GENERALIZED NET MODEL OF *E. COLI* GLYCOLYSIS CONTROL

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Abstract: In this paper a generalized net (GN) model that represents the *E. coli* glycolysis control through influence of glucose concentration and oxygen availability to metabolic intermediates is presented. Based on the described GN model of *E. coli* glycolysis control it is shown that different concentrations can lead the system into several directions. Using the apparatus of Generalized nets the specific intercellular molecular interactions - in this case - one of the most important and well known pathways present in all cells - glycolysis can be easily and efficiently represented.
Keywords: Generalized nets, *Escherichia coli*, Glycolysis control.

1.   Introduction

Modern biotechnology heavily depends on modified bacterial species for production of many important proteins, used in many fields of live. One of these bacterial species is *E. coli*. Cultivation of recombinant micro-organisms, e.g. *E. coli*, in many cases is the only economical way to produce pharmaceutical biochemicals such as interleukins, insulin, interferons, enzymes and growth factors. *E. coli* is still the most important host organism for recombinant protein production. It has several advantages which include easy for growing conditions and well learned genomics.

One of the most important processes in the bacterial or any cell is the glycolysis. Glycolysis is in the centre of the cell metabolic activity, taking part in anabolism and catabolism as well. It provides the cell with energy in the form of ATP, hydrogen protons in the form of NADH+ and many intermediate metabolites. The control of such a process is a complex and interconnected mechanism. Glycolysis has several levels of control which are implemented according to the cell's needs. Very important are the quantity of glucose and the levels of oxygen in the surrounding environment. Depending on one of these two factors bacterial cell can be in 5 functional states [13] which influence its development. Presences of secondary products in the environment such as acetate also influence the cell's development inhibiting it [6, 8, 18]. In some cases the cell

might intake Acetate in order to replenish its energetic reserves. This can be done by reversing the reactions that led to acetate formation and reenter it in the TCA cycle.

In this paper the apparatus of generalized nets is used to describe the glycolysis control through influence of glucose concentration and oxygen availability to metabolic intermediates. This is a mechanism in which bacterial cell can switch metabolic flow depending on the outside factors such as feeding rate. The apparatus of generalized nets can easily and efficiently represent the specific intercellular molecular interactions.

Recently attempts have been made to describe various models, optimization procedures, control loops considering bioprocesses, based on Generalized nets [10, 11, 12, 14, 15, 16, 17]. The theory of the Generalized nets (GN) [2, 3, 5] proved to be quite successful when applied to the description of the functioning of expert systems, machine learning and different technological processes. Up to now GNs are used as a tool for modelling of parallel processes in several areas [1, 2, 3, 4, 5] - economics, transport, medicine, computer technologies etc. Already GN are used to model genetic networks [7, 9]. Genetic networks are an approximation mathematical model that is consistent with the contemporary knowledge on gene-gene interactions. As in all models various assumptions on the nature of such relations are made (whether they will be expressed as differential equations, probabilistic distributions, topological criterion, etc. is a choice predetermined by the structure of the data implemented in the model).

2. *E. coli* glycolysis control

Glycolysis is used by both aerobic and anaerobic organisms. Glycolysis in human and bacteria are almost identical with respect to the enzymes employed, but differ by their uptake mechanism of glucose into the cell and the end product under anaerobic conditions.

Glycolysis is the process of converting glucose into pyruvate and generating small quantities of ATP (energy) and NADH (reducing power). It is a central pathway that produces important precursor metabolites: six-carbon compounds of glucose-6P and fructose-6P and three-carbon compounds of glycerone-P, glyceraldehyde-3P, glycerate-3P, phosphoenolpyruvate, and pyruvate. Acetyl-CoA, another important precursor metabolite, is produced by oxidative decarboxylation of pyruvate. When the enzyme genes of this pathway are examined in completely sequenced genomes, the reaction steps of three-carbon compounds from glycerone-P to pyruvate form a conserved core module, which is found in almost all organisms and which often corresponds to operon structures in bacterial genomes. Gluconeogenesis is a synthesis pathway of glucose from noncarbohydrate precursors. It is essentially a reversal of glycolysis with minor variations of alternative paths.

Key biochemical pathways in *E. coli* involved in the aerobic consumption of glucose and the synthesis of acetate, carbon dioxide and biomass are presented in Fig. 1 [6]. *E. coli* consumes glucose principally by a phosphotransferase system (PTS), which simultaneously generates pyruvate from PEP. Acetate is formed from pyruvate by pyruvate oxidase and phosphotransacetylase/acetate kinase. PEP carboxylase is the principal 'anaplerotic' pathway needed to replenish TCA cycle intermediates consumed for biomass formation. Not all pathways are indicated.

Glucose enters the cell and it is transformed by several enzymatic reactions to CO_2 and NADH, which eventually is transformed into energy through Oxidation-Reduction Reactions.

Throughout these enzymatic reactions many intermediate metabolites are formed. These metabolites may be used by the cell to form other different metabolites such as amino acids. This leads to branching in the metabolism of the cell. Depending on the quantity of incoming glucose a process called Overflow metabolism can be observed. When the concentration of glucose is high the cell stops using the TCA cycle and acquires energy through different pathway. This pathway produces relatively small amount of energy and acetate which is excreted. Production of acetate can be induced also by the lack of oxygen. Several enzymes in the TCA cycle are strongly oxygen sensitive.
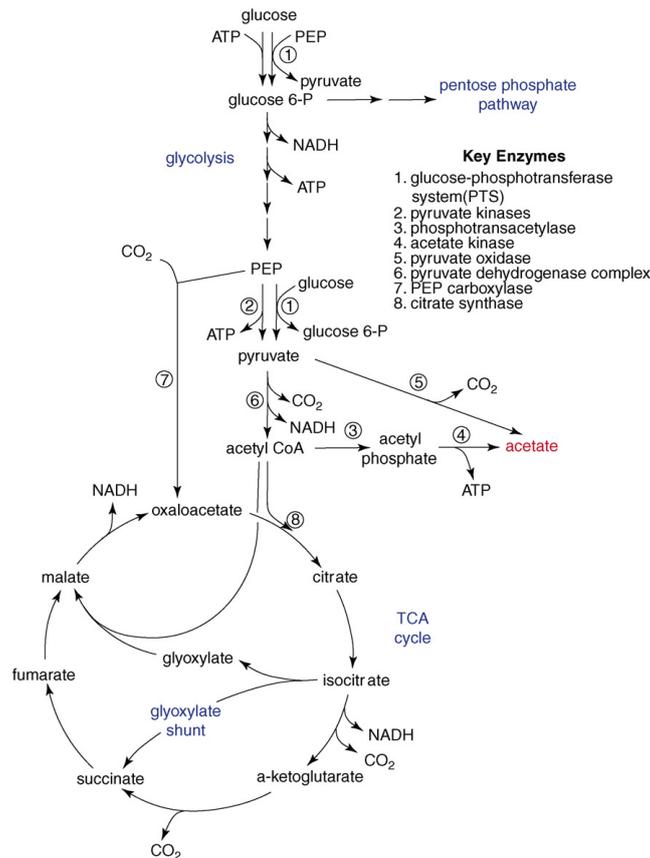

Figure 1: Key biochemical pathways in *Escherichia coli*

3. GN-model of *E. coli* glycolysis control

Initially, in places $l_1, l_2, l_3, l_4, l_7, l_{14}, l_{17}, l_{22}, l_{23}, l_{24}, l_{25}, l_{29}$, stay tokens α, β, γ, δ, ε, ν, η, θ, λ, ρ, φ, ω, with initial and current characteristics:
"Initial concentration of glucose",

"Initial concentration of ATP",
"Initial concentration of NAD^+",
"Initial concentration of $NADH$",
"Initial concentration of PEP",
"Initial concentration of Pyrovate",
"Initial concentration of Acetyl CoA",
"Initial concentration of Acetate",
"Initial concentration of CO_2",
"Initial concentration of Oxaloacetate",
"Initial concentration of O_2",
"Initial concentration of Ethanol".

The GN-model of *E. coli* glycolysis control is presented in Fig. 2.

Token α enters place l_1 with a characteristic "Concentration of glucose".

$$Z_1 = \langle \{l_1, l_2, l_{11}, l_{21}\}, \{l_2, l_6\}, \begin{array}{c|cc} & l_2 & l_6 \\ \hline l_1 & false & true \\ l_2 & true & true \\ l_{11} & true & W_1 \\ l_{21} & true & W_1 \end{array}, \wedge(l_1, l_2) \rangle.$$

where W_1 = "Aditional ATP molecules are needed".

In place l_6 the token obtains a characteristic "glucose transformation". Here glucose is activated and transformed into two identical tricarbon molecules.

The form of the second transition is:

$$Z_2 = \langle \{l_3, l_{30}\}, \{l_3, l_5\}, \begin{array}{c|cc} & l_3 & l_5 \\ \hline l_3 & true & W_2 \\ l_{30} & true & true \end{array}, \wedge(l_3) \rangle.$$

where W_2 = "Existance of token in place l_6 ".

In place l_5 the token obtains a characteristic "current concentration of NAD^+".

The form of the third transition is:

$$Z_3 = \langle \{l_4, l_{12}\}, \{l_4, l_8, l_9\}, \begin{array}{c|ccc} & l_4 & l_8 & l_9 \\ \hline l_4 & true & W_3 & true \\ l_{12} & true & W_3 & true \end{array}, \wedge(l_4) \rangle.$$

where W_3 = "No existance of token in place l_3 ".

In place l_8 the token obtains a characteristic "current concentration of $NADH$" and in place l_9 – a characteristic "Oxydation reduction phosphorylation".

The form of the fourth transition is:

$$Z_4 = \langle \{l_5, l_6, l_7\}, \{l_7, l_{10}, l_{11}, l_{12}, l_{13}\},$$

$$\begin{array}{c|ccccc} & l_7 & l_{10} & l_{11} & l_{12} & l_{13} \\ \hline l_5 & false & true & false & true & false \\ l_6 & true & true & false & false & false \\ l_7 & true & true & true & true & W_4 \end{array}, \wedge(l_5, l_6, l_7) \rangle.$$
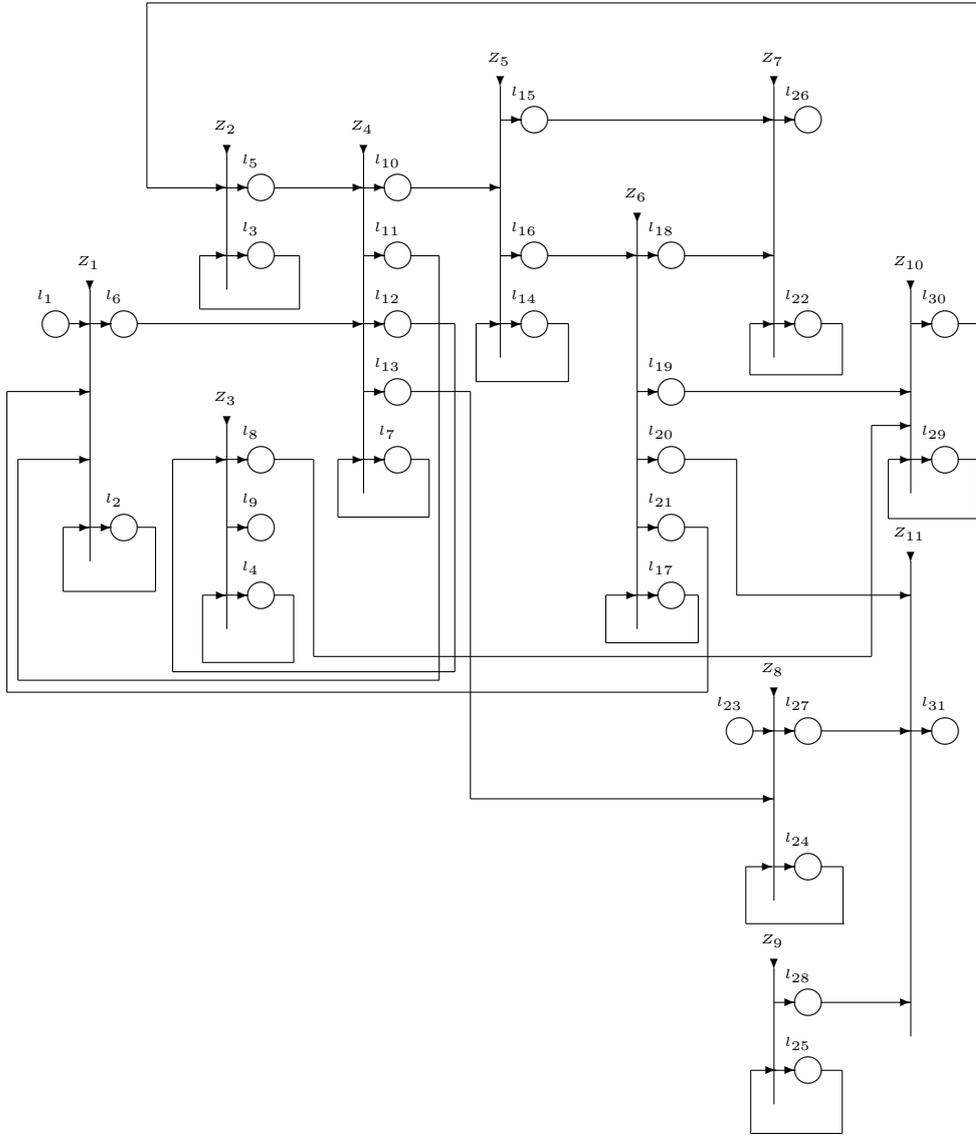
Figure 2: GN-model of *E. coli* glycolysis control

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where W_4 = "Low concentration of Oxaloacetate in place l_{24} ".

The tokens obtain the following characteristics:

in place l_{10} – "concentration of pyruvate";

in place l_{11} – "concentration of ATP";

in place l_{12} – "concentration of $NADH$";

in place l_{13} – "concentration of PEP".

The form of the fifth transition is:

$$Z_5 = \langle \{l_{10}, l_{14}\}, \{l_{14}, l_{15}, l_{16}\} \begin{array}{c|ccc} & l_{14} & l_{15} & l_{16} \\ \hline l_{10} & true & W_5 & W_6 \\ l_{14} & true & W_5 & W_6 \end{array}, \wedge(l_{10}, l_{14}) \rangle.$$

where

W_5 = "there is absence of O_2 concentartion in place l_{25}";

$W_6 = \neg W_5$.

The tokens obtain the following characteristics:

in place l_{15} – "concentration of acetate";

in place l_{16} – "concentration of Acetyl CoA".

In place l_{14} the token keeps the characteristics "concentration of pyruvate" from place l_{10}.

The form of the sixth transition is:

$$Z_6 = \langle \{l_{16}, l_{17}\}, \{l_{17}, l_{18}, l_{19}, l_{20}, l_{21}\}, \begin{array}{c|ccccc} & l_{17} & l_{18} & l_{19} & l_{20} & l_{21} \\ \hline l_{16} & true & W_5 & W_7 & W_8 & W_9 \\ l_{17} & true & W_5 & W_7 & W_8 & W_9 \end{array}, \wedge(l_{16}, l_{17}) \rangle.$$

W_7 = "Absence of NAD^+ concentartion in place l_3";

$W_8 = \neg W_7$;

W_9 = "Existance of token in place l_{18}".

The tokens obtain the following characteristics:

in place l_{19} – "concentration of ethanol";

in place l_{20} – "concentration of Acetyl CoA for TCA cycle".

The tokens keep the characteristics: in place l_{17} – "concentration of Acetyl CoA" from place l_{16};

in place l_{18} – "concentration of acetate" from place l_{15};

in place l_{21} – "concentration of ATP" from place l_{11}.

The form of the seventh transition is:

$$Z_7 = \langle \{l_{15}, l_{18}, l_{22}\}, \{l_{22}, l_{26}\}, \begin{array}{c|cc} & l_{22} & l_{26} \\ \hline l_{15} & true & true \\ l_{18} & true & true \\ l_{22} & true & true \end{array}, \wedge(l_{18}, l_{22}) \rangle.$$

In place l_{22} the token keeps the characteristics "concentration of acetate" from place l_{15}. In place l_{26} the token obtains a characteristics "concentration of excreted acetate".

The form of the eighth transition is:

$$Z_8 = \langle \{l_{13}, l_{23}, l_{24}\}, \{l_{24}, l_{27}\}, \begin{array}{c|cc} & l_{24} & l_{27} \\ \hline l_{13} & W_{10} & W_{10} \\ l_{23} & W_{10} & W_{10} \\ l_{24} & true & true \end{array}, \wedge(l_{13}, l_{23}, l_{24}) \rangle.$$

where $W_{10} = $ "Zero concentration in place l_{24} ".

In place l_{27} the token keeps the characteristics "concentration of Oxaloacetate" from place l_{24}.
The form of the ninth transition is:

$$Z_9 = \langle \{l_{25}\}, \{l_{25}, l_{28}\}, \begin{array}{c|cc} & l_{25} & l_{28} \\ \hline l_{25} & true & true \end{array}, \wedge(l_{25}) \rangle.$$

In place l_{28} the token keeps the characteristics "concentration of O_2" from place l_{25}.
The form of the tenth transition is:

$$Z_{10} = \langle \{l_8, l_{19}, l_{29}\}, \{l_{29}, l_{30}\}, \begin{array}{c|cc} & l_{29} & l_{30} \\ \hline l_8 & W_{11} & W_{11} \\ l_{19} & true & true \\ l_{29} & true & true \end{array}, \wedge(l_8, l_{19}, l_{29}) \rangle.$$

where $W_{11} = $ "Existance of token in place l_{29} ".

In place l_{30} the token obtains a characteristics "concentration of NAD^+.
The form of the eleventh transition is:

$$Z_{11} = \langle \{l_{20}, l_{27}, l_{28}\}, \{l_{31}\}, \begin{array}{c|c} & l_{31} \\ \hline l_{20} & true \\ l_{27} & true \\ l_{28} & true \end{array}, \wedge(l_{20}, l_{27}, l_{28}) \rangle.$$

4. Conclusion

In this paper generalized net model that represents the complex *E. coli* glycolysis control is presented. Described GN-model of *E. coli* glycolysis control, simulates the effect of glucose and oxygen on the metabolism of the bacteria. It is well shown that different concentrations can lead the system into several direction. The model demonstrates one of the most important and well known pathways present in all cells - glycolysis.

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