# DYNAMIC MODEL OF BIOCHEMICAL NETWORK OF *ZYMOMONAS MOBILIS* ADAPTATION FOR GLYCEROL CONVERSION INTO BIOETHANOL

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**Abstract**
One of the biodiesel production problems is occurrence of a significant quantity (about 10%) of the by-product – glycerol. This problem is offered to solve by adaptation of bacteria *Zymomonas mobilis*, which is notable for ethanol production facilities. To be able to process glycerine into ethanol using *Z.mobilis* bacteria, the bacteria must be modified. At the same time, computer modelling analysis is required to assess specific modification affectivity in interconnection with other processes in bacteria. Computer model results of simulated experiment to understand and predict that the cells and biological processes are essential to reduce the number of experiments. This in turn reduces the necessary financial resources and time, bio-medical biotechnology, pharmaceutical and environmental problems. The model describes conversion of glycerol into bioethanol in *Z.mobilis* bacterial cell. First phase of model creation is creation of a structure model based on biochemical reactions using computer software CellDesigner. On the second phase of model creation, kinetic parameters which are available in literature were identified. Using the databases KEGG, SABIO-RK, BRENDA, reactants, kinetic parameters and reaction equation types were defined. Dynamic model of *Z. mobilis* biochemical network was created using computer software COPASI. The dynamic model describes conversion of glycerol into bioethanol in *Z.mobilis* bacterial cell. In this time simulation data of the computer model of natural organisms are not to confirm laboratory experimental data. Simulation data of the computer model are not correct, to prevent this problem is required parameter estimation in computer software COPASI.
**Key words:** Computer modelling, *Zymomonas mobilis*, ethanol.

## Introduction

*Z mobilis* is undoubtedly one of the unique micro-bacteria in the world. It is known since 1912 as *Termobacterium mobilis*, *Pseudomonas Linder*, and finally as *Z.mobilis*. The first reviewing of their uniqueness was published in 1977 and 1988. *Z.mobilis* features manifest not only in biochemistry but also in growth, energy production, and response to the growing conditions. These features have caused great interest in science, biotechnology, and industrial areas. *Z.mobilis* is a bacterium which is notable for ethanol production facilities.

In the biodiesel production, washing process removes all water-soluble contaminants (methyl alcohol, glycerol, phosphates, etc.). One of the biodiesel production problems is generation of a significant amount (10%) of the by-product – glycerol. In order to process the glycerine into bioethanol using *Z.mobilis* bacteria, it must be modified. Biological experiments have revealed that by expressing bacterium *E. coli GlpF* and *GlpK* genes bacteria *Z.mobilis* is capable for processing glycerol into ethanol.

Biochemical reactions and process regulation network (Odzina et al., 2010) are too complicated to be able to predict the system's response without extensive computer modelling after changing any of its components. The aim of our research was to create a computer model of biological process by making simulation and analysing the results.

First phase of model creation was creation of the structure model based on biochemical reactions using computer software CellDesigner (Odziņa et al., 2010). On the second phase of model creation is stoichiometric analyses using computer software MatLab COBRA toolbox (Odzina et al., 2011), and then on the third step creating the dynamic model where kinetic parameters available in the literature are identified. Using the databases KEGG, SABIO-RK, BRENDA, reactants, kinetic parameters and reaction equation types were defined.

The dynamic model has characterized the conversion of glycerol into bioethanol inside the cell of bacteria *Z.mobilis*. The dynamic model has 22 reactions with 26 metabolites. The reactions are irreversible and reversible, and they work according to adapted Michaelis-Menten mechanism. The equation contains rate of reaction (*Vmax*), affinity constant (*Km*), and concentrations of metabolites. The problem is that databases do not feature all kinetic parameters suitable for bacteria *Z. Mobilis*. Therefore many kinetic parameters were taken from other organisms (as *E.Coli, S.cerevisiae*) whose structure resembles *Z. Mobilis*. Parameter estimation is performed to fit the model behavior with the one of laboratory experiments.

Computer model of *Z.mobilis* biochemical network was created using computer software COPASI (4.8.35.).

## Materials and Methods

COPASI is a software application for simulation and analysis of biochemical networks and their dynamics. COPASI is a stand-alone program that supports models in the SBML standard and can simulate their behavior using ODEs or Gillespie's stochastic simulation algorithm; arbitrary discrete

events can be included in such simulations. COPASI carries out several analyses of the network and its dynamics and has extensive support for parameter estimation and optimization. COPASI provides means to visualize data in customizable plots, histograms and animations of network diagrams (http://www.copasi.org/tiki-index.php?page=OD.Events&structure=OD).

Systems Biology Markup Language (SBML) is a modular language, with a core comprising a complete format that can be used alone. SBML is oriented towards representing biochemical networks common in research on a number of topics, including cell signaling pathways, metabolic pathways, biochemical reactions, gene regulation, and many others. Broken down into its constituents, this model contains a number of components: reactant species, product species, reactions, rate laws, and parameters in the rate laws. To analyze or simulate this network, additional components must be made explicit, including compartments for the species, and units on the various quantities. The top level of an SBML model definition simply consists of lists of these components: beginning of model definition: list of unit definitions, list of compartments, list of species, list of reactions, list of parameters, list of rules; end of model definition (Hucka, 2003).

The dynamic model of modified organism describes second parameters: unit definition, compartment, species, reaction, parameters and events. The meaning of each component:

*Unit definition*: A name for a unit used in the expression of quantities in the model. The units are: time unit is second (s), volume unit is liter (l), quantity unit is micromole (µmol).

*Compartment*: A container of the finite volume for substances. In SBML Level 1, a compartment is primarily a topological structure with a volume but no geometric qualities. The dynamic model has two compartments: cellular and extracellular.

*Species*: A substance or entity that takes part in a reaction. The dynamic model has 26 species, of which 23 species are into cellular compartment and 3 species are into extracellular compartment.

*Reaction*: A statement describing some transformation, transport or binding process that can change the amount of one or more species. For example, a reaction may describe how certain entities (reactants) are transformed into certain other entities (products). Reactions have associated rate laws describing how quickly they take place. The dynamic model has 22 reactions, of which 13 reactions are irreversible and 9 are reversible. In the dynamic model, part of the reactions are taken from publication Mehmet M. Altintas 'Kinetic Modeling to optimize pentose Fermentation in *Zymomonas mobilis'* (Altintas et al., 2006). From the publication take reactions which characterized Entner Doudoroff patway. Reactions which are not included in the E. Doudoroff pathway were taken from database - SABIO-RK (http://sabio.villa-bosch.de/index2.jsp), and kinetic parameter from other databases – KEGG (http://www.genome.jp/kegg/) and BRENDA (http://www.brenda-enzymes.org/).

Each reaction is described by an equation. An equation types are in Table 1.

Table 1

**Equations of the dynamic model**

| Reactions type | Reactions | Equation |
|---|---|---|
| Irreversible – one substrate, one product<br>Irreversible – one substrate, two products | G2P -> PEP<br><br>KDPG -> GAP + PYR | $\frac{Vmax \cdot \frac{KDPG}{KmKDPG}}{1+\frac{KDPG}{KmKDPG}}$ |
| Irreversible – two substrates, two products | PEP + ADP -> PYR + ATP | $\frac{Vmax \cdot \frac{PEP}{KmPEP} \cdot \frac{ADP}{KmADP}}{1+\frac{PEP}{KmPEP}+\frac{ADP}{KmADP}+\frac{PEP \cdot ADP}{KmPEP \cdot KmADP}}$ |
| Reversible – one substrate, one product | DOAP = GAP | $\frac{\frac{Vmaxf \cdot DOAP}{KmDOAP}}{1+\frac{DOAP}{KmDOAP}+\frac{GAP}{KmGAP}} - \frac{\frac{Vmaxr \cdot GAP}{KmGAP}}{1+\frac{DOAP}{KmDOAP}+\frac{GAP}{KmGAP}}$ |
| Reversible – two substrates, two products | BPG + ADP = G3P + ATP | $\frac{Vmaxf \cdot \frac{ADP \cdot BPG}{KmADP \cdot KmBPG} - Vmaxr \cdot \frac{ATP \cdot G3P}{KmATP \cdot KmG3P}}{1+\frac{ADP}{KmADP}+\frac{BPG}{KmBPG}+\frac{ADP \cdot BPG}{KmADP \cdot KmBPG}+\frac{ATP}{KmATP}+\frac{G3P}{KmG3P}+\frac{ATP \cdot G3P}{KmATP \cdot KmG3P}}$ |

*Parameters*: A quantity that has a symbolic name. The dynamic model has 26 initial concentrations and 85 kinetic parameters: the equation contains rate of reaction (Vmax, Vmaxf, Vmaxr), affinity constant (KmS, KmP) and concentrations of metabolites (for example, ATP, ADP).

*Events*: which can be viewed as a discrete conditional state transition of the model, consist of two required parts: a trigger, which causes the event, and at least one assignment, which modifies the model (http://www.copasi.org/tiki-index.php?page=OD.Events&structure=OD). The dynamic model has one event: glucose impulse after 200 seconds.

**Results and Discussion**

In Table 2 describes reactions of the dynamic model:

One of the principles of the dynamic model transactions: the system must be a steady state. A system in a steady state has numerous properties that are unchanging in time. The concept of steady state has relevance in many fields, in particular thermodynamics and economics. Steady state is a more general situation than dynamic equilibrium. If a system is in steady state, then the recently observed behavior of the system will continue into the future. In stochastic systems, the probabilities that various states will be repeated will remain constant. In many systems, steady state is not achieved until some time has elapsed after the system is started or initiated. This initial situation is often identified as a transient state, start-up or warm-up period. While a dynamic equilibrium occurs when two or more reversible processes occur at the same rate, and such a system can be said to be in steady state, a system that is in steady state may not necessarily be in a state of dynamic equilibrium, because some of the processes involved are not reversible.

The dynamic model has a steady state. If the dynamic model has a steady state, the fallowing steps can be performed: model analyses, time course and parameter estimation and optimization.

Table 2

**Reactions list of the dynamic model**

| No | Reactions Name | Equation |
|---|---|---|
| 1 | GAPD Glyceraldehyde-3-P dehydrogenase 1.2.1.12 (7) | GAP + NAD + Pi = BPG + NADH |
| 2 | G3PK 3-phosphoglycerate kinase 2.7.2.3 (8) | BPG + ADP = G3P + ATP |
| 3 | GPM Phospoglycerate mutase 5.4.2.1 (9) | G3P -> G2P |
| 4 | ENO Enolase 4.2.1.11 (10) | G2P -> PEP |
| 5 | PYRK Pyruvate kinase 2.7.1.40 (11) | PEP + ADP -> PYR + ATP |
| 6 | PYRD Pyruvate decarboxylase 4.1.1.1 (12) | PYR -> ACET + CO2 |
| 7 | ADH Alcohol dehydrogenase 1.1.1.1 (13_1) | ACET + NADH = ETOH + NAD |
| 8 | Triose Phosphate isomerase 5.3.1.1 (17) | DOAP = GAP |
| 9 | Glycerol-3-P dehydrogenase 1.1.1.94 (16) | GP + NAD = DOAP + NADH |
| 10 | Glycerol kinase 2.7.1.30 (15) | GL + ATP = GP + ADP |
| 11 | Oxygen consumption | NADH -> NAD |
| 12 | ATP -dissipiation | ATP -> ADP + Pi |
| 13 | GK Glucokinase 2.7.1.2 (2) | GLUC + ATP -> GLUC6P + ADP |
| 14 | GPD Glucose-6-P dehydrogenase 1.1.1.49 (3) | GLUC6P + NAD -> PGL + NADH |
| 15 | PGLS 6 phospogluconolactonase 3.1.1.31 (4) | PGL -> PG |
| 16 | PGD 6-phosphogluconate dehydratase 4.2.1.12 (5) | PG -> KDPG |
| 17 | KDPGA 2-keto-3-deoxy-6-phosphogluconate aldolase 4.1.2.14 (6) | KDPG -> GAP + PYR |
| 18 | ADH II Alcohol dehydrogenase 1.1.1.1 (13_2) | ACET + NADH = ETOH + NAD |
| 19 | GF Glucose Fascilitator (1) | GLUCout = GLUC |
| 20 | Ethanol export (14) | ETOH = ETOHout |
| 21 | Glycerin transport | GLout -> GL |
| 22 | EthanolOut evaporate | ETOHout -> |

Results from time course plots:

Plot 1 'Concentrations, Volumes, and Global Quantity Values' (Fig. 1); (x axis – s (second), y axis - µmol $s^{-1}$). In this model accumulate product GAP and GLUC6P. This problem in model is currently being addressed with parameter estimation methods, but in this moment are not good results. Other product achieves stability after two thousand seconds. Other species has low flow. The model aims to produce ethanol from glycerol and here you can see that the ethanol is produced, but not sufficiently and model still needs to improve.

Plot 2 'Reactions fluxes' (Fig. 2) (x axis – s (second), y axis - µmol $s^{-1}$). All reaction fluxes achieve stability flux after two thousand seconds.

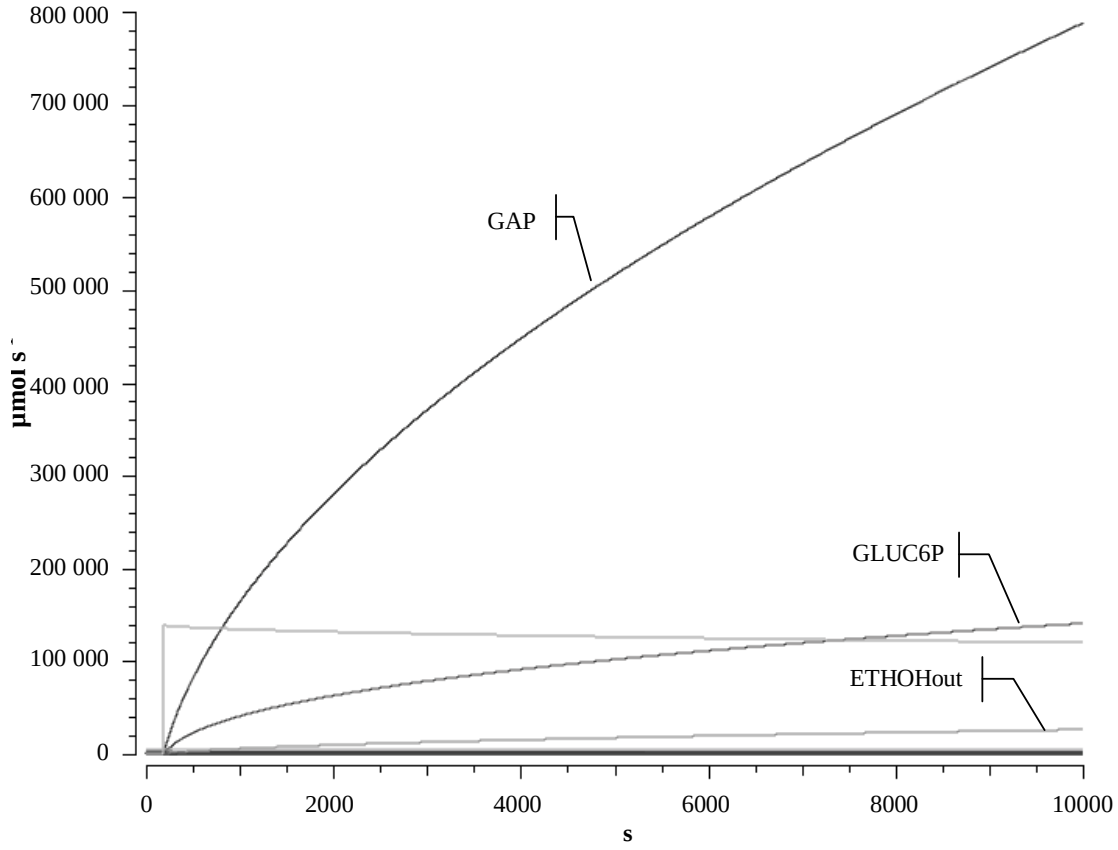


Figure 1. Concentrations, Volumes, and Global Quantity Values.

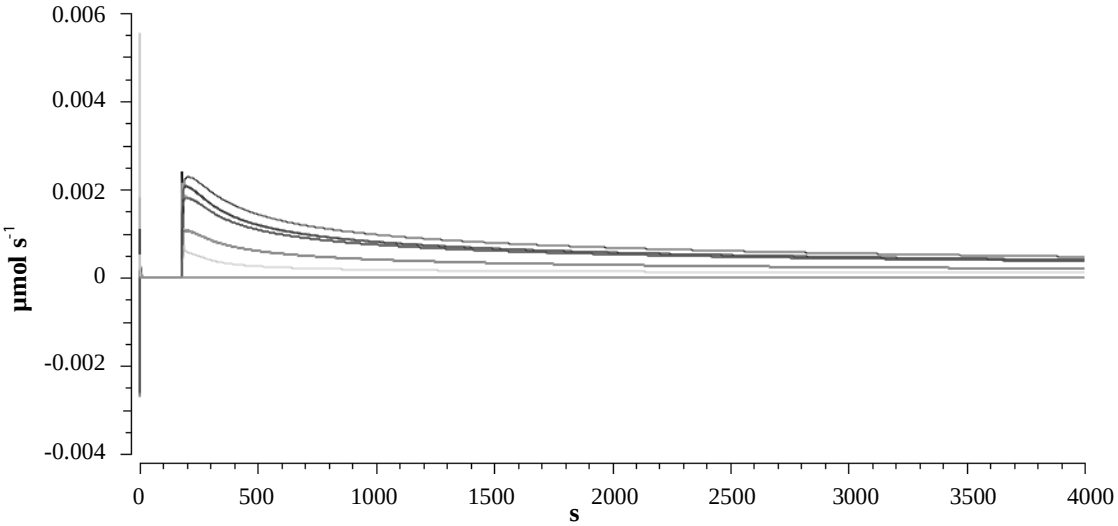


Figure 2. Reaction fluxes.

**Conclusions**

1. Results of simulated experiment to understand and predict that the cells and biological processes essential to reduce the number of laboratory experiments. Better results of the computer model simulation results can pruf in laboratory experiments. This in turn reduces the necessary financial resources and time, bio-medical biotechnology, pharmaceutical and environmental problems to be solved.
2. Programm Copasi is user friendly interface to create the dynamic model. The dynamic model has 22 reactions and 26 species.
3. The dynamic model describes conversion of glycerol into bioethanol in *Z.mobilis* bacterial cell. In this time simulation data of the computer model are not to confirm laboratory experimental data. Simulation data of the computer model are not correct, to prevent this problem is required parameter estimation in computer software COPASI.

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**References**

1. Altintas M.M., Eddy C.K., Zhang M., McMillan J.D., Kompala D.S. (2006) Kinetic Modeling to Optimize Pentose Fermentation in Zymomonas mobilis. Botechnology and Bioengineering Vol.94, No. 2, pp. 23.
2. BRENDA enzyms data base (2012) Available at: http://www.brenda-enzymes.org/, 10 October 2012.
3. Copasi User manual (2011) Available at: http://www.copasi.org/tiki-index.php?page=OD. Events&structure=OD, 10 March 2012.
4. Hucka M. (2003) The systems biology markup language (SBML): a medium for representation and exchange of biochemical network models. *Bioinformatics* 19, pp. 524–531.
5. KEGG Kyoto Encyclopedia of Genes and Genomes (1995-2012) Available at: http://www.genome.jp/kegg/, 10 October 2012.
6. Odziņa I., Rubina T., Rutkis R., Kalnenieks U., Stalidzans E. (2010) Structural Model of biochemical network of Zymomonas mobilis adaptation for glycerol conversion into bioethanol. 4-th international scientific conference "Applied Information and Communication Technologies 2010", Jelgava, LLU, CD, pp. 50–54.
7. Odziņa I., Rutkis R., Kalnenieks U. (2011) In silico engineering of biochemical network of *Zymomonas mobilis* adaption for Glycerol conversion into Bioethanol. 10th International Scientific Conference „Engineering for rural development", proceedings, Volume 10, Jelgava, LLU, pp. 272–275.
8. SABIO-RK - reactions, kinetic parameter (2012) Available at: http://sabio.villa-bosch.de/index2.jsp, 10 October 2012.