don't have enough data for compilation of mathematical model, which would involve all known intracellular processes for any microorganism or cell culture. Therefore authors of models have to make a set of assumptions, such as ignoring transportation or using stochastic approach. At the same time, today we can find enough works on modeling the properties of various biomolecules in different solutions (for example, [91]), and works on formalized physical description of specific processes, such as functioning of certain enzymes or complexes, also exist. Thus, in [92] quite detailed description of the process of photosynthesis is presented. By far modeling of intracellular processes goes in two lines: general processes and their subsequent clarification, and functioning of specific systems or even reactions with further compilation. Both approaches are schematically illustrated in Figure 2 made by analogy with "pyramid of the complexity of the living" presented in [93].

Obviously, both of the approaches described above in the long run will lead to creation of a complete model of microorganism or animal cell which will include even physicochemical processes associated with specific reactions and biomolecules, comprising conformational

changes. However, information we have today is not enough to create these models, nevertheless approaches for integration of various elements and sub-models into an integrated complex already exist.

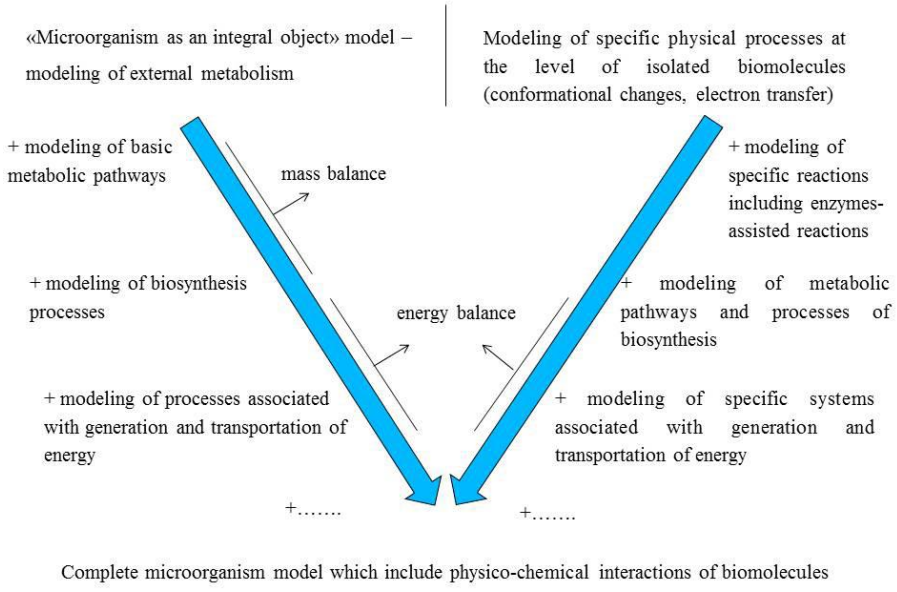


Figure 2. The main approaches to mathematical modeling of intracellular processes.

The first way has already been presented in work [3] where the whole model is made up of sub-models, each of which is responsible for its own process group. Thus, authors divided all processes into the groups by their functionality. Assuming the autonomy of sub-models, this approach gives an opportunity to combine different methods of modeling in each of the sub-models. In this case, an important task is to provide data exchange between the sub-models as it may occur, for example, that one sub-model will return results in quantitative terms (e.g., concentrations) and the other will be represented by Petri net and it will work with tokens which are converted into physical quantities in accordance with built-in set of rules.

Another approach is the spatial separation of processes, when each sub-model simulates specific system localized in space. Localization can be not strict in physical dimension, but, for example, attached to a specific organelle. With this method appears a problem of verifying the material flows between sub-models, but these flows are, in fact, a source of information of the intracellular transport. Creation of such a model is obviously a very difficult task, considering available information about intracellular processes and volumes of contained substances [94, 95]. On the other hand, after localization of sub-models in spatial system, spatial effects and their role in metabolism can be simulated.

To continue with this idea we can mention that in models divided into sub-model on the principle of the spatial localization further division into the estimated elements of sub-models on the principle of functionality is also theoretically possible.

The problem of uncertainty of modeled system leads to the analysis of possible use of external algorithms, which in any way would provide quantitative assessment of uncertain processes. Such an approximation will surely reduce the accuracy of the model. Use of

Poisson process as a model of RNA and proteins degradation described in work [3] can be an example of this approach. To date, there is no lack in algorithmic methods of dealing with complex data that are difficult to formalize, as in case there are experimental data only in a small part of the theoretically possible value. As an example we may cite artificial neural networks, which are actively used for so-called black-box modeling [96]. Black-box modeling is type of simulation where modeled system is represented as a black box and the goal is to define correlation between system output and input parameters through a variety of mathematical methods.

Apart from spatial effects an important model element is time tracking. For models similar to [3] tracking is conducted with reference to the known reaction rates. When using Petri nets track of time can be carried out, for example, in the following ways: by updating the set of rules, by artificial accelerating or slowing down movement of tokens along the arcs, or by implementing additional positions that would restrain tokens.

As mentioned earlier, the set of rules that governs model functioning is an integral part of Petri nets. But if the model contains several sub-models, there is a need to develop a set of rules for their interaction. Assumption of autonomy applied in [3] is quite effective if sub-model have been selected only by criterion of functionality. But it may be not effective enough in case of use of black-box as any of sub-models or in case of a spatial decomposition. The fact is that in a spatial decomposition each of sub-models may comprise component responsible for the material flow of matter from one sub-model to neighboring, which in turn causes a procedure for computing in the direction of the material flow motion. Thus, more serious system which will regulate functioning of various sub-models may be necessary.

Apart from approaches mentioned above solutions based on results of study of mass-tranfer in microstreams and nanostreams may appear. Today, this area of hydrodynamics is actively developing, and among recent publications [53, 54] should be mentioned. In first one the authors analyze the flow of binary fluids through a number of microcylinders located at a certain angle to the flow. By means of computer simulation, authors determine the parameters of such a system of microcylinders where it would be possible to separate components from their mixture. In work [54], authors are exploring such an interesting phenomenon as thermodiffusion. Research is also conducted by means of computer modeling. In the future, these works can help in the study of intracellular traffic of substances, and that of course will be reflected in the mathematical models of intracellular processes.

Conclusion

Development of mathematical models gives opportunity to simulate various types of experiments and choose those of them which have shown high-quality results for further experimental work. At the same time the process of modeling itself helps to organize and structure the definite volume of data and show the direction for further experimentations.

As is seen from above review, questions of modeling of intracellular processes are highly relevant nowadays. On the one hand, enough information about these processes has been accumulated in order to get an adequate model. On the other hand a lot of things still remain unknown, what makes possible using of the models as a tool in the research process. Growth in the number of works and the diversity of approaches to modeling in recent years only confirms foregoing thesis.

Consideration of contemporary models and approaches to modeling shows that today there is a need for implementation to the model of issues related to the intracellular substance transport. Currently we don't have enough data for complete mathematical description of these processes. However, in some cases, certain elements and approaches already can be used. For example, the work on non-equilibrium thermodynamics and dissipative systems is extremely interesting as a tool for modeling of systems of metabolic reactions in respect of diffusion processes.

In general we can expect that the concept of mathematical modeling of intracellular processes will bourgeon rapidly in the near future and we can expect new interesting models of both individual reacting systems and entire cells.

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