Abstract: Through the years the man has used microorganisms in the fermentation to produce different types from foods like being bread, yogurt, and cheeses among others, without knowing involved Biology in these processes. However, the cornerstone in the central metabolism, the dynamic behavior of the glycolytic pathway is not accurately known. The metabolic networks are difficult to represent in biochemistry, because complex relationships exist. Most of the kinetic models in biology are described by coupled non linear ordinary differential equation, with a tremendous numbers of equations. The main goal of this research modestly project is to get a global understanding of the dynamical behavior for one particular system, with four non linear ordinary differential equations proposed by Diaz Ricci in 2002, for all initial conditions and all values of the parameter. The methodology used was qualitative method for the Bifurcation analysis of dynamic systems. The results presented in this research, can be regarded as preliminary. From these it is possible to investigate further the dynamics of the system considering different situations to try to understand the behavior of glycolytic pathway of E. Coli.
1.- Introduction

Through the years the man has used microorganisms in the fermentation to produce different types from foods like being bread, yogurt, and cheeses among others, without knowing involved Biology in these processes. However, the cornerstone in the central metabolism, the dynamic behavior of the glycolytic pathway is not currently known.

One of the microorganisms better known and more used, at the moment in Biotechnology, is the bacterium Escherichia Coli also known as E. Coli. Currently it is used for methanol production as a biofuel.

E. Coli it is a Gram-negative bacterium that characterizes itself for being anaerobic facultative and is able to grow quickly to high densities in substrates of low costs.

E. Coli was the first organism whose genome was fully sequenced. Nevertheless the complexity of the biochemical processes that take place simultaneously makes very difficult the study of the influence of all the parameters and variables involved on the cellular metabolism to evaluate the influence of them in the yield and recombinant productivity of interest metabolites or proteins.

E. coli as any living cell, is extremely a well-organized autonomous systems that consist of a tremendous number of components that interact in complicated ways sustaining the processes of life [Centler F., et. al, 2006]. The key to understand their behavior is modeling their system organization [Cardelli, L., 2005].

Metabolism of living cells transforms substrates into metabolic energy, redox potential and metabolic end products that are essential to maintain cellular function. The flux distribution among the various biochemical pathways is determined by the kinetic properties of enzymes which are subject to strict regulatory control. [Varma A., Palsson B.O., 1993].

In the past decade different mathematical models for predicting and explaining various biochemical processes carried out for various microorganisms has been proposed. [Chassagnole C., 2002].

We can mention that the metabolic networks are difficult to represent in biochemistry, because complex relationships exist. For example, 483 reactions belong to a single pathway. Several processes were studied by considering the genetic map of E. Coli and published in 2000 in the journal Genome Research.
Table N° 1: List of all Known E. Coli Metabolic Pathways as Described by EcoCyc*

<table>
<thead>
<tr>
<th>Metabolic Pathway</th>
<th>Metabolic Pathway</th>
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<tbody>
<tr>
<td>(Dec)oxyribose phosphate metabolism</td>
<td>Isoleucine biosynthesis</td>
</tr>
<tr>
<td>3-Phenylpropionate and 3-(3-hydroxyphenyl)propionate</td>
<td>KDO biosynthesis</td>
</tr>
<tr>
<td>degradation</td>
<td>l-alanine degradation</td>
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<tr>
<td>Aerobic electron transfer</td>
<td>L-arabinose catabolism</td>
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<tr>
<td>Aerobic respiration, electron donors reaction list</td>
<td>L-cysteine catabolism</td>
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<td>Alanine biosynthesis</td>
<td>L-glyoxylate metabolism</td>
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<td>L-lactate degradaton</td>
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<td>Anaerobic respiration, electron acceptors reaction list</td>
<td>Leucine biosynthesis</td>
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<tr>
<td>Anaerobic respiration, electron donors reaction list</td>
<td>Lipid A precursor biosynthesis</td>
</tr>
<tr>
<td>Arginine biosynthesis</td>
<td>Lysine and diaminopimelate biosynthesis</td>
</tr>
<tr>
<td>Asparagine biosynthesis and degradation</td>
<td>Mannitol degradaton</td>
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<tr>
<td>Aspartate biosynthesis and degradation</td>
<td>Mannose and GDP- mannose metabolism</td>
</tr>
<tr>
<td>Betaine biosynthesis</td>
<td>Mannose catabolism</td>
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<tr>
<td>Biosynthesis of proto- and strethome</td>
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<tr>
<td>Betulin biosynthesis</td>
<td>Methionine biosynthesis</td>
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<tr>
<td>Carnitine metabolism</td>
<td>Methyl-donor molecule biosynthesis</td>
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<td>Carnitine metabolism, CoA-linked</td>
<td>Methylglyoxal metabolism</td>
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<td>Cobalamin biosynthesis</td>
<td>NAD phosphorylation and dephosphorylation</td>
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<td>Colanic acid biosynthesis</td>
<td>Nonoxidative branch of the pentose phosphate pathway</td>
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<tr>
<td>Cyanate catabolism</td>
<td>Nucleotide metabolism</td>
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<tr>
<td>Cysteine biosynthesis</td>
<td>O-antigen biosynthesis</td>
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<td>o-arabinoose catabolism</td>
<td>Oxidative branch of the pentose phosphate pathway</td>
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<td>o-galacturonic catabolism</td>
<td>Pantothenate and coenzyme A biosynthesis</td>
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<td>Peptidoglycan biosynthesis</td>
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<td>Pyridoxal 5′-phosphate biosynthesis</td>
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<td>Folk acid biosynthesis</td>
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<td>FormylFH biosynthesis</td>
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<td>Removal of superoxide radicals</td>
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<td>Thiamine biosynthesis</td>
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<td>Glutathione-glutaredox redox reactions</td>
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<tr>
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<td>Threonine catabolism</td>
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<td>Glycine biosynthesis</td>
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Most of the kinetic models in biology are described by coupled ordinary differential equations, and implement the appropriate methods to solve these systems. The
biochemical reaction very often involves a series of steps instead of a single one. Therefore, one of the biochemical research problems has been to capture or describe the series of steps, called **pathways**. [Chassagnole, C., 2006].

The main obstacle, to solve those differential equations, is the dimensionality of the parametric space, nonlinearity and ill-conditioned relations for parameter estimation. In this work such types of models are analyzed from the study of stationary states, periodic solutions and their bifurcations through the method of continuation. In particular the general study implies qualitative methods.

Our goal is to analyze and understand, the mechanisms of Glycolytic pathway of *Escherichia Coli* by dynamical systems techniques.

2. **Central Carbon Metabolism of E. Coli**

One of the main activities of the cell can be summarized in two points as:

1. The cell needs to find the necessary energy for its activity (catabolism).
2. The cell needs to produce simple molecules for its survival (anabolism)

These two activities are grouped under the name of metabolism [Chassagnole C., 2006]. The Central Carbon Metabolism of *E. coli* in general and specifically the glucose metabolism are well-known, well-studied and well-characterized topics; This metabolism can be described by several interconnected metabolic pathways as seen in Fig. Nº 1:
Fig. Nº 1: Simplified view of the Central Carbon Metabolism of E. Coli comprising (a) glycolysis and gluconeogenesis. (B) anaerotic reactions. (C) acetate formation and assimilation. (D) TCA cycle and E. Gluoxilate shunt. Arrows with broken lines indicate removal of metabolites for biosynthesis. The arrow with the dotted line indicates an anaplerotic reaction catalyzed by pyruvate carboxylase (an enzyme not present in wildtype E. Coli). From: S.Y. Lee (ed) Systems Biology and Biotechnology of Escherichia Coli. Springer Science+Business Media B. V. 2009. Pg. 379.

It is easy to notice that the metabolism of E. Coli involves varied and complex activities, in particular we will seek to study, for simplicity, the first stage “A” which includes the entry of exogenous glucose to Pyruvate become.

Our modeling problem is a small and modest brick in a general and challenger biochemical project.
Fig. Nº 2: Glycolytic Pathway, considering all the Enzymes involved. Zoom of block “A”
This stage can be represented by the following graph, which considers the simplified metabolic pathway.

![Graph of metabolic pathway](image)

Fig. Nº 3: The scheme includes all regulatory effects considered in this study. Abbreviations: G:Glucose; F6P:fructose 6 phosphate; FDP: fructose 1,6 disphosphate; PEP: phosphoenolpyruvate; PYR: pyruvate; ATP:Adenosyn triphosphate; ADP: adenoshyndiphosphate. [Diaz Ricci, 2000].

Given this simplified scheme is possible to construct a mathematical model including different enzymatic reactions in the Pentose Phosphate Pathway.

3.- Structure of the Model

The model is based on flux balances of the intermediate metabolites (Fig. Nº 3) proposed by Diaz Ricci. This model considers the dynamic of Embden-Meyerhof-Parnas pathway and pentose-phosphate pathway of E. Coli consists of mass balance equations for extracellular glucose and for the intracellular metabolites [Chassagnole C., 2002]. The Pentose Systems (PTS) consists in a complex of four proteins that transfer a phosphate group in a cascade reaction.

Taking into account the mass balance, the system can be described from a system of differential equations

\[
\frac{dC_j}{dt} = \sum_j \sigma_j v_j \quad (1)
\]

Were $j=\text{N}^0$ de metabolites considered; maximum value of $j$ depend on the model considered, it can be 100, 200, 400 or even more.
\( c_j \) denote the concentration of metabolite \( j \), (for example ADP, ATP, F6P, PEP, FDP).

\( \sigma_j \) is the maximum reaction rate, and

\( \nu_j \) is the saturation function of PTS system, depending of metabolite \( j \) considered.

Although the values that can take the substrates are very variable, from experimental studies in vivo for E. Coli, is possible to know maximum values admitted for Glu, ADP, ATP, PEP, FDP and PYR in Glycolytic Pathway. This values are ADP < 3mM; ATP < 3mM; F6P < 5mM; PEP < 1mM and PYR < 5mM.

One particular case will be study taken in account Fig. Nº 3 and (1). This case corresponds to the dynamical system model developed for the enzymes ADP, ATP, F6P, PEP and FDP by Diaz Ricci in 2000; considering constants of dissociations, number of protomers \( n=4 \), fractions of activities \( \langle R; T \rangle \), allosteric effectors (ADP and PEP), allosteric equilibrium \( \langle L \rangle \), values of dissociation constants (expressed in mM) and ATP glycolytic consumption rate constant.

In this model the independent variable is \( t \) (time), the dependents variables are the concentration of metabolite \( C_j \); the parameters are the maximum reaction rate \( \sigma_j \).

\[
\begin{align*}
\frac{dv}{dt} &= \sigma_2 v_2 - \sigma_4 v_4 - 2\sigma_3 \frac{x}{0.3+x} \\
\frac{dw}{dt} &= \sigma_1 \frac{y}{0.11+y} - \sigma_2 v_2 \\
\frac{dx}{dt} &= \sigma_2 v_2 - \sigma_3 \frac{x}{0.3+x} - 0.1 \times x \\
\frac{dy}{dt} &= 2\sigma_3 \frac{x}{0.3+x} - \sigma_1 \frac{y}{0.11+y} - \sigma_4 v_4
\end{align*}
\] (2)

Were \( x = FDP \); \( y = PEP \); \( v = ADP \); \( w = F6P \) and \( u = ATP \)

\[
v_1 = \frac{y}{0.11 + y}
\]

\[
v_2 = \frac{w \times \frac{u}{0.06} \times R^3 + L \times \frac{w}{25} \times \frac{u}{0.06} \times T^3}{R^4 + L \times T^4}
\]
We can see that the system (2) is composed by four nonlinear ordinary differential equations and four parameter \((\sigma_i)\).

**Objective:** The main goal of this research modestly project is to get a global understanding of the dynamical behavior of system (2) for all initial conditions and all values of the parameter.

### 4.- Methodology

As the model has four parameters to be estimated, it is possible to study the dynamics of the system (2) selecting a particular parameter and by fixing the other three, which will lead us almost infinite possibilities. In particular, based on previous numerical experience, we considered the variation of parameter \(\sigma_3\) in the interval \([0.1, 0.2]\). The results presented below, can be regarded as preliminary. From these it is possible to investigate further the dynamics of the system considering different situations to try to understand the behavior of **glycolytic pathway** of E. Coli.

\[
v_3 = \frac{x}{0.3 + x}
\]

\[
v_4 = \frac{(5 * y * v / 0.31 * 0.26)}{1 + \frac{y}{0.31}} + \frac{v}{0.26} + \frac{5 + V + y}{0.26 + 0.31}
\]

\[
L = L_0 \frac{(1 + \frac{y}{0.75})^4 (1 + \frac{v}{1.3})^4}{(1 + \frac{v}{0.025})^4 (1 + \frac{y}{1000})^4}; \quad L_0 = 4.10^6
\]

\[
R = 1 + \frac{w}{0.0125} + \frac{u}{0.06} + \frac{w * u}{0.0125 * 0.06}
\]

\[
T = 1 + \frac{w}{25} + \frac{u}{0.06} + \frac{w * u}{25 * 0.06}
\]
To study the dynamical system (2) we use Matlab and Auto software [Doedel E & Oldeman B, 2009], and qualitative methods. The steps that followed were:

1) Analyze the evolution of $x = \text{FDP}; y = \text{PEP}; v = \text{ADP}; w = \text{F6P}$ respect to time $t$. Analyze the possible existence of equilibrium, and in case of existing, if those equilibriums are stable or unstable with Matlab program.

2) Once the existence of orbits or equilibrium was established, explore the rest of the curve to identify other points of equilibrium using the method of continuation with the Auto software.

3) Plan future development strategies to understand the behavior of the glycolytic pathway

5.- Results

Step 1*: 1º) Analyze the evolution of $x = \text{FDP}; y = \text{PEP}; v = \text{ADP}; w = \text{F6P}$ respect to time $t$ using qualitative method, with Matlab program.

Considering the dynamic system (2), and through the program model2.m is possible to solve the system using the Matlab function “ode45” with known initial value (Diaz Ricci, 2002), and varying the parameter $\sigma_3$ in the interval $[0.1, 0.2]$ with ATP constant and the possible maximum values for ADP; PEP; FDP. Similar results were found regarding the time evolution for ADP, PEP, FDP and F6P.
We can observe that the evolution of \( v, w, x, \) and \( y \) respect to time \( t \) is cyclical, effect observed when we take a small rank of time, like Fig. N°5.

Fig. N° 4: Temporal evolution of ADP, F6P, PEP and FTP for \( S3=0.1 \). 2D Phase Diagrams for qualitative analysis. Rank of time \([0, 100\text{seg}]\).

To project at different levels we can observe periodic orbits.

Fig. N° 5: Temporal evolution of ADP, F6P, PEP and FTP for \( S3=0.1 \). 2D Phase Diagrams for qualitative analysis. Rank of time \([0, 30\text{seg}]\).

*This step was studied using “model2.m” (Appendix A), developed by Dr. Galan Vioque under Matlab software.
To find the period we plot the distance between two zeros of the distance function. After the transient time we assume that the system (2) in a periodic solution, considering 
\[ d(t) = \| u(t_f) - u(t_f + t) \|. \]

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<tr>
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<th>b)</th>
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<td><img src="image2.png" alt="Graph" /></td>
<td><img src="image3.png" alt="Graph" /></td>
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<tr>
<td>For ( \sigma_3 = 0.10 ); ( T = 2.81 )</td>
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<td>For ( \sigma_3 = 0.13 ); ( T = 2.43 )</td>
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<td>Evolution of Period ( T ) vs ( \sigma_3 )</td>
</tr>
</tbody>
</table>

**Fig. Nº 6:** Evolution of period \( T \) when the \( \sigma_3 \) parameter takes increasing values. The oscillations period depends of \( \sigma_3 \).
The periodic orbits found are very important and expected result in the physiological behavior, the energy consumption is oscillatory, this indicates that the particular selected model is suitable to study for the route of glycolic pathway E. Coli. The biological interpretation will be done with the interdisciplinary group of research belongs to the Facultad de Bioquimica, Quimica y Farmacia of Universidad Nacional de Tucumán.

**Step 2**) Considering the dynamic system (2), and through the program “model2.auto” develop in Auto software (Appendix B)

Auto software is a standard program to detect continuum equilibrium and periodic orbits, identify stability bifurcations by computing the Floquet multipliers.

Using Auto the results are presented in three ways: a) on the screen numerical data are displayed which identify possible equilibrium and their bifurcations. b) All the output is saved in three data files fort.7, fort.8 and fort.9, and c) graphics with bifurcations and solutions.

*In the rest of this section we show the results for a single continuation experiment*

a) Screen displayed for model2.auto for $\sigma_3$ parameter considering one initial point know, the equilibrium point, and the continuation method was applied for to explore the rest of the curve for identified other equilibrium points and specials bifurcations.

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```

**This step was studied using “model2.auto” (Appendix B), developed by Dr. Galan Vioque under AUTO software.**
We can observe that other EP (equilibrium point), one BP (branching point), four LP (limit point) and three PD (double period) were detected.

b) If now look at the values presented in the file fort.9 we can see the Floquet multipliers, and from their values to recognize the possible stability or unstability in one special point.
c) Graphics with Bifurcations and Solutions

Plot of Bifurcation for \( \sigma_3 \) selected parameter, equilibrium, double period and other interesting behavior are detected,

Evolution of period \( T \) when the \( \sigma_3 \) parameter takes increasing values. Considering the interval \([0.1, 0.2]\) the plot is similar to 1) of Fig.Nº 6

Zoom for a particular behavior detected

Fig. Nº 7 : Bifurcation plot. In a particular plot a) we can observe the behavior of the system, to analyze is possible get around the corner, in an orderly manner, taking into account the number of label for each point.
Non trivial orbit founded

Non trivial orbit founded in level 1.

Two orbit are plotted to compare the behavior system in levels 21 and 23.

Orbit evolution for several levels

Fig Nº 9: Non trivial orbits were detected
Selected level 30 and continuation the curve with auto, we founded:

**Fig. Nº 10**: Two Hopf bifurcations and a LP were detected.
Conclusions:

While studying the behavior of the model only with respect to a selected parameter we find a very rich dynamic behavior, we can mention periodic orbits, Hopf bifurcations, double periods (PD), limit points (LP), branching points (BP). Of course still we are far from fully understanding the dynamics of this system but we move one step in the knowledge of it. Biological interpretations are also necessary to advance in the study.

Finally based on preliminary results found, we can say: the study of this particular model opens a range of questions and possibilities for future research.
Appendix A. Matlab programs

Main_model2.m

```matlab
% Glycolytic model>
figure(1);clf; figure(2);clf
clear;

% parameter vector
% original values
% s1= 0.5;
% s2= 3.0;
% s3= 0.2;
% s4=3;
% u=1;
s1 = 0.1;
s2 = 3;
s3 = 0.20; %try moving s3 from 0.2 to 0.1 step 0.1 !!!!
s4 = 3;
u = 1;
par=[ s1; s2; s3; s4; u];

% regular integration
tspan = [0 100];
%var0 = [0.1; 0.035; 0.1; 0.012];
u0=[1 ; 1; 1; 1];
TOL=10^(-5);
options=odeset('RelTol',TOL,'AbsTol',TOL);
[t,u] = ode45(@model2, tspan, u0,options,par);

% Plot of the solution x and y z
figure(1);
subplot(431);plot(t,u(:,[1 ]))
ylabel('v')
subplot(434);plot(t,u(:,[2 ]))
ylabel('w')
subplot(437);plot(t,u(:,[3 ]))
ylabel('x')
subplot(4,3,10);plot(t,u(:,[4 ]))
```
ylabel('y')
xlabel('t')

% caux=sprintf('%3.2f',mu);
%
% title(strcat('van der Pol Eqn, \mu = ', caux))
subplot(332);plot(u(:,1),u(:,2))
xlabel('v')
ylabel('w')
subplot(335);plot(u(:,2),u(:,3))
xlabel('v')
ylabel('x')
subplot(338);plot(u(:,1),u(:,4))
xlabel('v')
ylabel('y')
subplot(3,3,3);plot(u(:,2),u(:,3))
xlabel('w')
ylabel('x')
subplot(3,3,6);plot(u(:,2),u(:,4))
xlabel('w')
ylabel('y')
subplot(3,3,9);plot(u(:,3),u(:,4))
xlabel('x')
ylabel('y')

% searching the limit cycle NOT ELEGANT
% EXCERCISE: improve with Newton
n=length(t); % last point
u0=u(n,:);
distance=[];
T=4;
tspan=[0:0.01: T];
[t,u] = ode45(@model2, tspan, u0,*,par);
n=length(t);
distance=[];
for k=1:n
    distance=[distance;t(k) norm(u(k,:)-u0)];
end
figure(2);
plot(t, distance(:,2))
xlabel('time'); ylabel('distance')
% locate the minimum of the distance away from
% the origin
[min,nmin]=min(distance(10:n,2));
T=t(9+nmin)
% write the numerical periodic orbit for the
% continuation with AUTO
sol=[t(1:9+nmin) u(1:9+nmin,:)];
save -ascii model2.dat sol

Note: ode45 is based on an explicit Runge-Kutta (4,5) formula, the Dormand-Prince pair. It is a one-step solver - in computing y(tn), it needs only the solution at the immediately preceding time point, y(tn-1). In general, ode45 is the best function to apply as a “first try” for most problems (Matlab v 7 Manual).

Model2.m

function dudt=model2(t,var,par)
% catalytic model 2
% v = ADP
% w = F6P
% x = FDP
% y = PEP
% parameters
s1 = par(1);
s2 = par(2);
s3 = par(3);
s4 = par(4);
u = par(5);
% constants
k1 = 0.11;
k3 = 0.3;
k5 = 0.1;
L0 = 4*10^6;
% variables
\begin{verbatim}
19  v = var(1);
20  w = var(2);
21  x = var(3);
22  y = var(4);
23  % auxiliary expressions
24  L = L0*( (1+y/0.75)*(1+v/1.3)/ ( ( 1+v/0.025)*(1+y/1000) ) )^4;
25  R = 1+(w/0.0125) + (u/0.06) +(w*u)/ (0.0125*0.06);
26  T = 1+(w/25)+(u/0.06)+(w*u)/(25*0.06);
27  v1 = y/(k1+y);
28  v2 = ((w/0.0125)\*u/0.06)*R^3 +L*(w/25)*(u/0.06)*T^3)/(R^4+L*T^4);
29  v3 = x/(k3+x);
30  v4 = (5*y*v/(0.31*0.26)) / (1+(y/0.31)+(v/0.26)+(5*v*y/(0.26*0.31)));
31  dudt = zeros(3,1);
32  dudt(1) = s2*v2 - s4*v4 - 2*s3*v3; % v' -> ADP
33  dudt(2) = s1*v1 - s2*v2; % w' -> F6P
34  dudt(3) = s2*v2 - s3*v3 - k5*x; % x' -> FDP
35  dudt(4) = 2*s3*v3 - s1*v1 - s4*v4; % y' -> PEP
\end{verbatim}

Appendix B. AUTO programs

Model2.f

!--------------------------------------------------------
! model2 :
!--------------------------------------------------------

SUBROUTINE FUNC(NDIM,U,ICP,PAR,IJAC,F,DFDU,DFDP)

C parameters

s1 = par(1)
s2 = par(2)
s3 = par(3)
s4 = par(4)

Draft versión
paru = par(5)

C constants

k1 = 0.11d0
k3 = 0.3d0
k5 = 0.1d0
L0 = 4*10**6

C variables

v = u(1)
w = u(2)
x = u(3)
y = u(4)

C auxiliary expressions

L = L0* ( (1+y/0.75)*(1+v/1.3)/((1+v/0.025)*(1+y/1000) ) )**4
R = 1+(w/0.0125) + (paru/0.06) + (w*paru)/ (0.0125*0.06)
T = 1+(w/25)+ (paru/0.06)+ (w*paru)/(25*0.06)
vl = y/(k1+y)
v2 = (w/0.0125)* (paru/0.06)*R**3+L*(w/25)* (paru/0.06)*T**3
v2 = v2/(R**4+L*T**4)
v3 = x/(k3+x)
v4 = (5*y*v/(0.31*0.26))/ (1+(y/0.31)+ (v/0.26)+ (5*v*y/(0.26*0.31)))

f(1) = s2*v2-s4*v4-2*s3*v3
f(2) = s1*v1-s2*v2
f(3) = s2*v2-s3*v3-k5*x
f(4) = 2*s3*v3-s1*v1-s4*v4

END SUBROUTINE FUNC

SUBROUTINE STPNT(NDIM,U,PAR,T)
!
! Parameter values for the starting orbit in model2.dat :
!
s1 = 0.5d0
s2 = 3.0d0
s3 = 0.2d0
s4 = 3.0d0
paru = 1.0d0
!
PAR(1) = s1
PAR(2) = s2
PAR(3) = s3
PAR(4) = s4
PAR(5) = paru
!
END SUBROUTINE STPNT
SUBROUTINE BCND
END SUBROUTINE BCND
SUBROUTINE ICND
END SUBROUTINE ICND
SUBROUTINE FOPT
END SUBROUTINE FOPT
SUBROUTINE PVLS
END SUBROUTINE PVLS

Model2.auto
#============
# model2
#============

pgm = 'model2'
print pgm, ": first run : a solution branch starting from numerical data"
model2=run('model2',c='model2.ini')
sv('t')
ch("IRS",2)
ch("ISW",-1)
ch("NPR",10)
ch("NMX",200)
run(s='t')
stop

print pgm, ": second run : switch branches at a period-doubling"
lor=lor+run(lor('PD1'),c='lor.2')
print pgm, ": third run : third run : another period-doubling"
lor=lor+run(lor('PD2'),c='lor.3')
save(lor,'lor')
c.model2
dat = 'model2'
NDIM = 4, IPS = 2, IRS = 0, ILP = 1
ICP = [3, 11]
NTST = 100, NCOL = 4, IAD = 3, ISP = 2, ISW = 1, IPLT = 0, NBC = 0, NINT = 0
NMX = 200, NPR = 50, MXBF = 10, IID = 2, ITMX = 8, ITNW = 7, NWTN = 7, JAC = 0
EPSL = 1e-08, EPSU = 1e-08, EPSS = 1e-06
DS = 0.1, DSMIN = 1e-10, DSMAX = 0.1, IADS = 1
NPAR = 6, THL = (11:0.0), THU = {}
UZR = (1:200.0), STOP = [UZ1]
Reference


Shiloach J., Rinas U. (2009), Glusose and Acetate Metabolism in E. Coli-System Level Analysis and Biotchnological Applications in Protein Production Processes. S.Y.
Lee (ed), Systems Biology and Biotechnology of Escherichia Coli. Springer Science-Business Media B.V. 18: 377-400