IN-SILICO MODULATE GLYCOLYTIC OSCILLATOR IN MODIFIED *E. COLI* TO CONTROL BIOPROCESSES OF INDUSTRIAL INTEREST

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Abstract

*Tryptophan (**TRP**) is an aromatic non-polar amino-acid essential, whose biosynthesis maximization is of high practical importance in industry, and medicine. On one hand, it is to underline that **TRP** synthesis is an oscillatory process strongly connected to the glycolysis through the **PEP** (phosphoenolpyruvate) node. On the other hand, it is well-known that glycolysis, under certain environmental conditions, displays autonomous oscillations of the glycolytic intermediates' concentrations thus reflecting the dynamics of the control and regulation of this major catabolic pathway with a major role in the cell central carbon metabolism (**CCM**) in living cells. Consequently, glycolysis model is the 'core' module of any systematic and structured model-based analysis of most of metabolic sub-process. By coupling two adequate reduced kinetic models for the glycolysis and **TRP** synthesis in the **E. coli** cells, adopted from literature, with the model of a semi-continuous bioreactor, this paper derives, for the first time, an **in silico** analysis of the optimal operating conditions of the bioreactor used for tryptophan synthesis, with accounting for the two interfering oscillatory processes. The paper also points-out the main factors influencing the glycolytic oscillations, by in silico (model-based) identifying some conditions, able to be modulated leading to occurrence of stable glycolytic oscillations in the E. coli cells, and **TRP** synthesis optimisation.*

Keywords: Reduced dynamic models; Glycolysis; Tryptophan synthesis; *Escherichia coli;* Oscillation occurrence; Bioreactor optimization

1. Introduction

When developing an industrial bioprocess, one essential engineering problem concerns the choice not only of the best bioreactor operating policy, but also the most suitable cell culture with genetically modified micro-organisms (GMO) to get the maximum reactor productivity for the target metabolite. As described in the literature, such GMO-s, can be *in-silico* (math model-based) design by using Systems Biology, Bioinformatics, and (Bio)Chemical Engineering tools [1].

In the last decades, there has been a continuous trend to replace complex chemical syntheses, energetically intensive and generating toxic wastes, with

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biosynthesis or biological processes to produce some fine chemicals or organic compounds in food, pharmaceutical, or detergent industry, using enzymatic or cell culture batch, semi-batch (fedbatch), fixed-bed, or fluidized-bed reactors.[1,2] This includes, among others, the production of monosaccharides derivatives, organic acids, alcohols, amino acids, and so on by using single-enzymatic or multi-enzymatic reactors, or the production of baker's yeast, food products and additives, recombinant proteins (enzymes and vaccines), and biopolymers by using bioreactors with cell cultures. The mentioned advantages of enzymatic synthesis, and of fermentation using cell cultures, over chemical catalytic processes are shortly underlined in Fig.1**.**

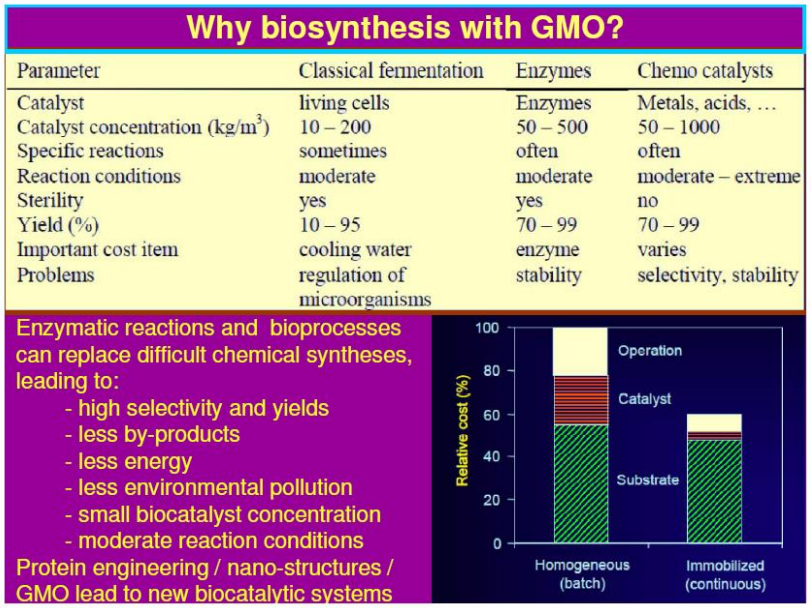

Fig. 1. Advantages of enzymatic synthesis, and of fermentation using cell cultures, over chemical catalytic processes. Structure of the production cost in the case of biosynthesis with free vs. immobilized enzymes

On the other hand, optimization of biological or enzymatic reactors requires adequate kinetic models of the biological / enzymatic process [1, 11]. This is why tremendous experimental and computational efforts have been invested in this respect, despite the high complexity of biological / cellular processes.

Glycolysis is an essential part of the cell metabolism. Glycolysis is the metabolic pathway that converts glucose (GLC) into pyruvate (PYR) (Fig. 2-4, 9). 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 numerous enzymatic syntheses into the cell [3].

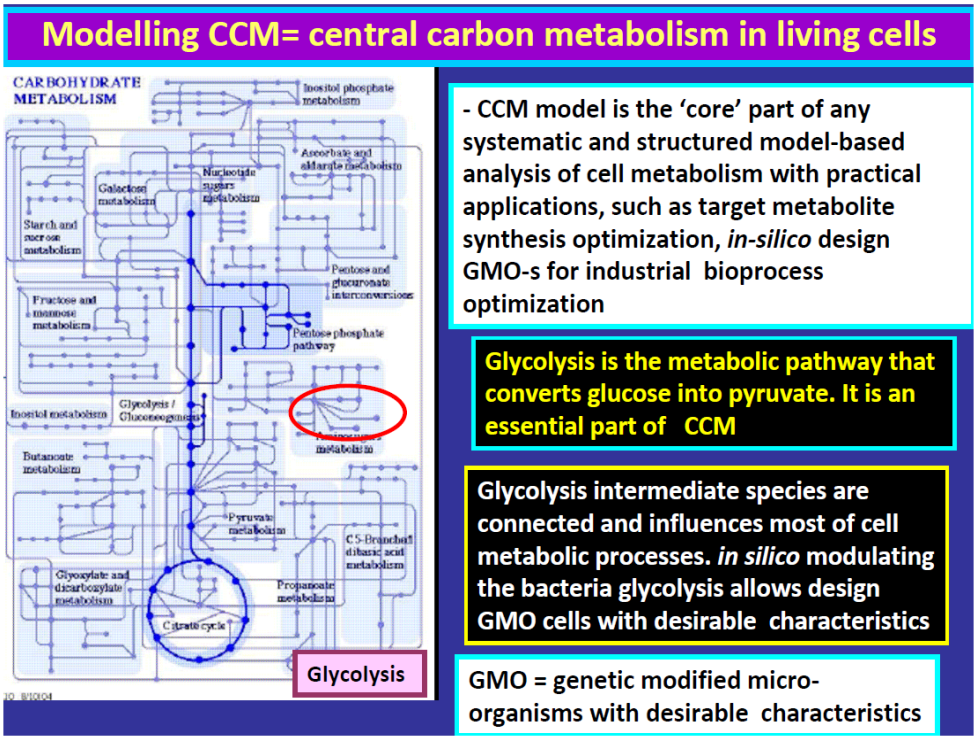

Fig. 2. Glycolysis pathway is the "core" of the CCM [**8**].

In fact, glycolysis, together with the phosphotransferase (PTS)-system for glucose transport into the cell, the pentose-phosphate pathway (PPP), and the tricarboxylic acid cycle (TCA), characterize the central carbon metabolism (CCM) [1,2,4,8] (Fig. 2, 3) which is responsible for all metabolic syntheses. The CCM model is the 'core' part of any systematic and structured model-based analysis of the cell metabolism with immediate practical applications, such as target metabolite synthesis optimization, *in-silico* re-programming of the cell metabolism to design new GMO-s, of practical applications in the biosynthesis industry, environmental engineering, and medicine [1-3]. As glycolysis is connected and influences most of cell metabolic processes, modulating the bacteria glycolysis based on lumped kinetic model is a classical but still of high interest subject [2,5].

By using the lumped dynamic model of Maria [4], this paper is aiming at identifying some conditions allowing modulating the characteristics (oscillation period, species concentration amplitude), of the glycolytic oscillations in *E. coli* cells. [3,5]. Such a model can be used, for instance to maximize the production of amino-acids (**TRP** here) by using immobilized GMO cells in a bioreactor [2].

2. Adopted kinetic model of glycolysis and TRP synthesis in *E. coli* prokaryotic bacteria

The adopted glycolysis kinetic model in *E. coli* (given in Table 2) is those proposed by Maria [4]**,** based on the reaction pathway presented in Fig.4 with the rate expressions given in Figs. 5-7. How glycolytic oscillations occur is shortly explained in section 3.

Aiming to maximize **TRP** production, by modulating the glycolysis characteristics by which **TRP** synthesis is closely connected through the **PEP** node (Fig.9), a **TRP** synthesis kinetic model it is also necessary.

Based on a simplified **TRP** synthesis pathway given in Fig. 9 and extended studies reviewed by **[**2,3,5,7**],** Bhartiya et al.[6] proposed a simplified kinetic model for the **TRP** synthesis given in the Table 4.

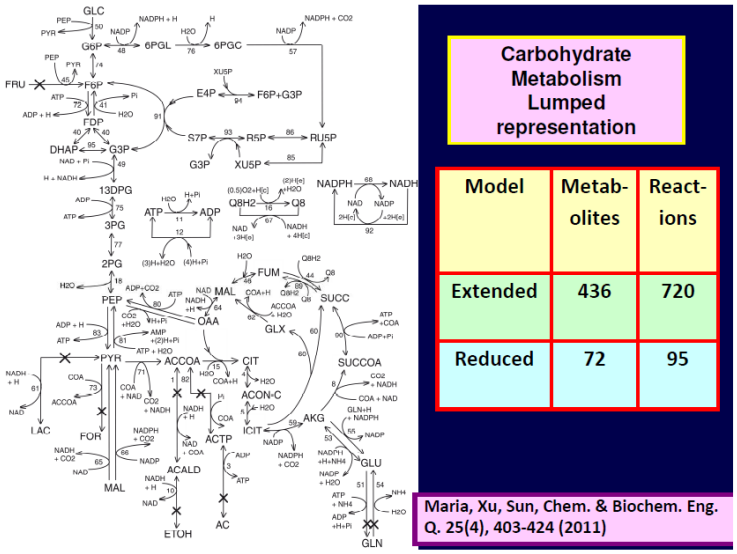

Fig. 3. Glycolysis and carbohydrate metabolism (CCM) model in *E.coli* of [9].

However, this adopted **TRP** model [6] (Table 1) suffered two modifications to match with the Bhartiya [6] experimental data, as suggested by Maria et al. [7]: I) The rate constant k_4 was re-estimated in order to fit the experimental curves of [6], that is the **OR**, **mRNA**, **T, E** species trajectories given in Figure 9 **(**stationary [**PEP]**s = 1 mM case), by using a classic estimator [7] and, II) To be connected to the glycolysis pathway (as displayed in Fig. 9), the **TRP** synthesis kinetic model of Table 4, was completed with terms accounting for the connection with the glycolysis through **PEP** node, **PEP** being explicitly included in the **TRP** synthesis rate (see the dc_T / dt term in Table 4; the nitrogen source in

the **TRP** balance is considered in excess and included in the k_4)[2-7]. Parameter estimation has been done by using the effective MMA of Maria [16].

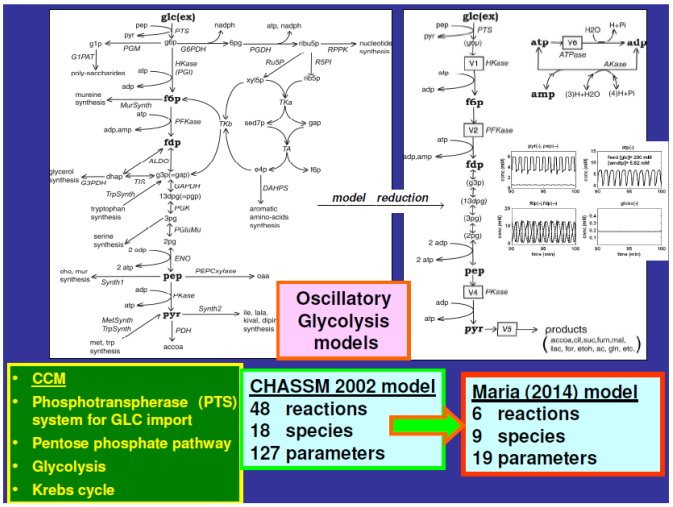

Fig. 4. A comparison of the glycolysis models of [10], and of [4]. The last one allows simulating glycolytic oscillations.

3. How glycolytic oscillations occur and simulation of some oscillation conditions

How glycolytic oscillations occur? Oscillations in chemical systems represent periodic state variable (i.e. species concentrations) transitions in time. According to Franck [12], 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 [12].

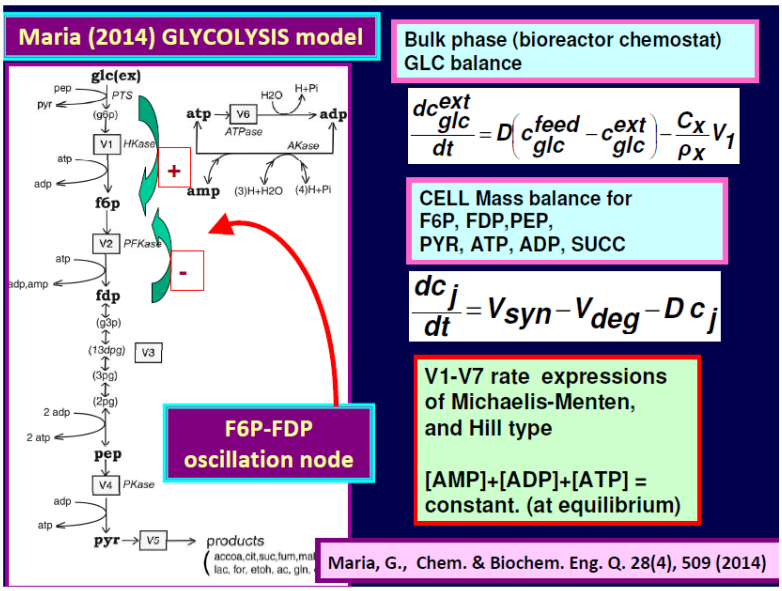

Fig. 5. Explanation of the regulatory loops simultaneously acting on the reaction V2, that produce glycolytic oscillations [3-5].

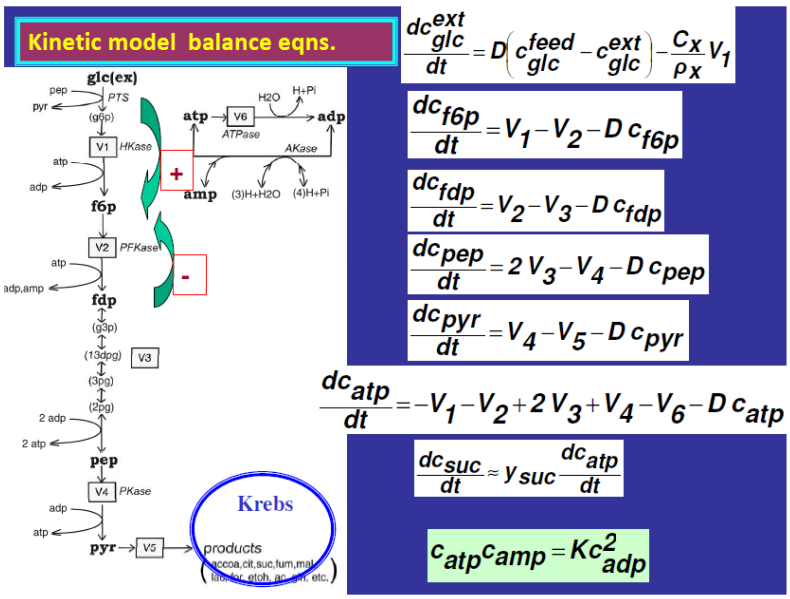

Fig. 6. The differential mass balance of the glycolysis kinetic model [4].

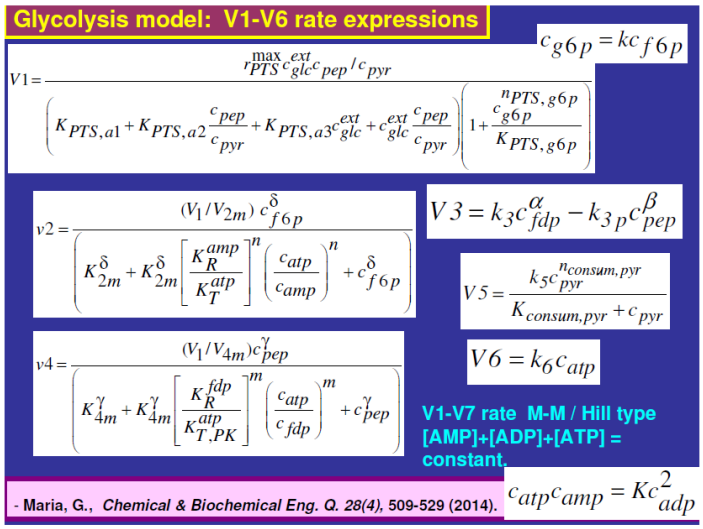

Fig. 7. Reaction rate expressions of the glycolysis kinetic model [4].

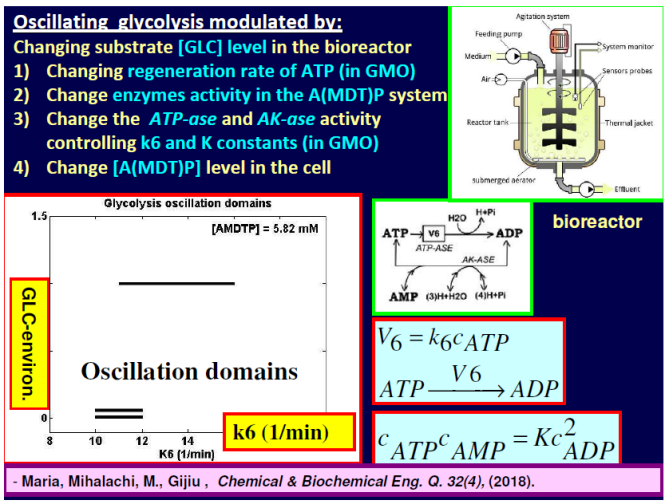

Fig. 8. Some oscillation domains of the glycolysis in *E. coli* in-silico simulated by using Maria [4] model. Suggestions to design GMO to modulate glycolytic oscillations [2,3,5]).

In the glycolysis case, as revealed by Termonia and Ross [13-15], 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 the Figs. 4-6). The glycolytic oscillation occurrence and characteristics (period, amplitude) are influenced by both external (environmental) and internal (genomic) factors [3, 4].

4. Bioreactor and cellular bioprocess structured model coupling glycolysis and TRP (tryptophan) synthesis in the *E. coli* cell

By adopting the glycolysis kinetic model of Maria [4], one can determine, by repeated simulations, the cell external and internal conditions leading to glycolytic oscillation occurrence, and conditions determining **TRP** synthesis maximization. In simulations, one considers the *E. coli* cell growing conditions of the semi-continuous bioreactor of Chassagnole et al. [10], given in Table 1 by using sparking air in excess, and necessary nutrients (for a cell culture equilibrated growth). The main mass balance equations of the bioreactor and glycolysis dynamic model are presented in Tables 2-3. To obtain the model solution with enough precision, a low-order stiff integrator ("ODE23S" routine) of the Matlab™ package was used.

Table 1

Nominal operating conditions of the tested semi-batch bioreactor (SBR) [2].

Parameter	Nominal value	Obs.
Biomass concentration (C_x)	8.7 gDW L^{-1} culture volume	adopted
Cell content dilution rate (μ)	$0.278 \times 10^{-4} - 1.667 \times 10^{-3}$ min^{-1} (adjusted to be identical to D)	To be optimized
Culture dilution rate, $D = F_L/V_L$	$0.278 \times 10^{-4} - 1.667 \times 10^{-3}$ min^{-1} (adjusted to be identical to μ)	To be optimized
Glucose feeding solution concentration C_{GLC}^{feed}	100–200, mM	200–400, mM (this paper).
Initial glucose concentration in the bioreactor $C_{GLC}^{ext}(t = 0)$	0.0557, mM (stationary value of Chassagnole et al., 2002)	1–5 mM (this paper)
Biomass density (ρ_x)	565.5 gDW (L cytosol)$^{-1}$	565.5 gDW (L cytosol)$^{-1}$
Measured [**AMDTP**]total	5.82 (mM)	5.82 (mM)

5. Simulation results

Simulations were made for the cell culture conditions given in Table 1**,** and for cells with [AMDTP]total = 5.82 mM.[3] Following the discussion in the previous chapter on oscillations occurrence, the influence of two main factors is studied here, that is:

i) [Glc]ext (related to the bioreactor operating conditions);
ii) $\boldsymbol{k_6}$ reaction rate (determined by the *ATP-ase* characteristics, related to the cell phenotype),
iii) all other reaction rate constants, and [AMDTP] level were kept unchanged during simulations of values given in Tables 2-3.

Simulations were conducted in an exhaustive way, by covering the ranges of the initial [Glc]ext = [0.01-1] mM (at $\boldsymbol{t = 0}$), and $\boldsymbol{k_6}$ = [0.1-20] 1/min. The results are plotted in Fig. 8. These simulation results lead to several model-based conclusions:

I) Oscillations are basically determined by the external level of [Glc] (triggering the glucose import into the cell) but also, for a certain [AMDTP], total energy

resources level in the cell (assumed to be quasi-constant in the present case study), are determined by the ATP to ADP conversion rate, and ATP regeneration rate (reflected here by k_6, and K constants of Table 2).

Table 2.

The glycolysis kinetic model of Maria [4] and its parameters.

Reaction	Parameters
$GLC + PEP \rightarrow F6P + PYR$ $PYR + ATP \rightarrow PEP + ADP + H$ $GLC + ATP \rightarrow F6P + ADP + H$	$c_{G6P} = kc_{F6P}$;
$V_1 = r_{PTS} = \frac{\rho_X}{C_X} \cdot \frac{r_{PTS}^{max} c_{GLC}^{ext} c_{PEP}/c_{PYR}}{\left(K_{PTS,a1} + K_{PTS,a2}\frac{c_{PEP}}{c_{PYR}} + K_{PTS,a3} c_{GLC}^{ext} + c_{GLC}^{ext}\frac{c_{PEP}}{c_{PYR}}\right)\left(1 + \frac{c_{G6P}^{n_{PTS,G6P}}}{K_{PTS,G6P}}\right)}$	$r_{PTS}^{max} = 308.8587$ $K_{PTS,a1} = 1.0260$ $K_{PTS,a2} = 3740.091$ $K_{PTS,a3} = 5911.072$ $K_{PTS,G6P}$ = absent $n_{PTS,G6P} = 0$
$F6P + ATP \rightarrow FDP + ADP + H$ $V_2 = r_{PFK} = \frac{(V_1/V_{2m})c_{F6P}^{\delta}}{\left(K_{2m}^{\delta} + K_{2m}^{\delta}\left[\frac{K_R^{AMP}}{K_T^{ATP}}\right]^n \left(\frac{c_{ATP}}{c_{AMP}}\right)^n + c_{F6P}^{\delta}\right)}$	$k = 5.8$ $\delta = 1.0437$ $V_{2m} = 0.062028$ $K_{2m} = 6.16423$ $K_R^{AMP} = 25\ \mu M$ $K_T^{ATP} = 60\ \mu M$
$FDP + 2ADP(+2NAD + 2P) \Leftrightarrow 2PEP + 2ATP(+2NADH + 2H + 2H2O)$ $V_3 = k_3 c_{FDP}^{\alpha} - k_{3p} c_{PEP}^{\beta}$	$k_3 = 73.63477$ $k_{3p} = 337.0371$ $\alpha = 0.05$ $\beta = 3$
$PEP + ADP + H \rightarrow PYR + ATP$ $V_4 = r_{PK} = \frac{(V_1/V_{4m})c_{PEP}^{\gamma}}{\left(K_{4m}^{\gamma} + K_{4m}^{\gamma}\left[\frac{K_R^{FDP}}{K_{T,PK}^{ATP}}\right]^m \left(\frac{c_{ATP}}{c_{FDP}}\right)^m + c_{PEP}^{\gamma}\right)}$	$\gamma = 1.33188$ $m = 4$ $V_{4m} = 0.13336$ $K_{4m} = 1.14644$ $K_R^{FDP} = 0.2$ Mm $K_{T,PK}^{ATP} = 9.3$ mM
$PYR \rightarrow products$ (ACCOA, CIT, SUCC, LAC, ETOH, AC, ...) $V_5 = \frac{k_5 c_{PYR}^{n_{consum,PYR}}}{K_{consum,PYR} + c_{PYR}}$	$k_5 = 693.3544$ $K_{consum,PYR} = 395.525$ $n_{consum,PYR} = 2.68139$
$ATP \rightarrow ADP + H$ $V_6 = k_6 c_{ATP}$	$k_6 = 12$ (can vary in the range of 0.1–4000)
Obs.: k_6 takes values according to the micro-organism phenotype (characteristics of the gene encoding the enzyme *ATPase* that catalyse this reaction).	
$2ADP \Leftrightarrow ATP + AMP$ $c_{ATP} c_{AMP} = K c_{ADP}^2$	$K = 1$
Obs.: Termonia and Ross (1981a, 1981b, 1982) indicated experimental evidence of a very fast reversible reaction catalysed by *AKase*, the equilibrium being quickly reached.	

II) Oscillations occur for <u>low</u> [Glc]ext but with a slow Glc import, due to relatively low k_6 constant values (i.e., a cell with a slow ATP conversion to ADP and ATP recovery);

III) By contrast, <u>high</u> levels of [Glc]ext, triggering high rate import, also produce glycolytic oscillations for larger values of k_6, due to the limited ATP recovery rate (k_6 being also related to the K constant governing the AMDTP pathway). Eventually, for too small, or too large k_6 values, the glycolysis reaches its steady-state.

Table 3

The dynamic model of the semi-continuous bioreactor of Maria et al. [2] and its parameters

(A) Species mass balance (glycolysis)	Auxiliary relationships, and parameters of Maria (2014), Maria et al. (2018a).
$\frac{dc_{GLC}^{ext}}{dt} = D(c_{GLC}^{feed} - c_{GLC}^{ext}) - \frac{C_x}{\rho_x}V_1$	
$\frac{dc_{F6P}}{dt} = V_1 - V_2 - \mu\, c_{F6P}$	i) Cell species initial concentrations are those measured by Chassagnole et al. (2002), that is (in mM):
$\frac{dc_{FDP}}{dt} = V_2 - V_3 - \mu\, c_{FDP}$	
$\frac{dc_{PYR}}{dt} = V_4 - V_5 - \mu\, c_{PYR}$	
	$c_{GLC}^{ext}(t=0) = 0.0557$ mM [reference value of Chassagnole et al. (2002), or 1–5 mM (this paper)]
	$c_{F6P}(t=0) = 0.600325977$,
	$c_{FDP}(t=0) = 0.272961814$,
	$c_{PEP}(t=0) = 2.67294507$
	$c_{PYR}(t=0) = 2.67061526$
	$c_{ATP}(t=0) = 4.27$
	ii) $c_{AMP} + c_{ADP} + c_{ATP} = c_{AMDTP}$ = constant (Termonia and Ross, 1981a, 1981b, 1982);
$\frac{dc_{ATP}}{dt} = -V_1 - V_2 + 2V_3 + V_4 - V_6 - \mu\, c_{ATP}$	iii) c_{ADP} results from solving the thermodynamic equilibrium relationship $c_{ATP}c_{AMP} = Kc_{ADP}^2$, that is: $c_{ADP}^2\frac{K}{c_{ATP}} + c_{ADP} - c_{AMDTP} + c_{ATP} = 0$
	iv) Products formation from **PYR** has been neglected in the model;
	v) Biomass concentration (C_x) is assumed to be quasi-constant.
	vi) D= bioreactor dilution.; μ = cell content dilution rate.
$\frac{dc_{PEP}}{dt} = 2V_3 - V_4 - \mu\, c_{PEP} - y_{trp}(2V_3)$	Completion with terms accounting for the PEP consumption in the TRP synthesis: $y_{trp} = {}^{r_{syn,trp}}/_{r_{syn,pep}} = 1/43.63$ (at **QSS**), from(Stephanopoulos and Simpson, 1997).

Table 4

TRP synthesis kinetic model [6] coupled with the glycolysis kinetic model [4] through PEP node [2,5].

(B) Species mass balance (TRP synthesis)	Parameters (Bhartiya et al., 2006)
$\frac{dc_{OR}}{dt} = k_1 c_{OT} C_1(T) - k_{d1}\, c_{OR} - \mu\, c_{OR}$	
$\frac{dc_{MRNA}}{dt} = k_2 c_{OR} C_2(T) - k_{d2}\, c_{MRNA} - \mu\, c_{MRNA}$	Initial values:
$\frac{dc_E}{dt} = k_3\, c_{MRNA} - \mu\, c_E$	
$\frac{dc_T}{dt} = k_4 c_{PEP} C_3(T)\, c_E - \frac{gT}{T+K_g} - \mu\, c_T$	
(the **PEP** term in the right side is accounting for the connection of **TRP** synthesis with the glycolysis)	$c_{OR}(t=0) = 0.01$, μM
	$c_{MRNA}(t=0) = 0.01$, μM
	$c_E\ (t=0) = k_3 c_{MRNA,o}/\mu$, μM
	$c_T\ (t=0) = 0.01$, μM
	$k_1 = 50$, 1/min.; $c_{OT} = 3.32$; nM
	$k_{d1} = 0.5$, 1/min.;
$C_1(T) = \frac{K_{i,1}^{n_H}}{K_{i,1}^{n_H} + T^{n_H}}$;	$k_4 = 0.059$; 1/min (Maria et al. (2018a)
$C_2(T) = \frac{K_{i,2}^{1.72}}{K_{i,2}^{1.72} + T^{1.72}}$	$g = 25$, μM/min.; $K_g = 0.2$, μM
	$k_2 = 15$, 1/min; $k_{d2} = 15$, 1/min
$C_3(T) = \frac{K_{i,3}^{1.2}}{K_{i,3}^{1.2} + T^{1.2}}$	$K_{i,1} = 3.53$, μM; $K_{i,2} = 0.04$, μM
	$K_{i,3} = 810$, μM; $n_H = 1.92$
	μ = Cell content dilution rate.
Obs. The nitrogen source in the **TRP** synthesis is considered in excess and included in the k_4 constant. To be connected to the glycolysis kinetic model, the **PEP** concentration kinetic trajectory generated by the glycolysis model was explicitly included in the **TRP** synthesis rate. Notations: **OR** = the complex between **O** and **R** (aporepressor of the **TRP** gene); **OT** = total TRP operon; **MRNA** = tryptophan mRNA during its encoding gene dynamic transcription, and translation; **E** = enzyme anthranilate synthase; **T** = **TRP** = tryptophan.	

IV) By contrast, high levels of [Glc]ext, triggering high rate import, also produce glycolytic oscillations for larger values of $\boldsymbol{k_6}$, due to the limited ATP recovery rate ($\boldsymbol{k_6}$ being also related to the ***K*** constant governing the AMDTP pathway). Eventually, for too small, or too large $\boldsymbol{k_6}$ values, the glycolysis reaches its steady-state.

V) The glycolytic oscillation domains in Fig. 8, plotted in terms of k_6 and [Glc]ext, are very narrow, revealing their high sensitivity with respect to the inducing factors, and their poor stability. As expected, such a result indicates that oscillations stability is also dependent on the micro-organism characteristics. For instance, by contrast, the glycolytic oscillations in yeast have been proved [3] to be very robust even in the presence of environmental noise, "oscillations being a side-effect of the trade-offs between robustness and regulatory efficiency of the feedback control of the auto-catalytic reaction network".

Simulation of the bioreactor dynamics with including coupled glycolysis and **TRP** kinetics models, revealed several conclusions relevant for TRP production maximization [2]:

I) The glucose initial concentration in the bioreactor, and its concentration in the feeding solution do not influence quantitatively the bioreactor performances (see Fig. 10);

II) On the contrary, the **TRP** production is strongly influenced by the bioreactor dilution. The maximum **TRP** production reaches the value of 0.47 (micro-M/min) for certain operating conditions ([GLC]o = 1 mM; dilution rate of 0.0003097 1/min) with quickly amortized oscillations (QAO) for glycolysis, and quasi-steady-state (QSS) regime for TRP synthesis (see Fig. 11)[2].

III) The bioreactor dilution (adjusted to be the same with the cell dilution rate) strongly influences the QSS / OSC (stable oscillations) regime of the cell bioprocess.

IV) In all cases, it is worth noting the firm evolution of the glucose level in the bulk-phase toward its steady-state (Fig. 10).

V) For the all set points investigated here and by [2], it is to remark the strong influence of the dilution (i.e. bioreactor dilution taken equal to the cell dilution) on the oscillatory behavior of the two cell sub-processes. While the glycolytic species present stationary or amortized oscillations, the **TRP** synthesis oscillations are very stable, even if of small amplitude, tending to QSS under some glycolytic/bioreactor conditions.

VI) Of course, other variables, not accounted in the model (cell characteristics reflected in the model constants) can influence the location of the problem solution. Subsequent experimental checks can validate this problem solution and, eventually, in the case of inconsistencies, they will lead to the model updating/completions for correcting its adequacy in order to perform futures bioreactor optimization analyses.

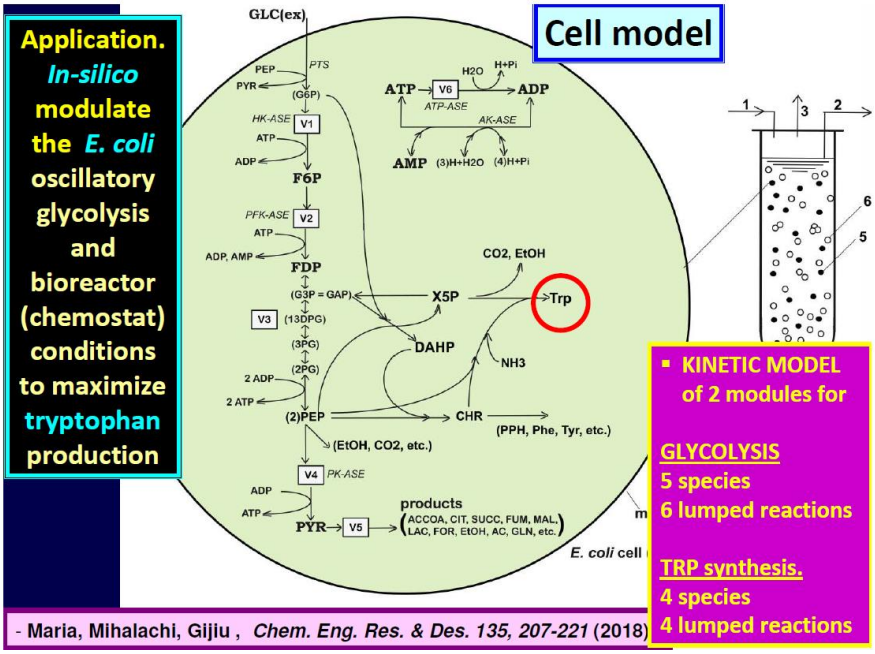

Fig. 9. Reaction pathway of glycolysis coupled with the **TRP** synthesis [2]. **TRP** synthesis kinetic model [6] coupled with the glycolysis kinetic model [4] through **PEP** node [2,5].

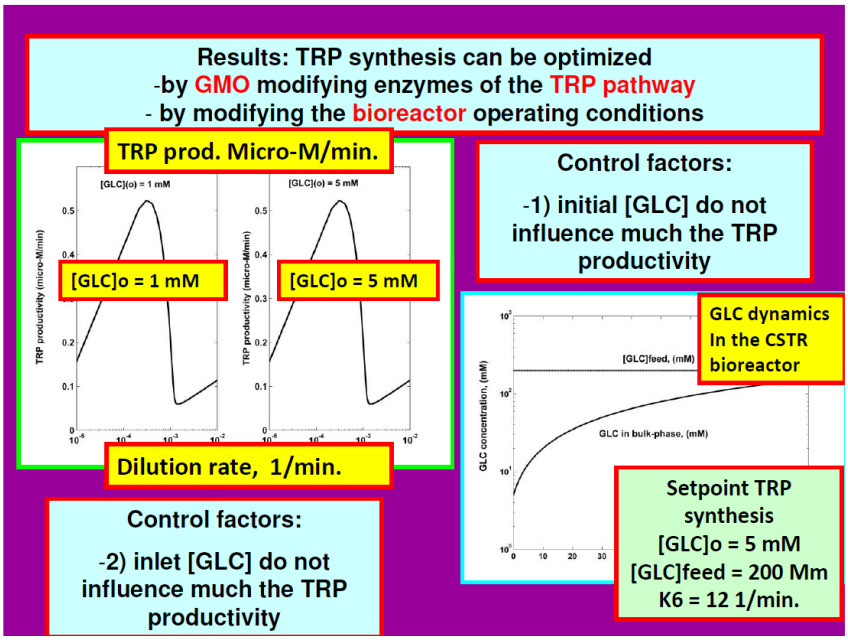

Fig. 10. In-silico evidence that tryptophan synthesis productivity is not influenced by the initial or inlet concentrations of glucose in the chemostat [2,3,5].

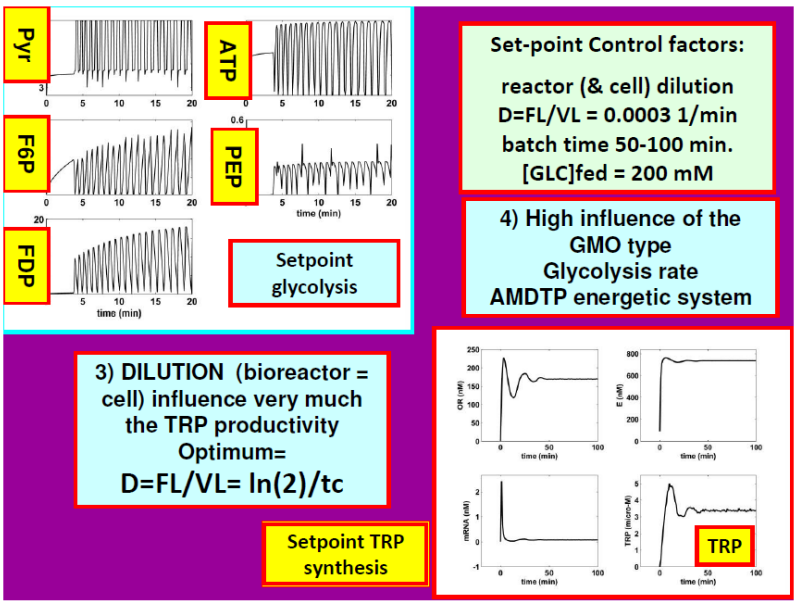

Fig. 11. In-silico determination of the optimal set-point (bioreactor and cell dilution) leading to maximization of the tryptophan synthesis [2,3,5].

6. Conclusions

The use of reduced kinetic models when modelling complex metabolic pathways is a continuous 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 / bioprocess optimization)[1]. As exemplified by the present case study, concerning *E. coli* glycolysis influence on the **TRP** synthesis, reduced kinetic models, of simple and easily adaptable structure to various cell cultures, can successfully be used for quick analyses of cell metabolism, such as the substrate utilization, oscillation occurrence, or structured interpretation of metabolic changes in modified cells.

The obtained results in the present paper prove, in a simple, but eloquent way that, beside cell phenotype characteristics (determinant for the **TRP** operon expression, and for the **ATP** regeneration engine), glycolysis is one of the major factors determining the **TRP** synthesis efficiency, and **TRP** maximization. So, to maximize the cell **TRP** production, glycolysis should be modulated to increase its speed.

This paper also proves in a relatively simple, yet eloquent way, how a lumped, but enough detailed and adequate dynamic model of essential cell metabolic processes (CCM) can support *in silico* engineering evaluations, if the

cell nano-scale metabolism (including metabolic by-products) will be somehow reflected by the bioreactor macro-scale dynamic model [2,3,7].

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Abbreviations and notations

13DPG, PGP	1,3-diphosphoglycerate	Glc, GLC	glucose
2PG	2-phosphoglycerate	GLCex, GLC(ex)	Glucose in the environment
3PG	3-phosphoglycerate	GLN	glutamine
AC	acetate	GRC	genetic regulatory circuits
AA	amino-acid	H	Hydrogen radical
ACCOA	acetyl-coenzyme A	HK-ase	hexokinase
AK-ase	adenylate kinase	LAC	lactate
AMDTP	adenosin-(mono)(di)(tri)phosphate lump	MAL	malate
ADP	adenosin-diphosphate	mM	Milli-molar
AMP	adenosin-monophosphate	MRNA	tryptophan mRNA during its encoding gene dynamic transcription, and translation
ATP	adenosin-triphosphate	NAD(P)H	nicotinamide adenine dinucleotide (phosphate) reduced
ATP-ase	ATP monophosphatase	O	**TRP** active gene
CCM	central carbon metabolism	OR	the complex between **O** and **R** (aporepressor of the **TRP** gene)
CIT	citrate	OT	total **TRP** operon
CHR	chorismat	P, Pi	Phosphoric acid
DAHP	3-Deoxy-D-arabino-heptulosonic acid 7-phosphate	PEP	phosphoenolpyruvate
DHAP	dihydroxyacetonephosphate	PFK-ase	phosphofructokinase
DW	dry-weight	PK-ase	pyruvate kinase
E	enzyme anthranilate synthase	Phe	Phenylalanine
ETOH	ethanol	PPP	pentose-phosphate pathway
F6P	fructose-6-phosphate	PPH	prephenate
FAD	Flavin adenine dinucleotide	PTS	phosphotransferase, or phosphoenolpyruvate: glucose phosphotransferase system

FADH	semiquinone form of the reduced FAD	PYR	pyruvate
FADH2	hydroquinone form of the reduced FAD	QSS	quasi-steady-state
FDP	fructose-1,6-biphosphate	R	aporepressor of the **TRP** gene
FOR	formate	SUCC, SUC	succinate
G3P, GAP	glyceraldehyde-3-phosphate	TCA	tricarboxylic acid cycle
G6P	glucose-6-phosphate	TF	transcription factors
GKase	glucokinase	T, TRP	tryptophan
GERM	gene expression regulatory module	Tyr	Tyrosine
		X5P	Xylulose 5-phosphate
c_j	species *j* concentration	**Indices**	O =initial; syn= synthesis
D	cell content dilution rate	Index s	Stationary (quasi-steady-state)
k_j , K_j	rate constants	y_{trp}	stoichiometric coeff.
t , t_c	Time, Cell cycle	**Super-script**	n = reaction order
QAO	quickly amortized oscillations	OSC	stable oscillations

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