CHEMICAL ENGINEERING TOOLS APPLIED TO SIMULATE SOME CONDITIONS PRODUCING GLYCOLYTIC OSCILLATIONS IN *E. COLI* CELLS

Gheorghe MARIA*[1], Marina MIHALACHI (MUSCALU)[2], Cristiana Luminita GIJIU [3]

Autonomous oscillations in glycolytic intermediates concentrations reflect the dynamics of control and regulation of this major catabolic pathway, and the phenomenon has been reported in a broad range of bacteria. Understanding glycolytic oscillations might therefore prove crucial for the general understanding of the cell metabolism regulation with immediate applications in medicine, industrial biosyntheses, or environmental engineering (in-silico re-programming of cell metabolism to design new micro-organisms). In this context, modelling bacteria glycolysis dynamics is a classical subject, but still of high interest. By using a kinetic model from literature, this paper is aiming at simulating some conditions leading to stable glycolytic oscillations in E. coli cells.

Keywords: reduced dynamic model; glycolysis; *Escherichia coli*; oscillations

Abbreviations and notations

13DPG, PGP	1,3-diphosphoglycerate	**FOR**	formate
		FUM	fumarate
2,3PG	2,3-phosphoglycerate	**G3P, GAP**	Glyceraldehyde,3,phosphat
AC	acetate	**G6P**	glucose-6-phosphate
AK-ASE	adenylate kinase	**GLC**	glucose
AMDTP	adenosin-(mono)(di)(tri)phosphate	**GLCex, GLC[ext]**	Glucose in the external environment
ATP	adenosin-triphosphate	**GLN**	glutamine
ATP-ASE	ATP monophosphatase	**HK-ASE**	hexokinase
CIT	citrate	**LAC**	lactate
DHAP	dihydroxyacetonephosphate	**MAL**	malate
ETOH	ethanol	**mTRM**	modified Termonia & Ross [11-12] model
F6P	fructose-6-phosphate	**NAD(P)H**	nicotinamide adenine dinucleotide (phosphate)

[1]* Professor, University POLITEHNICA of Bucharest, Romania, corresponding author: e-mail: gmaria99m@hotmail.com
[2] PhD student, Ing., University POLITEHNICA of Bucharest, Romania.
[3] Lecturer, University POLITEHNICA of Bucharest, Romania.

FDP	fructose-1,6-biphosphate	**Pi**	reduced Phosphoric acid
PEP	phosphoenolpyruvate	c_j	species j concentration
PFK-ASE	phosphofructokinase	C_x, ρ_x	biomass concentration and density
PK-ASE	pyruvate kinase	D	cell content dilution rate, identical to bioreactor dilution rate, F_L/V_L
PPP	pentose-phosphate pathway	F_L	liquid feed flow rate in the bioreactor
PTS	Phosphotransferase; **PEP-**glucose phosphotransferase system	$k_j, K_j, V_{2m}, V_{4m}, r_j^{max}$	Rate, and equilibrium constants
PYR	pyruvate	t	time
SUCC	Succinate (or **SUC**)	V_j	species j reaction rates
TCA	tricarboxylic acid cycle	V_L	bioreactor liquid volume
[.]	concentration	$\alpha, \beta, \gamma, \delta$	reaction orders

1. Introduction

Autonomous oscillations in the concentrations of glycolytic intermediates reflect the dynamics of control and regulation of this major catabolic pathway, and the phenomenon has been reported in a broad range of cell types [1]. Understanding glycolytic oscillations might therefore prove crucial for our general understanding of the cellular metabolism regulation and the interplay among different parts of metabolism as illustrated by the hypothesis that glycolytic oscillations play a role in complex pulsatile insulin secretion [2]. The key question in this context is the mechanism(s) of the oscillations, but despite much work over the last 40 years, it remains unsettled.

Besides, glycolysis, together with the phosphotransferase (PTS)-system for glucose transport into the cell, the pentose-phosphate pathway (PPP), and the tricarboxylic acid cycle (TCA), all together characterize the central carbon metabolism (CCM).

Modelling bacteria glycolysis is a classical subject but still of high interest, allowing *in silico* design of modified cells with desirable gene circuits and 'motifs' of practical applications in the biosynthesis industry, environmental engineering, and medicine [10,19]. Consequently, understanding and simulation of the cell characteristics and environmental conditions leading to stable glycolytic oscillations turns out to be an important step in the CCM analysis. To simulate the glycolysis in bacteria, a large number of glycolysis models, of a reduced or extended form, have been proposed over decades. The model

complexity [3] is in the range of 18-30 species, included in 48-52 reactions, with a total of 24-150 parameters. Most of these models are too complex for an easy utilisation, and rate constant identification. Besides, most of them, they cannot reproduce the glycolytic oscillations. Recently, Maria [3] proposed a reduced glycolysis model, denoted by $mTRM$, including only 9 species, involved in 7 lumped reactions, including 17 identifiable parameters. The $mTRM$ model was identified by using the dynamic experimental data of Chassagnole et al. [4]. The model has been proved to adequately reproduce the cell glycolysis under steady state, oscillatory, or transient conditions according to the defined glucose input flux, its environmental concentration, the total A(MDT)P cell energy resources, and cell phenotype characteristics (determining the activity of the $ATPase$ enzyme involved in the ATP utilization and its recovery system). The aim of this paper is to use the $mTRM$ model to simulate some conditions leading to the occurrence of stable glycolytic oscillations in the *E. coli* cells.

2. The kinetic model of glycolysis in the *E. coli* prokaryotic bacteria

Glycolysis (from an older term with the meaning of glucose degradation) is the metabolic pathway that converts glucose ($C_6H_{12}O_6$) into pyruvate ($CH_3COCOO^- + H^+$). The free energy released by the subsequent tricarboxylic acid cycle (**TCA**) originating from pyruvate is used to form the high-energy molecules **ATP** (adenosine triphosphate), and **NADH** (reduced nicotinamide adenine dinucleotide) that support the glycolysis and many enzymatic cell syntheses [5,6]. Glycolysis is a determined sequence of ten enzyme-catalyzed reactions. The intermediates provide entry points to glycolysis. For example, most monosaccharides, such as fructose or galactose, can be converted to one of these intermediates. The intermediates may also be directly useful. For example, the intermediate dihydroxyacetone (**DHAP**, an intermediate in the reaction of **f6p** conversion to **g3p** in **Fig. 1**) is a source of the glycerol that combines with fatty acids to form fat. Also, **NADPH** is also formed by the pentose-phosphate pathway (**PPP**), which converts glucose into ribose, which can be used in synthesis of nucleotides and nucleic acids. The **pep** is also the starting point for the synthesis of essential aminoacids such as tryptophan, cysteine, arginine, serine, etc. [7]. To model the dynamics and regulation of such a complex cell glycolytic process in detail is a very difficult task, if not impossible.
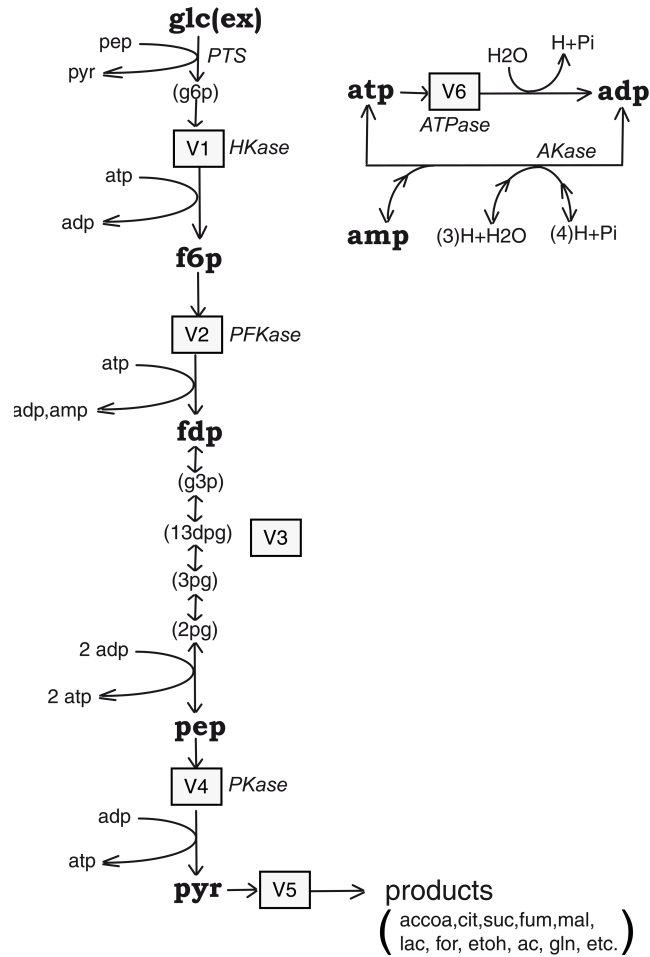
Fig. 1. Simplified reaction schemes of glycolysis in *E. coli* to base the reduced kinetic model of Maria [3], with including adenosin co-metabolites **ATP**, **ADP**, **AMP** synthesis. Species in parentheses are not explicitly included in the $mTRM$ model. Italic letters denote the enzymes. Squares include notations of enzymatic reactions. Notations: **glc(ex)**= glucose in the cell environment; **g6p**= glucose-6-phosphate; **f6p**= fructose-6-phosphate; *HK-ASE* – hexokinase; *PFK-ASE* – phosphofructokinase; *ATP-ASE* = **ATP** monophosphatase; **ADP** = adenosin-diphosphate; **ATP** = adenosin-triphosphate; **AMP** = adenosin-monophosphate; *AK-ASE* = adenylate kinase; Pi = Phosphoric acid; **fdp** = fructose-1,6-biphosphate; **g3p,gap**= glyceraldehyde-3-phosphate; **13dpg, pgp** = 1,3-diphosphoglycerate; **3pg** = 3-phosphoglycerate; **2pg** = 2-phosphoglycerate; **pep** = phosphoenolpyruvate; *PFK-ASE* = phosphofructokinase; **pyr** = pyruvate; **suc** = succinate.

Starting from an extended reaction pathway and model, and by applying lumping techniques [8-10,19] adapted from chemical engineering, Maria [3] proposed a reduced $mTRM$ model of only 9 species, 7 lumped reactions including

17 identifiable parameters. The $mTRM$ model was identified using experimental data and has been proved to adequately reproduce the cell glycolysis under steady state, oscillatory, or transient conditions according to the defined glucose input flux, its environmental concentration, the total **A(MDT)P** cell energy resources, and cell phenotype characteristics (concerning the enzyme *ATPase* involved in the **ATP** utilization and recovery system). The $mTRM$ kinetic model (rate expressions and parameters) is presented in **Table 1**.

3. How glycolytic oscillations occur

Oscillations in chemical systems represent periodic state variable (i.e. species concentrations) transitions in time.

According to Franck [13], spontaneous occurrence of self-sustained oscillations in chemical systems is due the coupled actions of at least two simultaneous processes. Oscillations sourced in a so-called "oscillation node" (that is a chemical species, or a reaction), on which concomitant rapid positive (perturbing) and slow negative (recovering) regulatory loops act. Because the coupling action between the simultaneous processes is mutual, the total coupling effect actually forms closed feedback loops for each kinetic variable involved. There exists a well-established set of essential thermodynamic and kinetics prerequisites for the occurrence of spontaneous oscillations, as following [13].

1) sustained oscillations can only occur in thermodynamically open systems far from equilibrium;
2) oscillatory systems always consist of more than one degree of kinetic freedom, i.e. the description of their temporal behaviour requires a corresponding set of simultaneous differential equations;
3) there exist extremely nonlinear relationships between the involved driving forces and driving fluxes or reactions;
4) oscillatory systems always contain unstable states;
5) oscillations are the result of mutual kinetic coupling between processes being otherwise independent from each other;

Table 1.

**The glycolysis kinetic model $mTRM$ of Maria [3] and its parameters (the units are in mM, min).**

Reaction	Parameters
$GLC + PEP \rightarrow F6P + PYR$ $PYR + ATP \rightarrow PEP + ADP + H$ $GLC + ATP \rightarrow F6P + ADP + H$	$c_{G6P} = kc_{F6P}$; $r_{PTS}^{max} = 308.8587$ $K_{PTS,a1} =$

$$V_1 = r_{PTS} = \frac{\rho_x}{C_x} \cdot \frac{r_{PTS}^{max} c_{GLC}^{ext} c_{PEP}/c_{PYR}}{B}$$

$$B = \left(K_{PTS,a1} + K_{PTS,a2} \frac{c_{PEP}}{c_{PYR}} + K_{PTS,a3}c_{GLC}^{ext} + c_{GLC}^{ext}\frac{c_{PEP}}{c_{PYR}} \right)\left(1 + \frac{c_{G6P}^{n_{PTS,G6P}}}{K_{PTS,G6P}} \right)$$

$K_{PTS,a2} = 3740.091$; $K_{PTS,a3} = 5911.072$; $K_{PTS,G6P} =$ absent ;

	1.0260
	$n_{PTS,G6P} = 0$
	$k = 5.8$

Reaction

$F6P + ATP \rightarrow FDP + ADP + H$

$$V_2 = r_{PFK} = \frac{(V_1/V_{2m})c_{F6P}^{\delta}}{\left(K_{2m}^{\delta} + K_{2m}^{\delta}\left[\frac{K_R^{AMP}}{K_T^{ATP}} \right]^n \left(\frac{c_{ATP}}{c_{AMP}} \right)^n + c_{F6P}^{\delta} \right)}$$

Parameters
$\delta = 1.0437$
$V_{2m} = 0.062028$
$K_{2m} = 6.16423$
$K_R^{AMP} = 25\ \mu M$
$K_T^{ATP} = 60\ \mu M$

Reaction

$2ADP \Leftrightarrow ATP + AMP$

$c_{ATP}c_{AMP} = Kc_{ADP}^2$

Obs.: Termonia & Ross [11-12] indicated experimental evidence of a very fast reversible reaction catalysed by *AKase*, the equilibrium being quickly reached.

Parameters
$K = 1$

Reaction

$FDP + 2ADP(+2NAD + 2P) \Leftrightarrow$
$2PEP + 2ATP(+2NADH + 2H + 2H2O)$

$$V_3 = k_3 c_{FDP}^{\alpha} - k_{3p} c_{PEP}^{\beta}$$

Parameters
$k_3 = 73.63477$
$k_{3p} = 337.0371$
$\alpha = 0.05$
$\beta = 3$

Reaction

$PEP + ADP + H \rightarrow PYR + ATP$

$$V_4 = r_{PK} = \frac{(V_1/V_{4m})c_{PEP}^{\gamma}}{\left(K_{4m}^{\gamma} + K_{4m}^{\gamma}\left[\frac{K_R^{FDP}}{K_{T,PK}^{ATP}} \right]^m \left(\frac{c_{ATP}}{c_{FDP}} \right)^m + c_{PEP}^{\gamma} \right)}$$

$K_R^{FDP} = 0.2$ Mm; $K_{T,PK}^{ATP} = 9.3$ mM

Parameters
$\gamma = 1.33188$
$m = 4$
$V_{4m} = 0.13336$
$K_{4m} = 1.14644$

Reaction

$PYR \rightarrow products(ACCOA, CIT, SUCC, LAC, ETOH, AC, ...)$

Parameters
$k_5 = 693.3544$

$$V_5 = \frac{k_5 c_{PYR}^{n_{consum,PYR}}}{K_{consum,PYR} + c_{PYR}}$$

$K_{consum,PYR} = 395.525$

$n_{consum,PYR} = 2.68139$
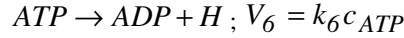
Reaction	Parameters
$ATP \rightarrow ADP + H$; $V_6 = k_6 c_{ATP}$	$k_6 = 4025.351$

Obs.: other values of k_6 are also possible according to the micro-organism phenotype (characteristics of the gene encoding the enzyme *ATPase* that catalyse this reaction).

Table 2.

The operating conditions of the Chassagnole et al. [4] bioreactor with *E. coli* cell cultures used to simulate the glycolytic oscillation occurrence.

Parameter	Value
Biomass concentration (C_x)	8.7 gDW L^{-1} culture volume
Cell content dilution rate (D)	0.001667 min^{-1}
Culture dilution rate (F_L/V_L)	0.001667 min^{-1} (adjusted to be identical to D)
Glucose feeding solution concentration [GLC]$_{feed}$	200, mM (this paper).
Biomass density (ρ_x)	565.5 gDW (L cytosol)$^{-1}$
Measured [AMDTP]total	5.82 (mM)

6)   once an oscillation occurs, it propagates in the whole reaction pathway;

7)   oscillations occur as a result of simultaneous feedback effects;

8)   feedback occurs when a process acts kinetically upon itself; it therefore consists basically in a closed chain of action which causes the well-known effects of self-enhancement in case of "positive feedback" and self-inhibition in case of "negative feedback" respectively, in a non-systemic, or systemic feedback;

Table 3.

The bioreactor and glycolysis mass balance equations for the kinetic model of Maria [3]. Cell species initial concentrations are those measured by Chassagnole et al. [4].

Species mass balance	Auxiliary relationships

$$\frac{dc_{GLC}^{ext}}{dt} = D\left(c_{GLC}^{feed} - c_{GLC}^{ext}\right) - \frac{C_x}{\rho_x}V_1$$

$c_{GLC}^{ext}(t=0) =$ **tried reference value (0.0557 mM, or 1 mM)**

$$\frac{dc_{F6P}}{dt} = V_1 - V_2 - D\,c_{F6P}$$

$c_{F6P}(t=0) =$ **6.00325977e-001, mM**

$$\frac{dc_{FDP}}{dt} = V_2 - V_3 - D\,c_{FDP}$$

$c_{FDP}(t=0) =$ **2.72961814e-001, mM**

i)

$c_{AMP} + c_{ADP} + c_{ATP} = c_{AMDTP}$
= constant; [11-12]

ii) c_{ADP} results from solving the thermodynamic equilibrium relationship

$c_{ATP}c_{AMP} = Kc_{ADP}^2$, that is:

$$c_{ADP}^2\,\frac{K}{c_{ATP}} + c_{ADP} - c_{AMDTP} + c_A$$

iii) product formation from **Pyr** has been neglected from the model.

$$\frac{dc_{PEP}}{dt} = 2\,V_3 - V_4 - D\,c_{PEP}\ ;\ c_{PEP}(t=0) =\textbf{2.67294507e+000, mM}$$

$$\frac{dc_{PYR}}{dt} = V_4 - V_5 - D\,c_{PYR};\ c_{PYR}(t=0) =\textbf{2.67061526e+000, mM}$$

$$\frac{dc_{ATP}}{dt} = -V_1 - V_2 + 2\,V_3 + V_4 - V_6 - D\,c_{ATP}\ ;\ c_{ATP}(t=0) =\textbf{4.27, mM}$$

9)   in chemical systems the systemic feedback is realized by reactions whose activation energy or rate constant depends on their own reaction products or reactants;

10)   the chemical oscillations exhibit positive and negative feedback simultaneously; according to the "principle of antagonistic feedback of chemical oscillators", the oscillations are understood as a consequence of an antagonistic interaction of a relatively fast acting positive feedback of labilizing tendency and a slower acting negative feedback of stabilizing recovering tendency;

11)   oscillations' occurrence and characteristics depend not only upon the presence of both kinds of feedback but also upon the correct ration of the time parameters of the feedback loops involved;

12)   the oscillatory system consists of two distinct loops of positive and negative feedbacks requiring at least two kinetic variables; each loop,

however, may contain several variables in series, all participating to the overall oscillatory process;

13)   the labilizing positive feedback manifests itself in pseudo-capacitive behaviour, and, sufficiently strong, it causes: instability, bistability, threshold behaviour, triggerability of state transitions; propagation phenomena;
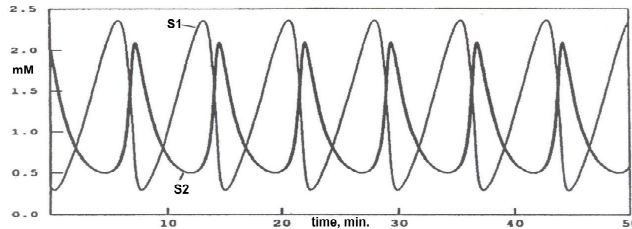

Fig. 2A. Experimental measured glycolytic oscillations in *E. coli* by Madsen et al. [1], and Schaefer et al. [15]. Notations: S1 = F6P; S2 = FDP.Time axis in minutes. Concentrations in mM.

In the glycolysis system case, extensive experiments (e.g. **Figs. 2A, 2B**) have revealed that self-sustained oscillations are reported in a broad range of cell types [1]. As revealed by Termonia & Ross [11-12] glycolytic oscillations occurrence is due to the antagonistic action of two processes on regulating the V2 reaction rate that converts **F6P** in **FDP** (see reaction scheme in **Fig. 3**). The glycolytic oscillation occurrence and characteristics (period) are influenced by both external and internal (genomic) factors, as following [14]:
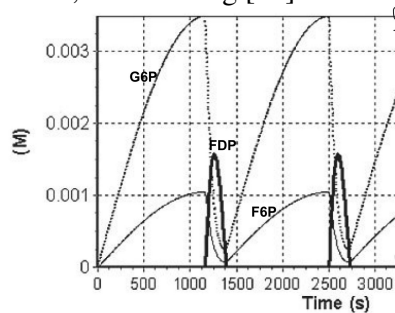

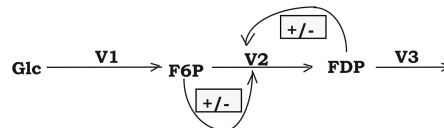Fig. 2B. Experimental measured glycolytic oscillations in E. coli by [15-16]. Concentrations in M.

Fig. 3. Chemical node inducing glycolytic oscillations (after [11-12]). ⊕, Θ denotes the feedback positive or negative regulatory loops. **Glc** = glucose; **F6P**= fructose-6-phosphate; **FDP** = fructose-1,6-biphosphate; **V1-V3** = reaction rates.

1) From one side it is the glucose import driving force through the phosphotransferase (PTS)-system (**Fig. 1**) regulated by the external concentration of glucose c_{GLC}^{ext} = [Glc]ext and the **PEP** and **PYR** levels;
2) On the other hand, it is the limited **A(MDT)P** cell energy resources, and iii) A limited **ATP** recovery rate (k6, K constants in the **Table 1**) due to the enzymes *ATP-ase* and *AK-ase* related to the bacteria genome and phenotype;
3) Glycolysis being a systemic process with a complex regulatory system, oscillations are also related to all the reaction rate and constants.
4) Among the glycolytic oscillation factors are to be mentioned: [**Glc**]ext; [**AMDTP**]total; k6.

4. Simulation of some oscillation conditions

By adopting the glycolysis kinetic model of Maria [3], one can determine by repeated simulations what are the cell external and internal conditions leading to oscillation occurrence, with a tremendous practical importance when determining the consequences on the metabolic syntheses connected to the glycolysis.

In the present study, one simulated the glycolysis occurrence in the *E. coli* cells growing conditions of the semi-continuous bioreactor of Chassagnole et al. [4] given in **Table 2** (using sparging air in excess, and necessary nutrients for a cell culture equilibrated growth. The bioreactor and glycolysis mass balance equations for the kinetic model of Maria [3] are presented in **Table 3.**

Simulations are made for cell culture conditions of **Table 2**, for cells with [**AMDTP**]total = 5.82 mM, only two factors being varied, that is: [**Glc**]ext, and k6. All other reaction rate constants are kept at the values given in **Table 1**.

Here are presented only the glycolytic oscillations obtained for k6 = 11 1/min with [**Glc**]ext = 0.0557 mM (**Fig. 4**), and [**Glc**]ex = 1 mM (**Fig. 5**).

By comparing the plots of these two figures, it is to remark that oscillation period takes values in the range of 0.5-0.9 min, being as smaller as [Glc]ext is smaller. Also, as expected, glycolytic oscillations are similar but of higher amplitudes as the **Glc** external levels are higher, compared to the experimentally determined glycolytic oscillation period of 0.2-1.7 min. [1,14,16], 1-180min. [17,18].
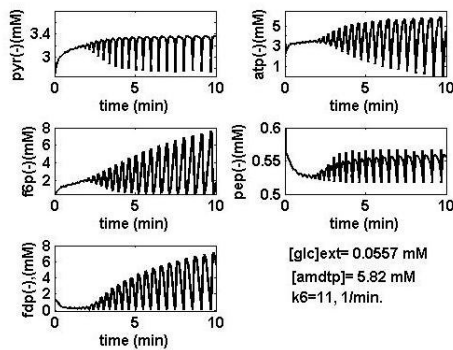
.

Fig. 4. Glycolytic stationary oscillations in *E. coli* for the operating conditions of **Table 2.** Identified parameters inducing oscillations are: **[Glc]ext = 0.0557** Mm**; k6 = 11** 1/min.
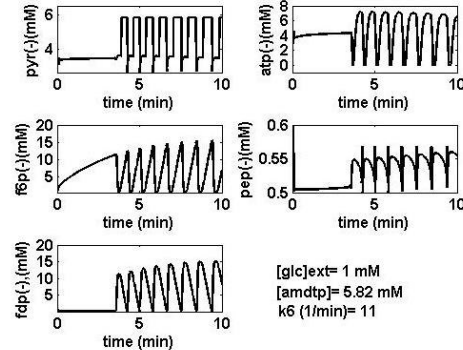
Fig. 5. Glycolytic stationary oscillations occurrence in *E. coli* for the bioreactor operating conditions of **Table 2**. Identified parameters inducing oscillations are: **[Glc]ext = 1** mM**; k6 = 11** 1/min**.** The **AMDTP** recovery system is characterized by the model of **Table 1** [3].

5. Conclusions

The use of reduced kinetic models when modelling complex metabolic pathways is a continuously challenging subject when developing structured cell simulators for various applications (flux analysis, target metabolite synthesis optimization, *in-silico* re-programming of the cell metabolism and design of new micro-organisms, bioreactor optimization). As exemplified by the *E. coli* glycolysis case study, the reduced $mTRM$ model, of simple and easily adaptable structure to various cell cultures, can be used in quick analyses of cell metabolism, such as the substrate utilization, oscillation occurrence, or structured interpretation of metabolic changes in modified cells. By extension, the glycolysis core model can be easily adapted to include any complex synthesis and regulatory pathway deriving from the main carbon uptake stream.

R E F E R E N C E S

[1] *M.F. Madsen, S. Dano, P.G. Sorensen,* On the mechanisms of glycolytic oscillations in yeast, FEBS Journal **272,** 2005, pp. 2648–2660.

[2] *K. Wierschem, R. Bertram,* Complex bursting in pancreatic islets: a potential glycolytic mechanism. J Theor. Biol., **228**, 2004, pp. 513–521.

[3] *G. Maria,* Insilico derivation of a reduced kinetic model for stationary or oscillating glycolysis in *E. coli* bacterium, Chem. Biochem. Eng. Q., **28**, 2014, pp.509-529.

[4] *C. Chassagnole, N. Noisommit-Rizzi, J.W. Schmid, K. Mauch, M. Reuss,* Dynamic modeling of the central carbon metabolism of Escherichia coli., Biotechnol. Bioeng.

79, 2002, pp.53-73.

[5] *S. Lubert,* Glycolysis, In: Biochemistry, Freeman and Co., New York, 1995, pp. 483–508.

[6] *C.K. Mathews, K.E. van Holde, K.G. Ahem,* Biochemistry, Prentice Hall, New Jersey, 1999.

[7] *K.A. Calhoun, J.R. Swartz,* Total amino acid stabilization during cell-free protein synthesis reactions, Journal of Biotechnology **123**, 2006, pp.193–203.

[8] *G. Maria,* A review of algorithms and trends in kinetic model identification for chemical and biochemical systems, Chem. Biochem. Eng. Q. **18**, 2004, pp.195-222.

[9] *G. Maria,* Relations between apparent and intrinsic kinetics of programmable drug release in human plasma, Chemical Engineering Science **60**, 2005, pp. 1709-1723.

[10] *G. Maria,* A review of some novel concepts applied to modular modelling of genetic regulatory circuits, Juniper publ, Newbury Park, CA (USA) 2017, https://juniperpublishers.com/ebook-info.php

[11] *Y. Termonia, J. Ross,* Oscillations and control features in glycolysis: Numerical analysis of a comprehensive model, Proceedings of the National Academy of Sciences of the USA, **78,** 1981a, pp.2952. http://dx.doi.org/10.1073/pnas.78.5.2952 (1998)

[12] *Y. Termonia, J. Ross,* Oscillations and control features in glycolysis: Analysis of resonance effects, Proceedings of the National Academy of Sciences of the USA, **78,** 1981b, pp.3563. http://dx.doi.org/10.1073/pnas.78.6.3563 (1998)

[13] *U.F. Franck,* Feedback kinetics in physicochemical oscillators, Ber. Bunsenges. Phys. Chem. **84**, 1980, pp.334-341.

[14] *P.O. Westermark, A. Lansner,* A model of phosphofructokinase and glycolytic oscillations in the pancreatic   -cell, Biophysical Journal, **85**, 2003, pp.126-139.

[15] *U. Schaefer, W. Boos, R. Takors, D. Weuster-Botz,* Automated sampling device for monitoring intracellular metabolite dynamics, Analytical Biochemistry **270** (1999) pp. 88. http://dx.doi.org/10.1006/abio.1999.4048 (2013)

[16] *A.S. Silva, J.A. Yunes,* Conservation of glycolytic oscillations in Saccharomyces cerevisiae, Genetics and Molecular Research, **3-5**, 2006, pp. 525-535.

[17] *M. Bier, B. Teusink, B.N. Kholodenko, H.V. Westerhoff,* Control analysis of glycolytic oscillations, Biophysical Chemistry, **62**, 1996, pp.15-24.

[18] *P. Rapp,* An atlas of cellular oscillators, J. exp. Biol., **81**, 1979, pp. 281-306.

[19] *G. Maria,* In-silico design of Genetic Modified Micro-organisms (GMO) of industrial use, by using Systems Biology and (Bio)Chemical Engineering tools, Juniper   publ,   Newbury   Park,   CA   (USA)   2018, https://juniperpublishers.com/ebook-info.php