

General Methodology for Metabolic Pathways Modeling and Simulation using Discrete Event Approach. Example on glycolysis of Yeast.

T. Antoine-Santoni¹, F. Bernardi¹, F. Giamarchi¹

¹University of Corsica - UMR CNRS 6134 Quartier Grossetti, BP 52 - 20250 Corte - FRANCE

{antoine-santoni, bernardi, giamarchi}@univ-corse.fr

Abstract—In the Bioinformatics research domain, the systemics approach (considering a cell as a system) is growing up very quickly. Most of the significant current researches focus on mathematical formalisms. However, the discrete event simulation domain is so mature nowadays, that it can be considered as the pending of differential equations in the continuous models domain. In this paper, we introduce a general methodology using the DEVS formalism in the bioinformatics domain and to apply it to the modeling and simulation of a metabolic pathway, the glycolysis.

Index Terms—DEVS, Methodology, Bioinformatic, Glycolysis

I. INTRODUCTION

Defenition of Bioinformatic is much debated in the literature, and many definitions can be found. For [1], "Bioinformatics encompass computer applications in biological sciences", and for [2], "Bioinformatics is conceptualizing biology in terms of macromolecules and then applying informatics techniques to understand and organize the information associated with these molecules, on a large scale". The definition we find most expressive is from the NCBI web site (The National Center for Biotechnology Information): "Bioinformatics is the field of science in which biology, computer science, and information technology merge into a single discipline". Modeling and simulation in bioinformatics are currently involved essentially in metabolomics and systeomics to study the essential biochemical processes like cellular signaling or metabolic pathways. These processes are usually analyzed using continuous differential equations or discrete event simulation.

To understand the functioning of metabolic pathways on the living cell level, we need to describe the behavior of this complex system. Representing metabolic pathway can be achieved using different formalisms. Two popular formalisms are the Π -calculus and Petri networks. They use some graphical representations to model cell behaviour and reaction. Recently, the DEVS discrete event formalism has been used in [3] to model an enzyme, the tryptophan synthetase or global metabolic pathway in [4]. DEVS, created by Zeigler, is a modular formalism for deterministic and causal systems. It allows a component-based approach for the design of complex systems. The hierarchical nature of DEVS makes it ideal for describing a system like metabolic pathways. Recent works

proves that modelling the metabolic pathway aspect is very important. A large number of metabolic pathways exists in a living cell and these particular systems play an important role. The discrete-event nature improves the execution performance of a model like this, due to the asynchronous nature of the events occurring in the cell [4]. However, defining a general methodology of modeling and simulation using an discrete event approach is very essential.

This paper is structured as follows: Section II includes a survey of the most popular formalisms in the metabolic pathway modelling field and a discussion on several advantages and disadvantages of the different approaches. Section III introduces the Discrete Event System Specification. Section IV describes the general methodology and our approach for the modeling of a metabolic pathway. Section V uses our DEVS-based methodology on the studied case: the glycolysis of the yeast. Different model of components of metabolic pathways are presented. Section VI presents the simulation aspects and their results. Section VII concludes this paper with an outlook on future research activities.

A. Related Works

1) *The Π -calculus*: The Π -calculus has been developed by Robin Miller as a formal language for concurrent computational processes. The syntax of the Π -calculus lets us represent processes, parallel composition of processes, synchronous communication between processes through channels, creation of fresh channels, replication of processes, and non-determinism. A process is an abstraction of an independent thread of control. A channel is an abstraction of the communication link between two processes. Processes interact with each other by sending and receiving messages over channels.

Let P and Q denote processes. Then

- $P \mid Q$ denotes a process composed of P and Q running in parallel.
- $a(x).P$ denotes a process that waits to read a value x from the channel a and then, having received it, behaves like P.
- $(x).P$ denotes a process that first waits to send the value x along the channel a and then, after x has been accepted by some input process, behaves like P.
- $(\nu a) P$ ensures that a is a fresh channel in P.

- $!P$ denotes an infinite number of copies of P , all running in parallel.
- $P + Q$ denotes a process that behaves like either P or Q .
- 0 denotes the inert process that does nothing.

The Π -calculus is a model of concurrent communicating processes based on the notion of naming. The Π -calculus can describe message passing in networks with the evolving topologies. Traditionally applied to computer systems, it was recently used to describe biochemical systems. Regev uses the Π -calculus to define the behaviour of the biochemical pathways [5]. The main contribution is to give the qualitative aspects of biochemical pathways [6].

This work is completed by the use of stochastic modeling as in [7] and [8], allowing interpreting an activity in observing the effects on its environment. The stochastic modeling allows to product either calculus or, by learning the behaviours, to carry out a task. These models allow describing the evolution state of a stochastic system according to the actions which are carried out.

2) *Petri Net*: One of the most current approaches in the Bioinformatics modeling field is the use of Petri Nets developed in the early 1960's. Petri Nets is a graphical oriented language of design, specification, simulation and verification of systems. This graphical formalism was used for the first time in the biologic systems by Reddy [9] and it is more and more used nowadays. ([10], [11], [12] give the overview of this domain research). Chen and Freier [13] show that Petri Nets allow to carry out a quantitative analyze of metabolic networks in addition to metabolic pathways to show their dynamic behaviour. Another approach, extension of this previous work, called, Hybrid Petri nets, has been developed.

The simplest kind of Petri Net is a bipartite digraph, i.e. a graph with two types of node and directed arcs which connect nodes of different types, as shown in Figure 1.

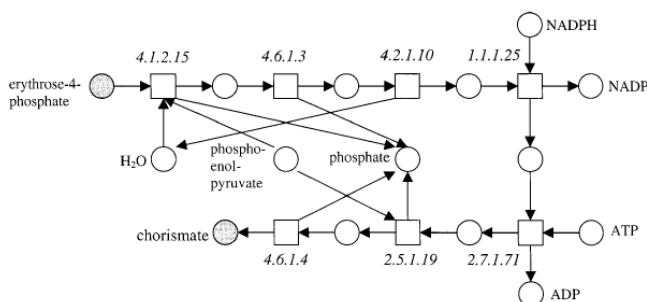


Fig. 1. A Petri Net representation of the shikimate pathway, a metabolic pathway from erythrose 4-phosphate to chorismate

The two types of node are called place nodes, represented as circles, and transition nodes, represented as boxes; hence this type of network is also known as a place-transition network. The arcs may be labelled with an integer weight, but unlabelled are assumed to have a weight equal to 1. Places may be marked by an integer number of tokens. The overall state of a system of n places is represented by a vector of size n consisting of the markings on each place. Edges connected to a transition node define sets of input places and output places for that transition. In a simple Petri Net, a transition

is enabled if all of its input places have a marking equal to or greater than the weight of the arc connecting that place to the transition. When a transition is enabled, it may be fired to remove a number of tokens from each input place equal to the weight of the connecting input arc, and create a number of new tokens at each output place equal to the weight of the connecting output arc. On Figure 1 (issued from [12]), Place nodes (circles) represent metabolites whereas transition nodes (boxes) represent reactions. Reactions are labelled by their EC numbers.

3) *Advantages and Disadvantages of the different approaches*: These formalisms present a user-friendly aspect since they use a graphical aspect understandable by a non-specialist. Petri Nets are proposed for the representation of the metabolism knowledge and for the dynamic behaviour simulation of reactions networks. They are presented like a good alternative to systems based on differential equations. The first advantage of the Π -calculus is to allow the describing of systems with mobile processes, i.e. systems which process numbers and communications links can vary in the course of time. It makes it possible to establish evidence of equivalence between models of distributed systems and to integrate the concept of locality and migrations during the calculations. The models using the Π -calculus formalisms are mathematically well defined as long as one remains faithful and transparent at the biological level. However, the complexity and the importance of biomolecular processes impose variations of Π -calculus very complex and difficult to implement.

Petri networks present a major disadvantage which can be associated to the concept of conflict, when a transition has a common entry, and the effective conflict, when a marking sensitizes two transitions.

The mathematical formalisms (Π -calculus, Bayesian networks and Boolean networks) allow a remarkable precision in the results that makes more comprehensible the studied system; the drafting of the evolution equations of a system makes it possible to analyse and underline some dysfunctions. The problem lies in the fact that the class of the models which one knows, in the current state of knowledge, to solve in an exact way is too small. Thus restrictive assumptions, approximations or terminals on values of measurements must be carried out.

II. DEVS FORMALISM

Based on systems theory, DEVS formalism was introduced by Professor B.P. Zeigler in the late 70s [14], [15]. It allows a hierarchical and modular way to model the discrete event systems. A system (or model) is called modular if it possesses the input and output ports permitting interaction with its outside environment. In DEVS, a model is seen as a "black box" S which receives and broadcasts messages on its input and output ports. DEVS defines two kinds of models: atomic models and coupled models, representing respectively the behavior and the internal structure of a part of a model.

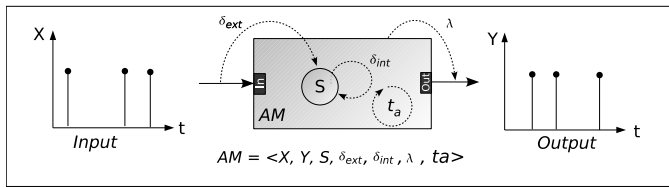


Fig. 2. DEVS atomic model

Figure 2 represents an AM atomic model with its output data Y calculated according to input data X . The AM atomic model has a state variable S that can be reached during the simulation. The functions δ_{ext} , λ , δ_{int} and t_a respectively allow the model's change of state when an external event occurs on one of those outputs (external transition function), the disposal of the output Y (output function), the model's change of state after having given an output (internal transition function) and finally the determination of the duration of the model's state (time advance function).

The coupled models are defined by a set of sub-models (atomic and/or coupled) and express the internal structure of the system's sub-parts thanks to the coupling definition between the sub-models.

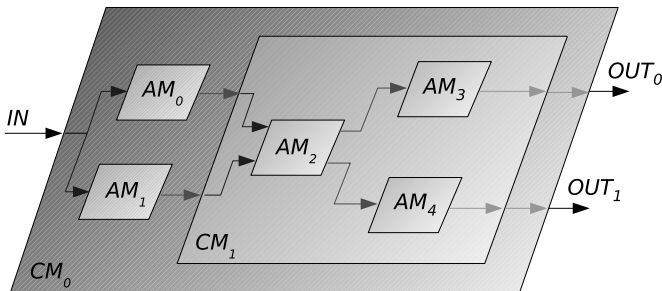


Fig. 3. DEVS coupled model

Figure 3 shows an example of the hierarchical structure of coupled model CM_0 which has an input port IN and two output ports OUT_0 and OUT_1 . It contains the atomic sub-models AM_0 , AM_1 and also the coupled model CM_1 . The latter can encapsulate other models such as atomic models AM_2 , AM_3 and AM_4 . A coupled model is specified through the list of its components (AM_0 , AM_1 , AM_2 , AM_3 , AM_4 and CM_1), the list of its internal couplings ($AM_0 \rightarrow CM_1$ and $AM_1 \rightarrow CM_1$), the list of the external input couplings ($IN \rightarrow AM_0$ and $IN \rightarrow AM_1$), the list of the external output couplings ($CM_1 \rightarrow OUT_0$ and $CM_1 \rightarrow OUT_1$) and the list of the sub-model's influence ($CM_1 = \{AM_0, AM_1\}$ or CM_1 and influenced by AM_0 and AM_1).

DEVS formalism is mainly used for the description of discrete event systems. It constitutes a powerful modeling and simulation tool permitting a system modeling on several levels of description as well as the definition of the models' behaviors. One of DEVS formalism's important properties is that it automatically provides a simulator for each model. DEVS establishes a distinction between a system modeling and a system simulation so as any model can be simulated without the need for a specific simulator to be implemented. Each atomic model is associated with a simulator in charge

of managing the component's behavior and each coupled model is associated with a coordinator in charge of the time synchronization of underlying components.

The DynDEVS formalism, a DEVS extension [3] has been used for the study of the Tryptophan synthetase, an enzyme catalyzing the L-Tryptophan synthesis. Based on a continuous model of the tryptophansynthetase, a discrete model has been developed. This model defines this enzyme like a discrete event model, with the states and the discrete event releasing the states transitions. Then, this model is coupled with the model which describes the dynamic of the metabolites in the cytoplasm.

III. GENERAL METHODOLOGY OF MODELING OF METABOLIC PATHWAYS

In the following part, we detail our general modeling methodology.

A. Analytical phase: identification of the essential actors

We can divide a metabolic way in 4 principal actors: substrate, enzyme, product, reaction. We define here 4 principal groups according to their actions and their respective constitutions, but from which we determine sub-groups as follows:

1. Substrate: starting molecule, molecule becoming again Co-substrate, Co-substrate.
2. Enzyme: as many groups as there are enzymes to model in the way metabolic
3. Reaction: 3 important reactions exist: the fixing of the enzyme and the substrate (fixing), the transformation of the substrat into product (transformation) and release of the product (release);
4. Product: there will be two destinies for the product in a metabolic way: either it is the final product and it will leave the metabolic pathway, or this product will be re-used in the way and will become again a substrate in the system.

The essential concept of our modeling approach is the following relation:



where:

- $[S]$ is the concentration in substrate ,
- $[E]$ is the concentration in enzyme ,
- $[P]$ is the concentration in product .

The factors that will be taken into account are:

1. The speed, which will be a function of the concentrations in enzyme and substrate. Indeed, the release of the events will be done according to these reaction speeds.
2. Enzymatic characteristics: the maximum speed of reaction V and the constant K_m , the hierarchy of the reactions: the successive order of the events which intervene in the catabolism or the anabolism of the studied way.

B. Synthetic phase: the table of identification

The identification of the actors of the metabolic way is carried out by a list indexing all the elements to be modeled according to 4 great groups. This list, called a table of

identification, as shown on I, is evolutionary and can vary according to information which one has at one precise moment and the elements that one wants to use in modeling; its rules of construction are as follows:

1. A compound is classified among the 4 great groups (naturally a reaction is not a real entity in a strict sense of the term, but we will see thereafter the utility to consider it this way);

2. Some characteristics are assigned: Co-substrate, true substrate, molecule starting, molecules intermediate, final product, enzyme, etc.;

3. The possible roles and actions of actors are defined;

4. A passage order is established with the aim of defining a hierarchy in the future modeling;

5. More information on each actor of the way metabolic are added. Let us note that it is important to reveal the structure of substrates and products in chemical formula because that will be useful for modeling.

C. Some precise detail

One of the important points of our approach is to understand that we consider a metabolic way as a system. Indeed, it can appear aberrant for a biologist to want to separate the concept of enzyme and the concept of reaction. However this distinction is essential in our approach for two reasons:

- First of all, because it would be necessary, in the contrary case, to define for each enzyme its specific characteristics with respect to each substrate, which would make the modeling almost impossible.
- Second, if this distinction is not carried out, our position is on a lower level of abstraction, i.e. the enzyme (it isn't the goal). Our modeling will thus be carried out on the level of the metabolic way in its globality. In our approach, each class (each actor) will represent a coupled model DEVS. Each object of the class will be associated with an atomic model using the characteristics of the table of identification.

IV. APPLICATION ON THE METABOLIC PATHWAY, GLYCOLYSIS

A. The Glycolysis

Glycolysis as shown Figure 4 on proceeds in the cytoplasm of practically all the organizations and cells, which they are aerobic or anaerobic. Its assessment is simple : a molecule of glucose will be divided into two molecules of pyruvate. under anaerobic conditions, the pyruvate is then transformed to regenerate of NAD⁺ ; it is thus formed products of fermentation like lactate and ethanol. Under conditions, glycolysis is the only possibility leading to the synthesis of ATP (the largest stock of chemical energy) starting from ADP and inorganic phosphate.

B. Specificities of glycolysis

We know that we have 10 enzymes for this metabolic way. However, only 9 of them will have as a role to generate only one product. Indeed, the aldolase, the fourth enzyme will

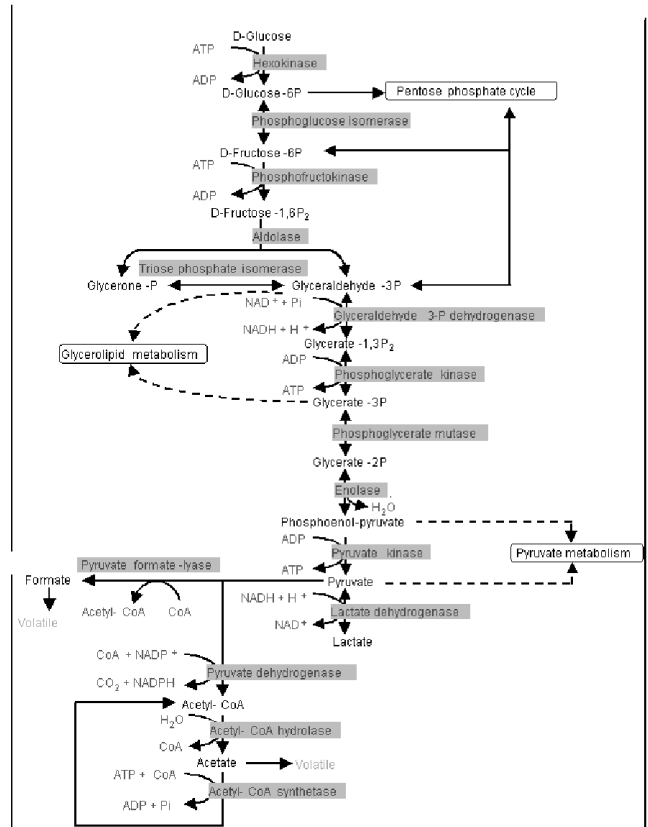


Fig. 4. Glycolysis

produce in fact two products; we define a particular atomic model (reusable) which provides two products to different times. In addition, glycolysis consists of 10 reactions and three stages of control which are controlled by enzymes with an allosteric behavior.

This problem is solved by the use of results of experiments in [16] carried out on the glycolysis of yeast, which enable us to have the concentrations in substrates and products, but also of all the characteristics of the enzymes acting in glycolysis. This enables us to be able to assign to all our models, describing the enzymes, the data necessary to be able to carry out their action.

Two other aspects enter in account: concept of michaelian enzymes and allosteric enzymes. Indeed, a michaelian enzyme won't have the same behavior as an allosteric one, this last requiring a co-substrate to function. We propose to solve this problem by using an atomic model DEVS, named Co-Substrate, which provides to the non-michaelian enzymes, the cosubstrates necessary to their actions (ATP, ADP, NAD⁺). In this manner, the calculation of time does not require the recourse of the differential equations.

C. Modeling aspects

According to the concepts of our approach of modeling, we define three coupled models (Substrate, Reaction, Enzyme), all composed only by of atomic models. We also define an atomic model Product that should have been a coupled model

| Name | Type | [X] | Characteristic | PassageOrder | Other essential characteristic |
|----------------|-----------|-----|----------------|--------------|--------------------------------|
| Glucose | Substrate | 100 | C6H12O6 | 1rst | |
| Aldolase | Enzym | 53 | 4.1.2.13 | 4st | Vmax = 0.12 |
| Transgluco-G6P | Reaction | "" | Need ATP | 1rst | |

TABLE I

SHORT EXAMPLE OF IDENTIFICATION TABLE

according to our approach, but since it is made up only of one very simple atomic model, we choose to define it as an atomic model.

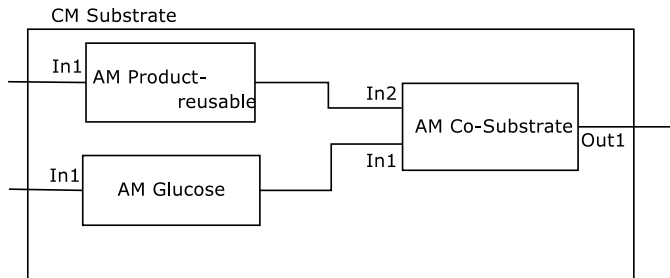


Fig. 5. The Substrate CM

The first atomic model of this coupled model is the starting molecule of the metabolic way (AM Glucose) as shown on Figure 5. The input port of this model corresponds to the data of the molecule's concentration of the corresponding in the metabolic way, while the output port is coupled to AM Co-substrate which represents the factors necessary in the metabolic reactions (these factors will not influence the reaction speed, but are necessary so that the reaction occurs).

Let us note that the state S of this model is placed in mode off until the end of modeling once this one launched.

The second model is called Product-reusable and corresponds to the products resulting from a reaction and which will be re-used like substrate in the way. Let us specify that this model is a model of management and that it does not rise from the Product class. This model will be under operation during almost all the simulation of the model. Its output port is coupled to AM Co-Substrate which will provide necessary information according to data reaching it.

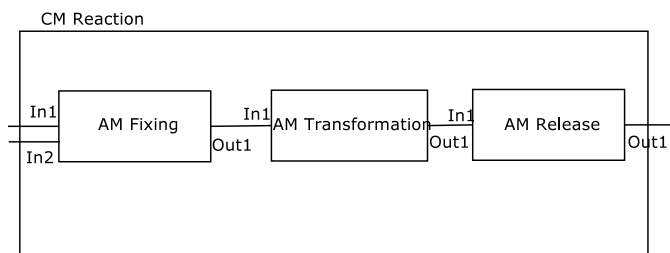


Fig. 6. Reaction Coupled Model

Each one of the three types of reactions previously identified (Fixing, Transformation and Release) will be represented by a different atomic model as shown on Figure 6.

AM Fixing comprises two input ports: a first for the start molecule and the intermediate molecules and a second for "the entry" of the enzyme. This model is used as junction between

the substrate and the enzyme, confirming our point of view different from the one commonly adopted in biology. The role of this model is thus to receive the substrate, ready to be transformed, and to select the necessary enzyme by sending a message towards the CM Enzym.

Once this selection carried out, the substrate-enzyme association is sent towards the AM Transformation in which the reaction will be modelled and the time will be calculated. Finally, the AM Release simply sends the product towards the CM Product.

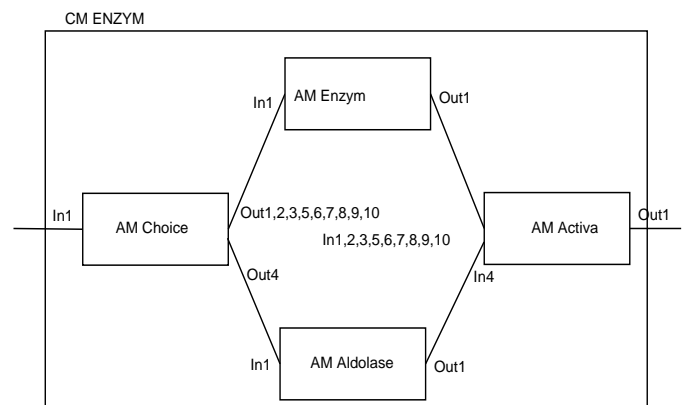


Fig. 7. Enzym Coupled Model

The coupled model illustrated on Figure 3 comprises three types of atomic models. The AM Choice allows the analysis of information coming from the model coupled Reaction (concept of specificity). From this information, this model will select the corresponding enzyme. Information concerning this selected enzyme are sent by the AM Activation towards CM Reaction. The number of enzymes is specified in this coupled model. For each input in the table of identification an atomic model presenting a an input port coupled with the AM Choice and an output port coupled to the AM Activation will be specified.

This very simple model receives the product and tests it. If it is a final product, it leaves the way and, on the contrary case, it is redirected towards the CM Substrate.

These 4 models are coupled between them as illustrated on Figure 8. The CM Substrate has 2 input ports: in1 comes from the messages generator and in2 coming from CM Produced. It has an output port out1 transporting the data of the first molecule and the intermediate substrates towards the CM Reaction. This last thus receives two input ports, in1 coupled with CM Substrate and in2 corresponding to the entry of the enzyme on an input port for the selection and an output port for the coupling Enzyme-Substrate.

Finally, the CM Product has an input port for the arrival of the product coming from CM Reaction and two output ports, out1 for the final products towards the exterior of the way,

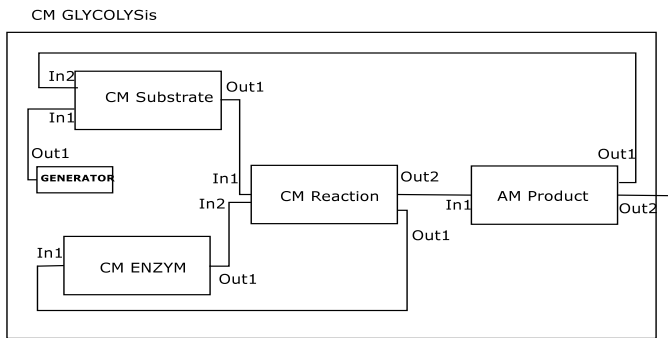


Fig. 8. Glycolysis Coupled Model : definition of the coupling

and out2 towards CM Substrate for the products which will become again substrate in the metabolic pathway.

D. Simulation and Results

For this simulation, we make the choice to use a DEVS simulator written in Python [17].

Results we obtained, presented in the TABLE II and TABLE III show the validity of our approach:

- The phenomenon of catabolism and the succession of the stages of glycolysis are perfectly modeled;
- The enzymatic phenomenon of specificity is reproduced;
- The role of the Aldolase which has a particular function compared to the other enzymes, is highlighted at the time of simulation;
- Time corresponding to reaction speeds appear with each transformation of a substrate into product and are expressed in second.

We bring a precision on reaction speeds. We do not have reference values for the reaction times, however these values are calculated according to biochemical equations. We also see, on the TABLE II and TABLE III, two cycles from simulation. These two cycles represent the two molecules synthesized by the aldolase, which go each one realized a cycle in glycolysis.

Figure 9 presents an example of simulation. The simulation time appears clearly. This example shows how time changes of value. Time value changes when substrate is in AM Trans which corresponds to a substrate-product transformation in the cell.

```

***** CLOCK: 0.000000

INTERNAL TRANSITION: A2 (Fix)
New State: None
New State: None
Output Port Configuration:
port0: < molecule = Glucose, structure = C66012H, concent =
100.000000, = ATP, caract= start molecule, enzym = 0, konste
= 0.000000, vmax = 0.000000, nbre = 1 >
Next scheduled internal transition at time 1000000.000000
port1: None
Next scheduled internal transition at time 1000000.000000

EXTERNAL TRANSITION: A4 (Trans)
Input Port Configuration:
port0: < molecule = Glucose, structure = C66012H, concent =
100.000000, = ATP, caract= start molecule, enzym = 0, konste
= 0.000000, vmax = 0.000000, nbre = 1 >
New State: None
Next scheduled internal transition at time 0.124008
ROOT DEVS' OUTPUT PORT CONFIGURATION:
port0: None

***** CLOCK: 0.124008

INTERNAL TRANSITION: A4 (Trans)
New State: None
Output Port Configuration:
port0: < molecule = Gluco6P, structure = C606H11P, concent =
2.450000, need = , caract= 2nd molecule, enzym = Hexokinase
2.7.1.1, konste = 3.800000, vmax = 0.840000, nbre = 1 >
Next scheduled internal transition at time 1000000.124008

EXTERNAL TRANSITION: A3 (Lib)
Input Port Configuration:
port0: < molecule = Gluco6P, structure = C606H11P, concent =
2.450000, need = ATP, caract= 2nd molecule, enzym =
Hexokinase 2.7.1.1, konste = 3.800000, vmax = 0.840000, nbre
= 1 >
New State: None
Next scheduled internal transition at time 0.124008

ROOT DEVS' OUTPUT PORT CONFIGURATION:
port0: None

```

Fig. 9. Example of simulation

To represent time, we choose a graphical representation as shown on Figure 10. There are 13 reaction products representing 13 transformation steps of simulation. We can see on the TABLE II and TABLE III, 15 time values. Indeed, first step is the glucose and last step is the last pyruvate molecule on output port.

| Substrate | Product | Enzym | Co-substrate | Time |
|-----------------|-----------------|--|--------------|-------|
| Glucose | Gluc6P | Hexokinase 2.7.1.1 | ATP | 0,124 |
| Gluc6P | fruc6P | glucose-6-Phosphate isomerase 5.3.1.9 | | 0,136 |
| fruc6P | fruc1-6biP | 6-phosphofructokinase 2.7.1.11 | ATP | 0,467 |
| fruc1-6biP | acetoneP | aldolase | | 0,469 |
| acetoneP | Glycera3P | triose-phosphate-isomerase 5.3.1.1 | | 0,469 |
| Glycera3P | 1-3biPGlycerate | glyceraldehyde_3-phosphate-deshydrogenase 1.2.1.12 | NAD+ | 0,472 |
| 1-3biPGlycerate | 3PGlycerate | phosphoglycerate-kinase 2.7.2.3 | ADP | 0,486 |
| 3PGlycerate | 2PGlycerate | Phosphoglycerate-mutase 5.4.2.1 | | 0,487 |
| 2PGlycerate | Phosphoenol | phosphopyruvate-hydratase 4.2.1.11 | | 0,571 |
| Phosphoenol | pyruvate | pyruvatekinase 2.7.1.40 | ADP | 0,605 |

TABLE II

TABLE OF RESULTS AFTER THE FIRST CYCLE OF SIMULATION

| Substrate | Product | Enzym | CoSubstrate | Time |
|-----------------|-----------------|--|-------------|-------|
| Glycera3P | 1-3biPGlycerate | glyceraldehyde_3-phosphate-deshydrogenase 1.2.1.12 | NAD+ | 0,472 |
| 1-3biPGlycerate | 3PGlycerate | phosphoglycerate-kinase 2.7.2.3 | ADP | 0,486 |
| 3PGlycerate | 2PGlycerate | Phosphoglycerate-mutase 5.4.2.1 | | 0,487 |
| 2PGlycerate | Phosphoenol | phosphopyruvate-hydratase 4.2.1.11 | | 0,571 |
| Phosphoenol | pyruvate | pyruvatekinase 2.7.1.40 | ADP | 0,605 |

TABLE III

TABLE OF RESULTS AFTER THE SECOND SIMULATION CYCLE

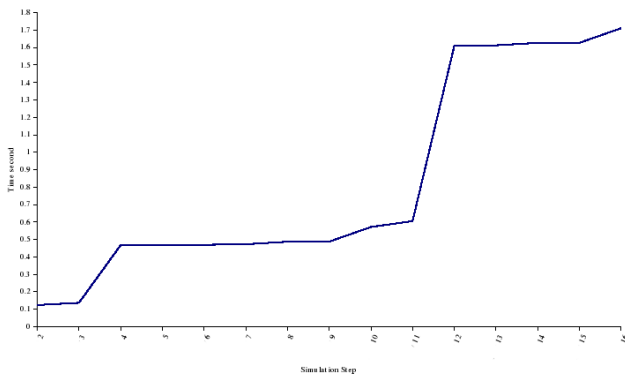


Fig. 10. Product formation as function step simulation

V. CONCLUSION

Our essential goal was to define a general methodology for the modeling of metabolic pathways. Using this method, we can create our various models and simulate them. Final results match our waiting. Indeed we obtained the representation of a catabolic way with its various stages. Enzymatic specificity is obviously distinguished during simulation and the roles of each enzyme during degradation appear clearly, as for example the aldolase which presented the biggest problem during modeling. It's difficult to distinguish between time reaction and time simulation but the reaction evolution is represented. However our methodology allows representing time evolution of reaction in time.

The principal perspective of this work is the development of a methodology for the automatic generation of models directly from biological specifications by using a methodology of meta-modeling.

REFERENCES

- [1] T. Attwood and D. Parry-Smith, *Introduction to Bioinformatics. Cell and Molecular Biology in Action*. 1999. Prentice Hall.
- [2] G. D. Luscombe, N. and M. Gerstein, "What is Bioinformatics? A Proposed Definition and Overview," *Methods in Medical Informatics*, vol. 40, pp. 346–358, 2001.
- [3] D. Degenring, M. Röhl, and A. Uhrmacher, "Discrete Event Multi-Level Simulation of Metabolite Channeling," *Elsevier Biosystems*, vol. (to appear), 2003.
- [4] W. G. Djafarzadeh, R. and M. T., "DEVS modeling and simulation of the cellular metabolism by mitochondria," in *Proceeding of the 2005 DEVS Integrative M and S Symposium, Spring Simulation Conference*, pp. 55–62, 2005.
- [5] A. Regev, W. Silverman, and E. Shapiro, "Representation and Simulation of Biochemical Processes using the pi-Calculus Process Algebra," in *Proceedings of the 6th Pacific Symposium on Biocomputing* (World Scientific press, ed.), pp. 459–470, 2001.
- [6] F. Tang, "Applications of the Pi-Calculus in Biology," *Biology Journal Club*, vol. 20, no. 1, 2003.
- [7] C. Priami, "Stochastic pi-Calculus," *Computer Journal*, vol. 38, no. 7, pp. 578–589, 1995.
- [8] C. Priami, A. Regev, E. Shapiro, and W. Silverman, "Application of a Stochastic Name-Passing Calculus to Representation and Simulation of Molecular Processes," *Information Processing Letters*, vol. 80, pp. 25–31, 2001.
- [9] V. Reddy, "Modeling Biological Pathways - A Discrete Event Systems Approach," 1994. M.Sc. Thesis, University of Maryland, Institute for Systems Research.
- [10] R. Höfstadt, "Petri Nets and the Simulation of Metabolic Networks," *In Silico Biology*, vol. 3, no. 3, pp. 321–322, 2003.
- [11] H. Matsuno, Y. Tanaka, H. Aoshima, A. Doi, M. Matsui, and S. Miyano, "Biopathways Representation and Simulation on Hybrid Functional Petri Net," *In Silico Biology*, vol. 3, no. 32, pp. 389–404, 2003.
- [12] J. Pinney, D. Westhead, and G. McConkey, "Petri Net Representations in Systems Biology," *Biochemical Society Transactions*, vol. 31, no. 6, pp. 1513–1515, 2003.
- [13] M. Chen and A. Freier, "Petri Net Based Modelling and Simulation of Metabolic Networks in the Cell," in *Proceedings of the 2002 Bioinformatics Research and Education Workshop*, 2002. Hinxtion,UK.
- [14] B. P. Zeigler, *Theory of Modeling and Simulation*. Academic Press, 1976.
- [15] B. P. Zeigler, H. Praehofer, and T. G. Kim, *Theory of Modeling and Simulation, Second Edition*. Academic Press, 2000.
- [16] B. Teusink, J. passarge, C. Reijenga, E. Esgalhad, C. Van der Weijden, M. Schepper, M. Walsh, B. Bakker, K. Van Dam, H. westerholff, and J. Snoep, "Can yeast glycosylis be understood in terms of in vitro kinetics of the constituent enzymes. Testing biochemistry," *European Journal of Biochemistry*, vol. 267, no. 1, pp. 5313–5329, 2000.
- [17] J.-S. Bolduc and H. Vangheluwe, "pythonDEVS : A modeling and simulation package for classical hierarchal DEVS," in *Technical Report, MSDL, University of McGill*, 2001.