BIOCHEMICAL REACTIONS MODELS USING THE PETRI NETWORK APPROACH

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În aceast articol sunt propuse modele computaţionale pentru reacţii chimice ireversibile, reversibile, bimoleculare şi cinetica enzimelor cu un singur substrat, bazate pe formalismul reţea Petri diferenţială. Căile metabolice de semnalizare conţin reacţii biochimice în care substraturile sunt catalizate enzimatic şi sunt transformate în produse biochimice active. Reacţiile enzimatice sunt descrise cantitativ prin ecuaţii diferenţiale ordinare, în modelul propus de reţea Petri. Specificitatea reacţiilor biochimice sunt captate în modelul propus de reţea Petri. Studiul simulării arată validarea calitativă a acurateţii modelului propus de reţea Petri, cu rezultate bazate pe un studiu de caz pentru cinetica enzimelor.

In this paper we propose computational models for irreversible, reversible, bimolecular and single substrate enzyme kinetics based on the differential Petri network formalism. Metabolic signalling pathways contain biochemical reactions in which substrates are enzymatically catalyzed and turn them into active biochemical products. The enzyme reactions are described quantitatively through ordinary differential equations (ODEs) in the proposed Petri network model. The specificity of the biochemical reactions are captured in the proposed Petri network model. The simulation study shows qualitative validation of the dependability of the proposed Petri network model with case study results for enzyme kinetics.

Keywords: Enzyme kinetics, computational model, Petri network, ordinary differential equations, penicillin

1. Introduction

Metabolic network modelling and simulation is an important step in complex biological system development and allows for the comprehension of the molecular mechanisms that take place in the cell. The metabolic network modelling breaks down metabolism pathways into their respective reactions and enzymes, and analyzes them within the perspective of the entire network. Quantitative models of biochemical networks are a central component of modern systems biology. Building and managing these complex models is a major challenge that can benefit from the application of formal methods adopted from theoretical computing science [1].

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Many approaches exist for modelling metabolic network. Some of the approaches have constructs that are specifically tailored for modelling only the biological aspects. Few of these approaches have tools which facilities the study and analysis of the dynamic behaviour of the system together with the discrete elements and presents dependable characteristics. Biochemical reaction systems have by their very nature three distinctive characteristics [2]: 1) they are inherently bipartite, i.e. they consist of two types of players, the species and their interactions; 2) they are inherently concurrent, i.e. several interactions can usually happen independently and in parallel and 3) they are inherently stochastic, i.e. the timing behaviour of the interactions is governed by stochastic laws. So it seems to be a natural choice to model and analyse them with a formal method, which shares exactly these distinctive characteristics: the Petri networks.

There are three major ways of modelling biochemical networks (qualitative, stochastic and continuous) in the context of Petri nets [3]. The qualitative time-free description is the most basic, with discrete values representing levels of concentrations [4]. In the stochastic description, discrete values for the amounts of species are retained, but a stochastic rate is associated with each reaction [5]. A continuous model describes amounts of species using continuous values and associates a deterministic rate with each reaction [6]. Many other authors have studied and described particular biologic Petri models ([7], [8], [9]). Two important surveys on applying Petri nets for biochemical networks are [10] and [11], offering a rich choice of further reading pointers, among them numerous case studies. Finally, is important to mention the most used framework for representation of biochemical network models [12].

The main advantages of using Petri networks are: intuitive modelling style, mathematically founded analysis techniques, coverage of structural and behavioural properties as well as their relations, integration of qualitative and quantitative analysis techniques and reliable tool support.

This paper presents preliminary results in modelling and simulation of biochemical metabolic pathways with the Petri network formalism which translates biological and chemical processes to Petri network formalisms and uses Petri network based tools to study the behaviour of these processes. The example models the Penicillin N production.

2. Petri network formalism for studying system dynamics

The Petri networks are used extensively for the representation and the simulation of concurrent discrete-event dynamic systems. A Petri network is a directed bipartite graph, in which nodes represent transitions, which are events that may occur, and places, which are seen as conditions, and directed arcs that describe which places are pre- and/or post- conditions for which transitions. [13]

Tokens that are placed in places signify that the condition that represents the place holds. The placement of tokens in the network, called marking, defines the network's state. The Petri network can be simulated by moving tokens according to a firing rule; when all the places with arcs leading to a transition have a token, the transition is enabled, and may fire, by removing a token from each input place and adding a token to each output place. The results of the simulation can be plotted as graphs, or otherwise analyzed.

The Petri networks can also be structurally examined to verify desired system properties, such as boundedness and liveness. Boundedness guarantees that in every place of the network, the number of tokens is always less than some finite number, for example there is no toxic accumulation of metabolites. Liveness guarantees that all transitions can be enabled. Another type of analysis involves determining whether we can move from one state of the system to another state, for example there are met the conditions for taking place the enzyme-substrate reaction from the specific metabolic pathway.

3. Particular models for enzyme kinetics

Almost all processes in a biological cell need enzymes to occur at significant rates. Since enzymes are selective for their substrates and speed up only a few reactions from among many possibilities, the set of enzymes in a cell determines which metabolic pathways occur in that cell. This study is limited to the Michaelian enzymes.

In biochemistry, a metabolic pathway is the series of chemical reactions that occurs in a cell. In each pathway, a principal species is modified by chemical reactions. Enzymes catalyze these reactions, and often require additional minerals, vitamins and other factors in order the reactions to take place. Pathways may be very elaborate because of the many chemicals that are involved. The entire collection of pathways that exists in the cell is called the metabolic network. The importance of the pathways is that they maintain the homeostasis of the organism.

The metabolism modifies the initial molecule and shapes it into an end product. The end product can be stored by the cell, used as a metabolic product or used to initiate another metabolic pathway. A molecule called a substrate enters a metabolic pathway depending on the needs of the cell and the availability of the substrate. An increase in concentration of anabolic and catabolic end products would slow the metabolic rate for that particular pathway.

4. Enzyme kinetics modelling with ordinary differential equations

Most of the biological cells' dynamic behaviour may be reduced to the biochemical reactions which take place within, may be reduced to the way in

which molecules like genes, proteins and RNA interact, catalyze the reactions and contribute to the good functioning of the cells.

The simplest chemical reaction is the first order irreversible reaction, presented in Equation 1, in which k_1 represents the rate at which substance **S** is converted in substance **P**.

$$S \xrightarrow{k_1} P$$
$$\frac{dS(t)}{dt} = -k_1 S(t) \tag{1}$$
$$\frac{dP(t)}{dt} = k_1 S(t)$$

Equation 1 First order irreversible reaction

The first order reversible reaction is presented in Equation 2.

$$S \xleftrightarrow{k_1,k_2} P$$
$$\frac{dS(t)}{dt} = -k_1 S(t) + k_2 P(t) \tag{2}$$
$$\frac{dP(t)}{dt} = k_1 S(t) - k_2 P(t)$$

Equation 2 First order reversible reaction

Both reactions presented in Equation 1 and in Equation 2 are linear. The simplest non-linear chemical reaction is the bimolecular reaction presented in Equation 3.

$$S_1 + S_2 \xrightarrow{k_1} P$$
$$\frac{dS_1(t)}{dt} = -k_1 S_1(t) S_2(t)$$
$$\frac{dS_2(t)}{dt} = -k_1 S_1(t) S_2(t) \tag{3}$$
$$\frac{dP(t)}{dt} = k_1 S_1(t) S_2(t)$$

Equation 3 Bimolecular reaction

The kinetics presented in Equation 1, in Equation 2 and in Equation 3 is mass-action kinetic law type. The equations may be more complicated like the case of the enzyme-substrate chemical reactions presented in Equation 4 and in Equation 5

Enzyme kinetics is the investigation of how enzymes **E** bind substrates **S** and turn them into products **P**. Equation 4 shows most of the enzyme kinetics characterized by single-substrate reactions based on Michaelis–Menten–Henri kinetics [14].

$$E + S \xleftrightarrow{k_1,k_{1r}} ES \xrightarrow{k_2} E + P \tag{4}$$

Equation 4 Single-substrate mechanism for enzyme kinetics

Enzyme reactions take place in two stages. In the first stage, the substrate **S** binds reversibly to the enzyme **E**, which forms the enzyme-substrate complex **ES**. The enzyme-substrate complex **ES** is sometimes called the Michaelis complex. The enzyme E then catalyzes the chemical step in the reaction and releases the product **P**. k_1 represents the constant rate at which the enzyme-substrate complex **ES** is obtained. k_{1r} represents the constant rate at which the enzyme-substrate complex **ES** dissociate back in enzyme **E** and substrate **S**. k_2 represents the rate constant at which the enzyme-substrate complex **ES** dissociates in enzyme **E** and product **P**. k_2, also called k_{cat} or the turnover number, is the maximum number of enzymatic reactions catalyzed per second.

The set of ordinary differential equations used to model the enzyme kinetics described in the above paragraph are presented below in Equation 5.

$$\begin{aligned}
\frac{dE(t)}{dt} &= -k_1 \cdot E(t) \cdot S(t) + (k_{1r} + k_2) \cdot ES(t) \\
\frac{dS(t)}{dt} &= -k_1 \cdot E(t) \cdot S(t) + k_{1r} \cdot ES(t) \\
\frac{dES(t)}{dt} &= k_1 \cdot E(t) \cdot S(t) - (k_{1r} + k_2) \cdot ES(t) \\
\frac{dP(t)}{dt} &= k_2 \cdot ES(t)
\end{aligned} \tag{5}$$

Equation 5 Ordinary differential equations for enzyme kinetics

Michaelis-Menten kinetics relies on the law of mass action, which is derived from the assumptions of free diffusion and thermodynamically-driven random collision. However, many biochemical or cellular processes deviate significantly from these conditions, because of macromolecular crowding, phase-separation of the enzyme/substrate/product, or one or two-dimensional molecular movement. This is why the time variation of the enzyme-substrate product concentration is not assumed in the differential equations presented in Equation 5 to be zero.

Several enzymes can work together in a specific order, creating metabolic pathways. In a metabolic pathway, one enzyme takes the product of another enzyme as a substrate. After the catalytic reaction, the product is then passed on to another enzyme. Sometimes more than one enzyme can catalyze the same reaction in parallel, this can allow more complex regulation: with for example a low constant activity being provided by one enzyme but an inducible high activity from a second enzyme.

Enzymes determine what steps occur in these pathways. Without enzymes, metabolism would neither progress through the same steps, nor be fast enough to serve the needs of the cell.

In the following we will synthesize the philosophy in using Petri models, which is the possibility to build a complex network with simple circuits which can be extracted from a dedicated library. Fig. 1 represents three simple reactions representing as follows: a) simple enzymatic reaction, Michaelis-Menten kinetics; b) reversible enzymatic reaction, Michaelis-Menten kinetics; b) an enzymatic reaction, mass action kinetics. Fig. 2 illustrate how we can obtain a more complex model from simple ones: two enzymatic reactions, mass action kinetics, building a cycle.

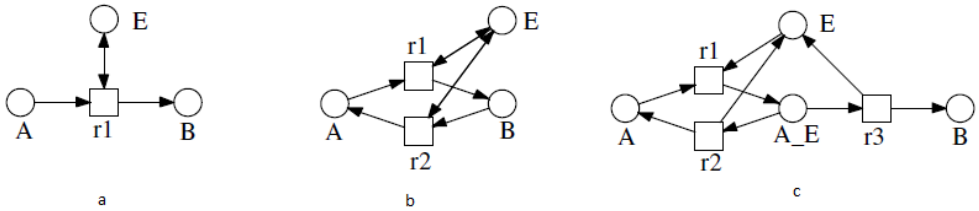

Fig. 1 Petri net components for some typical basic structures of biochemical reactions

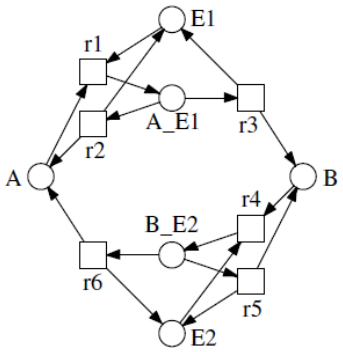

Fig. 2 Building a complex Petri model from basic structures

5. Enzyme kinetics modelling with Petri network

Differential Petri networks are a kind of Petri networks which contains differential places and differential transitions [15].

If all the markings and all the arc weights were non-negative, the behaviour of a differential Petri network could be modelled by a hybrid Petri network with special speeds for continuous transitions [16].

Differential predicate transition Petri networks are a special type of Differential Petri networks that can monitor sets of differential equations associated with specific places. In particular, when a token is produced in a place

p, the corresponding set of differential equations is activated and continuous variables associated with the produced token can evolve.

Enabling functions are associated with transitions and depend on the continuous values associated to the tokens located in the input places of the corresponding transition.

Junction functions, associated with transitions too, allow modifying and communicating the values of the continuous variables to the tokens produced in the output places of the fired transitions.

A differential predicate transition Petri network is composed of the following elements:

- $N = <P, T, Pre, Pos>$ is a Petri network, where P is the set of places, T the set of transitions, Pre and Pos are the matrix that defines arcs connecting places to transitions and transitions to places.
- $X = \{X_{p_1}, X_{p_2}, X_{p3}, \ldots\}$ is the set of continuous variables.
- X_{p_i} is a vector of variables (which belong to X) associated with each place p_i. Each marking in the place p_i is actually an instance of X_{p_i}.
- e_i is an enabling function associated with each transition t_i. This function enables or not the firing of t_i according to the values of X_{p_i} which are in the input places of t_i.
- j_i is a junction function associated with each transition t_i. When considering the firing of transition t_i, this function defines the values of vectors X_{p_i} which belong to the output places of t_i.
- f_i is a differential equation system associated with each place p_i. This system defines the variables evolution associated with tokens in the place p_i.
- M_0 is the initial marking, specifying the number of tokens in each place and the variables values associated to each token for the initial time (t = 0 s).

The proposed Petri network model which uses the differential Petri networks is presented below.

Variables' vector for the places p_1 and p_2 for the enzyme kinetics model is defined as following:

- For the place p_1, the variables' vector $X_{p_1} = (E_1, S_1, ES_1)$ contains the enzyme E_1, substrate S_1 and enzyme-substrate complex ES_1 concentrations.

System's equations are defined by f_1 and f_2, based on the ordinary differential equations presented in Equation 2 and are partitioned as follows:

- f_1:

$$\frac{dE_1(t)}{dt} = -k_1 \cdot E_1(t) \cdot S_1(t) + (k_{1r} + k_2) \cdot ES_1(t)$$
$$\frac{dS_1(t)}{dt} = -k_1 \cdot E_1(t) \cdot S_1(t) + k_{1r} \cdot ES_1(t) \quad (6)$$
$$\frac{dES_1(t)}{dt} = k_1 \cdot E_1(t) \cdot S_1(t) - (k_{1r} + k_2) \cdot ES_1(t)$$

- f_2:

$$\frac{dP_2(t)}{dt} = k_2 \cdot ES_2(t) \quad (7)$$

The enabling function e_1 associated with the fire's enabling of the transition t_1 is defined as upper or lower bounds for the enzyme, substrate and enzyme-substrate complex concentration values as follows:

$$E_1(t) \le treshold_1$$
$$S_1(t) \le treshold_2 \quad (8)$$
$$ES_1(t) \ge treshold_3$$

The threshold values, $treshold_1$, $treshold_2$ and $treshold_3$, depend on the metabolic pathway characteristics.

The junction function j_1 associated with the firing of the transition t_1 is defined as the same value concentration for enzyme-substrate complex in each place p_1 and p_2:

$$ES_1(t) = ES_2(t) \quad (9)$$

Petri network modelling of the enzyme kinetics is presented in Fig. 3 below. In this model, the place p_1 models the first dynamic step of the single-substrate mechanism for enzyme kinetics from Equation 4, which is the forming of the enzyme-substrate complex **ES** in the presence of enzyme and substrate levels of concentration**.** The place p_2 models the second dynamic step of the single-substrate mechanism for enzyme kinetics from Equation 4, which is the forming the product **P** in the presence of the enzyme-substrate complex level of concentration obtained from the previous place, place p_1.

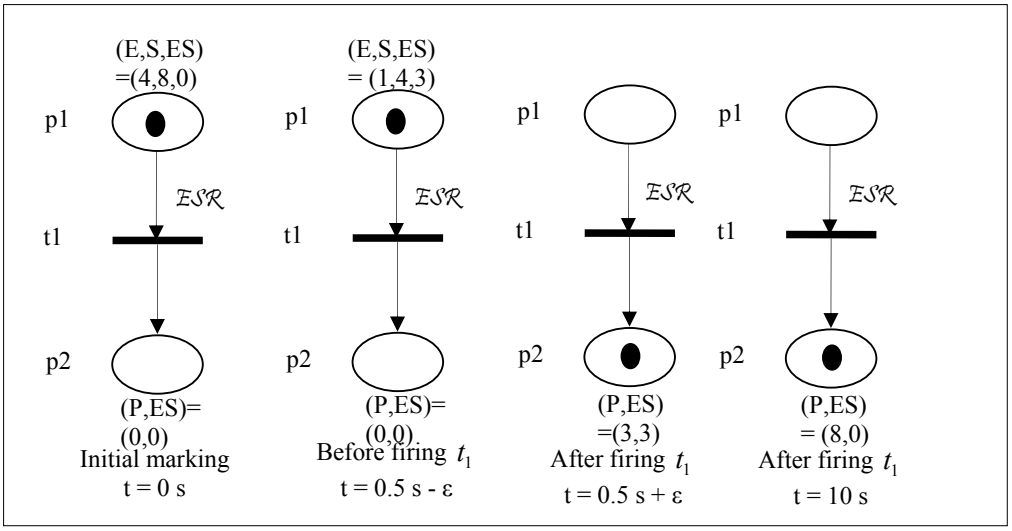

Fig. 3 Petri network model of single substrate enzyme kinetics (*ESR*)

The novelty of this Petri network for enzyme kinetics is the approach of partitioning the ODEs to places, which holds actually the dynamic information of the biochemical process.

6. Enzyme kinetics modelling with the computational objects

The proposed computational framework for modelling enzyme kinetics contains object for modelling irreversible reactions, reversible reactions, bimolecular reactions and single substrate enzyme reactions.

The numerical inputs for the enzyme-substrate reaction *(ESR)* of the computational model are the initial marking which contains the initial species concentration values, the constant rates (*k* values) and the thresholds values (*thres* values): $M_0, k_1, k_{1r}, k_2, treshold_1, treshold_2, treshold_3$. The computational model is presented in Fig. 4 (d).

Using the same approach as for modelling single substrate enzyme kinetics, the Petri network model of irreversible reaction *(IR)* have as numerical inputs of the computational model the initial marking which contains the initial species concentration values, the constant rate (*k* value) and the threshold values (*thres values*): $M_0, k_1, treshold_1, treshold_2$. The computational model in presented in Fig. 4 (a).

The numerical inputs for the reversible reaction *(RR)* of the computational model are the initial marking which contains the initial species concentration values, the constant rate (*k* value) and the thresholds values (*thres* values):

$M_0, k_1, treshold_1, treshold_2, treshold_3$. The computational model in presented in Fig. 4 (b).

Also, the numerical inputs for the bimolecular reaction *(BR)* of the computational model are the initial marking which contains the initial species concentration values, the constant rates (*k* values) and the thresholds values (*thres* values): $M_0, k_1, k_{1r}, treshold_1, treshold_2, treshold_3$. The computational model in presented in Fig. 4 (c).

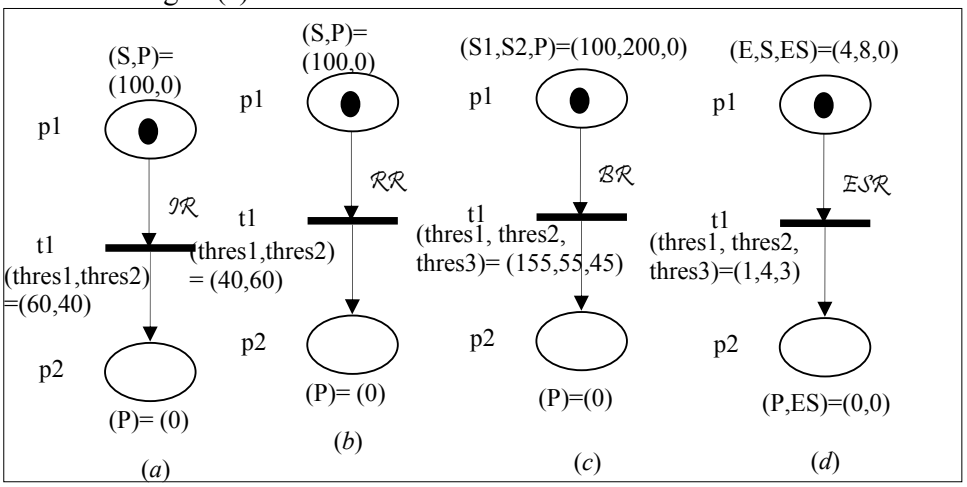

Fig. 4 Computational Petri network models for the irreversible reaction (a), reversible reaction (b), bimolecular reaction (c) and enzyme-substrate reaction (d)

The computational models presented in Fig. 4 may be used for modelling and simulating biochemical pathways. There is an actual interest to computationally visualize the static and dynamic events that take place in biochemical reactions. The proposed computational objects express this static and dynamic information in a way suitable to be used for visual modelling which the standard approaches, i.e. differential equation solving.

The standard approach only, i.e. differential equation solving, does not capture the discrete information,, which is the biological meaning. The graphical display of the curves, solutions of the differential equation system does not self contain any biological information. Biologists may add to the mathematical model captured through the differential equation systems, the discrete information which consists of the particularities of the biological system using the proposed approach. These particularities of the biological system are captured in the Petri network model.

7. Using the Petri network model for penicillin and cephalosporin biosynthesis

Penicillin is a large group of natural or semi synthetic antibacterial antibiotics derived directly or indirectly from strains of fungi of the genus Penicillium and other soil-inhabiting fungi grown on special culture media.[17].

Penicillin is a secondary metabolite of fungus Penicillium that is produced when growth of the fungus is inhibited by stress. It is not produced during active growth. Production is also limited by feedback in the synthesis pathway of penicillin.

Cephalosporins are a large group of broad-spectrum antibiotics obtained from Acremonium, formerly Cephalosporium, a genus of soil-inhabiting fungi. Cephalosporins are similar in structure and antimicrobial action to penicillin. The cephalosporins have been classified according to general features of antimicrobial activity, with successive generations having increasing activity against gram-negative organisms and decreasing activity against gram-positive organisms. (Dorland's, 2007)

The core set of chemical reactions that generate penicilins and cephalosporins are presented in equation 10.

$$\begin{aligned}
&3\,\mathrm{ATP} + \mathrm{L\text{-}2\text{-}aminohexanedioate} + \mathrm{L\text{-}cysteine} + \mathrm{L\text{-}valine} + \mathrm{H_2O} = \\
&3\,\mathrm{AMP} + 3\,\mathrm{diphosphate} + \mathrm{N\text{-}[L\text{-}5\text{-}amino\text{-}5\text{-}carboxypentanoyl]\text{-}L\text{-}cysteinyl\text{-}D\text{-}valine} \\
&\mathrm{N\text{-}[(5S)\text{-}5\text{-}amino\text{-}5\text{-}carboxypentanoyl]\text{-}L\text{-}cysteinyl\text{-}D\text{-}valine} + \mathrm{O_2} = \\
&\mathrm{isopenicillin\ N} + 2\,\mathrm{H_2O} \\
&\mathrm{isopenicillin\ N} = \mathrm{penicillin\ N} \\
&\mathrm{phenylacetyl\text{-}CoA} + \mathrm{isopenicillin\ N} + \mathrm{H_2O} = \\
&\mathrm{CoA} + \mathrm{penicillin\ G} + \mathrm{L\text{-}2\text{-}aminohexanedioate}
\end{aligned} \tag{10}$$

A *N-(5-amino-5-carboxypentanoyl)-L-cysteinyl-D-valine* synthase or EC 6.3.2.26 is an enzyme that catalyzes the first chemical reaction from Equation 6. The 5 substrates of this enzyme are *ATP* ( ), *L-2-aminohexanedioate* (, obtained through Lysine degradation), *L-cysteine* (($C_3H_7NO_2S$), *L-valine* ( ), and water ( ), whereas its 3 products are *AMP* ($C_{10}H_{14}N_5O_7P$), *diphosphate* ($P_2H_4O_7$) and *N-[L-5-amino-5-carboxypentanoyl]-L-cysteinyl-D-valine* ($C_{14}H_{25}N_3O_6S$). This enzyme belongs to the family of ligases, specifically those forming carbon-nitrogen bonds

as acid-D-amino-acid ligases (peptide synthases). The systematic name of this enzyme class is L-2-aminohexanedioate:L-cysteine:L-valine ligase (AMP-forming, valine-inverting). Other names in common use include L-delta-(alpha-aminoadipoyl)-L-cysteinyl-D-valine synthetase, ACV synthetase, and L-alpha-aminoadipyl-cysteinyl-valine synthetase. This enzyme participates in penicillin and cephalosporin biosynthesis.

Isopenicillin-N synthase (IPNS) is a non-heme iron-dependent enzyme belonging to the oxidoreductase family. This enzyme catalyzes the formation of *isopenicillin-N* from δ-(L-α-aminoadipoyl)-L-cysteinyl-D-valine as it can be seen in the second chemical equation from **Error! Reference source not found.**. This reaction is a key step in the biosynthesis of penicillin and cephalosporin antibiotics. The active site of most isopenicillin-N synthases contains an iron ion.

An *isopenicillin-N epimerase* or EC 5.1.1.17 is an enzyme that catalyzes the third chemical reaction from **Error! Reference source not found.**. This enzyme contains pyridoxal phosphate. Epimerization at C-5 of the 5-amino-5-carboxypentanoyl group to form *penicillin N* is required to make a substrate for EC 1.14.20.1, deactoxycephalosporin-C synthase, to produce cephalosporins. Forms part of the penicillin biosynthesis pathway. Hence, this enzyme has one substrate, *isopenicillin N*, and one product, *penicillin N* ($C_{14}H_{21}N_3O_6S$). This equation is used for the simulation with the proposed Petri network model for single substrate enzyme kinetics. This enzyme belongs to the family of isomerases, specifically those racemases and epimerases acting on amino acids and derivatives. The systematic name of this enzyme class is penicillin-N 5-amino-5-carboxypentanoyl-epimerase. This enzyme participates in penicillin and cephalosporin biosynthesis.

An *isopenicillin-N N-acyltransferase* or EC 2.3.1.164 is an enzyme that catalyzes the fourth chemical reaction from **Error! Reference source not found.**. The 3 substrates of this enzyme are *phenylacetyl-CoA* ($C_{29}H_{42}N_7O_{17}P_3S$), *isopenicillin N*, and water (H_2O), whereas its 3 products are *CoA* ($C_{21}H_{36}N_7O_{16}P_3S$), *penicillin G* ($C_{16}H_{18}N_2O_4S$), and *L-2-aminohexanedioate* ($C_6H_{11}NO_4$). This enzyme belongs to the family of transferases, specifically those acyltransferases transferring groups other than aminoacyl groups. The systematic name of this enzyme class is acyl-CoA:isopenicillin N N-acyltransferase. Other names in common use include acyl-coenzyme A:isopenicillin N acyltransferase, and isopenicillin N:acyl-CoA: acyltransferase. This enzyme participates in penicillin and cephalosporin biosynthesis.

8. Case studies and simulations

The third chemical reaction from **Error! Reference source not found.** is modelled with the Petri network model proposed in Section 6. The enzyme **E** is

the *isopenicillin-N epimerase*, the single substrate catalyzed enzymatic **S** is the *isopenicillin N* and the obtained product **P** is *penicillin N* ($C_{14}H_{21}N_3O_6S$).

The differential equations from the Petri network model for enzyme kinetics are solved by utilizing MATLAB library functions, for example solver ode113. The solver uses a variable order Adams-Bashforth-Moulton PECE solver (Predictor-Evaluation-Corrector-Evaluation). It is more efficient than the one-step solver based on explicit Runge-Kutta formula, which uses the Dormand-Prince pair, at stringent tolerances and when the function is particularly expensive to evaluate. The used Adams-Bashforth-Moulton PECE solver is a multistep solver, which means that it needs the solutions from several preceding time points to compute the current solution.

The absolute tolerance used is 1.0E-6 and the relative tolerance used is 0.0010 for a time horizon of 10 seconds.

The simulation uses the proposed Petri net model from Section 3.2 with the initial marking $M_0 = (E, S, ES) = (4,8,0)M$ and the threshold values are $treshold_1 = 1$, $treshold_2 = 4$, $treshold_3 = 3$. All these concentration values are expressed in Molarity, M. The values used for the constants involved in the reactions are $k_1 = 2\ \left(M \cdot s\right)^{-1}$, $k_{1r} = 1\ s^{-1}$ and $k_2 = 1.5\ s^{-1}$. These values depend on the reaction specifics.

In the Fig. 5 below it is represented the concentration-time variation of the enzyme **E**, substrate **S**, enzyme-substrate complex **ES** and product **P**. It can be seen that the initial degradation of the enzyme and substrate generates the enzyme-substrate complex, modelled in the Petri network from Fig. 3 as place p_1 with ODEs f_1. The maximal concentration value of the enzyme-substrate complex corresponds to the minimal concentration value of the enzyme, which corresponds to the enabling function e_1.

This is the moment when the transition t_1 is fired in conformance with the junction function j_1. After this point the concentration value of the enzyme increases before setting to its steady state and the concentration value of the enzyme-product complex decreases exponentially to zero, modelled in the Petri network from Fig. 3 as place p_2 and ODE f_2. The occurrence of the enzyme-substrate complex **ES** boosts the increase of the concentration value for the product **P**.

The partitioning of the plot from Fig. 5 captures the Petri net modelling of the Michaelian enzyme kinetics in the p_1 place and in the p_2 place, presented in Fig. 3.

The concentration-time variation for first order irreversible reaction with $k_1 = 1,\ S(0) = 100, P(0) = 0$ is represented in Fig. 6 below. The threshold values are $threshold_1 = 60, threshold_2 = 40$.

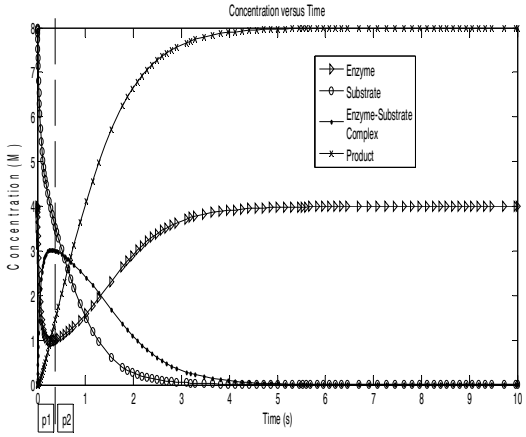

Fig. 5 Concentration-time variance of single substrate enzyme kinetics

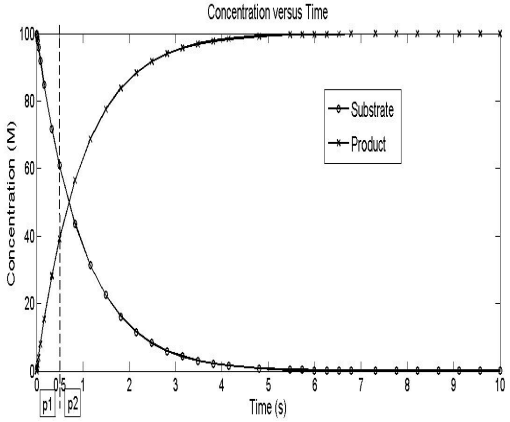

Fig. 6 Concentration-time variance of the first order irreversible reaction

The concentration-time variation for first order reversible reaction with $\boldsymbol{k_1 = 3},\ \boldsymbol{k_2 = 1},\ \boldsymbol{S(0) = 100, P(0) = 0}$ is represented in Fig. 7 below. The threshold values are $threshold_1 = 40, threshold_2 = 60$.

The concentration-time variation for the bimolecular reaction with $k_1 = 0.001,\ S_1(0) = 100,\ S_2(0) = 200, P(0) = 0$ is represented in Fig. 8 below. The threshold values are:

$threshold_1 = 155, threshold_2 = 55, threshold_3 = 45$.

Also, the partitioning of the plots from the above figures captures the Petri net modelling of the reactions kinetics in the p_1 place and in the p_2 place. The time moment for the models from Fig. 6 to Fig. 8 is taken in the vicinity of the intersection point of the product and substrate concentration values (P=S).

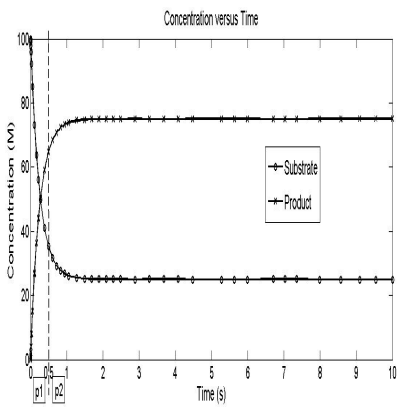

Fig. 7 Concentration-time variance of the first order reversible reaction

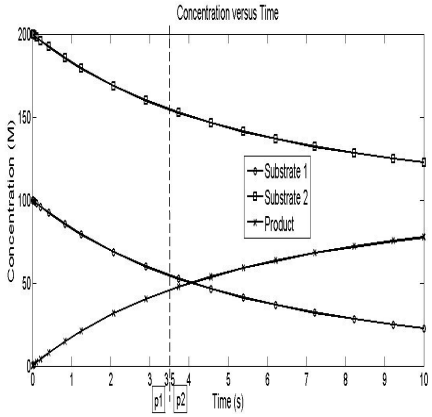

Fig. 8 Concentration-time variance of the bimolecular reaction

The biological meaning of the vicinity of the intersection point stresses that the amount of the product concentration **P** is starting to be more than the amount of the substrate concentration, so the product is beginning to play a biological meaning in the biological system. Until that moment, the product **P** does not play any important biological role, for the models from Fig. 5 to Fig. 9.

9. Conclusions and further work

The novelty of this Petri network model for enzyme kinetics is the approach of partitioning the ODEs to places, which holds actually the dynamic information of the biochemical reaction and the dependability assessment of the model.

The proposed Petri network model for enzyme kinetics may be used in metabolic signalling pathway modelling. The model may be extended in order to incorporate exceptions of the single substrate enzyme reactions, like cooperative binding of substrate to the active site in allosteric regulation, and also to incorporate multi-strata enzyme reactions like ternary complex or ping–pong.

The dependability of the proposed Petri network model for single substrate enzyme kinetics may be increased by incorporating fault tolerance mechanisms and ensuring more experimental results compliance. Also fractal approaches may be employed for modelling enzyme kinetics [18].

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