MELISSA

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Dynamic simulator of the 3 connected compartments : Rhodobacter, Nitrifying and Spirulina

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TABLE OF CONTENTS

Abbreviations or notations:
AcOH : Acetic acid
CO_2 : carbon dioxide (gaseous or solvated)
EPS : exopolysaccharide
HPC : Higher Plant Chamber
NH_3 : ammonia (gaseous or solvated)
NO_2 : nitrite ion
NO_3 : nitrate ion
N_s : Nitrosomonas
N_b : Nitrobacter
PC : phycocyanin
XA : active biomass

Note :
In a figure, a graph ij is located by its row i and its column j.

1. INTRODUCTION

A first global simulator was already build a few years ago. It described the behaviour of the 5 compartments (Crew, Liquefying, Rhodobacter, Nitrifying and Spirulina) of the open loop of MELISSA at the time. It was based on 13 stoechiometric equations for each of whom a key element was completely consumed, the other compounds being assumed to be in sufficient quantities.

Since then, the modelling of a few compartments has been improved taking into account the thermodynamic equilibria (gas/liquid, dissociation in water), the chemical kinetics, a few mineral limitations and a hydraulic flow (rather complex in the case of the nitrifying column). Here, these 3 compartments (Rhodobacter, Nitrifying and Spirulina) are connected in order to study the dynamic interactions between them and with the environment.

2. DESCRIPTION OF THE SIMULATOR

The simulator is composed of the 3 above compartments. These 3 basic components are designed pr_rhodo, pr_nitri and pr_spiru in the figure 1a and their parameters are set in intialization files (given in annex). They can be linked between them and with the environment in various ways. The figures 1b and 1c show a possible set of links. The gas and liquid coming into Rhodobacter are supposed to come from compartment 1 (Liquefying); its outgoing gas and solid (biomass) are removed (the gas could be sent to the HPC, if any) while the liquid goes into the Nitrifying. The gas coming into this one comes from the consumer compartment; the nitri-biomass is removed while the gas and solid flows enter the Spirulina. These links can be changed easily enough for the needs of a particular study.

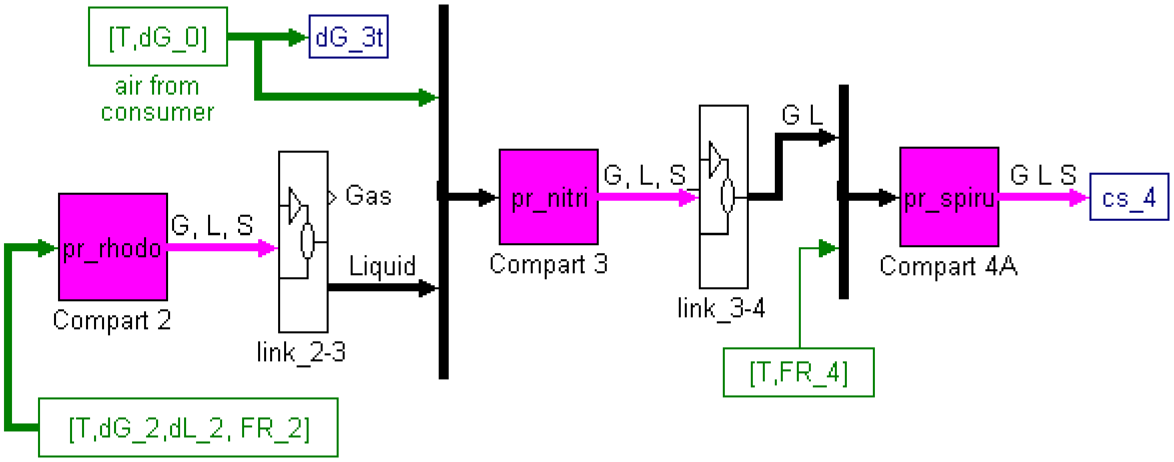

Figure 1a : Simulator of the 3 connected compartments

The modelling of the compartments is detailed in annex. An example of the links is shown in the figures 1b and 1c where the ‘Matlab® function’ store the concentrations correctly in the input vector from the output vector and take into account the variations of the concentrations due to pH and temperature.

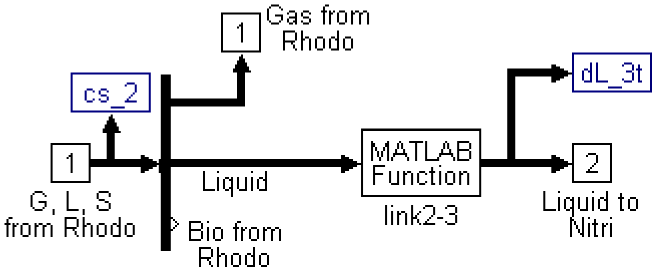

Figure 1b : Liquid link from Rhodo to Nitri

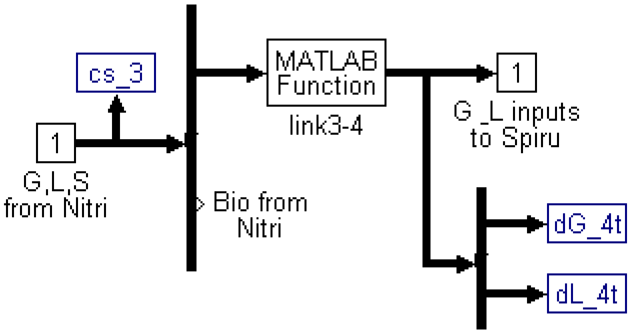

Figure 1c : Gas and Liquid link from Nitri to Spiru

State of modelling of the compartments :

1_ The Rhodobacter compartment is simulated according to the latest version of the first principles model of TN 45.1 by LGCB : the only source of carbon is the acetate ion.

2_ The model of the nitrifying column is limited to TN 27.1 and TN 27.2 by LGCB and does not take into account :

- the inhibitory effect of NO_2^- and NO_3^- on the Nitrobacter growth (introduced in TN 27.3);
- the biofilm diffusion model (introduced in TN 27.3) ;
- the metabolism and growth of Nitrosomonas and Nitrobacter in presence of organic matter (introduced in TN 32.1) .

3_ The Spirulina compartment is simulated according to the first principles model of TN 19.1 and 19.2 (Version 1, issues 0 and 1, January 1997) by LGCB. A Monod term for the phosphate $\frac{C_P}{K_P + C_P}$ has been added in the production rates of the active biomass (XA), of the phycocyanin (PC) and of the vegetative biomass (XV) in order to limit the kinetics when the phosphate concentration is going to zero (detail in annex A3.1.2.).

As an additional information to the TN 19.1 and 19.2, it is mentioned that the synthesis of glycogen is represented by the following global chemical reaction :

$$CO_2 + 0.827\ H_2O + 0.008\ H_2SO_4 \rightarrow C\ H_{1.67}\ O_{0.71}\ S_{0.008} + 1.0745\ O_2$$

Sizing of the compartments :

These compartments are supposed to destroy the waste of one man and the carbonic gas of 3 rats and to furnish the oxygen for 3 rats.

The actual Spirulina compartment is performing these specifications. So its volume (77 l) and the incoming gas and liquid flow rates are unchanged.

The size of the nitrifying column is reviewed in order to allow a good oxidation of ammonia into nitrate with a very low production of nitrite as it explained in annex A4.2 .

The volume of the Rhodobacter compartment is merely increased in the ratio of the new flow rate of liquid to keep unchanged the residence time. Its volume is 42.5 l to fit the incoming liquid flow rate of 0.77 l/h.

3. EXAMPLE OF SIMULATION

The simulation is done with the links explained above (figure 1).

The input data of Rhodo are :

- The concentrations of NH_3 and AcOH in the incoming liquid are those produced by the waste of 1 man. They are extrapolated from the previous TN 35.1 (ADERSA) of the global simulator where the wastes are those of 3 men and where the conversions are supposed to be complete. They are such that the corresponding flow rates are : 0.0718 mol/h of NH_3 total and 0.0118 mol/h for AcOH total (the flow rate of AcOH of TN35.1 has been increased with the flow rate of butyric acid multiplied by 2 to take into account the relative stoechiometry of C atom of these both source of carbon).
- The sulphate and phosphate concentrations in the incoming flow are extrapolated from those of TN 19.3 (LGCB), Appendix 7, Figure 8 and have been chosen to avoid mineral limitation (Sulphate : $2.02\ 10^{-3}$ mol/l (0.2 g/l) and phosphate : $3.95\ 10^{-3}$ mol/l (0.4 g/l)).
- The initial concentration of biomass is 0.6 g/l;
- The concentrations of the incoming gas and liquid flows are constant throughout the simulation.
- The initial light flux is such that the reactor is steady state (about 16.0 W/m^2) and is set at 31 W/m^2 at t=20 h to consume nearly all the acetic acid.

As shown in the figure 1, the nitrifying column receives the air from the consumer compartment. The gas inputs of the column, constant throughout the simulation, are :

- The CO_2 production rate of the team is the one of 3 rats, i.e. 0.12 mol/h ($4\ 10^{-2}$ mol/h per rat; according to TN47.3 p.8 by UAB).
- The O_2 concentration of the incoming gas flow is 21 % molar fraction.

- The incoming gas flow rate is 180 l/h (TN 43.110 p.26, UAB).

The concentrations in the incoming liquid are those of the output of Rhodo (particularly the O_2 concentration is null). The incoming liquid flow rate is 77 10^{-2} l/h (as in Rhodo).

The gas and liquid inputs of the Spiru are the gas and liquid outputs of Nitri.

The simulated horizon is 600 h.

The results of the simulation are gathered in 3 groups of figures (mass balance; concentrations at output of the compartment; production rate of the whole compartment) for each of the compartments. All details about mass balance are explained in annex A1.5 .

The group of mass balance (figures 2 to 4) allows to follow the variation of the incoming flow rates of each atom C, N, S and P throughout the simulated open loop. It has to be reminded that the biomass produced by a compartment is not entering the following one, which explains that the incoming flow rate decreases from a compartment to the following one (except for atom C between Rhodo and Nitri because of CO_2 gas entering Nitri from the consumer compartment).

Rhodo :

When the light flux increases (from 16 to 31 W/m^2) at time t=20h, the consumption rate of AcOH increases instantaneously from 7 10^{-3} to 11 10^{-3} mol/h (graph 21 of figure 8). So do the variation rates of the other substrate (NH_3) and of the products (CO_2 and biomass). Beyond that time, the rates vary slowly with the increasing of the biomss concentration; the discontinuity observed in this evolution at time t=180h in not explained (the mean efficient intensity ε_J of the light law could be at the origin of this phenomenon).

At the end of the simulation (600 h), a new steady state is reached, corresponding to the light flux of 31 W/m^2. The output concentration of AcOH is then 2.6 10^{-4} mol/l (graph 31 of figure 5).

Because of the light flux step, the consumption rate of NH_3 increases slightly (graph 12 of figure 8) and the output concentration of NH_3 decreases slightly (graph 21 of figure 5).

Nitri :

The consequences on Nitri (which are weak because its ‘mixotrophic’ behaviour is not yet simulated) are that the variation rates of the biomass Ns and Nb become negative(graph 41 of figure 9) and then the production rate of NO_3 and the consumption rates of O_2 and CO_2 decrease slightly(graphs 22, 11 and 12 of figure 9).

Spiru :

The consequences on Spiru are very light : it can be noted a 1 % increasing of Glycogen production. The variations of the concentrations of CO2 and O2 (graphs 11 and 21 of figure 7) are due to variations of the inputs coming from Nitri but not due to a change in the functioning of Spiru itself.

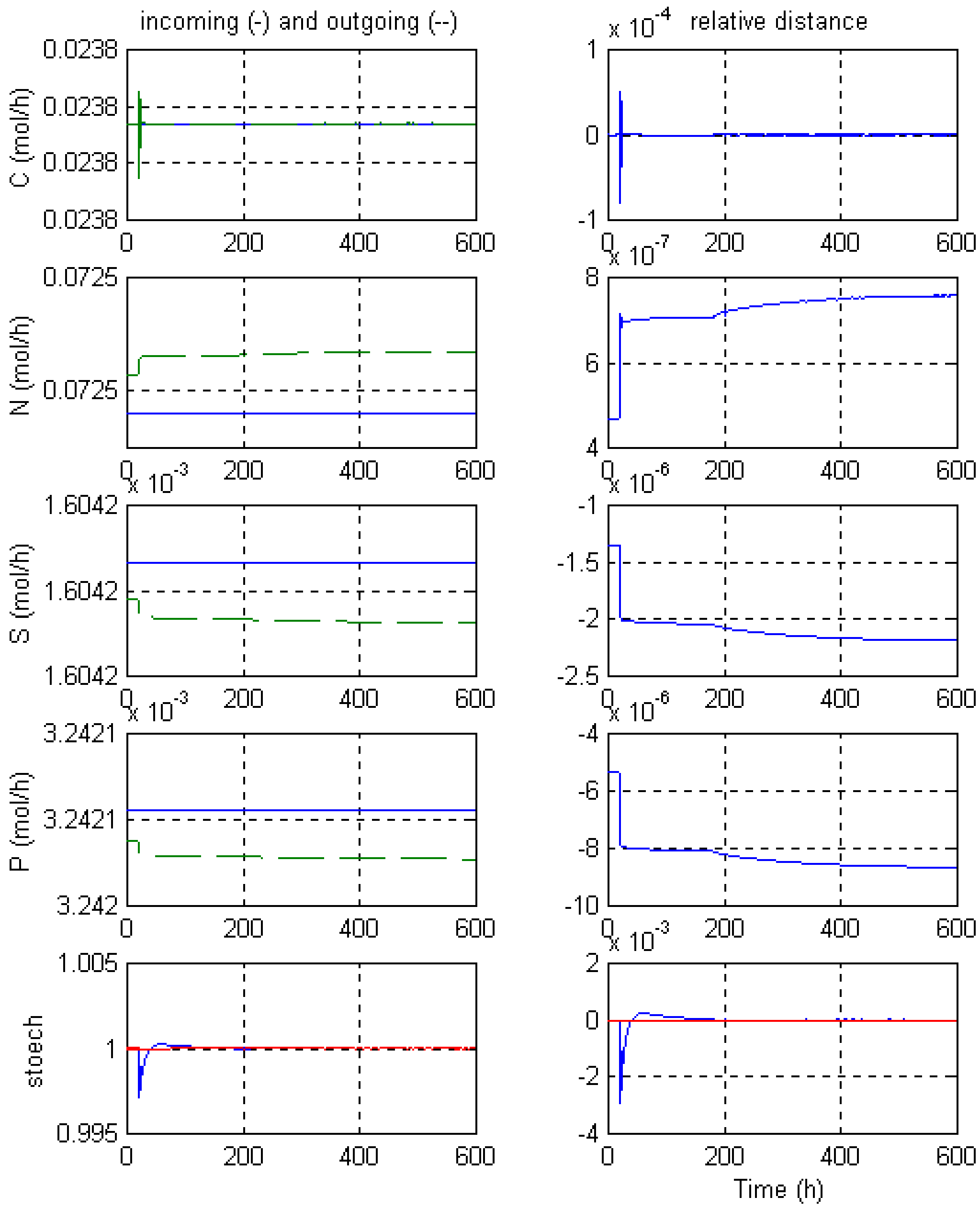

Figure 2 : Mass balance and stoechiometry of Rhodo

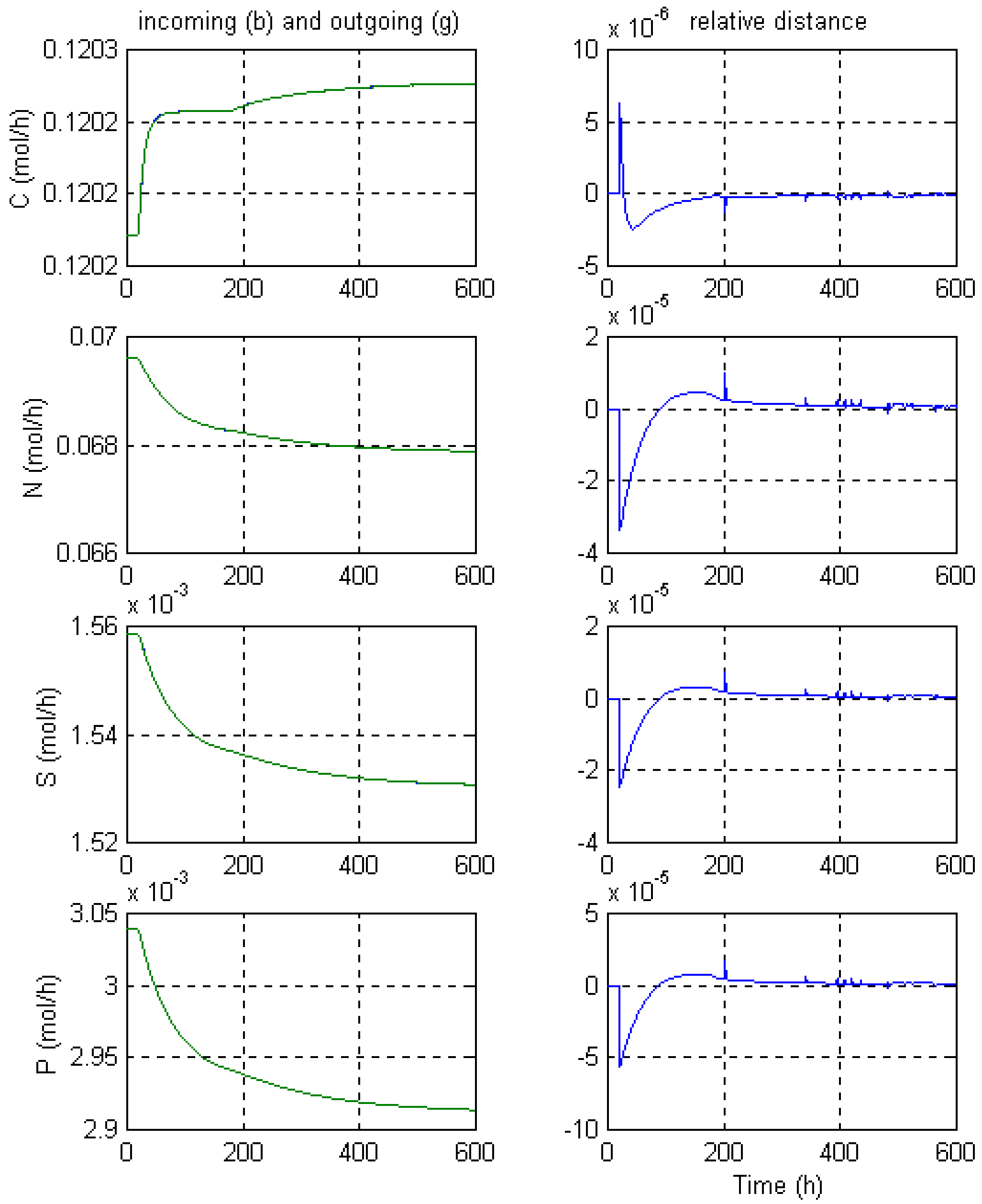

Figure 3 : Mass balance of Nitri

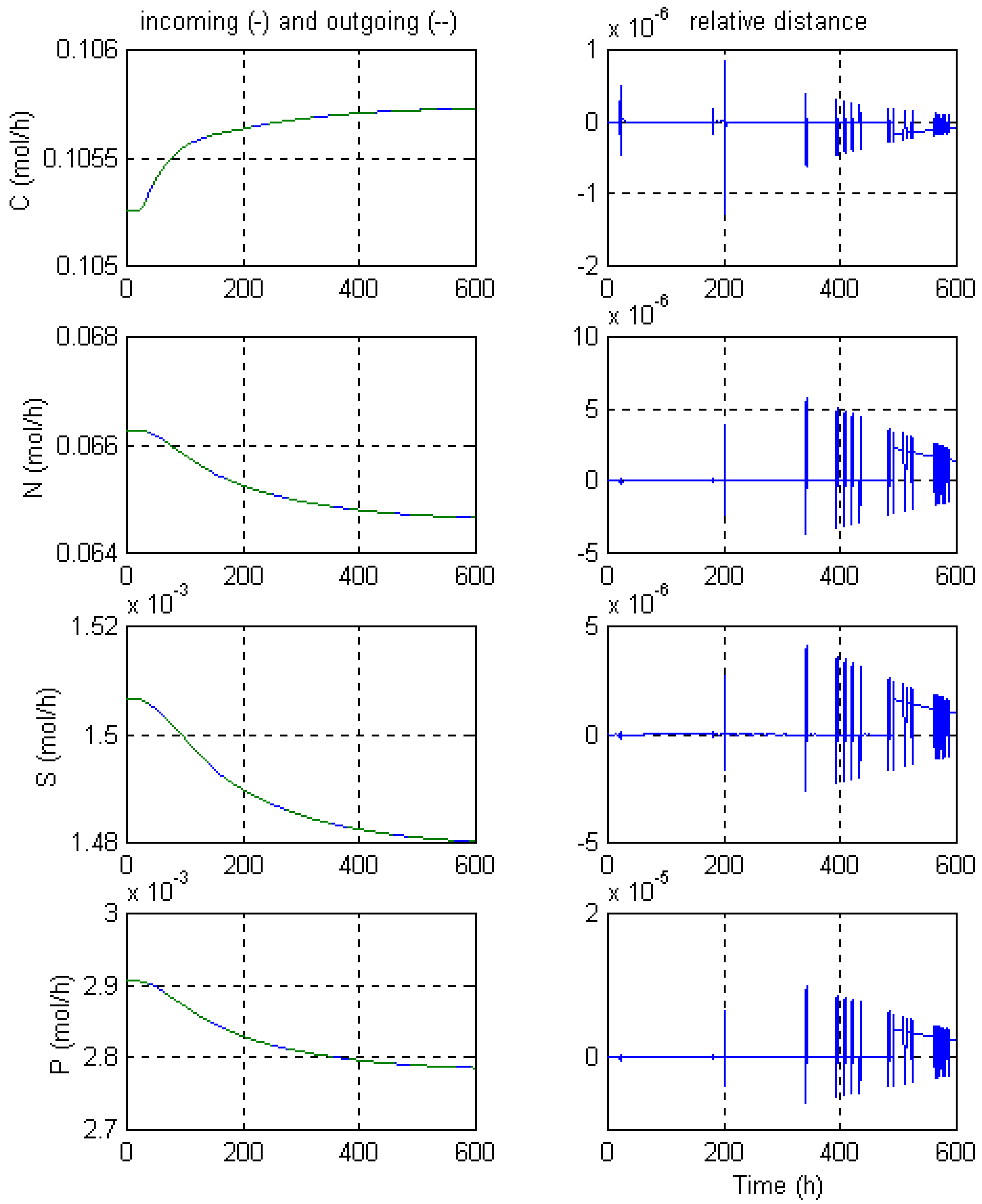

Figure 4 : Mass balance of Spiru

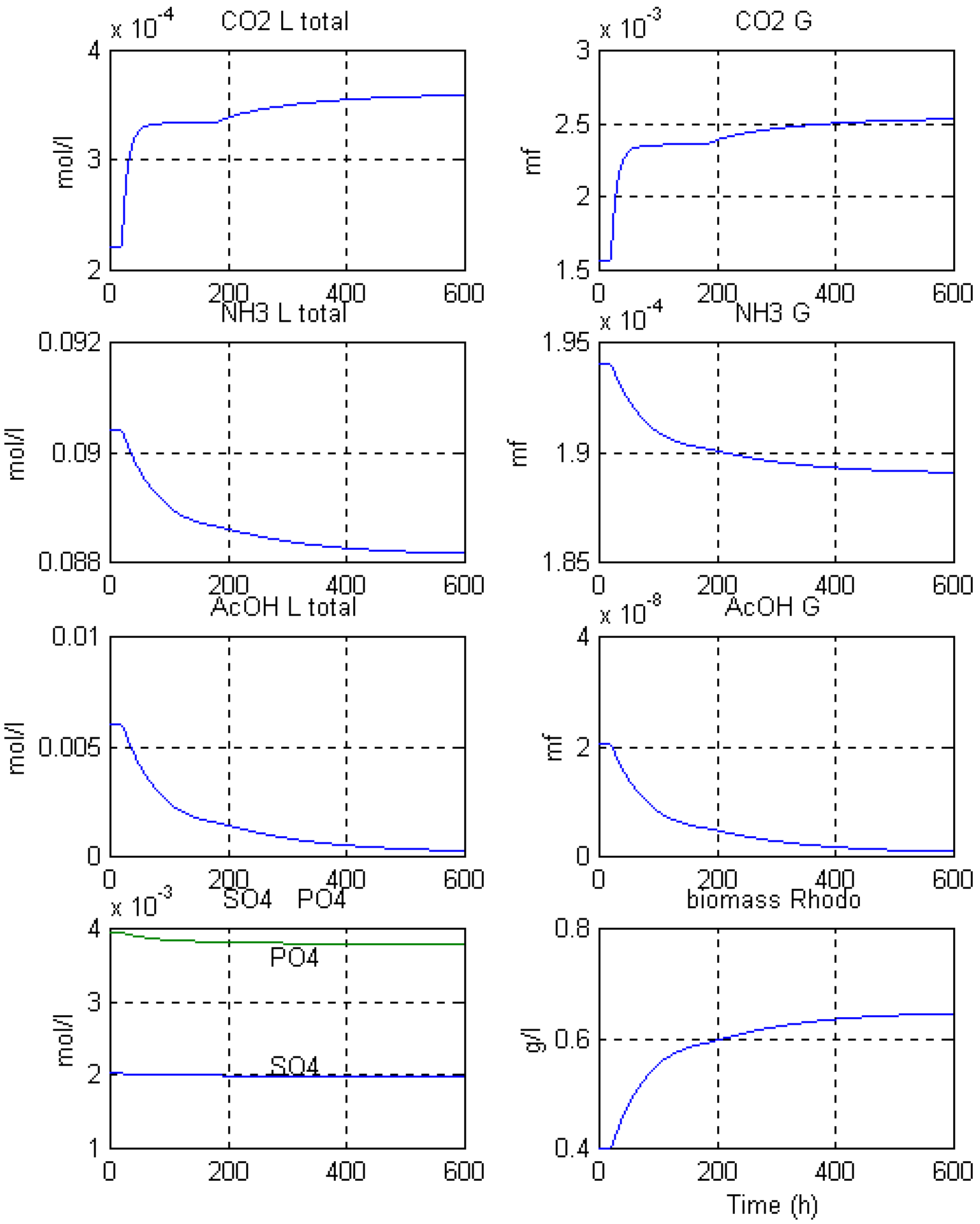

Figure 5 : Concentrations at output of Rhodo

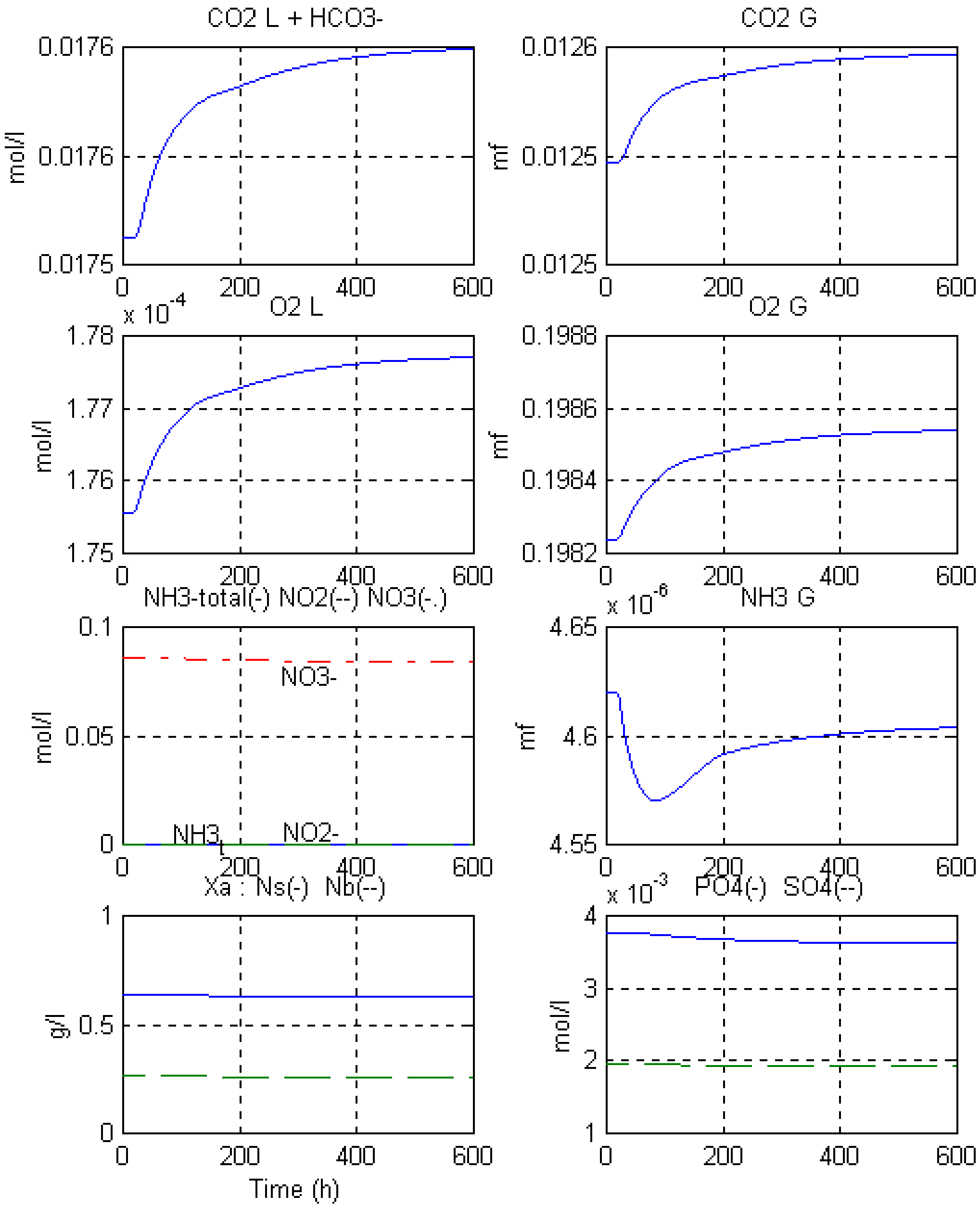

Figure 6 : Concentrations at output of Nitri

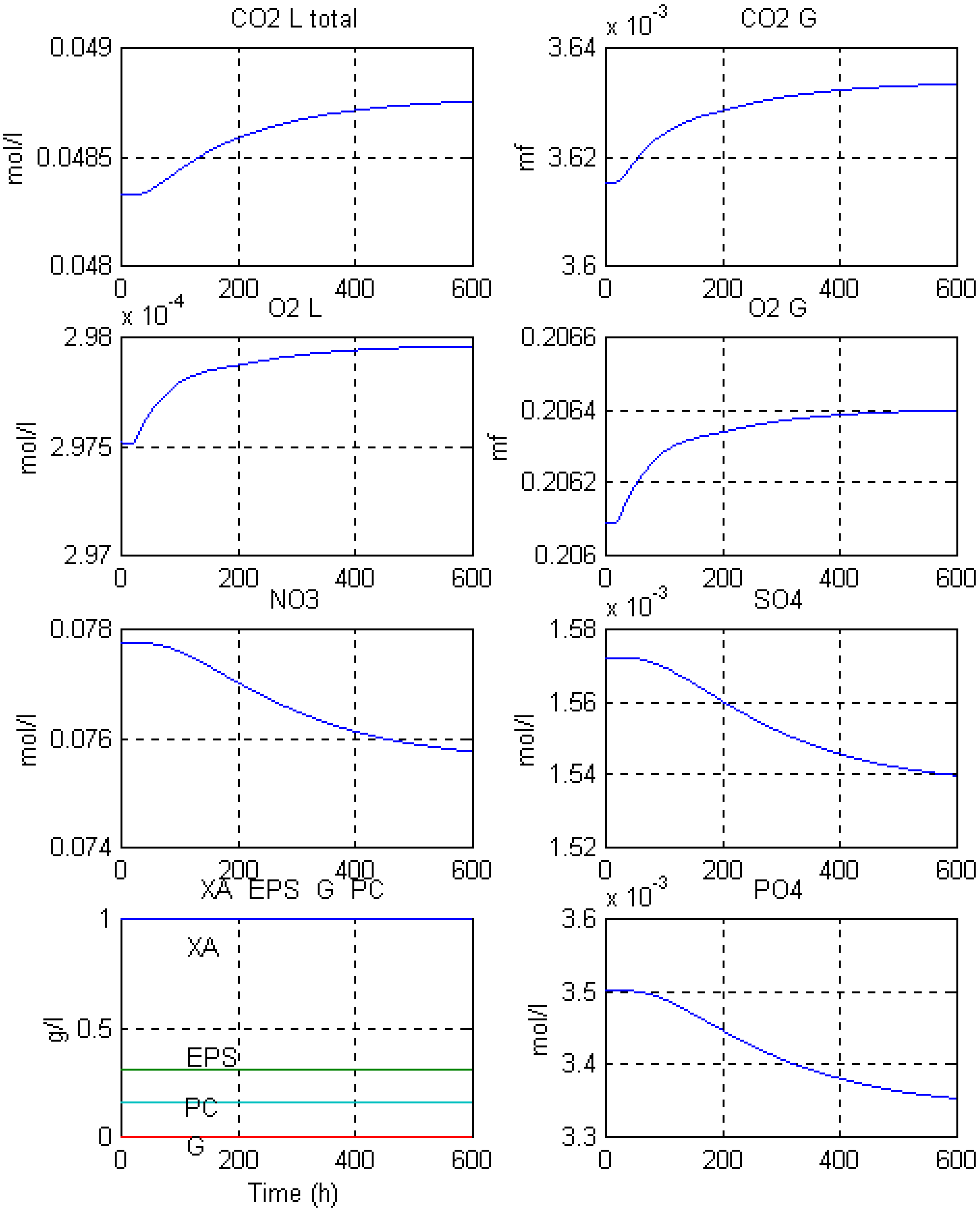

Figure 7 : Concentrations at output of Spiru

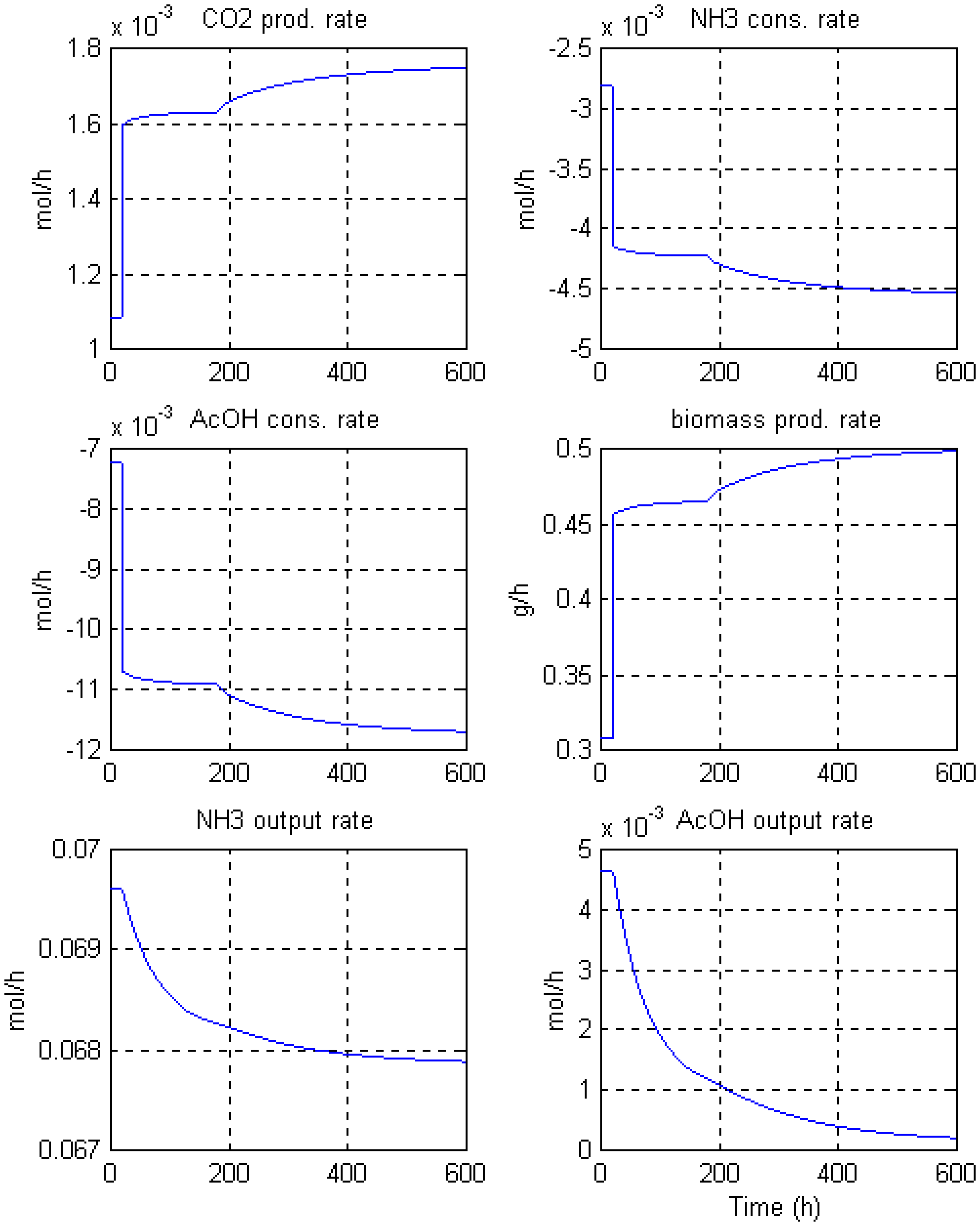

Figure 8 : Production rates of Rhodo
positive rate = production rate
negative rate = consumption rate

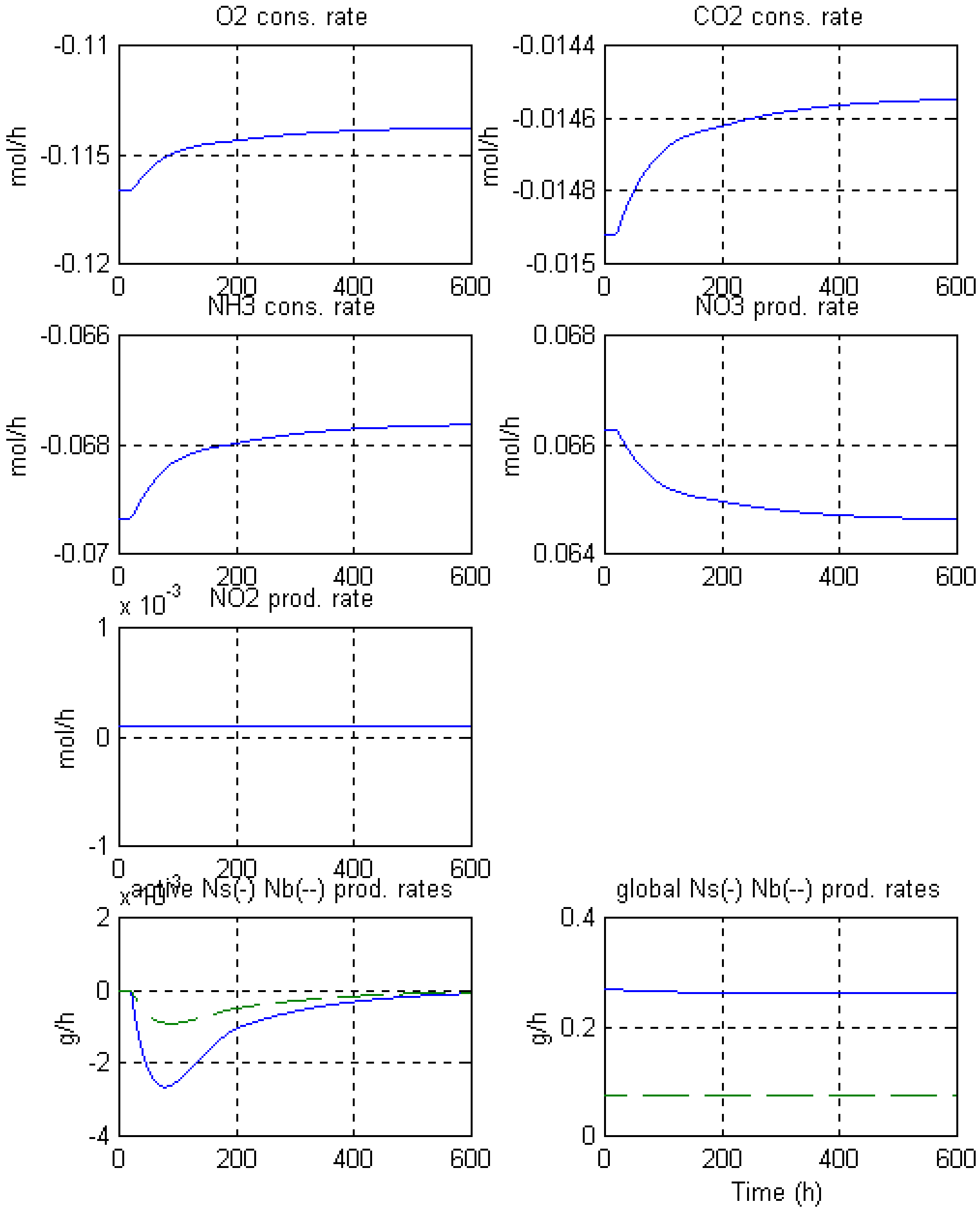

Figure 9 : Production rates of Nitri
positive rate = production rate
negative rate = consumption rate

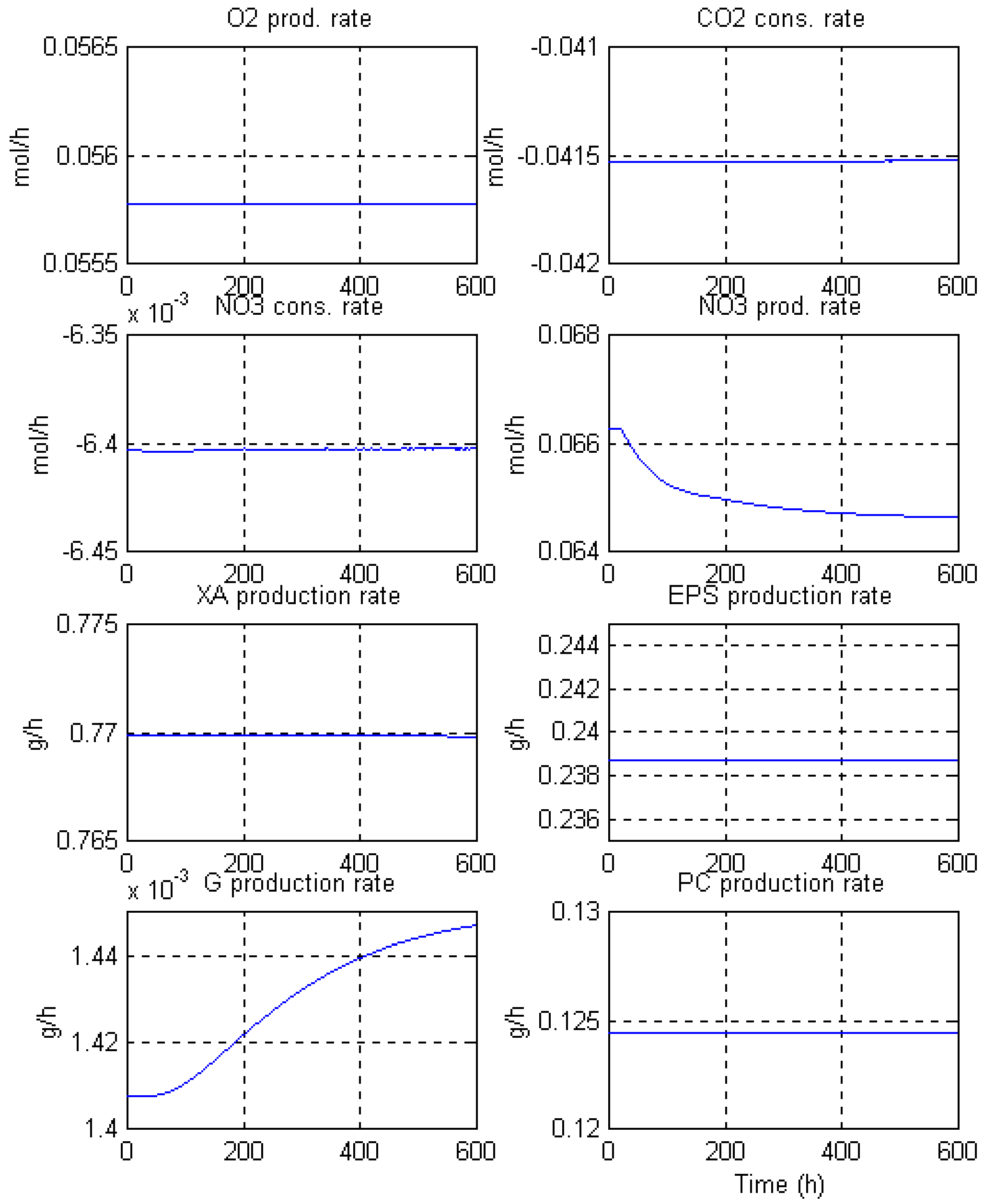

Figure 10 : Production rates of Spiru
positive rate = production rate
negative rate = consumption rate

4. CONCLUSION

The present simulator of the 3 connected compartments Rhodobacter, Nitrifying and Spirulina constitutes a main step towards a global dynamic simulator. It is built in a modular way : the links between the compartments and the environment (sources or sinks of material) can be modified easily enough. The compartments themselves are described with a maximum of parameters, which allow to change the number of substrates or the stoechiometry or the kinetics.

The elaboration of this simulator has been done step by step, each compartments being simulated and tested separately to make the building reliable. This procedure will be kept for further development in order to take into account the evolution of the first principles models, as soon as it appears.

Nevertheless improvements are still necessary : better presentation of the global results; shorter running time.

Apart from the study, two interesting results are worth being mentioned : the sizing of the nitrifying column with regard to the minimization of the nitrite production and the location of the main flux of oxygen in the different parts of the column (annex A4.2 and A4.3).

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ANNEX

MODELLING OF THE COMPARTMENTS

A1. GENERAL MODELLING OF THE PHOTOBIOREACTORS : THE RHODOBACTER AND SPIRULINA COMPARTMENTS

A1.1. Introduction

A1.1.1 General remarks

From the physico-chemical point of view, 3 phases have to be taken into account : gas, liquid and solid. Nevertheless the solid compound (biomass) is assumed to be equally distributed throughout the liquid and, from the modelling point of view, there is no difference between the concentration of a compound in the liquid phase and the one in the solid phase. So, for modelling, the compounds are parted into 2 groups :

- The bi-phasic compounds which are present in gas and liquid phases and are tied by a gas/liquid equilibrium law. Depending on the compartment, they are some among O_2, CO_2 NH_3 and AcOH.
- The mono-phasic compounds which are only present in the liquid under a solvated or solid form. They are nitrate, sulphate, phosphate and biomass.

The water, whose concentration is assumed constant in each of the gas and liquid phases, is treated apart. The consumption rate of water by the formation of biomass represents about 10^{-3} of the total water flow rate and is neglected.

In the mathematical relations of the following study, the concentrations are expressed in mol/l.

A1.1.2. Notation

The notation is the one used each time there is equilibrium between gas and liquid phases. It was already used in previous Technical Notes (TN44.2, TN48.1 and TN48.2) and is recalled hereafter. For each compound involved in the reactor (figure A1.1) :

a : molar concentration in the gas phase

b : molar concentration in the liquid phase of the molecular form

c : molar concentration at the thermodynamic equilibrium

d_G : molar concentration in the incoming gas flow

d_L : molar concentration in the incoming liquid flow of the molecular form

q_G : gas flow rate (1/h)

q_L : liquid flow rate (1/h)

r : mean volumetric production or consumption rate (mol/1/h)

K : volumetric transfer coefficient in liquid phase (notation K_{La} in TN 27.1)

k : dissociation constant of acid/base equilibrium

In the liquid phase, the concentration of a molecular form, x, and the one of its ionic form x' are linked by the relation which implies the dissociation constant k :

$x' = k \cdot x$

$$\text{with} \quad k = \frac{K_{a1}}{[H^+]} \cdot \left(1 + \frac{K_{a2}}{[H^+]}\right) \qquad \text{for di-acid as } CO_2 \text{ solvated}$$

$$k = \frac{K_{a1}}{[H^+]} \qquad \text{for mono-acid as AcOH}$$

$$k = \frac{K_b \cdot [H^+]}{K_e} \qquad \text{for base as } NH_3$$

$$k = 0 \qquad \text{for the other compounds}$$

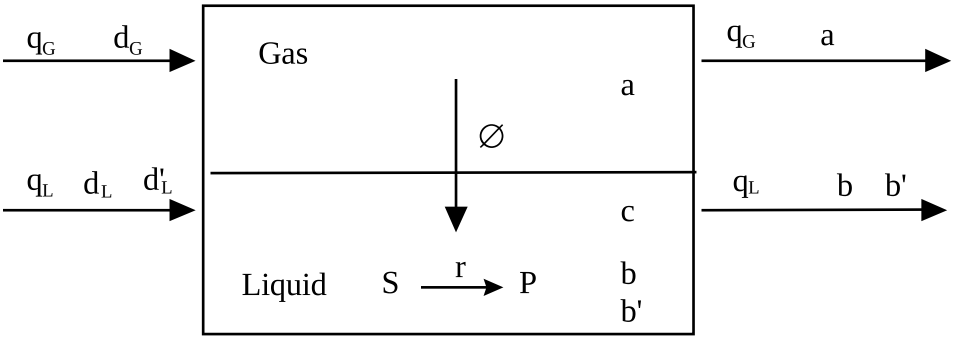

Figure A1.1 : General flow sheet of a reactor
(S = substrate , P = product)

A1.2. Initial system of equations

A1.2.1. Bi-phasic compounds

It is assumed that the temperature and the pH are constant, that the thermodynamic equilibrium is reached for the dissociation reactions (CO_2) and that the gases are perfect.

Gas/liquid equilibrium

$$a = \alpha \cdot c$$

$$\text{with} \quad \alpha = \frac{k_p}{n_0 \cdot V_M} \qquad \text{(A1.1)}$$

n_0 : number of mole in a litre of water ($n_0 = 55.56$ mol/l)

V_M : molar volume under 1 atmosphere and 309 K

Flux Φ from gas to liquid

$$\Phi = K \cdot (c - b) \qquad \text{(A1.2)}$$

Mass balance in the gas

$$V_G \cdot \frac{da}{dt} = -\Phi \cdot V_L + q_G \cdot (d_G - a) \qquad \text{(A1.3)}$$

Mass balance in the liquid

$$V_L \cdot \frac{db}{dt} = (r + \Phi) \cdot \frac{V_L}{1+k} + q_L \cdot (d_L - b) \qquad \text{(A1.4)}$$

A1.2.2. Mono-phasic compounds

Mass balance in the liquid

$$V_L \cdot \frac{db}{dt} = q_L \cdot (d_L - b) + V_L \cdot r \qquad \text{(A1.5)}$$

A1.3. Solution of the system of equations

The system of equations of the bi-phasic compounds can be simplified.
Taking into account (A1.1) and (A1.2), the Laplace transform of (A1.3) is :

$$\left(1 + \beta \cdot \frac{V_G}{q_G} \cdot p\right) \cdot a = \beta \cdot d_G + \alpha \cdot (1 - \beta) \cdot b$$

$$\text{with} \quad \beta = \frac{1}{1 + \frac{K \cdot V_L}{\alpha \cdot q_G}} \qquad \text{(A1.6)}$$

$$p : \text{Laplace variable}$$

The variable $\frac{V_G}{q_G}$ represents the residence time of the gas in the reactor. As the volume of gas V_G is nearly null, this time is very short compared to the other time constants of the system.
Moreover $\beta \le 1$

then the time constant $\beta \cdot \frac{V_G}{q_G}$ is negligible and (A1.6) becomes :

$$a = \beta \cdot d_G + \alpha \cdot (1 - \beta) \cdot b$$

$$\text{with} \quad \beta = \frac{1}{1 + \frac{K \cdot V_L}{\alpha \cdot q_G}} \qquad \text{(A1.7)}$$

That means that the concentrations in gas, a, are pure static functions of d_G and b.

Taking into account (A1.1), (A1.2), (A1.4) and (A1.7), the expression of the concentrations in liquid, b, becomes :

$$\frac{db}{dt} = -\left(\frac{q_L}{V_L} + \delta\right) \cdot b + \frac{\delta}{\alpha} \cdot d_G + \frac{q_L}{V_L} \cdot d_L + \frac{1}{1+k} \cdot r$$

$$\text{with} \quad \delta = \frac{K \cdot \beta}{1+k} \qquad \text{(A1.8)}$$

$$\beta = \frac{1}{1 + \dfrac{K \cdot V_L}{\alpha \cdot q_G}}$$

<u>Note :</u> in order to allow simulations with q_G null, δ has to be set to 0 when the incoming flow rate of gas is null.

A1.4. Mathematical relations of the simulator

The process is simulated by means of a System function of Matlab®. The core of a System function is the state system which is composed of the differential equations describing the process. In the present case the global state system is composed of 2 different groups of equations : one group for the bi-phasic compounds and another one for the mono-phasic compounds.

A1.4.1. State system of the bi-phasic compounds

From (A1.8) and (A1.7), the state system of the bi-phasic compounds is :

$$\begin{aligned} \dot{X} &= A \cdot X + B \cdot U \\ Y &= C \cdot X + D \cdot U \end{aligned} \qquad \text{(A1.9)}$$

where :

- X is the column vector of the concentrations of the bi-phasic compounds in the liquid phase (depending on the compartment, they are some among O_2, CO_2 NH_3 and AcOH);
- U is the column vector of the inputs (concentrations of the bi-phasic compounds in the incoming gas and liquid phases, variation rates of the bi-phasic compounds in liquid phase);
- Y is the column vector of the outputs (concentrations of the bi-phasic compounds in the outgoing gas and liquid phases);
- A, B, C and D are constant matrices if the gas and liquid flow rates are constant, or time variable matrices if these flow rates depend on time. They are detailed further for each for the Rhodo and Spirulina compartments.

A1.4.2. State system of the mono-phasic compounds

From (A1.5), the state system of the mono-phasic compounds is composed of the following differential equations:

$$\frac{db_i}{dt} = \frac{q_L}{V_L} \cdot \left(d_{Li} - b_i\right) + r_i \qquad \text{(A1.10)}$$

where i is the index of the mono-phasic compounds.

A1.5. Checking the mass balance

Checking the mass balance of each atom (C N S and P) is a way of checking the programme itself of the simulator. The checking can not be done for the atoms H and O because the consumption of water is neglected in the production of the biomass.

Definitions :

For the checking of the mass balance, the incoming molar flow rate is defined as the flow rate entering into the reactor and the outgoing flow rate is defined as the sum of the derivative of quantity inside the reactor and of the flow rate which goes out of the reactor.

Given a reactor with a volume V. Given q the volumetric flow rate passing through the reactor. Given x and y the concentrations of an atom in the incoming flow and in the reactor, respectively.

Then the incoming flow rate is defined as the product $q \cdot x$

and the outgoing flow rate is defined as the sum of the two products $V \cdot \frac{dy}{dt} + q \cdot y$

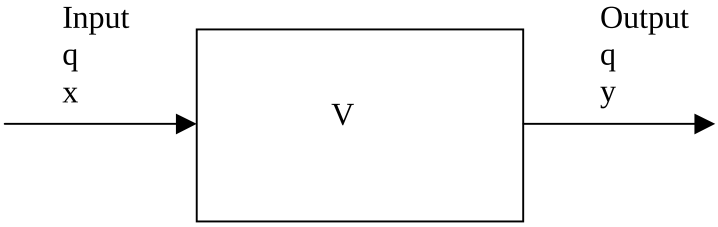

Figure A1.2 : Definition of incoming and outgoing molar flow rates

Checking the mass balance consists in checking that $q \cdot x$ is equal to $V \cdot \frac{dy}{dt} + q \cdot y$ at any moment.

Given for each atom C, N, S and P :

c_{Gi} : molar concentration in the incoming gas phase
c_{Li} : molar concentration in the incoming liquid phase
c_G : molar concentration in the reactor or outgoing gas phase
c_L : molar concentration in the reactor or outgoing liquid phase
c_S : molar concentration in the reactor or outgoing solid phase
r_{Ti} : total molar flow rate in the incoming gas and liquid phases
r_T : total molar flow rate in the reactor and outgoing flows (gas and liquid)

The total incoming molar flow rate, i.e. the molar flow rate of the concerned atom before the transformation, is expressed by :

$$r_{Ti} = q_G \cdot c_{Gi} + q_L \cdot c_{Li} \qquad \text{(A1.11)}$$

The total outgoing molar flow rate, i.e. the flow rate of the concerned atom after transformation in the reactor, is expressed by :

$$r_T = V_G \cdot \frac{dc_G}{dt} + q_G \cdot c_G + V_L \cdot \frac{dc_L}{dt} + q_L \cdot c_L + V_L \cdot \frac{dc_S}{dt} + q_L \cdot c_S \qquad (A1.12)$$

Given c_m the concentration in the liquid and solid phases of the reactor :

$$c_m = c_L + c_S \qquad (A1.13)$$

the relation (2) becomes :

$$r_T = V_G \cdot \frac{dc_G}{dt} + q_G \cdot c_G + V_L \cdot \frac{dc_m}{dt} + q_L \cdot c_m \qquad (A1.14)$$

Given the matrices M_{Gj}, M_{Lj} and M_{Sj} whose coefficients are the stoechiometric composition in atom j (C, N, S or P) of the Gas, Liquid and Solid phases. Theses matrices are detailed for each compartment in Tables A2. and A3.3 .

The molar concentrations previously defined are expressed by :

$$\begin{aligned} c_{Gij} &= d_G \cdot M_{Gj} \\ c_{Lij} &= d_L \cdot M_{Lj} \\ c_{Gj} &= a \cdot M_{Gj} \\ c_{Lj} &= b \cdot M_{Lj} \\ c_{Sj} &= b_S \cdot M_{Sj} \end{aligned} \qquad (A1.15)$$

where :

- d_G and d_L are general notation of the molar concentrations of the different phases Gas and Liquid of the incoming flow;
- a, b and b_S are the molar concentrations of the different phases Gas, Liquid and Solid of the outgoing flow.

In this system (A1.15), d_G, d_L, a, b and b_S are row vectors (depending on time) and M are column matrices.

Checking the mass balance consists in checking that the incoming flow rate computed by (A1.11) is equal to the outgoing flow rate computed by (A1.14) :

$$r_{Ti} = r_T \qquad (A1.16)$$

And, if the incoming flow rate is not null, the relative distance is expressed by :

$$\delta = \frac{r_T - r_{Ti}}{r_{Ti}} \qquad (A1.17)$$

A2. SIMULATOR OF THE RHODOBACTER COMPARTMENT

A2.1. Mathematical relations of the simulator

A2.1.1 State system of the bi-phasic compounds

The general state system of the bi-phasic compounds is described by (A1.9) and is recalled hereafter :

$$\begin{aligned} \dot{X} &= A \cdot X + B \cdot U \\ Y &= C \cdot X + D \cdot U \end{aligned} \qquad \text{(A2.1)}$$

where :

- X is the column vector of the concentrations of the bi-phasic compounds in the liquid phase (for the Rhodobacter compartment, they are CO_2 , NH_3 and AcOH);
- U is the column vector of the inputs (concentrations of the bi-phasic compounds in the incoming gas and liquid phases, variation rates of the bi-phasic compounds in liquid phase);
- Y is the column vector of the outputs (concentrations of the bi-phasic compounds in the outgoing gas and liquid phases);
- A, B, C and D are constant matrices if the gas and liquid flow rates are constant, or time variable matrices if these flow rates depend on time.

<u>Detailed description of these vectors and matrices for the Rhodobacter compartment:</u>

Note : Z^t is the transpose matrix of the matrix Z.

The indices 1 to 3 are associated to CO_2 , NH_3 and AcOH, respectively.

The variables δ_1 , δ_2 and δ_3 are defined in (A1.8) for CO_2 , NH_3 and AcOH, respectively.

The variables k_1 , k_2 and k_3 are the dissociation constants for CO_2 , NH_3 and AcOH, respectively.

$$\begin{aligned} X^t &= [b_1 \;\; b_2 \;\; b_3] \\ U^t &= [d_{G1} \;\; d_{G2} \;\; d_{G3} \;\; d_{L1} \;\; d_{L2} \;\; d_{L3} \;\; r_1 \;\; r_2 \;\; r_3] \\ Y^t &= [a_1 \;\; a_2 \;\; a_3 \;\; b_1 \;\; b_2 \;\; b_3] \end{aligned} \qquad \text{(A2.2)}$$

$$A=\begin{pmatrix} -\left(\frac{q_L}{V_L}+\delta_1\right) & 0 & 0 \\ 0 & -\left(\frac{q_L}{V_L}+\delta_2\right) & 0 \\ 0 & 0 & -\left(\frac{q_L}{V_L}+\delta_3\right) \end{pmatrix}$$

$$B=\begin{pmatrix} \frac{\delta_1}{\alpha_1} & 0 & 0 & \frac{q_L}{V_L} & 0 & 0 & \frac{1}{1+k_1} & 0 & 0 \\ 0 & \frac{\delta_2}{\alpha_2} & 0 & 0 & \frac{q_L}{V_L} & 0 & & \frac{1}{1+k_2} & 0 \\ 0 & 0 & \frac{\delta_3}{\alpha_3} & 0 & 0 & \frac{q_L}{V_L} & 0 & 0 & \frac{1}{1+k_3} \end{pmatrix}$$

$$C=\begin{pmatrix} \alpha_1\cdot(1-\beta_1) & 0 & 0 \\ 0 & \alpha_2\cdot(1-\beta_2) & 0 \\ 0 & 0 & \alpha_3\cdot(1-\beta_3) \end{pmatrix} \qquad \text{(A2.3)}$$

$$D=\begin{pmatrix} \beta_1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & \beta_2 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & \beta_3 & 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

A2.1.2. State system of the mono-phasic compounds

The general state system of the mono-phasic compounds is described by (A1.10) and is recalled hereafter :

$$\frac{db_i}{dt}=\frac{q_L}{V_L}\cdot\left(d_{Li}-b_i\right)+r_i \qquad \text{(A2.4)}$$

where i is the index of the compounds defined in the table A2.1 .

Index i	Compound
1	Sulfate
2	Phosphate
3	Active biomass

Table A2.1 Index of the mono-phasic compounds

For the biomass (i = 3), the input concentration is null.

The production rate of the biomass has been established in TN 45.1 (LGCB).
Although the mineral limitation has not been studied yet for Rhodobacter, a Monod type limiting factor is introduced in the present simulator in order to avoid the sulphate concentration going below zero.
So the mean volumetric growth rate of biomass is expressed by :

$$r_X = R_X \cdot \frac{C_S}{K_S + C_S} \qquad \text{(A2.5)}$$

where R_X is the mean volumetric growth rate under light limitation (section A2.1.4).

A2.1.3. Variation rates of the compounds in the liquid phase

The variation rates of CO_2, NH_3, AcOH, SO_4 and PO_4 depend on the stoechiometry of the production of biomass and on its production rate :

$$r_C = M_S \cdot r_X \qquad \text{(A2.6)}$$

where :

- r_C is the vector of variation rates of CO_2, NH_3, AcOH, SO_4 and PO_4 ;
- M_S is the matrix of the stoechiometry of production of biomass (table A2.1).

	For 1.8505 mol of biomass
CO_2	0.1495
NH_3	-0.38749
AcOH	-1
SO_4	-0.006292
PO_4	-0.02813

Table A2.2 : Matrix of stoechiometry for the production of 1.8505 mol of biomass
Source : TN 39.1 p. 10

A2.1.4. Improvement of the computation of the mean growth rate of biomass

According to the first principles model, the mean volumetric biomass growth rate, is :

$$R_X = k \cdot \langle r_{X3} \rangle$$

with k equal to the minimum of the 2 terms : $\left\lfloor q, \ \frac{1}{1 + x'^2_3 - x^2_3} \right|$

$$\langle r_{X3} \rangle = 2 \,.\, \mu_M \,.\, C_X \,.\, J$$

$$J = {}_{Y3}\ g \,.\, dx \qquad\qquad Y_3 = [0 \quad x'_3] \cup [x_3 \quad 1]$$

$$g = \frac{I_\Sigma}{K_J + I_\Sigma} . x$$

$$I_\Sigma = F_0 . \frac{1}{x} . \frac{2\,ch[\delta . x]}{ch\,\delta + \alpha . sh\,\delta}$$

with :

$x = \frac{r}{R}$: relative abscissa

R : radius of the photobioreactor

r : abscissa (locates the local cylindrical surface at distance r from the axis of the photobioreactor)

F_0 : mean incident light flux

α and δ : function of the biomass concentration and defined in TN 45.1

x'_3 and x_3 being the roots of $I_\Sigma = E_J$

It has been noted that the method used for the calculation of x'_3 and x_3 in the model based predictive control (TN 38.3) causes small discontinuities of the mean growth rate of the biomass when the integration interval Y_3 varies of one step from one moment t to the next moment t+dt. It has no inconvenience for the control performance. But this problem has to be cancelled in the simulator : these discontinuities have no physical basis.

Calculation of the roots x'_3 and x_3 :

On the interval $[0 \quad 1]$ the function I_Σ is first decreasing and then increasing. Its single minimum is given by :

$$x_{min} = \frac{z_{min}}{\delta}$$

where z_{min} is the solution of $th(z) = \frac{1}{z}$

The roots x'_3 and x_3 exist only and if only $I_\Sigma(x_{min}) \le \varepsilon_J$.

Then $x'_3 \in \left]0 \quad x_{min}\right]$ and $x_3 \in [x_{min} \quad 1]$

They are solution of

$$ch(\delta \cdot x) = a \cdot x \quad \text{with} \quad a = \frac{\varepsilon_J \cdot (ch(\delta) + \alpha \cdot sh(\delta))}{2 \cdot F_0}$$

The solutions z_{min}, x'_3 and x_3 are obtained by dichotomy with absolute tolerances of 10^{-6}, 10^{-6} and 10^{-4}, respectively, which are necessary to avoid discontinuity of the mean growth rate of biomass.

A2.2. Checking the mass balance and the stoechiometric ratios

Mass balance :

The stoechiometric composition of each of the compounds are gathered in the following table A2.3 .

Matrices M_{Gj}				
j	1	2	3	4
atom	C	N	S	P
CO_2 in gas	1	0	0	0
NH_3 in gas	0	1	0	0
AcOH in gas	2	0	0	0

Matrices M_{Lj}				
j	1	2	3	4
atom	C	N	S	P
CO_2 in liquid	$1+k_{CO2}$	0	0	0
NH_3 in liquid	0	$1+k_{NH3}$	0	0
AcOH in liquid	$2(1+k_{AcOH})$	1	0	0
SO_4	0	0	1	0
PO_4	0	0	0	1

Matrices M_{Sj}				
j	1	2	3	4
atom	C	N	S	P
Biomass Rhodo	1	0.2094	0.0034	0.0152

Table A2.3 : Matrices of stoechiometric compositions for the compounds involved in the Rhodobacter compartment

The checking has been done for different kind of simulations : light flux constant or not, concentrations of the incoming flows (gas and liquid) constant or not, gas and liquid flow rates null (i.e. batch conditions) or not. The figure A2.1 shows the variables r_{Ti}, r_T and δ of the relations (A1.16) and (A1.17) in the case of the simulation described in the paragraph A2.3.
For each of the atom C, N, S and P the outgoing flow rate r_T is equal to incoming flow rate r_{Ti} within a relative distance δ under 10^{-7} for C (except at the light step where it is equal to 10^{-5}) and 10^{-8} for the other atoms, which is due to numerical noise in computation of the approximate derivative of c_m in the expression (A1.14) of r_T.

Stoechiometric ratios :

Given the chemical reaction (R) where the biomass X and the products B_j are made from the substrates A_i :

$$\alpha_i\ A_i \rightarrow X + \beta_j\ B_j \tag{R}$$

The production/consumption rates are bound by :

$$\frac{d[X]}{dt} = \frac{1}{\beta_j} \cdot \frac{d[B_j]}{dt} = -\frac{1}{\alpha_i} \cdot \frac{d[A_i]}{dt}$$

For a general compound A_k (product or substrate), associated with a signed stoechiometric coefficient α_k (positive for a product and negative for a substrate), this relation becomes (with the notation r for a production rate) :

$$r_X = \frac{1}{\alpha_k} \cdot r_k$$

Given the reference stoechiometric coefficients α_k^r of the reaction (R)

and given the simulated stoechiometric coefficients α_k^s calculated from $\alpha_k^s = \frac{r_k}{r_X}$ for each compound k,

the stoechiometric ratios s_k are defined by :

$$s_k = \frac{\alpha_k^s}{\alpha_k^r}$$

Theoretically

$$s_k = 1 \qquad \forall k \quad \text{and} \quad \forall t$$

The graph of the bottom right hand side corner shows, for the compounds CO_2, NH_3 and AcOH, the ratios s_k. This ratios are equal to 1, as expected, excepted at the flux light step variation where it decreases to 0.99 for CO_2, probably because of simplification of the model or of numerical noise.

As the mass balance, these ratios constitute a checking of the software of the simulator.

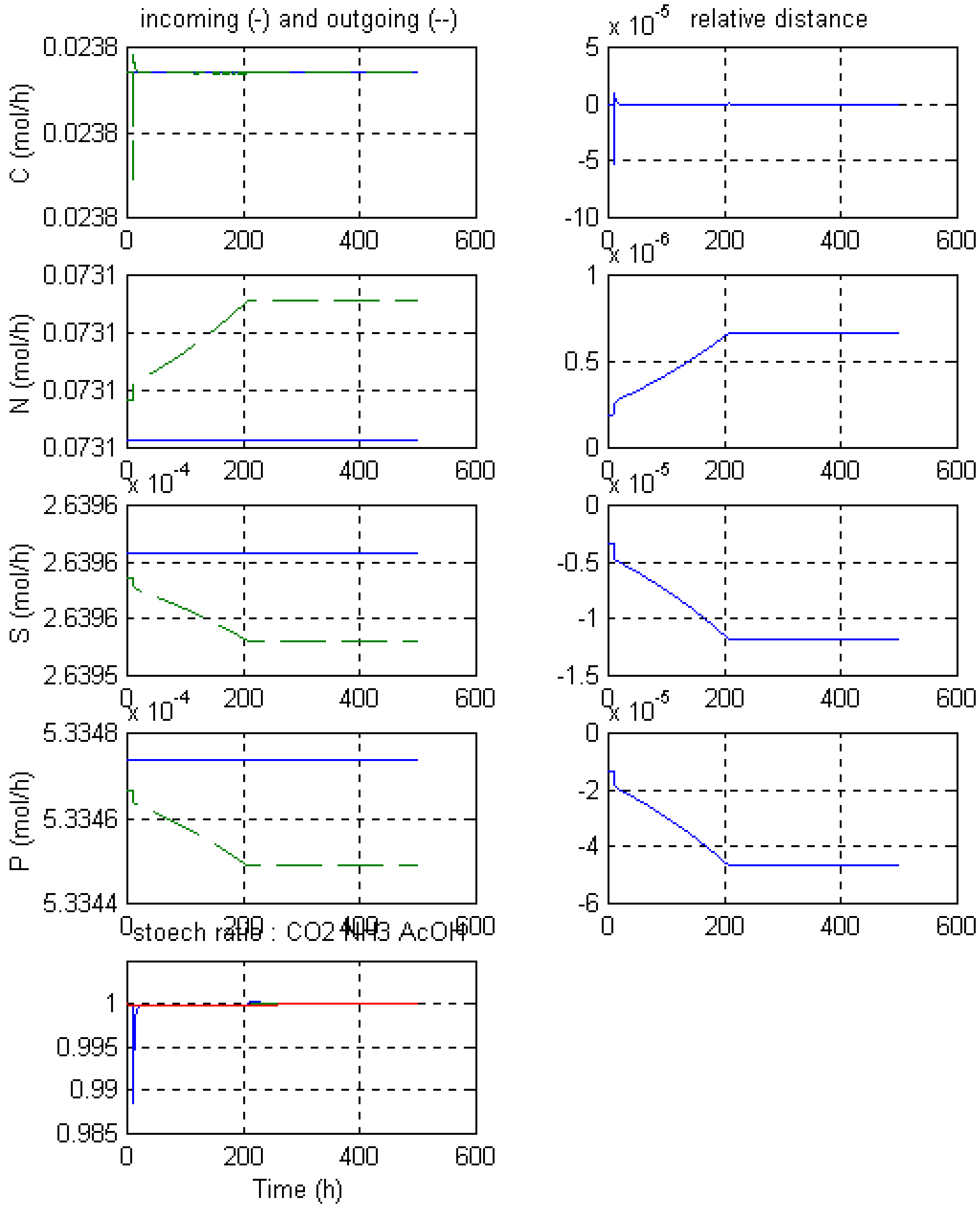

Figure A2.1 : Checking the mass balance of the atoms C, N, S and P and the stoechiometric ratios s_k of the compounds CO_2, NH_3 and AcOH

Left hand side column : incoming flow = blue line - ; outgoing flow = green line - -
Right hand side column : relative distance δ expressed in relation (A1.17)

A2.3. Simulation results

A simulation has been done with the following data, in the case of the actual reactor :

- The volume of the reactor is 7 l.
- the incoming gas flow rate is 18 l/h (extrapolated from the gas flow rate (180 l/h) of the Spirulina compartment whose volume is 77 l); the inert gas is supposed to be N_2 or He.
- the incoming liquid flow rate is 0.127 l/h ,such that the residence time is 55 h, as in TN 43.8 of UAB (Preliminary connection between 3 compartments).
- the concentrations of NH_3 and AcOH in the incoming liquid are those produced by the waste of 1 man. They are extrapolated from the previous TN 35.1 (ADERSA) of the global simulator where the wastes are those of 3 men and where the conversions are supposed to be complete. They are such that the corresponding flow rates are : 0.0724 mol/h of NH_3 total and 0.0119 mol/h for AcOH (the flow rate of AcOH in TN35.1 has been increased of the flow rate of butyric acid multiplied by 2 to take into account the stoechiometry of C atom).
- The sulphate and phosphate concentrations in the incoming flow are extrapolated from those of TN 19.3 (LGCB), Appendix 7, Figure 8 and have been chosen to avoid mineral limitation (Sulphate : 0.2 g/l and phosphate : 0.4 g/l).
- The initial concentration of biomass is 1 g/l;
- The concentrations of the incoming gas and liquid flows are constant all along the simulation.
- The initial light flux is such that the system is steady state (about 40 W/m^2) and is set at 100 W/m^2 at t=10 h.

The figure A2.2 shows the concentrations in the outgoing gas and liquid flows and the figure A2.3 the production rates (N.B. : the rates are signed and a negative production rate actually means that the concerned compound is consumed).

The production of biomass increases from 0.12 g/l up to 0.44 g/h at time t=200 h. Then the concentration of biomass (about 2.8 g/l) is such that all the light energy is absorbed; so the production stops increasing. Beyond that point, the biomass concentration still increases during about 160 h, which represents about 3 times the residence time.

This test shows that for the present conditions with a light flux equal to 100 W/m^2, the conversion is 84% for AcOH and 6% for NH_3.

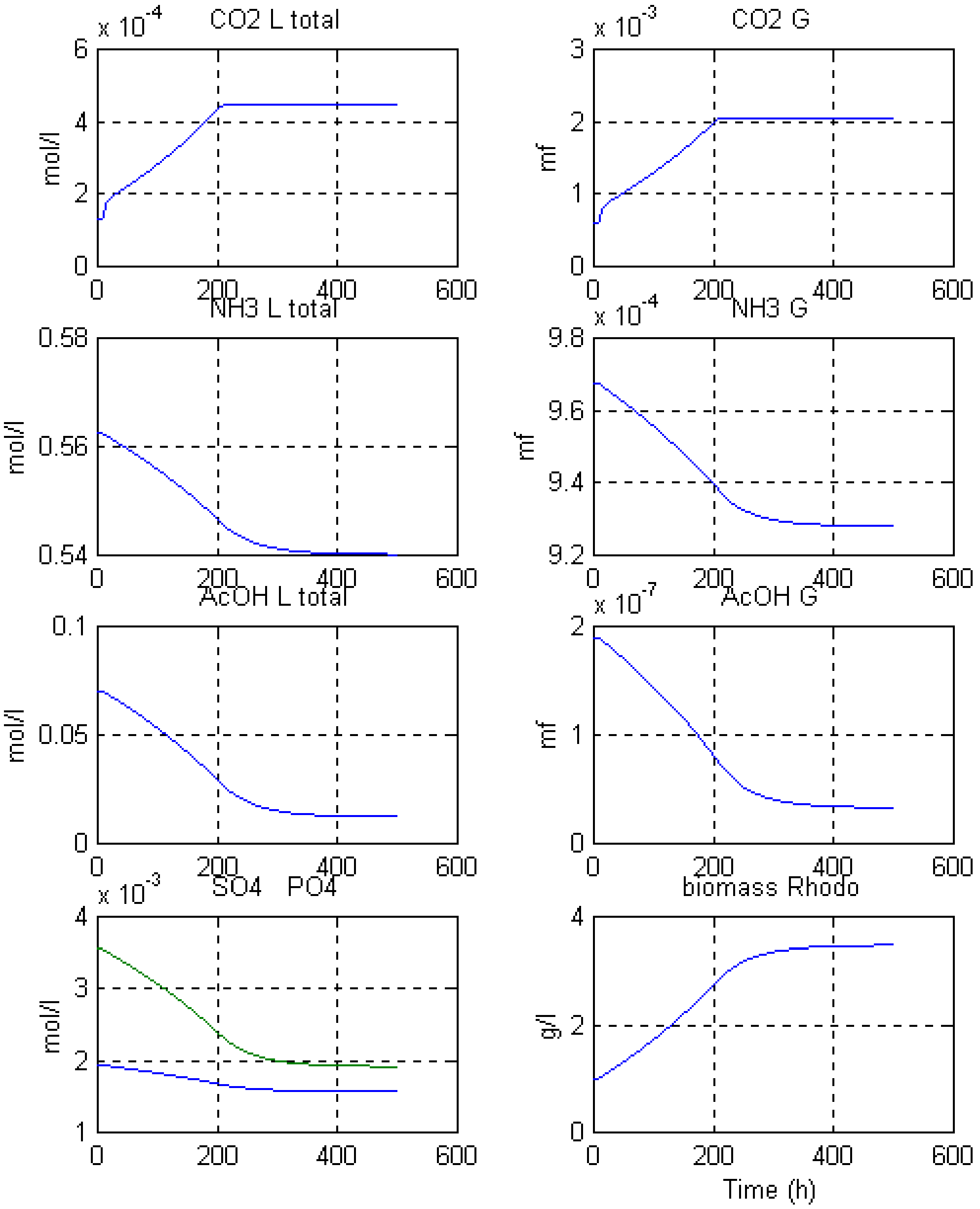

Figure A2.2 : Step of light flux at time t=10 h.
Concentrations in the reactor

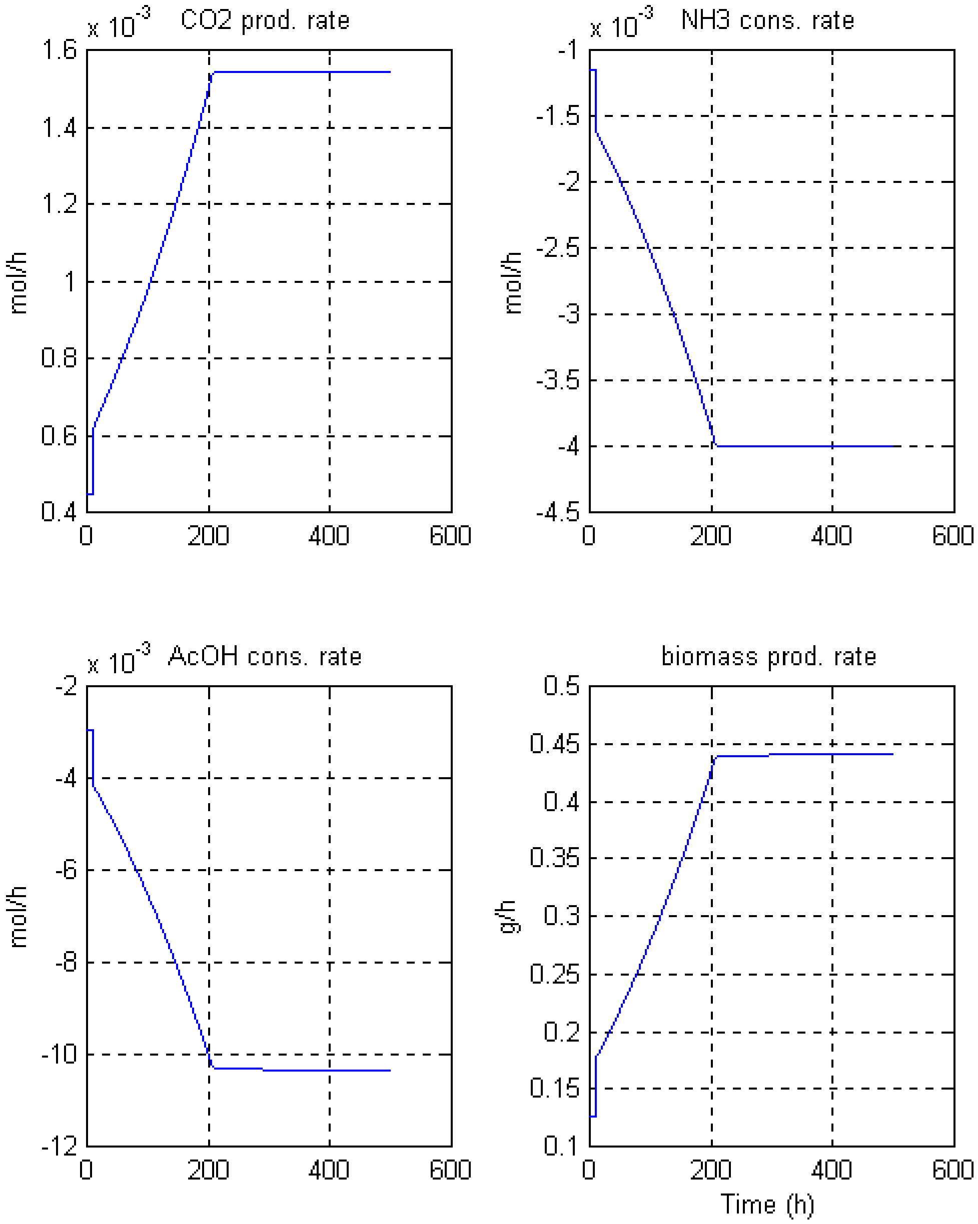

Figure A2.3 : Step of light flux at time t=10 h.
Production rates

A2.4. Simulation parameters

All the parameters of the simulation are recalled in the following Matlab® file :

```
%***********************************************************************
%       Photoheterotrophic compartment (Rhodobacter)                   *
%       Version 1     November 2000                                    *
%                                                                      *
%       State system according to TN 54.2                              *
%                                                                      *
%                                                                      *
%       i_sim.m : Initialization of the simulation                     *
%                                                                      *
%       . no mineral limitation                                        *
%       . no carbon limitation                                         *
%       . no decay of biomass                                          *
%       . waste of 1 man (data computed from TN 35.1, ADERSA)          *
%       . carbon source : acetate                                      *
%       . anaerobic condition done by means of inert gas : N2 or He    *
%       . light limitation law according to TN 45.1; April 1999        *
%                                                                      *
%***********************************************************************

clear all

arret = 0;
% Simulation parameters
        tdeb=0;
        tmin=1e-6;
        tmax=.2;
        prec=1e-3;
        tfin=1;    % h
        nbptx=ceil(tfin/1e-2);

% Simulation horizon
        dt = .5;        % (h) simulation period of the inputs
        T = [0:dt:tfin]';
        [m,n] = size(T);

% Type of test
% ------------
typtst = 1;     titre = 'Constant concentrations of the inputs';
typtst = 2;     titre = 'Light flux step';

% General parameters of the simulated process
% --------------------------------------------
NG = 3;         % number of bi-phases substrates (in the L and G phases) : CO2 NH3
CH3COOH
NL = 2;         % number of mono-phase substrates in the liquid phase : SO4, PO4
NS = 1;         % number of compounds of the biomass : biomass Rhodo
NI = 2*NG+NL+1; % nb of inputs of the process (photobio reactor)
NX = NG+NL+NS;  % nb of coef of state vector X
NO = NG+NX;     % nb of outputs of the process (photobio reactor)

% Parameters of the PBR
% ---------------------
RT = .048;   % radius of the reactor (m)
VL = 7 ;     % volume of liquid (l)
Gin = 18;    % incoming gas flow rate (l/h) (same ratio as in Spirulina)
             % if Gin is time variable, move 'statesys' into the S-function
'pr_rhodo'
             % in order to compute the state system at each sampling period
```

```
tau = .0181; % (h) same residence time as in TN43.8, UAB
Fin = tau*VL;% incoming liquid flow rate (l/h); if Fin variable, do as for Gin)
fI = .6;     % illuminated surface fraction (ADERSA meeting on May 12th 1999)
Temp = 303;  % K (TN35.1 ADERSA)
pH = 7;      % TN35.1 ADERSA
FRmin = 1;   % (W/m2)
FRmax = 400; % (W/m2)

% Gas/liquid transfer parameters (same as for Spirulina : TN 43.110 p.26, UAB)
% ------------------------------
KLa = 12;    % CO2 NH3 CH3COOH (1/h)
% Partition coefficients
% ----------------------
kpartN = [1853.1;   11.349; 2.6414e-2];% CO2 NH3 CH3COOH (T=303K,non ionic form)
                                       % computed from TN35.1,ADERSA
% Dissociation coefficient (for CO2)
% ----------------------------------
Ka = [4.6271e-7; 5.1173e-11];               % at T=303K, from TN 35.1,ADERSA
KCO2 = Ka(1)/10^(-pH)*(1+Ka(2)/10^(-pH)); % [HCO3-]+[CO3--] = KCO2 * [CO2]solvated
% Dissociation coefficient (for NH3)
% ----------------------------------
Kb = 1.6916e-5;         % basicity cst at T=303K, from TN 35.1,ADERSA
Ke = 1.4376e-14;        % ionic product at T=303K, from TN 35.1,ADERSA
KNH3 = Kb*10^(-pH)/Ke;  % [NH4+] = KNH3 * [NH3]solvated
% Dissociation coefficient (for AcOH)
% -----------------------------------
Ka = 1.7478e-5;         % at T=303K, from TN 35.1,ADERSA
KAc = Ka/(10^(-pH));    % [AcO-] = KAc * [AcOH]
% Dissociation coefficient (for H3PO4) (unused)
% -----------------------------------
Ka = 6.166e-8;                    % (at T=298K, from TN 27.2,LGCB)
xx = 10^(-pH)/Ka;   KPO4 = 1+xx;  %[PO4]total = f([HPO4=])
% Dissociation vector for the biphase substrates
% ----------------------------------------------
Kdis = [KCO2; KNH3; KAc]; % CO2 NH3 CH3COOH
% Gas/liquid thermodynamical equilibrium constants
% ------------------------------------------------
VM = 22.4 * Temp / 273;  % molar volume of perfect gas (l/mol)
alpha = kpartN / 55.56 / VM;
% Parameters of the RHODO first principles model (LGCB TN 45.1)
% -------------------------------------------------------------
Ea=270;   % mean Schuster mass absorption coefficient (m2/kg) (TN 45.1 p.15)
Es=370;   % mean Schuster mass scattering coefficient (m2/kg) (TN 45.1 p.15)
muM=.15;  % max. specific growth rate for acetate     (1/h)   (TN 45.1 p.18)
KJ=15;    % half saturation constant                  (W/m2)  (TN 45.1 p.5)
EpsJ=.01; % mean efficient intensity                  (W/m2)  (TN 45.1 p.11)
q_rhod=3.2;    % max ratio of V2/V3                   (no dim)(TN 45.1 p.17)
% Computation of the state system of the biphasic compounds
% ---------------------------------------------------------
[A2,B2,GG1,GG2] = statesys(NG, NL, Gin, Fin, VL, alpha, Kdis, KLa);
% Stoechiometry of the reactions of production of biomass
% -------------------------------------------------------
  % 1. mol substrate/1.8505 mol biomass Rhodo (from TN 39.1 p.10)
    Yx1  =  [ .1495  ;    % CO2
             -.38749 ;    % NH3
             -1      ;    % CH3COOH
             -.006292;    % H2SO4
             -.02813 ];   % H3PO4
    Yx1 = Yx1/1.8505;

  % change of unit of Yx1 :
    Matom = [12;   1;      16;    14;    32;    31];  % C H O N S P atomik mass
    %        C     H       O      N      S      P
```

```
    Scomp = [1     1.5951  .3699  .2094  .0034 .0152]; % stoechio composition
biomass Rhodo
    Mbio = Scomp*Matom;
  % 2. mol substrate/g biomass
    Yx1 = Yx1 ./ (ones(NG+NL,1)*Mbio'); % (mol substrate / g biomass)
% Limiting factors associated to mineral limitation (similar to XA of Spirulina)
% -------------------------------------------------
KSSO4 = 2.5e-4/(Matom(5)+4*Matom(3));  % mol/l
% Vector of indices in state vector
% ---------------------------------
vNB = 1:NG;               % indices of Biphasic compounds
vNM = NG+1:NG+NL;         % indices of Monophasic compounds
vNS = NG+NL+1:NG+NL+NS;   % indices of Solid compounds (biomass components)
% Initial concentrations in the reactor
% -------------------------------------
  %1. Gas phase :
    fm_CO2_0 = 0;   % no CO2 (molar fraction)
    fm_H2O_0 = 4.129e-2;    % H2O (molar fraction)
    if Fin > 0
      cNH3_t = .2172/3/Fin;   % NH3 (molar concentration of N total in liquid
phase)
                              % from waste of 1 man according to TN35.1, ADERSA
      cAc_t = 3.563e-2/3/Fin; % AcOH (molar concentration of C total in liquid
phase)
                              % from waste of 1 man according to TN35.1, ADERSA
    else
      cNH3_t = .2172/3/tau/VL;  % NH3 (molar concentration of N total in liquid
phase)
                                % from waste of 1 man according to TN35.1, ADERSA
      cAc_t = 3.563e-2/3/tau/VL;% AcOH (molar concentration of C total in liquid
phase)
                                % from waste of 1 man according to TN35.1, ADERSA
    end
    fm_NH3_0 = cNH3_t/(1+KNH3)*kpartN(2)/55.56;      % NH3 (molar fraction)
    fm_Ac_0  = cAc_t /(1+KAc) *kpartN(3)/55.56;      % AcOH (molar fraction)
    fm_N2_0 = 1 - fm_CO2_0 - fm_NH3_0 - fm_Ac_0 - fm_H2O_0;  % N2
    % conc. in gas phase (perfect gases at T='Temp' Kelvin and P=1 atm)
    cN2 = fm_N2_0 / VM;         % mol/l
    cH2O = fm_H2O_0 / VM;       % mol/l
  %2. Liquid phase :
  cCO2_0 = fm_CO2_0 / kpartN(1) * 55.56;       % mol/l
  cNH3_0 = fm_NH3_0 / kpartN(2) * 55.56;       % mol/l
  cAc_0  = fm_Ac_0  / kpartN(3) * 55.56;       % mol/l
  cSO4_0  = .2/(Matom(5)+4*Matom(3));          % mol/l (from TN19.3,Appendix
7,Figure 8,LGCB)
  cPO4_0  = .4/(Matom(6)+4*Matom(3));          % mol/l (from ? )
  %3. Solid phase (biomass) according to TN38.3,ADERSA) :
  cX_0 = 1.;        % g/l

% Solution of th(z) = 1/z
% for the determination of the minimum of the function Isigma in rx_rhodo
% =======================================================================
[zmin_I, ii] = dichoto('tanh', '1/', 1, 2, 100, 1e-6);

% Initial light flux (W/m2) for steady state point
% ------------------------------------------------
x10 = FRmin;  x20 = FRmax;
aa = Fin/VL/(cSO4_0/(KSSO4+cSO4_0))*cX_0;
FR0 = dichoto2(x10, x20, 100, 1e-1, ...
               cX_0, fI, RT, Ea, Es, muM, KJ, EpsJ, q_rhod, zmin_I, aa)
%FR0 = 50;           % incident light flux (W/m2)
% Initial state system for steady state point
% -------------------------------------------
  % Computation of the limiting factors due to mineral limitation
```

```
  % (assumed to be similar to Spirulina)
    KlX = cSO4_0/(KSSO4+cSO4_0);
  % Computation of the growth rates RX under light limitation
    RX = rx_rhodo(cX_0, FR0, fI, RT, Ea, Es, muM, KJ, EpsJ, q_rhod, zmin_I);
  % Computation of the biomass growth rates r under mineral limitation
    rX = RX * KlX;
  % Computation of the variation rates r of chemical compounds
    ri = Yx1 * [rX];
  % Initial state system of the biphasic compounds
    u0= [[fm_CO2_0; fm_NH3_0; fm_Ac_0] / VM; cCO2_0; cNH3_0; cAc_0; ri(vNB)];
    XG0 = -inv(A2)*B2*u0;
  % Initial state system of the monophasic compounds
    if Fin > 0
      XL0 = [cSO4_0; cPO4_0] + VL/Fin*ri(vNM);
    else
      XL0 = [cSO4_0; cPO4_0];
    end
X0 = [XG0; XL0; cX_0]
%Temporal inputs
%---------------
CG0 = [fm_CO2_0; fm_NH3_0; fm_Ac_0] / VM;                % mol/l
CL0 = [cCO2_0; cNH3_0; cAc_0; cSO4_0; cPO4_0]; % mol/l

dG = ones(size(T))*CG0';     % incoming gas [CO2,NH3,AcOH] conc (mol/l)
dL = ones(size(T))*CL0';     % incoming liq [CO2,NH3,AcOH,H3PO4,H2SO4] conc (mol/l)
FR = ones(size(T))*FR0;

if typtst == 2
  t0 = 10;
  ind = find(T>=t0);
  FR(ind) = 50.4*ones(size(ind));
end

% Mass balance checking
% =====================
% Matrices of stoechiometric composition of each of compounds :
% atom :      C        N        S        P
MSG =   [     1        0        0        0;   % CO2 Gas
              0        1        0        0;   % NH3 Gas
              2        0        0        0];  % AcOH Gas
MSL =   [ 1+Kdis(1)    0        0        0;   % CO2 Liquid
              0     1+Kdis(2)   0        0;   % NH3 Liquid
         2*(1+Kdis(3)) 0        0        0;   % AcOH Liquid
              0        0        1        0;   % SO4
              0        0        0        1];  % PO4
MSS = Scomp(:,[1,4,5,6]);                     % biomass
MSS = MSS ./ (Mbio*ones(1,4));  % atom/g biomass

% Saving the specific variables of the Rhodobacter compartment
% ==============================================================
X0_2 = X0;       NL_2 = NL;       NG_2 = NG;       NS_2 = NS;
NI_2 = NI;       NX_2 = NX;       NO_2 = NO;
A2_2 = A2;       B2_2 = B2;       GG1_2 = GG1;  GG2_2 = GG2;
KSSO4_2 = KSSO4;
Kdis_2 = Kdis;
vNB_2 = vNB;  vNM_2 = vNM;  vNS_2 = vNS;
Fin_2 = Fin;  Gin_2 = Gin;  VL_2 = VL;
Yx1_2 = Yx1;
fI_2 = fI;
RT_2 = RT;
Ea_2 = Ea;       Es_2 = Es;       muM_2 = muM;
KJ_2 = KJ;       EpsJ_2  = EpsJ;
T_2 = T;         dG_2 = dG;       dL_2 = dL;       FR_2 = FR;
cH2O_2 = cH2O;   cN2_2 = cN2;
```

```
MSG_2 = MSG; MSL_2 = MSL; MSS_2 = MSS;

if arret
% break
end
disp(' *** Rhodo - End of initialization ***')
```

A3. SIMULATOR OF THE SPIRULINA COMPARTMENT

A3.1. Mathematical relations of the simulator

A3.1.1 State system of the bi-phasic compounds

The general state system of the bi-phasic compounds is described by (A1.9) and is recalled hereafter :

$$\begin{aligned} \dot{X} &= A \cdot X + B \cdot U \\ Y &= C \cdot X + D \cdot U \end{aligned} \qquad \text{(A3.1)}$$

where :

- X is the column vector of the concentrations of the bi-phasic compounds in the liquid phase (for the Spirulina compartment, they are O_2 and CO_2);
- U is the column vector of the inputs (concentrations of the bi-phasic compounds in the incoming gas and liquid phases, variation rates of the bi-phasic compounds in liquid phase);
- Y is the column vector of the outputs (concentrations of the bi-phasic compounds in the outgoing gas and liquid phases);
- A, B, C and D are constant matrices if the gas and liquid flow rates are constant, or time variable matrices if these flow rates depend on time.

Detailed description of these vectors and matrices for the Spirulina compartment:

Note : Z^t is the transpose matrix of the matrix Z.

The indices 1 and 2 are associated to O_2 and CO_2, respectively.

The variables δ_1 and δ_2 are defined in (A1.8) for O_2 and CO_2, respectively.

The variables k_1 and k_2 are the dissociation constants for O_2 and CO_2, respectively.

$$\begin{aligned} X^t &= [b_1 \ \ b_2] \\ U^t &= [d_{G1} \ \ d_{G2} \ \ d_{L1} \ \ d_{L2} \ \ r_1 \ \ r_2] \\ Y^t &= [a_1 \ \ a_2 \ \ b_1 \ \ b_2] \end{aligned} \qquad \text{(A3.2)}$$

$$A = \begin{pmatrix} -\left(\frac{q_L}{V_L} + \delta_1\right) & 0 \\ 0 & -\left(\frac{q_L}{V_L} + \delta_2\right) \end{pmatrix}$$

$$B = \begin{pmatrix} \frac{\delta_1}{\alpha_1} & 0 & \frac{q_L}{V_L} & 0 & \frac{1}{1+k_1} & 0 \\ 0 & \frac{\delta_2}{\alpha_2} & 0 & \frac{q_L}{V_L} & 0 & \frac{1}{1+k_2} \end{pmatrix} \qquad \text{(A3.3)}$$

$$C = \begin{pmatrix} \alpha_1 \cdot (1-\beta_1) & 0 \\ 0 & \alpha_2 \cdot (1-\beta_2) \end{pmatrix}$$

$$D = \begin{pmatrix} \beta_1 & 0 & 0 & 0 & 0 & 0 \\ 0 & \beta_2 & 0 & 0 & 0 & 0 \end{pmatrix}$$

A3.1.2. State system of the mono-phasic compounds

The general state system of the mono-phasic compounds is described by (A1.10) and is recalled hereafter :

$$\frac{db_i}{dt} = \frac{q_L}{V_L} \cdot \left(d_{Li} - b_i\right) + r_i \qquad \text{(A3.4)}$$

where i is the index of the compounds defined in the table A3.1 .

Index i	Compound
1	Nitrate
2	Sulfate
3	Phosphate
4	Active biomass
5	Vegetative biomass
6	Exopolysaccaride
7	Phycocyanin
8	Chlorophyll

Table A3.1 Index of the mono-phasic compounds

For the constituents of the biomass ($4 \le i \le 8$), the input concentration is null.

The production rates of the components of the biomass are those established in TN 19.2 (LGCB), taking into account the modification (on June 5th, 1998) of the growth rate of the EPS (exopolysaccaride).

In agreement with their author and in order to avoid negative concentration of PO_4, a Monod term for PO_4 has been added to the expressions of $\langle r_{XA} \rangle, \langle r_{PC} \rangle$ and $\langle r_{XV} \rangle$. They are listed hereafter in (A3.5) :

$$
\begin{aligned}
&\langle r_{XT} \rangle = \langle R_{XA} \rangle + \langle R_{EPS} \rangle \\
&\langle r_{XA} \rangle = \langle R_{XA} \rangle \cdot \frac{C_N}{K_N + C_N} \cdot \frac{C_S}{K_S + C_S} \cdot \frac{C_P}{K_P + C_P} \\
&\langle r_{PC} \rangle = z_{PC} \cdot \langle R_{XA} \rangle \cdot \left(\frac{C_N}{K_N + C_N} \cdot \frac{C_S}{K_S + C_S} \cdot \frac{C_P}{K_P + C_P} - \left(\frac{K_N}{K_N + C_N} + \frac{K_S}{K_S + C_S} + \frac{K_P}{K_P + C_P} \right) \right) \\
&\langle r_{XV} \rangle = \langle R_{XA} \rangle \cdot \left(\frac{C_N}{K_N + C_N} \cdot \frac{C_S}{K_S + C_S} \cdot \frac{C_P}{K_P + C_P} + \frac{C_{PC}}{K_{PC} + C_{PC}} \cdot \left(\frac{K_N}{K_N + C_N} + \frac{K_S}{K_S + C_S} + \frac{K_P}{K_P + C_P} \right) \right) \\
&\langle r_{EPS} \rangle = \langle r_{XT} \rangle - \langle r_{XV} \rangle \\
&\langle r_G \rangle = \langle r_{XV} \rangle - \langle r_{XA} \rangle \\
&\langle r_{CH} \rangle = z_{CH} \cdot \langle r_{XA} \rangle
\end{aligned}
\qquad \text{(A3.5)}
$$

As the reactions of production of EPS and G are not reversible, the rates $\langle r_{EPS} \rangle$ and $\langle r_G \rangle$ cannot be negative. The adequate protection is set in the software.

A3.1.3. Variation rates of the compounds in the liquid phase

The variation rates of O_2, CO_2, NO_3, SO_4 and PO_4 depend on the stoechiometry of the production of XA, EPS and G (glycogen) and on their production rates r_b :

$$r_C = M_S \cdot r_b \qquad \text{(A3.6)}$$

where :

- r_C is the vector of variation rates of O_2, CO_2, NO_3, SO_4 and PO_4;
- M_S is the matrix of the stoechiometry of production of XA, EPS and G (table A3.2) ;
- r_b is the vector of production rates of XA, EPS and G.

$$r_b = \begin{bmatrix} \langle r_{XA} \rangle \\ \langle r_{EPS} \rangle \\ \langle r_G \rangle \end{bmatrix}$$

	XA	**EPS**	**G**
O_2	1.437	0.96	1.0745
CO_2	-1	-1	-1
NO_3	-0.1921	0	0
SO_4	-0.0052	-0.015	-0.008
PO_4	-0.0063	0	0

Table A3.2 : Matrix of stoechiometry of production of biomass
Source : TN 19.2 p. 6 and Thesis of JF Cornet

A3.2. Checking the mass balance

The stoechiometric composition of each of the compounds are gathered in the following table A3.3 .

Matrices M_{Gj}				
j	1	2	3	4
atom	C	N	S	P
O_2 in gas	0	0	0	0
CO_2 in gas	1	0	0	0

Matrices M_{Lj}				
j	1	2	3	4
atom	C	N	S	P
O_2 in liquid	0	0	0	0
CO_2 in liquid	$1+k_{CO2}$	0	0	0
NO_3	0	1	0	0
SO_4	0	0	1	0
PO_4	0	0	0	1

Matrices M_{Sj}				
j	1	2	3	4
atom	C	N	S	P
XA	1	0.1921	0.0052	0.0063
EPS	1	0	0.015	0
G	1	0	0.008	0

Table A3.3 : Matrices of stoechiometric compositions for the compounds involved in the Spirulina compartment

The checking has been done for different kind of simulations : light flux constant or not, concentrations of the incoming flows (gas and liquid) constant or not, gas and liquid flow rates null (i.e. batch conditions) or not. The figure A3.1 shows the variables r_{Ti}, r_T and δ of the relations (A1.16) and (A1.17) in the case of the simulation described in the paragraph A3.3.1 with no mineral limitation.

For each of the atom C, N, S and P the outgoing flow rate r_T is equal to incoming flow rate r_{Ti} within a relative distance δ under 10^{-7} for C (except at the light step where it is equal to 10^{-5}) and 10^{-8} for the other atoms, which is due to numerical noise in computation of the approximate derivative of c_m in the expression (A1.14) of r_T.

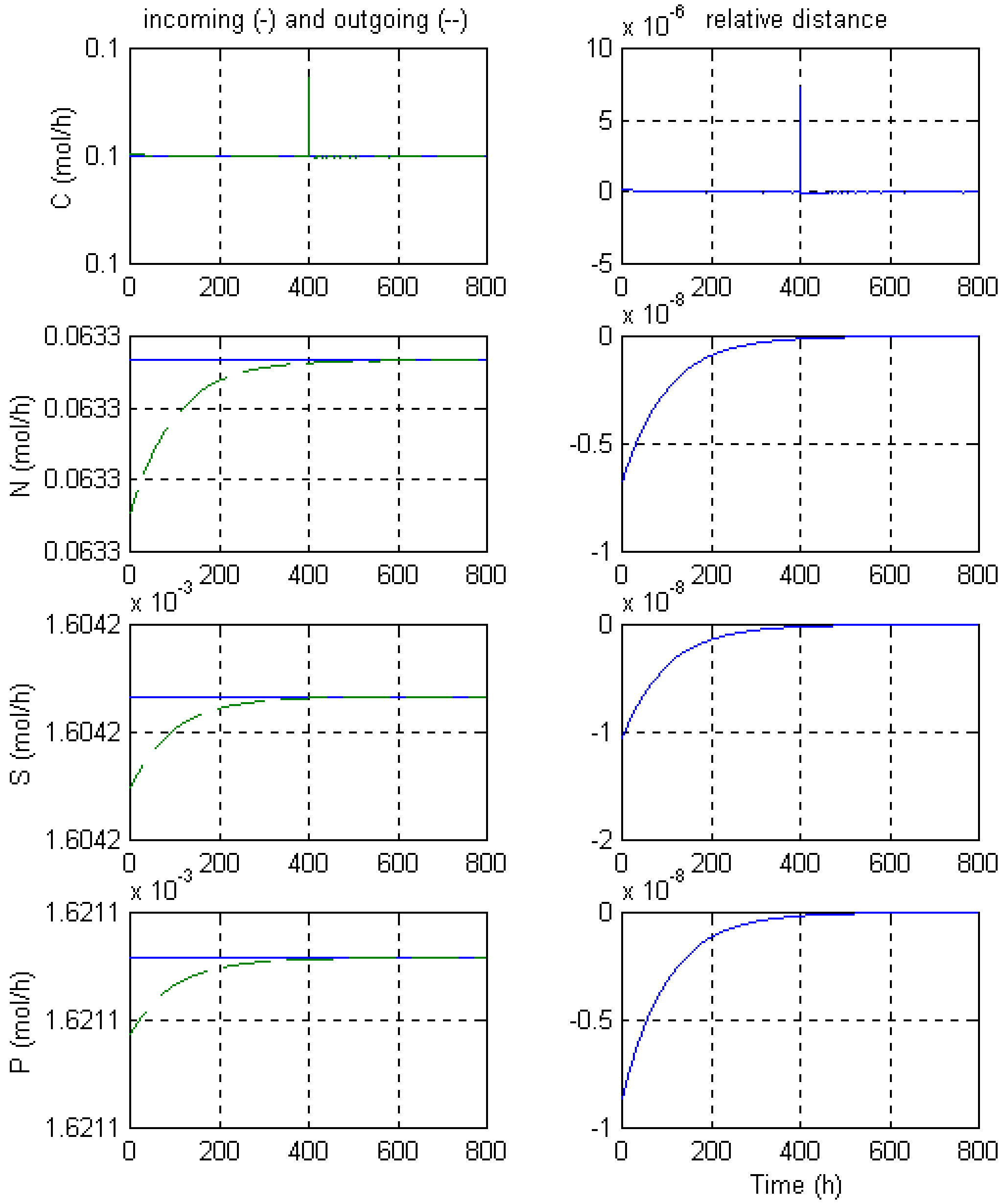

Figure A3.1 : Checking the mass balance of atom C, N, S and P
Left hand side column : incoming flow = blue line - ; outgoing flow = green line - -
Right hand side column : relative distance δ expressed in relation (A1.17)

A3.3. Simulation results

A3.3.1. Simulation with no mineral limitation

The simulation without mineral limitation has been done with the following data, representative of a real case :

- the CO_2 production rate of the team is the one of 4 rats, i.e. 4 10^{-2} mol/h.
- the O2 concentration of the incoming gas flow is 21 % molar fraction.
- the incoming gas flow rate is 180 l/h (TN 43.110 p.26, UAB).
- the incoming liquid flow rate is 77 10^{-2} l/h ,such that the residence time is 100 h.
- the O2 concentration in the incoming liquid flow is null.
- the concentrations of CO_2 and NO_3 in the incoming liquid are those produced by the waste of 1 man. They are extrapolated from the previous TN 35.1 (ADERSA) of the global simulator where the wastes are those of 3 men and where the conversions are supposed to be complete. They are such that the corresponding flow rates are : 0.1 mol/h of CO2 total and 0.06 mol/h for NO3.
- The sulphate and phosphate concentrations in the incoming flow and the initial concentration of biomass are those of TN 19.3 (LGCB), Appendix 7, Figure 8 :
  - Sulphate and phosphate : 0.2 g/l;
  - XA = 0.1 g/l;
  - XV = XA;
  - EPS=.02 g/l.
- The concentrations of the incoming gas and liquid flows are constant all along the simulation.
- The light flux is 50 W/m^2 at the beginning and is set at its maximum, 223 W/m^2, in the middle of the simulation, at t=400 h. That way, the simulation is composed of 2 steady states at 50 and 223 W/m^2 (Recall : the time constant is 100 h).

The figure A3.2 shows the concentrations in the outgoing gas and liquid flows and the figure A3.3 the production rates (N.B. : the rates are signed and a negative production rate means in fact that the concerned compound is consumed).

Nearly no glycogen is produced : the G production rate (bottom left hand side of fig. A3.3) is very low (about 0.3 % of XA production rate). The total production rate of biomass is 0.7 g/h at 50 W/m^2 and 1.7 g/h at 223 W/m^2, for a liquid flow rate of 0.77 l/h.

The CO2 consumption is 0.03 and 0.07 mol/h at 50 W/m^2 and 223 W/m^2 , respectively. Compared to the input flow rate of 0.1 mol/h, the conversion of CO2 into biomass is 30 % and 70 % for these 2 values of the light flux.

In the same way, the NO_3 consumption is about 0.005 and 0.01 mol/h at 50 W/m^2 and 223 W/m^2 , respectively. Compared to the input flow rate of 0.06 mol/h, the conversion of NO_3 into biomass is 8.5 % and 17 % for these 2 values of the light flux, which is the expected coefficient of conversion.

A3.3.2. Simulation of a nitrate limitation

For this test, the conditions of the previous simulation (without any limitation) are maintained except that the initial NO_3 concentration in the reactor is low at the starting and that the NO_3 concentration of the incoming liquid is near to zero.

The figures A3.4 and A3.5 show the temporal evolution of the concentrations in the reactor and of the production rates. The main features are :

- Due to its limitation and its consumption by production of XA, $\left[NO_3^-\right]$ falls to 0 in the graph 31 (row 3, column 1) of the figure A3.4 . A x zoom would show that it happens in less than 2 hours.
- Graph 31 of figure A3.5 : $\left\langle r_{XA}\right\rangle$ falls to 0 at the same speed as $\left[NO_3^-\right]$ because of the limiting factor binding the 2 variables in (A3.5).
- Graph 42 of figure A3.5 : the PC is consumed ($\left\langle r_{PC}\right\rangle$ is negative due its expression in (A3.5)) until there is no more PC in the reactor (graph 41 of figure A3.4). Due to this consumption, the concentration of PC decreases faster than the dilution alone would imply (the dilution rate can be observed on the evolution of $[CO_2]$ in graph 11 of figure A3.4).
- As $\left\langle R_{XA}\right\rangle$, $\left\langle R_{EPS}\right\rangle$ tends to 0 at the same speed as [PC]. So according to their expressions in (A3.5), $\left\langle r_{EPS}\right\rangle$ and $\left\langle r_G\right\rangle$ have the same behaviour as $\left\langle r_{PC}\right\rangle$. This is confirmed in the graphs 32 and 41 of figure A3.5 .

So it can be concluded that the simulation runs as expected.

A3.3.3. Simulation of a phosphate limitation

For this test too, the conditions of the simulation with no mineral limitation are maintained except that the initial PO_4 concentration in the reactor is low at the starting and that the PO_4 concentration of the incoming liquid is near to zero.

The figures A3.6 and A3.7 show the temporal evolution of the concentrations in the reactor and of the production rates. The main features are very similar to the ones of the previous simulation because of the symmetry of the chemical reactions and of the expressions of the production rates in (A3.5) for NO_3 and PO_4 :

- Due to its limitation and its consumption by production of XA, $[PO_4]$ falls to 0 in the graph 42 (row 4, column 2) of the figure A3.6 in about 20 hours.
- Graph 31 of figure A3.7 : $\left\langle r_{XA}\right\rangle$ falls to 0 at the same speed as$[PO_4]$ because of the limiting factor binding the 2 variables in (A3.5).
- Graph 42 of figure A3.7 : the PC is consumed ($\left\langle r_{PC}\right\rangle$ is negative due its expression in (A3.5)) until there is no more PC in the reactor (graph 41 of figure A3.6). Due to this consumption, the concentration of PC decreases faster than the dilution alone would imply (the dilution rate can be observed on the evolution of $[CO_2]$ in graph 11 of figure A3.6).
- As $\left\langle R_{XA}\right\rangle$, $\left\langle R_{EPS}\right\rangle$ tends to 0 at the same speed as [PC]. So according to their expressions in (A3.5), $\left\langle r_{EPS}\right\rangle$ and $\left\langle r_G\right\rangle$ have the same behaviour as $\left\langle r_{PC}\right\rangle$. This is confirmed in the graphs 32 and 41 of figure A3.7 .

Here too, the simulation runs as expected.

A3.3.4. Simulation of a sulphate limitation

For this test too, the conditions of the simulation with no mineral limitation are maintained except that the initial SO_4 concentration in the reactor is low at the starting and that the SO_4 concentration of the incoming liquid is near to zero.

The figures A3.8 and A3.9 show the temporal evolution of the concentrations in the reactor and of the production rates. The main features are :

- Due to its limitation and its consumption by production of XA, EPS and G, $[SO_4]$ falls to 0 in the graph 32 (row 3, column 2) of the figure A3.8 in about 5 hours.
- Graph 31 of figure A3.9 : $\langle r_{XA} \rangle$ falls to 0 at the same speed as$[SO_4]$ because of the limiting factor binding the 2 variables in (A3.5).
- Graph 42 of figure A3.9 : the PC is produced first and then consumed as long as there is PC in the reactor (graph 41 of figure A3.8).
- Although $[SO_4]$ is null after t=5 h, [PC] is not null. So $\langle R_{XA} \rangle$ and $\langle R_{EPS} \rangle$ are not null and the production of XT goes on (as it is observed on a real process where there is still production of EPS and G without S atom). Consequently the productions of EPS and G are not null and the consumption of SO_4 continues. Then $[SO_4]$ becomes negative.

So the present simulator is not correct when $[SO_4]$ is too low. As this situation will be rarely encountered on a real process, it has been decided not to make the simulator still more complex by inserting added chemical reactions where EPS and G are produced without S atom. In order to avoid the case of negative concentration of SO_4 , a message is delivered and the simulation stops.

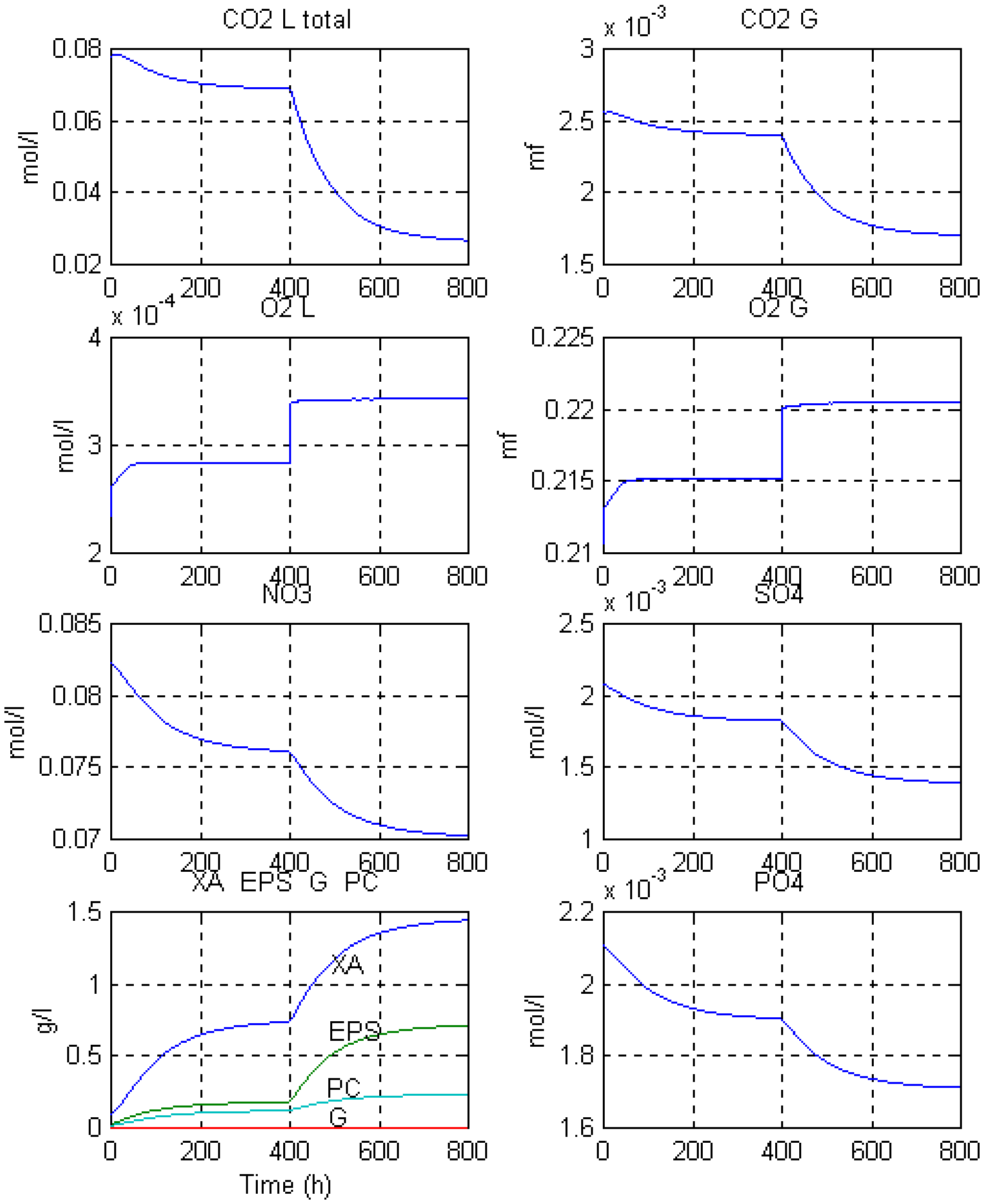

Figure A3.2 : Constant input concentrations ; light flux step at t=400 h
Concentrations in the reactor
XA : blue line
EPS = dark green line
G : red line
PC : pale green line

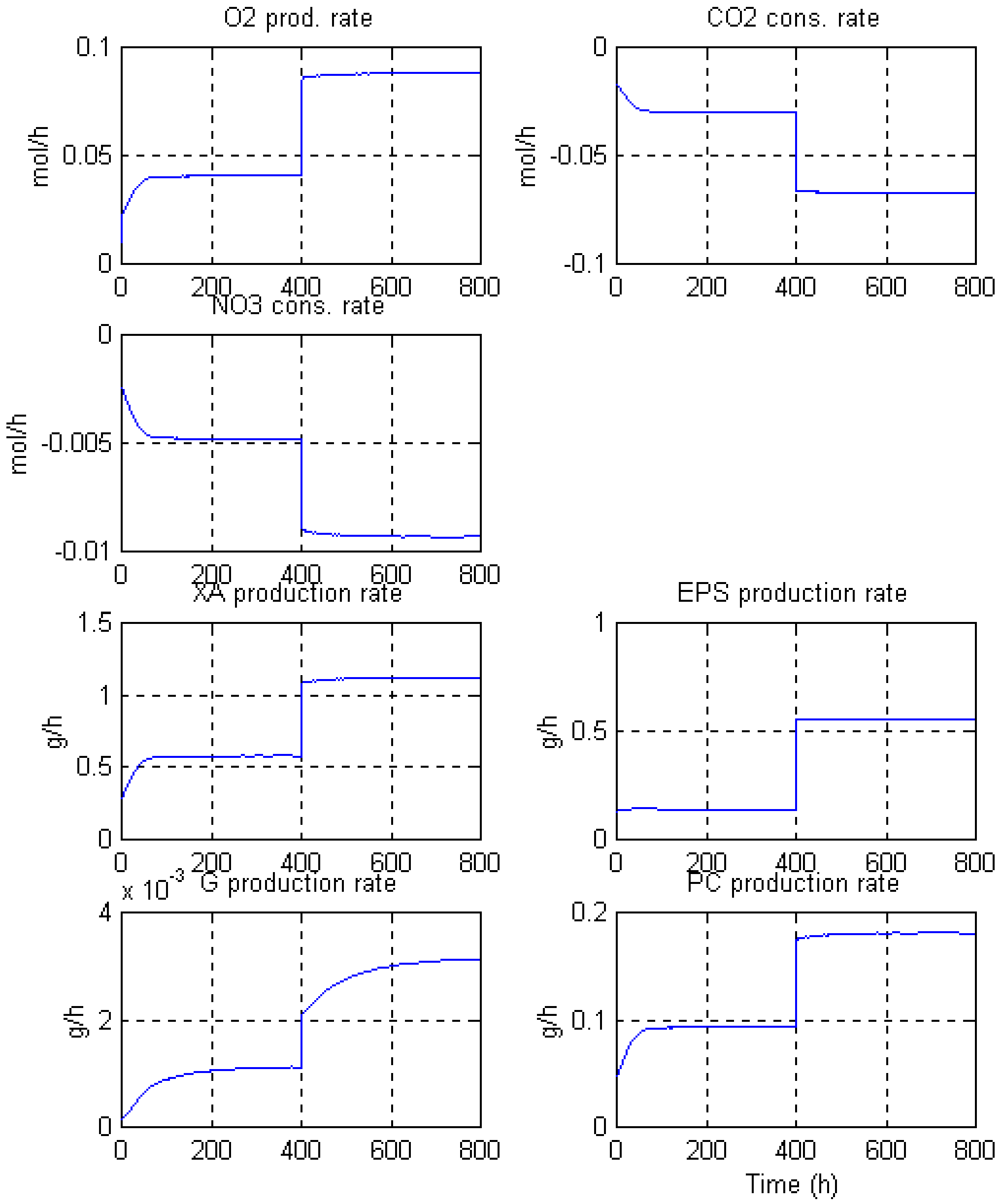

Figure A3.3 : Constant input concentrations ; light flux step Production rates

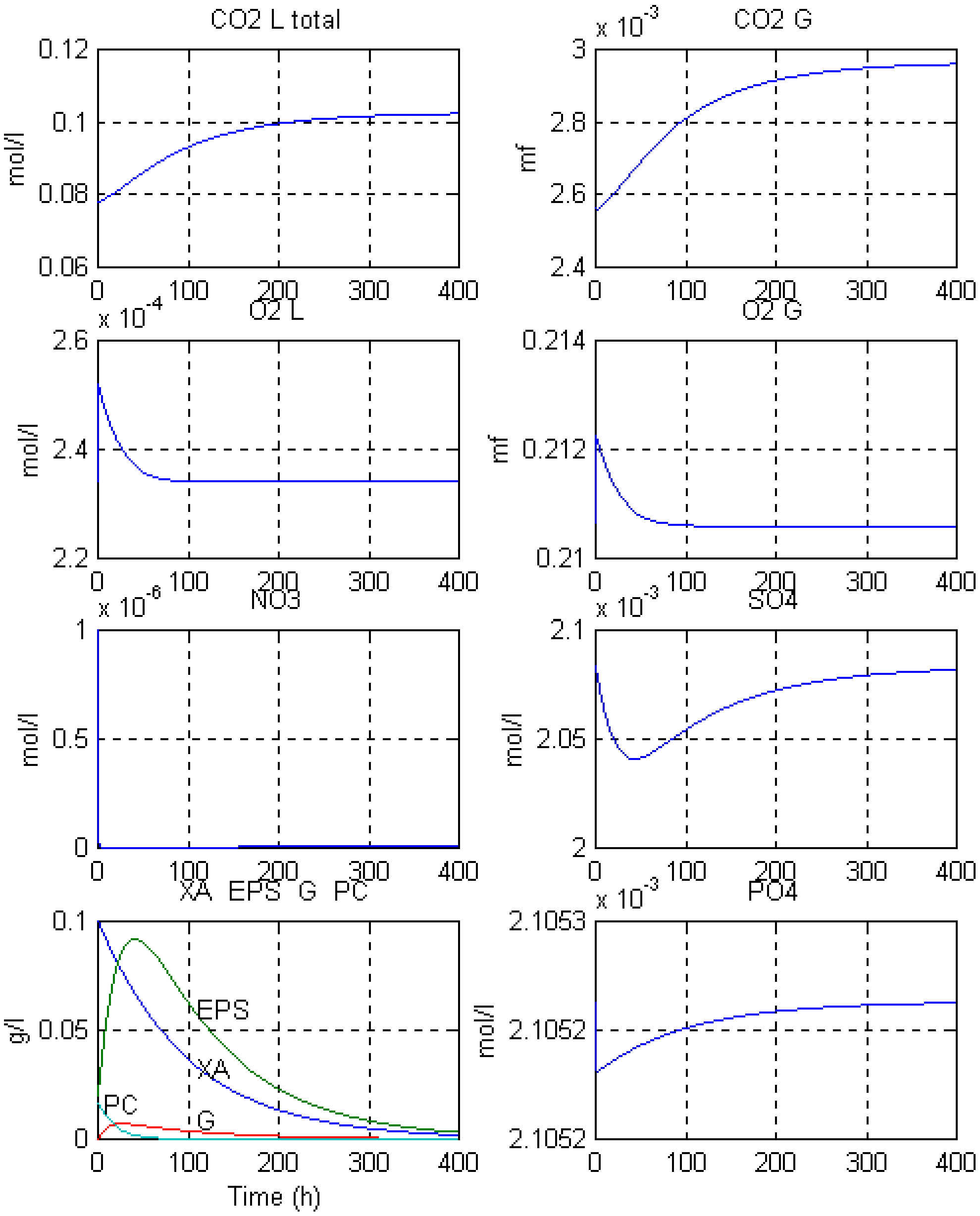

Figure A3.4 : NO_3 limitation at light flux equal to 50 W/m^2
Concentrations in the reactor
XA : blue line
EPS = dark green line
G : red line
PC : pale green line

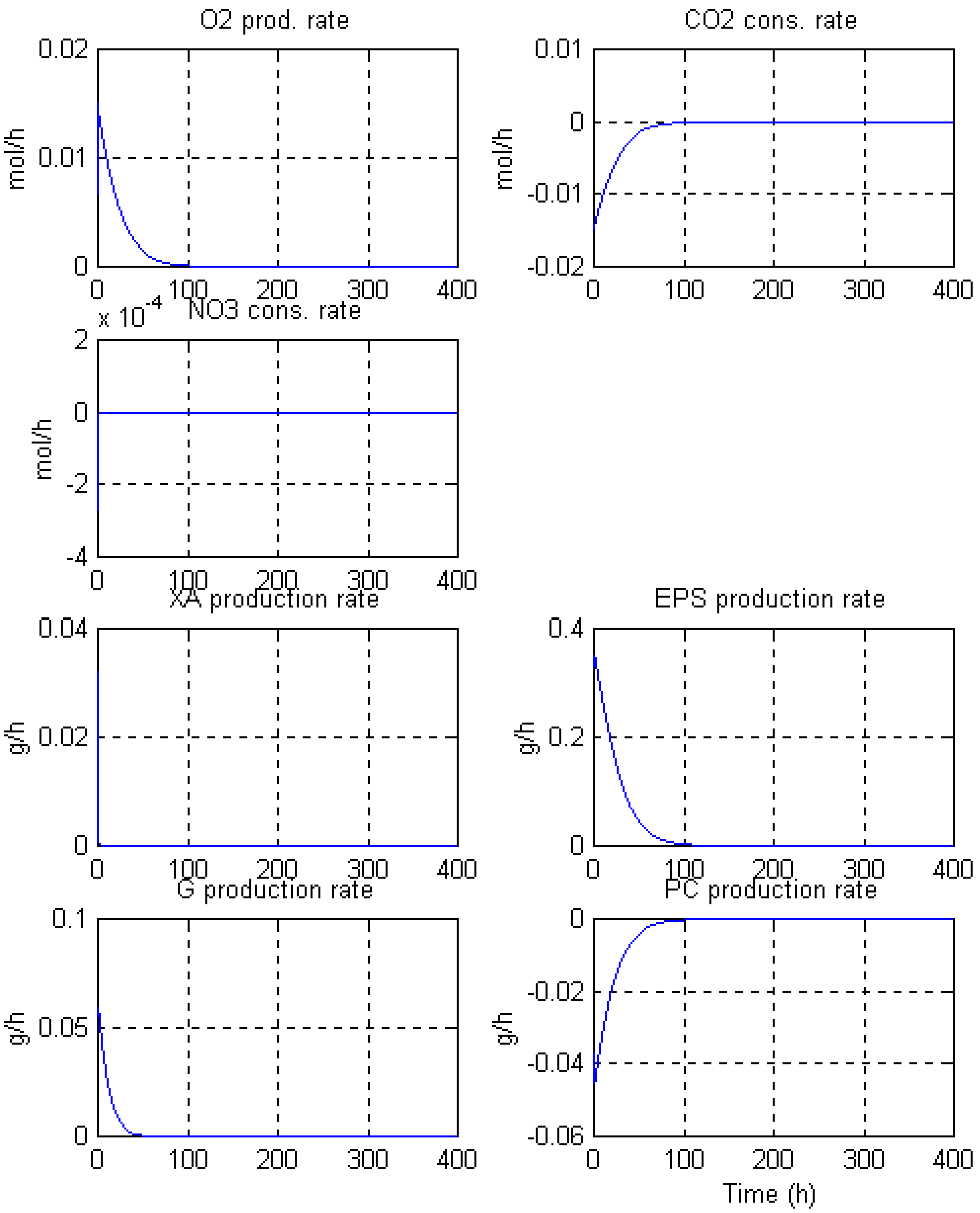

Figure A3.5 : NO_3 limitation at light flux equal to 50 W/m^2
Production rates

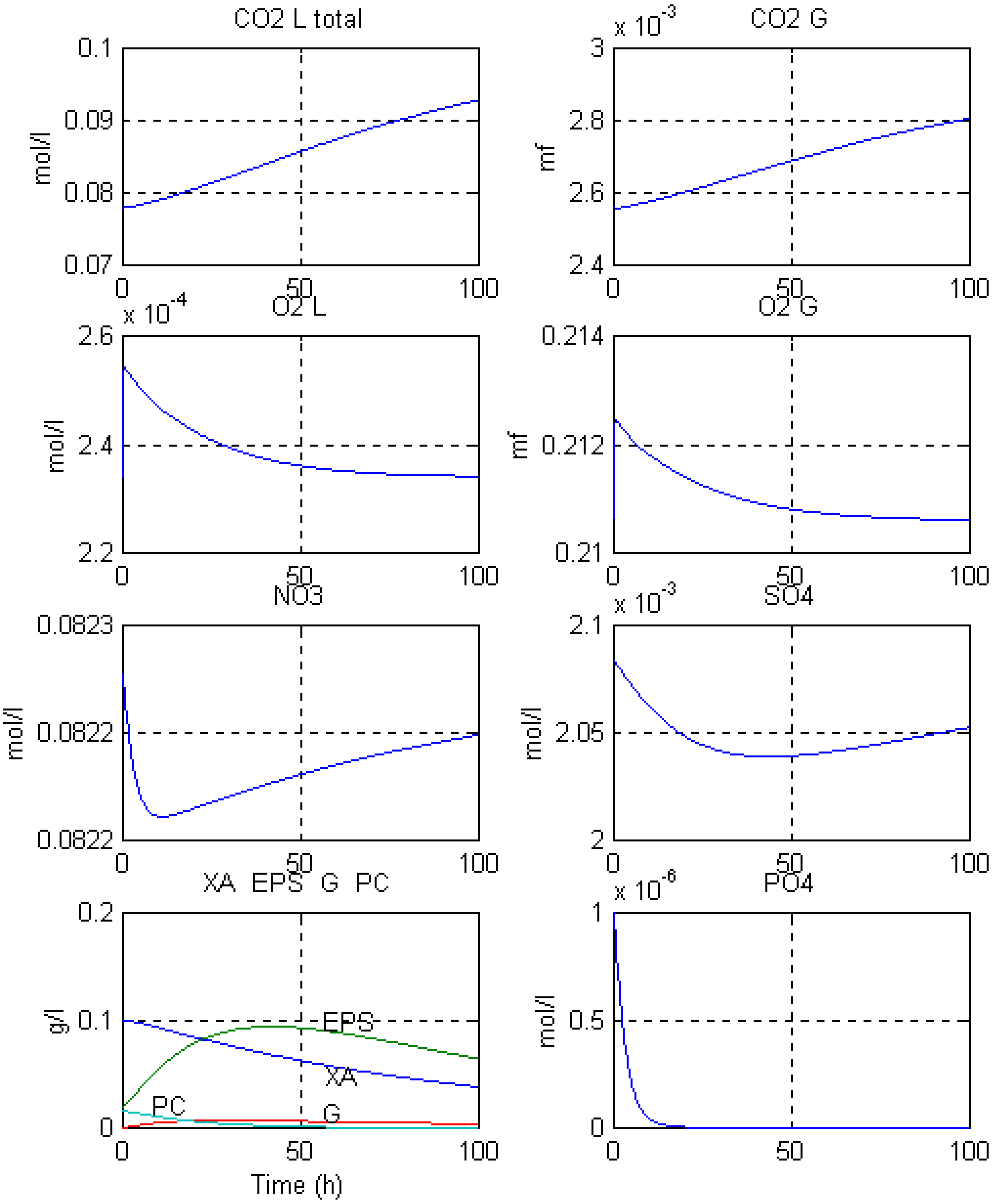

Figure A3.6 : PO_4 limitation at light flux equal to 50 W/m^2
Concentrations in the reactor
XA : blue line
EPS = dark green line
G : red line
PC : pale green line

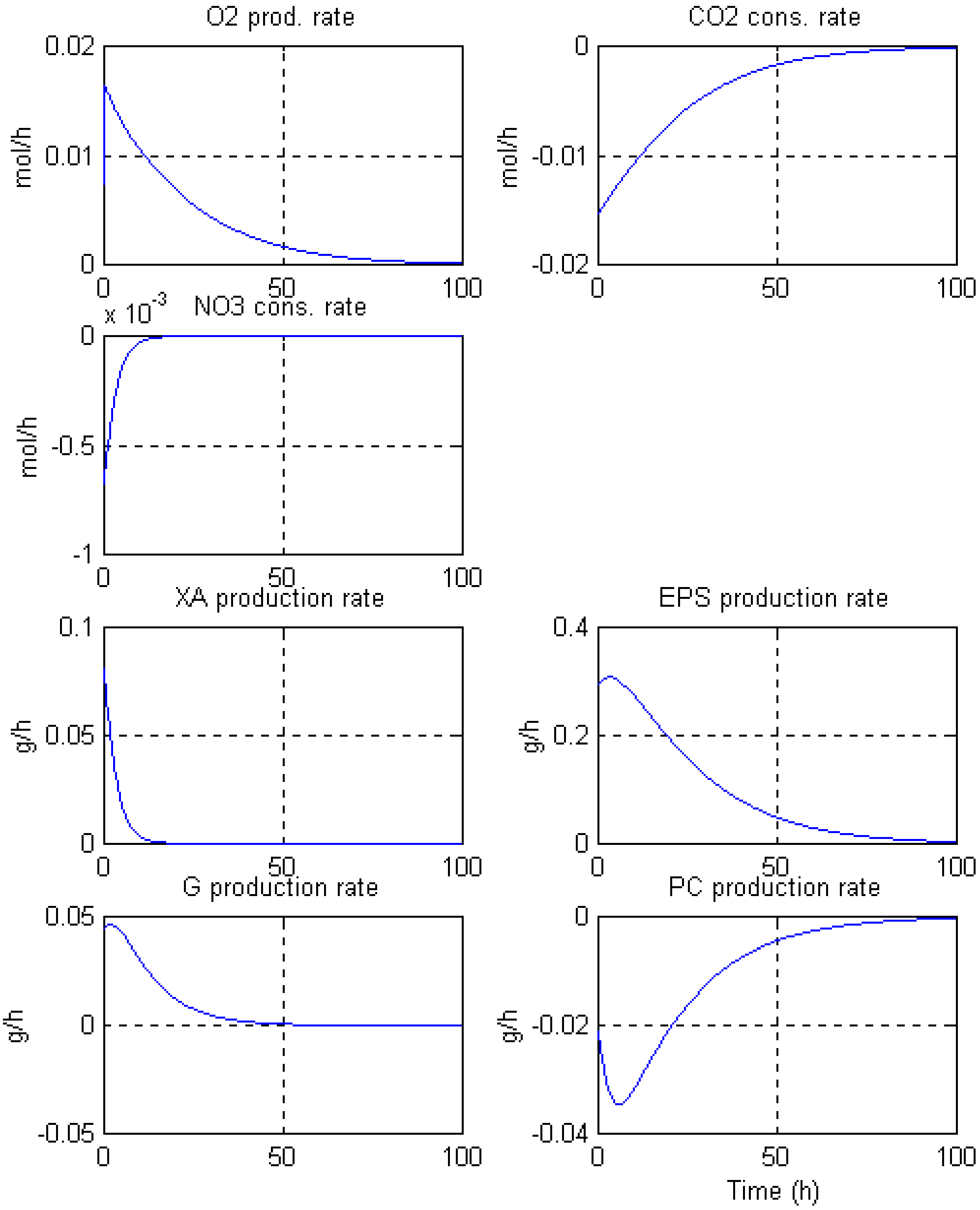

Figure A3.7 : PO_4 limitation at light flux equal to 50 W/m^2
Production rates

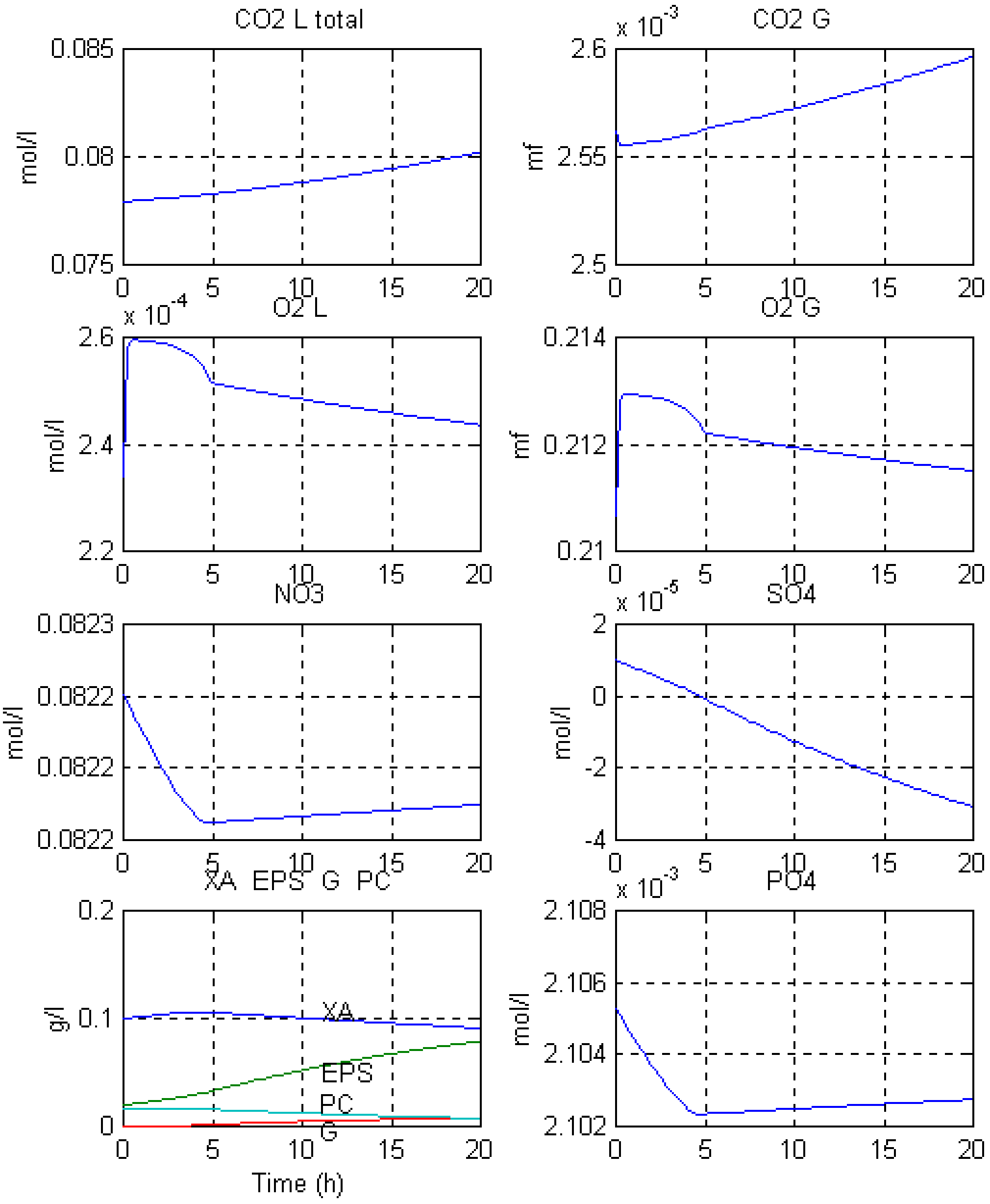

Figure A3.8 : SO_4 limitation at light flux equal to 50 W/m^2
Concentrations in the reactor
XA : blue line
EPS = dark green line
G : red line
PC : pale green line

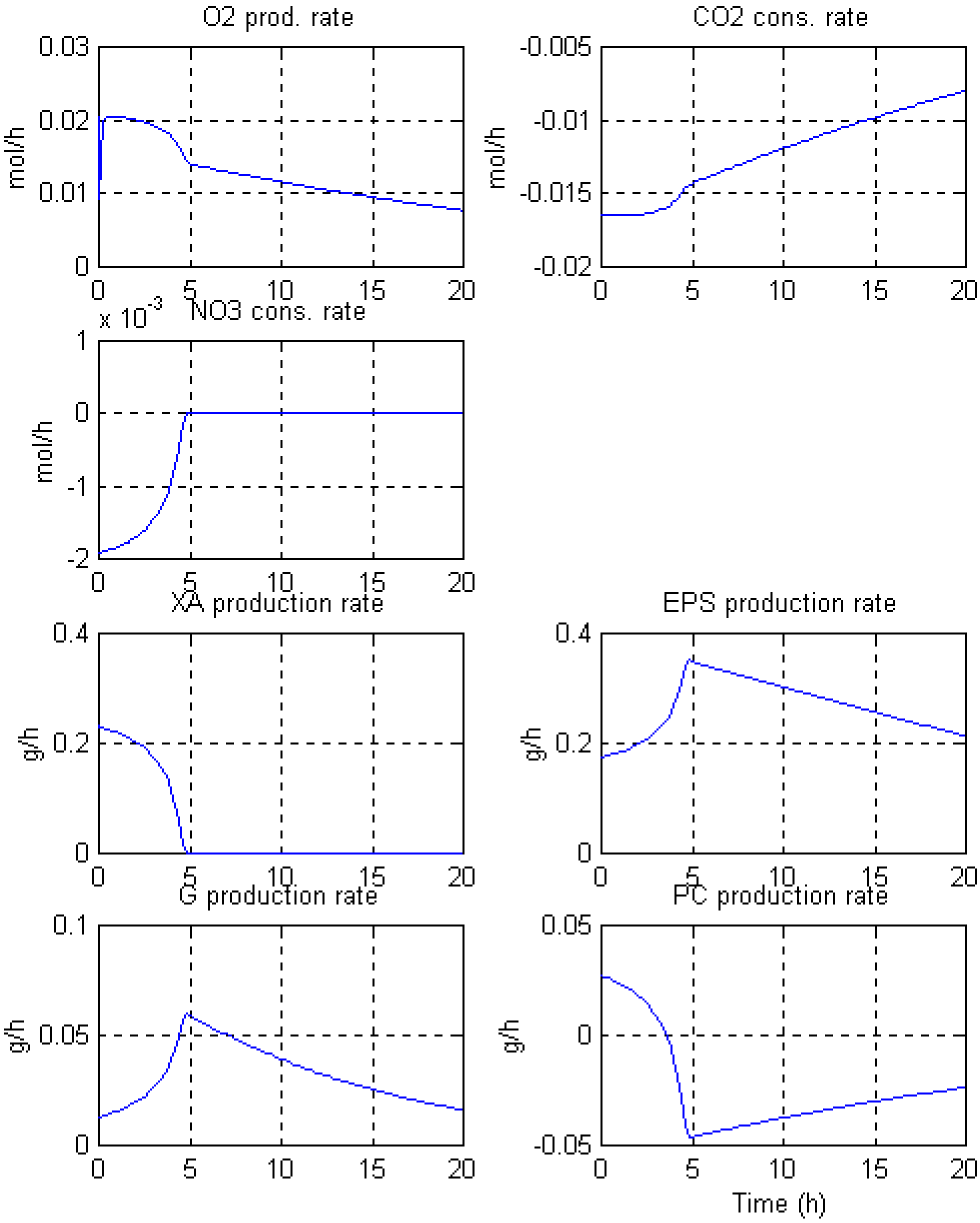

Figure A3.9 : SO_4 limitation at light flux equal to 50 W/m^2
Production rates

A3.4. Simulation parameters

All the parameters of the simulation are recalled in the following Matlab® file :

```
%*******************************************************************
%       Photoautotrophic compartment (Photosynthesis)             *
%       Version 1     November 2000                               *
%                                                                 *
%       State system according to TN 54.2                         *
%                                                                 *
%                                                                 *
%       i_sim.m : Initialization of the simulation                *
%                                                                 *
%       . mineral limitation (NO3 SO4)                            *
%       . no carbon limitation (conclusion of TN 32.4, LGCB)      *
%       . CO2 production of 4 rats (.04 mol/h)                    *
%       . waste of 1 man (data computed from TN 35.1, ADERSA)     *
%       . NH3 is not a substrate (not consumed by biomass)        *
%                                                                 *
%*******************************************************************

 clear all

arret = 0;
% Simulation parameters
        tdeb=0;
        tmin=1e-3;
        tmax=.2;
        prec=1e-3;
        tfin=800;    % h
        nbptx=ceil(1.2*tfin/tmin);

% Simulation horizon
        dt = .5;        % (h) simulation period of the inputs
        T = [0:dt:tfin]';
        [m,n] = size(T);

% Type of test
% ------------
typtst = 3;     titre = 'Steps of input concentr. and of light';
typtst = 1;     titre = 'Constant concentrations of the inputs';
typtst = 4;     titre = 'Test of mineral limitation : NO3 limitation';
typtst = 6;     titre = 'Test of mineral limitation : PO4 limitation';
typtst = 5;     titre = 'Test of mineral limitation : SO4 limitation';
typtst = 2;     titre = 'Constant concentr. of the inputs; light step';

% General parameters of the simulated process
% -------------------------------------------
NG = 2;         % number of bi-phases substrates (in the L and G phases) : O2, CO2
NL = 3;         % number of mono-phase substrates in the liquid phase:NO3, SO4, PO4
NS = 5;         % number of compounds of the biomass : XA, XV, EPS, PC, CH
NI = 2*NG+NL+1; % nb of inputs of the process (photosynthetic reactor)
NX = NG+NL+NS;  % nb of coef of state vector X
NO = NG+NX;     % nb of outputs of the process (photosynthetic reactor)

% Parameters of the PBR
% ---------------------
RT = .076;   % radius of the reactor (m)
VL = 77;     % volume of liquid (l) (from TN43.110 p.4, UAB)
Gin = 180;   % incoming gas flow rate (l/h) (from TN 43.110 p.26, UAB)
             % if Gin is time variable, move 'statesys' into the S-function 'pr_spiru'
             % in order to compute the state system at each sampling period
tau = .01;   % (h) residence time as in the photobioreactor
Fin = tau*VL;% incoming liquid flow rate (l/h) (if Fin variable, do as for Gin)
fI = .688;   % Illuminated surface fraction (TN43.110 p.4, UAB)
Temp = 309;  % K
pH = 9.5;
```

```
% gas/liquid transfer parameters (TN 43.110 p.26, UAB)
% ------------------------------
KLa = 12;    % O2 CO2 NH3   (1/h)
% partition coefficients
% ----------------------
kpartN = [4.9856e4; 2135.7; 13.539];   % O2 CO2 NH3 (T=309K,non ionic form)
                                       % computed from TN35.1,ADERSA
                                       % (for extension to NH3 when NH3=substrate)
kpartN = [4.9856e4; 2135.7];           % O2 CO2 (T=309K,non ionic form)
% dissociation coefficient (for CO2)
% ----------------------------------
Ka = [4.882e-7; 5.661e-11];               % at T=309K, from TN 35.1,ADERSA
KCO2 = Ka(1)/10^(-pH)*(1+Ka(2)/10^(-pH)); % [HCO3-]+[CO3--] = KCO2 * [CO2]solvated
% dissociation coefficient (for NH3)
% ----------------------------------
Kb = 1.7241e-5;         % basicity cst at T=309K, from TN 35.1,ADERSA
Ke = 2.1900e-14;        % ionic product at T=309K, from TN 35.1,ADERSA
KNH3 = Kb*10^(-pH)/Ke;  % [NH4+] = KNH3 * [NH3]solvated
% dissociation coefficient (for H3PO4)
% ------------------------------------
Ka = 6.166e-8;                    % (at T=298K, from TN 27.2,LGCB)
xx = 10^(-pH)/Ka;   KPO4 = 1+xx;  %[PO4]total = f([HPO4=])
% dissociation vector for the biphase substrates
% ----------------------------------------------
Kdis = [0; KCO2; KNH3]; % O2  CO2  NH3 (for extension to NH3 when NH3=substrate)
Kdis = [0; KCO2];       % O2  CO2
% liquid/gas thermodynamical equilibrium constants
% ------------------------------------------------
VM = 22.4 * Temp / 273;  % molar volume of perfect gas (l/mol)
alpha = kpartN / 55.56 / VM;
% parameters of the Spirulina growth (TN19.2, LGCB)
% -------------------------------------------------
zCH = .01;  % mass biotic fraction of CH
zPC = .162; % mass biotic fraction of PC
Ea=872;     % global absorption mass coef. (m2/kg)
Es=200;     % global scattering mass coef. (m2/kg)
muM=.45;    % max growth rate for biomass in relation to phycocyanins (1/h)
muEPS=1.852;% max growth rate for EPS in relation to phycocyanins (1/h)
Kj=20;      % half satur. cste for energy to active biomass (W/m2)
KjEPS=750;  % half satur. cste for energy to EPS synthesis (W/m2)
Fmin=1;     % min incident light energy flux (W/m2)

% Computation of the state system of the biphasic compounds
% ---------------------------------------------------------
[A2,B2,GG1,GG2] = statesys(NG, NL, Gin, Fin, VL, alpha, Kdis, KLa);
% Stoechiometry of the reactions of production of biomass
% -------------------------------------------------------
  % 1. mol substrate/mol biomass
  %             XA       EPS     Glycogen
    Yx1  =  [ 1.437     0.96      1.0745;    % O2
             -1        -1        -1     ;    % CO2
             -0.1921    0         0     ;    % NO3-
             -0.0052   -0.015    -0.008 ;    % H2SO4
             -0.0063    0         0     ];   % H3PO4

  % change of unit of Yx1 :
    Matom = [12;   1;      16;    14;    32;   31];  % C H O N S P atomik mass
    %        C     H       O      N      S     P
    Scomp = [1     1.566  .405   .1921  .0052 .0063; % stoechio composition XA
             1     1.65   .95     0     .015   0   ; % stoechio composition EPS
             1     1.67   .71     0     .008   0  ]; % stoechio composition G
    Mbio = Scomp*Matom;
  % 2. mol substrate/g biomass
    Yx1 = Yx1 ./ (ones(NG+NL,1)*Mbio'); % (mol substrate / g biomass)
% Limiting factors associated to mineral limitation
% -------------------------------------------------
KSNO3 = 5e-4/(Matom(4)+3*Matom(3));    % mol/l
KSSO4 = 2.5e-4/(Matom(5)+4*Matom(3));  % mol/l
KSPO4 = KSSO4;                         % mol/l (according to JFC)
KSPC = .06;                            % g/l
% Vector of indices in state vector
```

```
% ---------------------------------
vNB = 1:NG;               % indices of Biphasic compounds
vNM = NG+1:NG+NL;         % indices of Monophasic compounds
vNS = NG+NL+1:NG+NL+NS;   % indices of Solid compounds (biomass components)
% Initial concentrations in the reactor
% -------------------------------------
  %1. Gas phase :
    % 1.1. Case of CO2
    % the production flow rate of CO2 is 4e-2 mol/h (4 rats)
    rCO2 = .04;   % mol/h
    fm_CO2_0 = rCO2*VM/Gin;   % CO2 (molar fraction)
    % 1.2. Other gases
    fm_O2_0  = .21;         % O2  (molar fraction)
    fm_H2O_0 = 5.796e-2;    % H2O (molar fraction)
    fm_N2_0 = 1 - fm_O2_0 - fm_CO2_0 - fm_H2O_0;    % N2
    % conc. in gas phase (perfect gases at T='Temp' Kelvin and P=1 atm)
    cN2 = fm_N2_0 / VM;         % mol/l
    cH2O = fm_H2O_0 / VM;       % mol/l
  %2. Liquid phase :
  cO2_0   = fm_O2_0 / kpartN(1) * 55.56;       % mol/l
  if Fin > 0
    cCO2_0  = (.3/3-rCO2)/(1+Kdis(2))/Fin;   % mol/l (solvated CO2 for 1 man,from TN35.1 p.11)
    cNO3_0  = .19/3/Fin;                     % mol/l (for 1 man,from TN35.1)
  else
    cCO2_0  = (.3/3-rCO2)/(1+Kdis(2))/tau/VL;% mol/l (solvated CO2 for 1 man,from TN35.1 p.11)
    cNO3_0  = .19/3/tau/VL;                  % mol/l (for 1 man,from TN35.1)
  end
  if typtst == 4
    cNO3_0 = 1e-6;
  end
  cSO4_0  = .2/(Matom(5)+4*Matom(3));           % mol/l (from TN19.3,Appendix 7,Figure 8,LGCB)
  if typtst == 5
    cSO4_0 = 1e-5;
  end
  cPO4_0  = .2/(Matom(6)+4*Matom(3));           % mol/l (from ? (idem SO4))
  if typtst == 6
    cPO4_0 = 1e-6;
  end
  %3. Solid phase (biomass) according to TN19.3,Appendix 7,Figure 8,LGCB) :
  cXA_0 = .1;        % g/l
  cXV_0 = cXA_0;     % g/l
  cEPS_0 = .02;      % g/l
  cPC_0 = zPC*cXA_0; % g/l
  cCH_0 = zCH*cXA_0; % g/l
% Initial light flux
% ------------------
FR0 = 50;           % incident light flux (W/m2)
%Temporal inputs
%---------------
CG0 = [fm_O2_0; fm_CO2_0] / VM;                % mol/l
CL0 = [cO2_0; cCO2_0; cNO3_0; cSO4_0; cPO4_0]; % mol/l

dG = ones(size(T))*CG0';     % incoming gas [O2,CO2] conc (mol/l)
dL = ones(size(T))*CL0';     % incoming liq [O2,CO2,NO3,H3PO4,H2SO4] conc (mol/l)
FR = ones(size(T))*FR0;
if typtst == 2
  ind = find(T>400);
  FR(ind) = 223*ones(size(ind));
elseif typtst == 3
  ind = find(T>10);
  dG(ind,:) = 0.5*dG(ind,:);
  dL(ind,:) = 0.5*dL(ind,:);
  ind = find(T>20);
  FR(ind) = 223*ones(size(ind));
elseif typtst == 4
  ind = find(T>1);
  dL(ind,3) = 0.01*dL(ind,3);
elseif typtst == 5
  ind = find(T>1);
  dL(ind,4) = 0.01*dL(ind,4);
elseif typtst == 6
  ind = find(T>1);
```

```
  dL(ind,5) = 0.01*dL(ind,5);
end
% Initial state system
% --------------------
X0 = [cO2_0; cCO2_0; cNO3_0; cSO4_0; cPO4_0; cXA_0; cXV_0; cEPS_0; cPC_0; cCH_0];

% Mass balance checking
% =====================
% Matrices of stoechiometric composition of each of compounds :
% atom :   C        N        S        P
MSG =   [  0        0        0        0;   % O2  Gas
           1        0        0        0];  % CO2 Gas
MSL =   [  0        0        0        0;   % O2  Liquid
         1+Kdis(2)  0        0        0;   % CO2 Liquid and ionic forms
           0        1        0        0;   % NO3
           0        0        1        0;   % SO4
           0        0        0        1];  % PO4
MSS = Scomp(:,[1,4,5,6]);                  % XA, EPS, G
MSS = MSS ./ (Mbio*ones(1,4));  % atom/g biomass

% Saving the specific variables of the Spirulina compartment
% ==========================================================
X0_4 = X0;     NL_4 = NL;     NG_4 = NG;     NS_4 = NS;
NI_4 = NI;     NX_4 = NX;     NO_4 = NO;
A2_4 = A2;     B2_4 = B2;     GG1_4 = GG1;   GG2_4 = GG2;
KSPC_4 = KSPC; KSNO3_4 = KSNO3;
KSSO4_4 = KSSO4;                  KSPO4_4 = KSPO4;
Kdis_4 = Kdis;
vNB_4 = vNB;   vNM_4 = vNM;   vNS_4 = vNS;
Fin_4 = Fin;   Gin_4 = Gin;   VL_4 = VL;
Yx1_4 = Yx1;
fI_4 = fI;
zPC_4 = zPC;   zCH_4 = zCH;
RT_4 = RT;
Ea_4 = Ea;     Es_4 = Es;     muM_4 = muM;   muEPS_4 = muEPS;
Kj_4 = Kj;     KjEPS_4 = KjEPS;                  Fmin_4  = Fmin;
T_4 = T;           dG_4 = dG;     dL_4 = dL;     FR_4 = FR;
cH2O_4 = cH2O; cN2_4 = cN2;
MSG_4 = MSG;   MSL_4 = MSL;   MSS_4 = MSS;

if arret
% break
end
disp(' *** Spiru - End of initialization ***')
```

A4. SIMULATOR OF THE NITRIFYING COMPARTMENT

A4.1. Introduction

This simulator is described in TN 44.2 by ADERSA. The fundamental equations of this first principles model come from TN's 27.1 and 27.2 by LGCB.

In these technical notes, the volumes of the part A, B and C of the column are 1.48, 6.17 and 0.45 litres, respectively. Due to the beads in part B, the volume ratios of gas and liquid are 0.04 and 0.33, respectively .

In the present study, the column has to transform the waste of one man, which is much more than in the case of the above TN's. So the volume has to be increased. The following paragraph shows that a minimum volume is required.

A4.2. Minimum volume of the column

The liquid going from Rhodo into Nitri is O_2 free and the required O_2 for NH_3 oxidation is brought by the gas phase. For any biphasic compound, and for O_2 particularly, the total flux, expressed in mol/h (and not in kg/m^3/h), between the gas and liquid phases is :

$$\varphi = V \cdot K_{La} \cdot (c - b) \qquad \text{(A4.1)}$$

with V : volume of liquid
K_{La} : volumetric transfer coefficient in liquid phase
c : molar concentration at thermodynamic equilibrium in liquid phase
b : molar concentration in liquid phase

Given φ' the flux of O2 required by the oxidation, the necessary condition to avoid a limitation of the consumption of this compound is :

$$\varphi \geq \varphi' \quad \forall t \qquad \text{(A4.2)}$$

where t is the time.

For a biphasic compound, the relation between its molar fraction in the gas phase, y, and its molar concentration in the liquid phase, c, is (demonstration in TN 44.2 annex 1) :

$$c = \frac{n_0}{k_p} \cdot y \qquad \text{(A4.3)}$$

with n_0 : number of mole in a litre of water
k_p : partition coefficient of gas/liquid equilibrium

So the minimum volume of liquid is expressed by :

$$\boxed{V \geq \frac{1}{K_{La} \cdot \left(\frac{n_0}{k_p} \cdot y - b \right)} \cdot \varphi'} \qquad \text{(A4.4)}$$

The concentration b is determined from the value of the limiting concentration : a concentration is supposed not limiting when it is greater than ten time its limiting value.

Numerical application :

The flow rate of NH_3, produced by one man and to be treated by the column, is 0.0682 mol/h (27.8 g NH_3 per day).

Based on the simplified rule that 2 moles of O_2 are required for the transformation of 1 mole of NH_3, the flow rate of O_2 is 0.136 mol/h (52.4 g O_2 per day).

According to TN 27.2 p. 4, the limiting concentrations of O_2 for the growth of Ns and Nb are $5.05\ 10^{-6}$ mol/l and $1.7\ 10^{-5}$ mol/l, respectively.
So the values of the parameters of (A4.4) are :

- $K_{La} = 51$ h-1;
- $n_0 = 55.56$ mol/l
- $k_p = 4.6\ 10^4$;
- y = .21 (molar concentration of O_2 in air coming from the consumer compartment);
- b = $5.05\ 10^{-5}$ mol/l or $1.7\ 10^{-4}$ mol/l, depending on the limiting condition;
- $\varphi' = 0.136$ mol/h .

So two possible minimum volumes of liquid :
$V_{Ns} = 13.3$ l and $V_{Nb} = 31.7$ l, depending on the limiting condition.
Of course, the minimum volume is the volume of liquid of the part B (fixed bed) without the beads.

The choice of the minimum volume has an effect on the production rate of nitrite, as it is illustrated in the following simulations.

To be as complete as possible, each simulation gives two figures, the first one for the concentrations in part B and the second one for the production rates of the whole column. The 2 first hours of each simulation are steady state, which explains the differences in the biomass concentrations from one simulation to another one (graphs 41 of the figures A4.1 , A4.3 and A4.5). The O_2 and CO_2 are in excess (graphs 22 and 12 of the same figures) in order to be not responsible of any limitation. The fixed bed is assumed to be equivalent to 1 tank.

The first simulation (figures A4.1 and A4.2) is done with the limiting growing condition of Ns (V_{Ns} = 13.3 l). The graph 31 of fig. A4.2 shows that NO_2 production rate increases up to 0.005 mol/h and then decreases to $1.22\ 10^{-4}$ mol/h during a transient of about 5 h.

The second simulation (figures A4.3 and A4.4) is done with the limiting growing condition of Nb (V_{Nb} = 31.7 l). The graph 31 of fig. A4.4 shows that NO_2 production rate increases up to 0.007 mol/h and then decreases to $1.06\ 10^{-4}$ mol/h during a transient of about 5 h. Despite a higher volume ($V_{Nb} > V_{Ns}$) the peak of nitrite is bigger because the better availability of O_2 is first used by the producer of nitrite (which is not limited by the O_2 concentration) but at steady state the production of nitrite is lower.

The third simulation (figures A4.5 and A4.6) is done with a volume equal to 9 l (lower than the one based on the limiting growing condition of Ns). The graph 31 of fig. A4.6 shows that NO_2 production rate increases up to 0.011 mol/h. Then it decreases slowly to $4.29\ 10^{-3}$ mol/h (not visible on the figure). At output of the column, the concentration of nitrite reaches 10^{-2} mol/l (graph 31 of figure A4.5).

The performances of these 3 columns are gathered in the following table A4.1 :

Liquid Volume of the fixed bed	**NH_3 consumption**		**NO_2 production**		**NO_3 production**	
	mol/h	**molar %**	**mol/h**	**molar %**	**mol/h**	**molar %**
9	$6.69\ 10^{-2}$	98.1	$4.29\ 10^{-3}$	6.3	$6.05\ 10^{-2}$	88.7
13.3	$6.75\ 10^{-2}$	98.9	$1.22\ 10^{-4}$	0.18	$6.49\ 10^{-2}$	95.2
31.7	$6.78\ 10^{-2}$	99.4	$1.06\ 10^{-4}$	0.16	$6.50\ 10^{-2}$	95.3

Table A4.1 : Yield of the column versus the liquid volume of the fixed bed
The molar percentages are computed with regard to the incoming flux of NH_3

The volumes 13.3 and 31.7 l imply nearly the same performance. Both of them produce nearly the same quantity of nitrite and nitrate. Nevertheless the first one produces less biomass as it consumes less ammonia. From this point of view, it could be the best choice. Moreover it is the lesser bulky.
The volume of 9 l is far too small as it implies a production of nitrite 35 times higher than with the 2 previous volumes.

It is demonstrated that the sizing of the column is crucial regarding the production of nitrite. As a consequence, the right determination of K_{La} in the part B (fixed bed) is crucial too.
On the other hand, the value of K_{La} in the parts A and C has no importance for the yield of the column as it will be sawn hereafter.

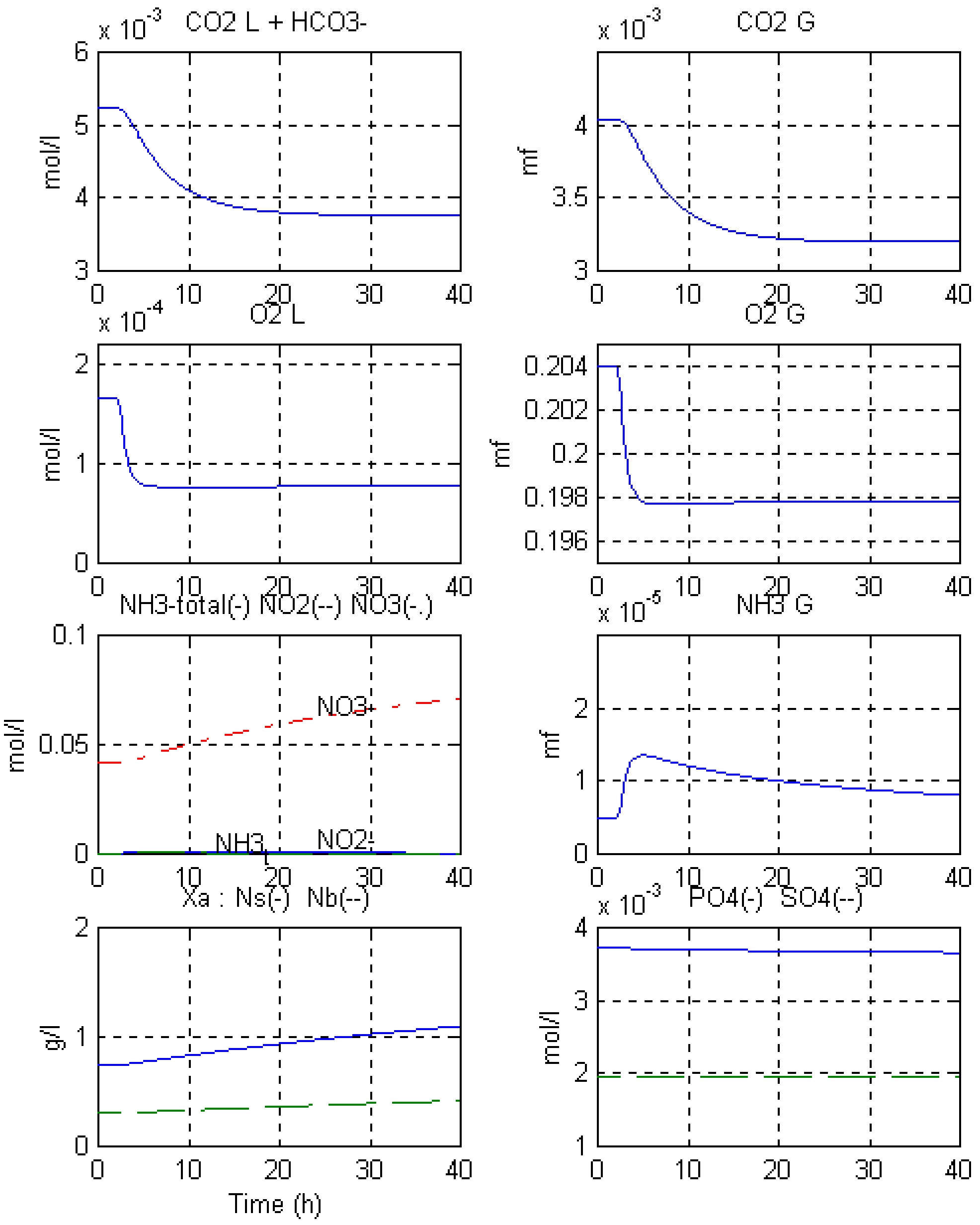

Figure A4.1 : Volume of liquid of the fixed bed : 13.3 l
Concentrations in part B

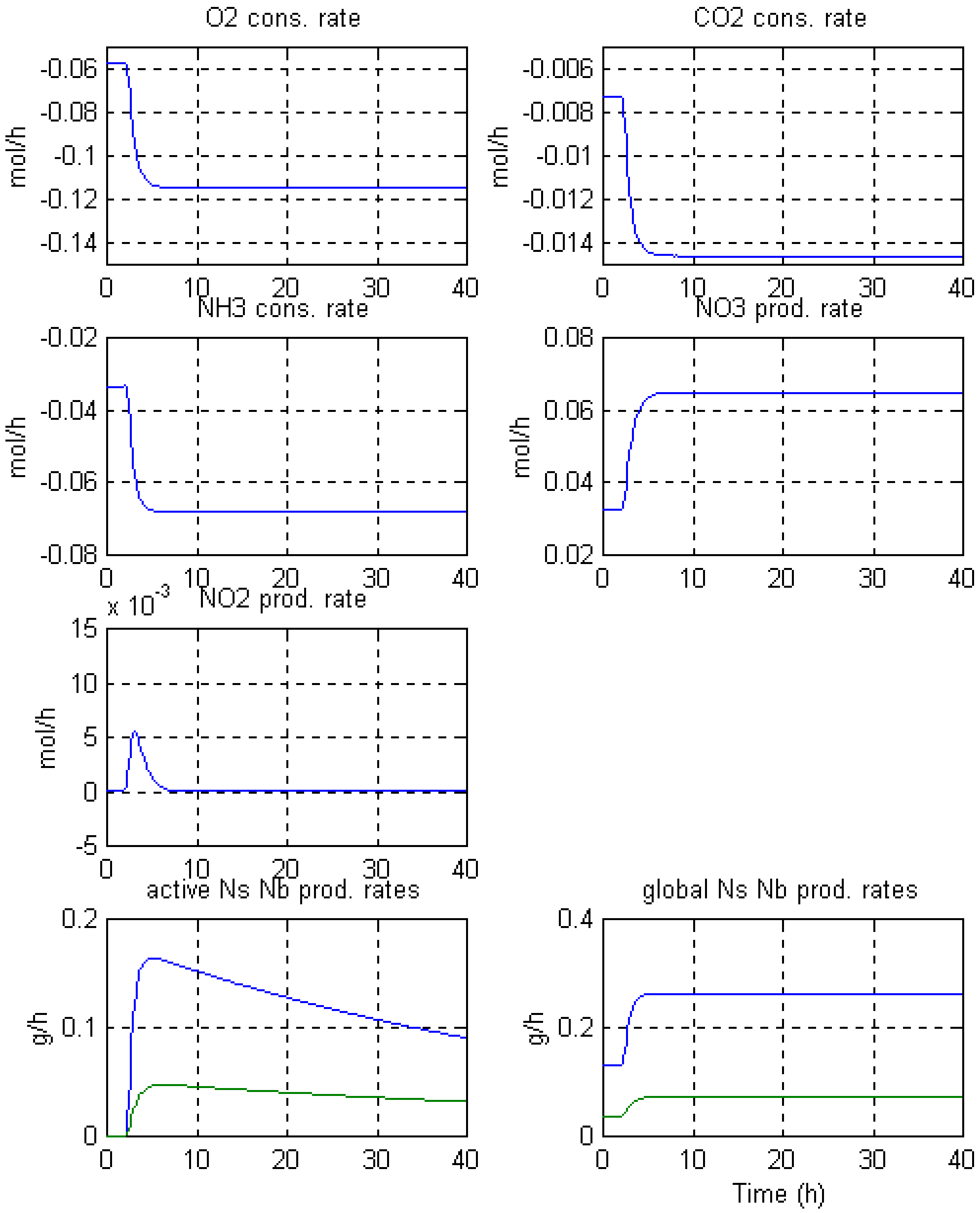

**Figure A4.2 : Volume of liquid of the fixed bed : 13.3 l
Consumption rates of the whole column**

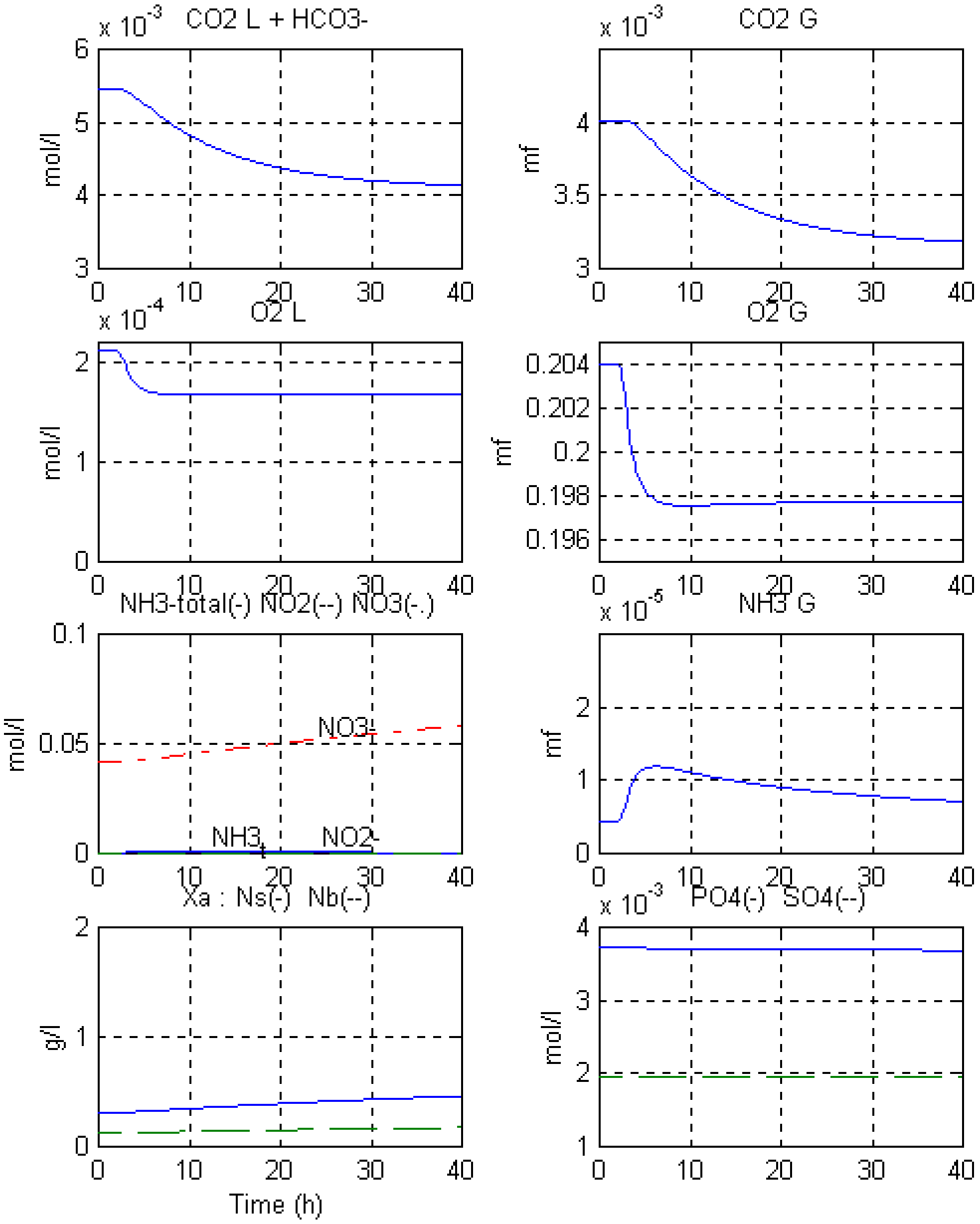

Figure A4.3 : Volume of liquid of the fixed bed : 31.7 l
Concentrations in part B

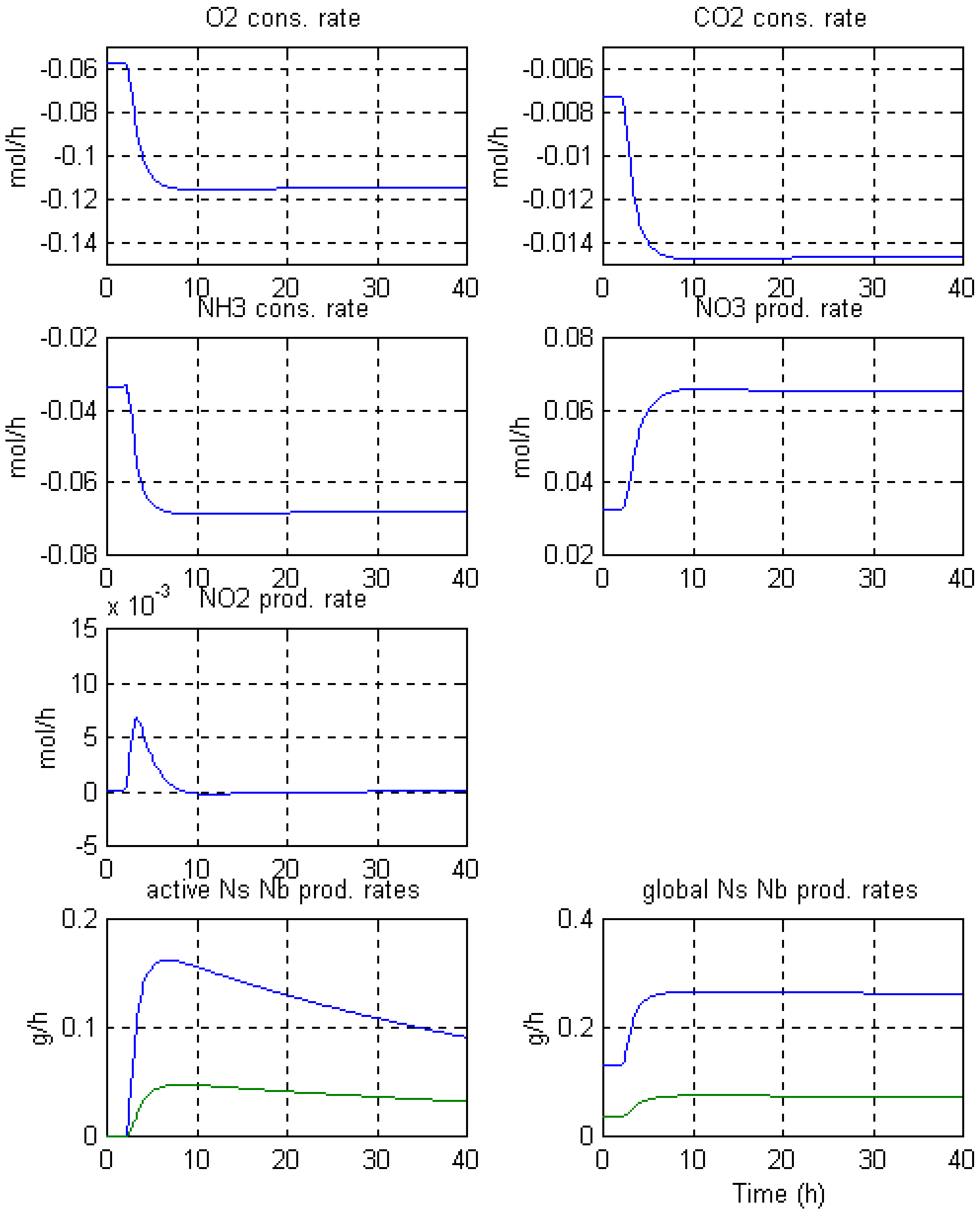

Figure A4.4 : Volume of liquid of the fixed bed : 31.7 l
Consumption rates of the whole column

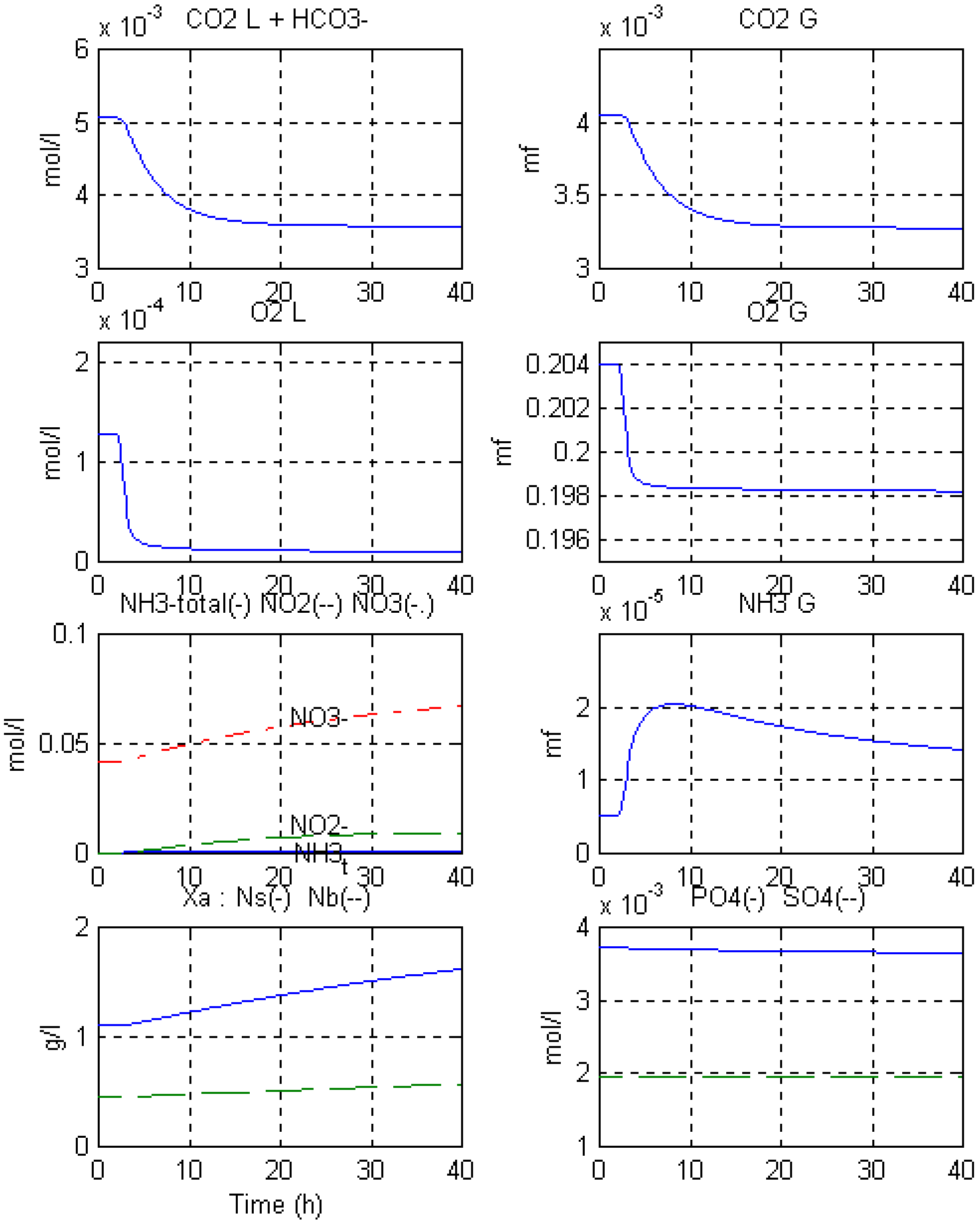

Figure A4.5 : Volume of liquid of the fixed bed : 9 l
Concentrations in part B

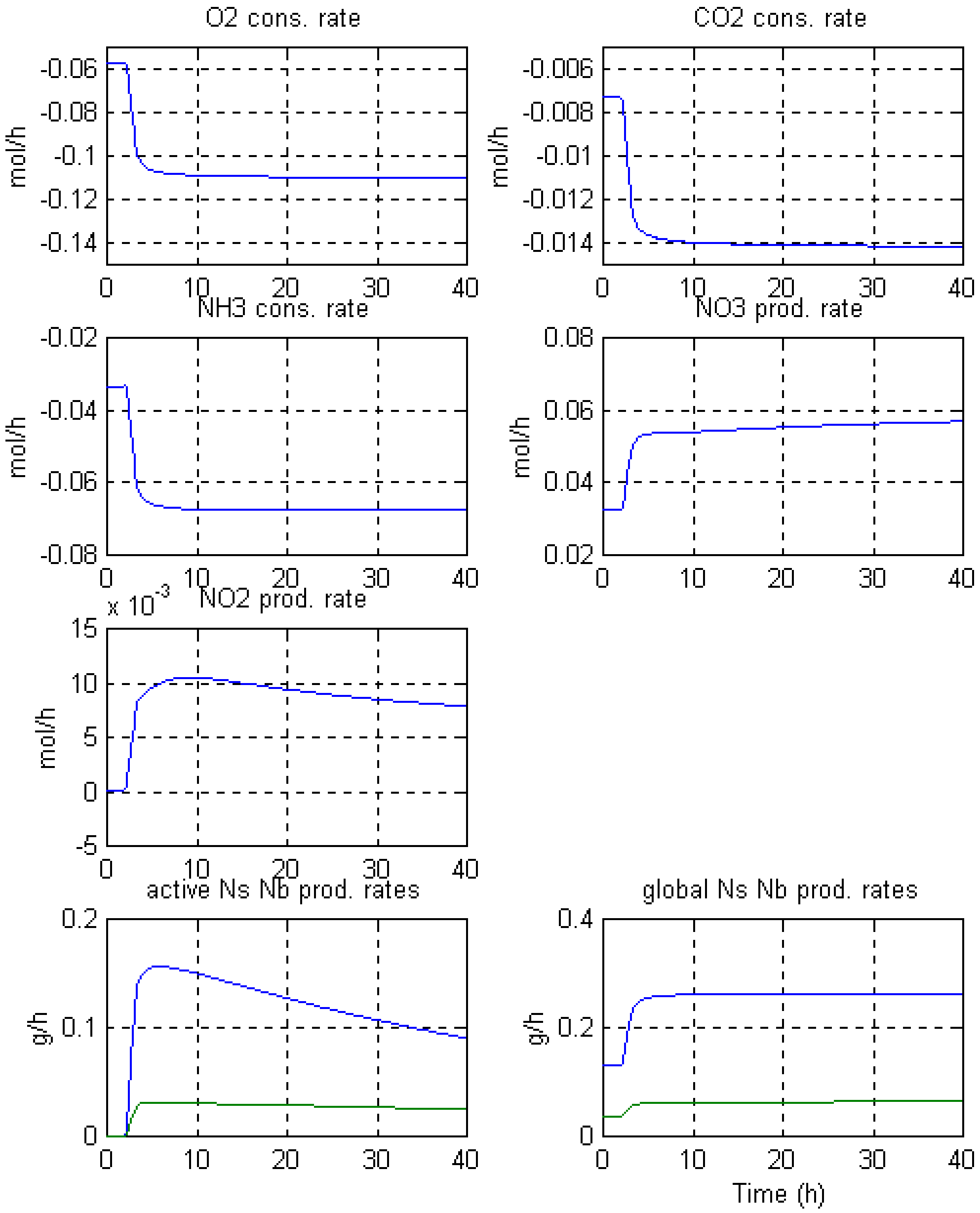

Figure A4.6 : Volume of liquid of the fixed bed : 9 l
Consumption rates of the whole column

A4.3. Location of the main flux of O_2 in the column

The determination of K_{La} in the different parts of the column is not easy. So another approach is to compute the contribution of each part in the transfer of O_2 from the gas into the liquid. For this computation, the value of K_{La} is the same in each part so that its influence is uniform throughout the column.

The following simulation allows to check that the flux of O_2 is the most important in the fixed bed where O_2 is consumed : about 94 % of the total flux is realised in part B (fig. A4.7).

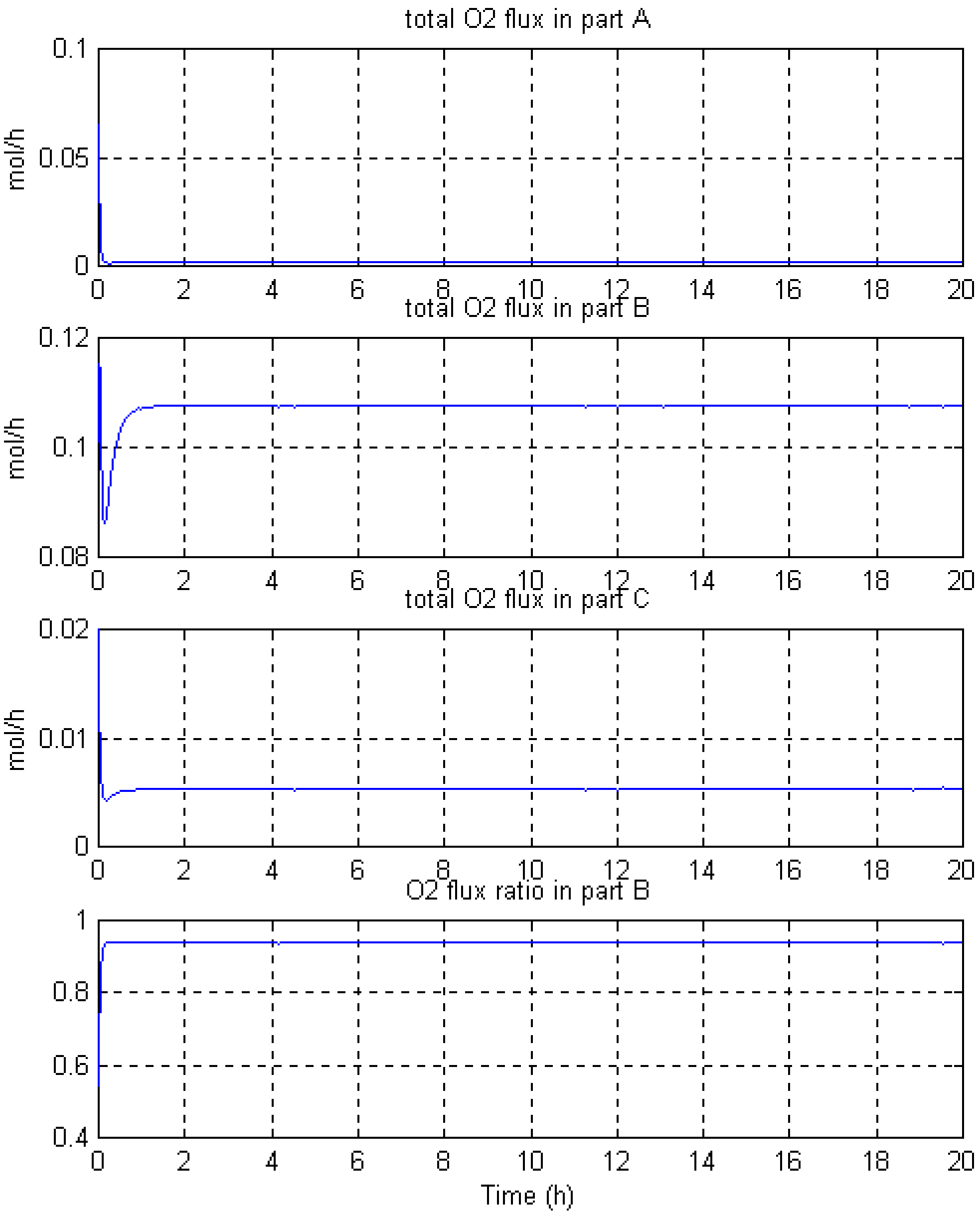

Figure A4.7 : Breakdown of flux of O2 among the different part of the column

The flux of O_2 is 0.002, 0.114 and 0.005 mol/h in the parts A, B and C respectively. Despite the same value of K_{La} in these different parts, the main flux occurs in the fixed bed where O_2 is consumed. (Except during a very short transient (5 mn) where the flux is relatively important in the parts A and C because O_2 solvated is null at starting of the simulation and it has to reach its thermodynamic equilibrium).

A4.4. Simulation parameters

All the parameters of the simulations are recalled in the following Matlab® file :

```
%***********************************************************************
%       Nitrifying column                                              *
%       Version 2.2     November 2000                                  *
%       for the 3 connected compartments : 2, 3 and 4a                 *
%                                                                      *
%       State system according to TN 44.2                              *
%          . dissociation of CO2 and NH3                               *
%          . column = linear combination of tanks                      *
%                                                                      *
%       Modifications in March 2000                                    *
%          . vector KlNs and KlNb moved into scalar                    *
%          . Monod type law for maintenance in 'irate.m'               *
%                                                                      *
%                                                                      *
%       i_sim.m   Initialization of the simulation                     *
%                                                                      *
%***********************************************************************

 clear all

arret = 0;
% Simulation parameters
        tdeb=0;
        tmin=1e-8;
        tmin=1e-3; % 4.04.00
        tmax=.2;
        prec=1e-6;  % necessary accuracy to avoid numerical unstability
        tfin=20;
        nbptx=ceil(1.2*tfin/tmin);
        nbptx = 100000;

% Simulation horizon
        dt = .05;        % (h) simulation period of the inputs
        dt = 1.;         % (h) simulation period of the inputs (10.03.00)
        T = [0:dt:tfin]';
        [m,n] = size(T);

% type of test
% ------------
typtst = 1;     titre = 'Constant inputs;Approximate steady state ';
                %At starting : no G/L thermodynamical equilibrium
                %              G coming from consumer
                %              L coming from Rhodo

% Column parameters
%------------------
    % 1.Parameters of the previous column (until TN 48.2)
        NB = 1;         % number of stirred tanks in part B (fixed bed)
        VA = 1.48;      % volume of part A (l)
        VB = 6.17;      % volume of part B (l)
        VC = 0.45;      % volume of part C (l)
        epsL = .33;     % volume ratio of liquid
        epsG = .04;     % volume ratio of gas
        epsT = epsG + epsL;
```

```
        Gin = .03*60;   % incoming gas volumetric rate (l/h)
        Fin = .0028*60; % incoming liq volumetric rate (l/h)
        RG = 1*99.0;    % recycling ratio of gas
        RL = 1*6.42;    % recycling ratio of liquid
        fG = 0;         % ratio of backward flow in a tank (gas phase)
        fL = 0;         % ratio of backward flow in a tank (liquid phase)

    % 2.Parameters of the column for TN 54.2
        Vmin = 31.7;    % (l) volume min of the part B (fixed bed)
        coef_V=Vmin/epsL/VB;% multiplicative coef. of the vol of previous column
        VA = VA*coef_V; % adapted volume of part A (l)
        VB = VB*coef_V; % adapted volume of part B (l)
        VC = VC*coef_V; % adapted volume of part C (l)
        Gin = Gin*coef_V;% adapted incoming gas volumetric rate (l/h)
        Fin = Fin*coef_V;% adapted incoming liq volumetric rate (l/h)

        % The G and L flow rates of the Spirulina compart are set to the other compartments
        % The volumes of these latest ones are modified to keep their residence time unchanged
        VL_4 = 77;         % volume of liquid of Spiru Reactor (l) (from TN43.110 p.4, UAB)
        Gin_4 = 180;       % incoming gas flow rate (l/h) (from TN 43.110 p.26, UAB)
        tau_4 = .01;       % (1/h) to have residence time as in the actual reactor
        Fin_4 = tau_4*VL_4;% incoming liquid flow rate (l/h)
        % modif. of the L and G flow rates to fit the flow of Spirulina Compart
        coef_L = max(0,(1+RL)*Fin/Fin_4 - 1);
        Fin = Fin_4;                % l/h
        coef_G = max(0,(1+RG)*Gin/Gin_4 - 1);
        Gin = Gin_4;       % l/h
        % modif. of the recycling ratio RG to keep constant '(1+RG)*Gin'
        RG = RG*coef_G;
        % modif. of the recycling ratio RL to keep constant '(1+RL)*Fin'
        RL = RL*coef_L;

        VnB = VB / NB;  % volume of an equivalent stirred tank

% general parameters
% ------------------
        NG = 3;         % number of bi-phases substrates (in the L and G phases)
        NL = 4;         % number of mono-phase substrates in the liquid phase
        NS = 2;         % number of strains of bacteria (Ns and Nb)
        NX = NG+NL+2*NS;% nb of coef of state vector Xi for any tank
        NO = NX+NG;     % nb of outputs for any tank
        NI = 2*NG + NL;
        NV = NI * (NB + 2);

% kinetic parameters
% ------------------
        Nls = 4;                        % nb of limiting substrates
        Nis = 2;                        % nb of inhibitory substrates

        %          Ns         Nb
        mumax = [5.7e-2 ;   3.6e-2];    % max specific growth rate (1/h)
        maint = [3.38e-3;   7.92e-3];   % maintenance coeffcient (1/h*(mol/gbio))

        KlNs  = [5.05e-6           ;    % limiting substrate for growth : O2   (mol/l)
                 6.625e-5          ;    % limiting substrate for growth : NH3  (mol/l)
                 1e-10            ];    % no limitation with HCO3- for growth  (mol/l)

        KlNb  = [           1.7e-5 ;    % limiting substrate for growth : O2   (mol/l)
                            3.6e-4 ;    % limiting substrate for growth : NO2- (mol/l)
                            1e-10 ];    % no limitation with HCO3- for growth  (mol/l)

        In =    [1e30       1e30   ;    % inhibitory substrate : NO2- (mol/l)
                 1e30       1e30]  ;    % inhibitory substrate : NO3- (mol/l)

        KmNs  = KlNs(1:2,1);         % limiting substrate for maintenance : O2; NH3   (mol/l)
        KmNb  = KlNb(1:2,1);         % limiting substrate for maintenance : O2; NO2-  (mol/l)

% stoechiometric parameters
% -------------------------
                                        % inverse of yield of biosynthesis :
                                        % (mol substr./mol biomass)
        Yx1  =  [-5.4269   -6.5106 ;    % O2
```

```
                 -1        -1      ;    % HCO3-
                 -4.5341   -0.1994 ;    % NH3
                 4.3347    -15.1714;    % NO2-
                 0          15.1714;    % NO3-
                 -0.0089   -0.0089 ;    % HPO4--
                 -0.0035   -0.0035];    % SO4--
               % 4.3099    -0.0248 ;    % H+   (unused for simplification)
               % 1          1     ];    % HO-  (unused for simplification)

                                        % inverse of yield of maintenance :
                                        % (mol substr./mol maintenance substr.)
        Ym1  =  [-1.5      -0.5    ;    % O2
                 0          0      ;    % HCO3-
                 -1         0      ;    % NH3
                 1         -1      ;    % NO2-
                 0          1      ;    % NO3-
                 0          0      ;    % HPO4--
                 0          0     ];    % SO4--

        % change of unit of Yx1 :
        Matom = [12;   1;      16;    14;   32;  31]; %C H O N S P atomik mass
        Scomp = [1     1.6147 .3906 .1994 .0035 .0089]; % stoechio composition
        M_nitri = Scomp*Matom;
        Yx1 = Yx1 / M_nitri; % (mol substrate / g biomass)

        Yx(1,1) = 1/Yx1(3,1);   % yield for (global) growth of Ns (g X/mol NH3)
        Yx(2,1) = 1/Yx1(4,2);   % yield for (global) growth of Nb (g X/mol NO2-)

% gas/liquid transfer parameters
% ------------------------------
        KLa = 1*[51; 51; 500];    % O2 CO2 NH3   (1/h)
% partition coefficients
% ----------------------
        kpartN = [4.599e4; 1853.1; 11.349  ];   % O2 CO2 NH3 (T=303K,non ionic form)
                                                % computed from TN35.1,ADERSA
% dissociation coefficient (for CO2)
% ----------------------------------
        Ka = 4.627e-7;          % at T=303K, from TN 35.1,ADERSA
        KCO2 = Ka / 1e-8;       % [HCO3-] = KCO2 * [CO2]solvated
% dissociation coefficient (for NH3)
% ----------------------------------
        Kb = 1.6916e-5;         % basicity cst at T=303K, from TN 35.1,ADERSA
        Ke = 1.4376e-14;        % ionic product at T=303K, from TN 35.1,ADERSA
        KNH3 = Kb * 1e-8 / Ke;  % [NH4+] = KNH3 * [NH3]solvated
% dissociation coefficient (for H3PO4)
% ------------------------------------
        Ka = 6.166e-8;          % (at T=298K, from TN 27.2,LGCB)
        xx = 1e-8/Ka;   KPO4 = 1+xx;       %[PO4]total = f([HPO4=])
% dissociation vector for the biphase substrates
% ----------------------------------------------
        Kdis = [0; KCO2; KNH3]; % O2  CO2  NH3
% liquid/gas thermodynamical equilibrium constants
% ------------------------------------------------
        VM = 22.4 * 303 / 273;  % molar volume (l/mol)
        alpha = kpartN / 55.56 / VM;
% initial concentrations in the column
% ------------------------------------
  %1. Gas phase (coming from the consumer compartment) :
        fm_O2_0  = .21;         % O2  (molar fraction)
        % the production flow rate of CO2 is 4e-2 mol/h (4 rats)
        rCO2 = .04;   % mol/h
        fm_CO2_0 = rCO2*VM/Gin;   % CO2 (molar fraction)
        fm_H2O_0 = 4.129e-2;    % H2O (molar fraction)
        C_NH3_t = .0886;        % NH3 (molar conc. of N total in liquid from Rhodo)
        fm_NH3_0 = 0;           % NH3 (molar fraction) : no NH3 from consumer
        fm_N2_0 = 1 - fm_O2_0 - fm_CO2_0 - fm_NH3_0 - fm_H2O_0;    % N2
        % conc. in gas phase (perfect gases at T=303 K and P=1 atm)
        CN2 = fm_N2_0 / VM;         % mol/l
        CH2O = fm_H2O_0 / VM;       % mol/l
  %2. Liquid phase (from Rhodo):
        C_O2_0   = 0;                % mol/l
        C_CO2_0  = 3.33e-4/(1+KCO2); % mol/l (CO2 solvated)
```

```
        C_NH3_0  = C_NH3_t/(1+KNH3); % mol/l (NH3 solvated)
        C_NO2_0  = 0;                % mol/l
        C_NO3_0  = 0;                % mol/l
        C_PO4_0  = 3.8e-3;           % mol/l (PO4 as in Rhodo compartment)
        C_SO4_0  = 2e-3;             % mol/l (SO4 as in Rhodo compartment)
        C_XNs_0  = 1.2;              % g/l
        C_XNb_0  = .25;              % g/l
%Temporal input :
%----------------
    CG0 = [fm_O2_0; fm_CO2_0; fm_NH3_0] / VM;        % mol/l
    CL0 = [C_O2_0; C_CO2_0; C_NH3_0; C_NO2_0; C_NO3_0; C_PO4_0; C_SO4_0];
    CX0 = [C_XNs_0; C_XNb_0; C_XNs_0; C_XNb_0];

    CG = [1; 1; 1] .* CG0;
    CL = [1; 1; 1; 1; 1; 1; 1] .* CL0;

    dG = ones(size(T))*CG'; % incoming G [O2,CO2,NH3] conc (mol/l)
    dL = ones(size(T))*CL'; % incoming L [O2,CO2,NH3,NO2,NO3,HPO4,SO4] conc (mol/l)

% Computation of the state system of the nitrifying compartment
%--------------------------------------------------------------
    [Ae,Be,Ce,De,E] = statesys(NG, NL, NB, Gin, Fin, RG, RL, fG, fL, ...
                               VA, VnB, VC, epsL, epsG, epsT, ...
                               alpha, Kdis, KLa);

% Initialization of the state vector
%-----------------------------------
        %X0 = CL0;
        %      O2 L       CO2 L            NH3        NO2    NO3      PO4     SO4
        X0 = [1.6e-4; 4e-3/(1+KCO2); 1.4e-4/(1+KNH3); 2e-4; C_NH3_t; C_PO4_0; C_SO4_0];  % all
NH3 transformed into NO3
        X0s = X0;  % approximate steady state
        for ii = 1:NB+1
          X0 = [X0; X0s];
        end
        X0 = [X0; 0; 0; 0; 0];
        for ii = 1:NB
          X0 = [X0; CX0];
        end
        X0 = [X0; 0; 0; 0; 0];

% Initialization of vector of indices
%------------------------------------
    iO2  = 1:NG+NL:(NB+1)*(NG+NL)+1; % indices of O2 L in the state vector
    iCO2 = 2:NG+NL:(NB+1)*(NG+NL)+2; % indices of HCO3- in the state vector
    iNH3 = 3:NG+NL:(NB+1)*(NG+NL)+3; % indices of NH3 L in the state vector
    iNO2 = 4:NG+NL:(NB+1)*(NG+NL)+4; % indices of NO2- in the state vector
    iSub = 1:(NG+NL)*(NB+2);         % ind. of subs. in state vector
    %iSubO= 1:(2*NG+NL)*(NB+2);       % ind. of subs. in output vector
    iXNs = 1 : 2*NS : 2*NS*(NB+1) + 1;
    iXNs = iXNs + (NG+NL)*(NB+2); % ind. of act. biomass Ns in the state vector
    iXNb = iXNs + 1;              % ind. of act. biomass Nb in the state vector
    iXag = (NG+NL)*(NB+2) + 1 :(NG+NL+2*NS)*(NB+2); % ind. of active and global
    indG0 = 1:NG;         indG = indG0;
    indL0 = NG+1:2*NG+NL; indL = indL0;
    for ii = 1:NB+1
      indG = [indG, indG0+ii*NI];   % ind. of Gas concen. in the output vector
      indL = [indL, indL0+ii*NI];   % ind. of Liq concen. in the output vector
    end

        if 0   % printing the following values in a file
         %diary res1
          NB
          ch = '     A       Bn        C'
          VG_c
          VL_c
          tauL_c
          ch = '     O2      CO2       NH3'
          K=KLa'
          k=kpartN'
          Ka = Kdis'
          tau1_A = tau1_c(1,:)
```

```
          tau2_A = tau2_c(1,:)
          tau2_tau1_A = tau2_tau1_c(1,:)
          tau1_Bn = tau1_c(2,:)
          tau2_Bn = tau2_c(2,:)
          tau2_tau1_Bn = tau2_tau1_c(2,:)
          tau1_C = tau1_c(3,:)
          tau2_C = tau2_c(3,:)
          tau2_tau1_C = tau2_tau1_c(3,:)
          teta2_A = teta2_c(1,:)
          teta2_tau1_A = teta2_tau1_c(1,:)
          teta2_Bn = teta2_c(2,:)
          teta2_tau1_Bn = teta2_tau1_c(2,:)
          teta2_C = teta2_c(3,:)
          teta2_tau1_C = teta2_tau1_c(3,:)
         %diary off
        end

        % Checking the mass balance
        % 1. Matrices of stoechiometric composition of each compounds :
        % atom : C         N         S         P
        MSG = [  0         0         0         0       ;  % O2  Gas
                 1         0         0         0       ;  % CO2 Gas
                 0         1         0         0       ]; % NH3 Gas
        MSL = [  0         0         0         0       ;  % O2
               1+Kdis(2)   0         0         0       ;  % CO2 L and ionic forms
                 0      1+Kdis(3)    0         0       ;  % NH3 L and ionic forms
                 0         1         0         0       ;  % NO2
                 0         1         0         0       ;  % NO3
                 0         0         0         1       ;  % HPO4
                 0         0         1         0      ];  % SO4
        MSS = [Scomp(:,[1,4,5,6]);Scomp(:,[1,4,5,6])];    % biomass Ns and Nb
        MSS = MSS ./ ([M_nitri;M_nitri]*ones(1,4));       % atom/g biomass

        % 2. matrices of volume of tanks
        Vg = [ VA*epsG/epsT;
               VnB*epsG*ones(NB,1);
               VC*epsG/epsT];
        Vl = [ VA*epsL/epsT;
               VnB*epsL*ones(NB,1);
               VC*epsL/epsT];

        % Weight vector of the noise on the derivative of the state vector
        WX0 = 0*5*[0    0    1e-6   1e-6    1e-6  0    0];
        WX = WX0;
        for ii = 1:NB+1
          WX = [WX, WX0];
        end

        % Weight vector of the noise on the output vector
        WYG0 = 0*5*[0    0    0 ];
        WYL0 = 0*5*[0    0    1e-5/(1+KNH3)   0    1e-5  0    0];
        WYG = WYG0;
        WYL = WYL0;
        for ii = 1:NB+1
          WYG = [WYG, WYG0];
          WYL = [WYL, WYL0];
        end

% Saving the specific variables of the Nitrifying compartment
% ============================================================
X0_3 = X0;     NL_3 = NL;     NG_3 = NG;     NS_3 = NS;
NB_3 = NB;     NX_3 = NX;     NO_3 = NO;     NI_3 = NI;     NV_3 = NV;
Kdis_3 = Kdis;
Fin_3 = Fin;   Gin_3 = Gin;
cH2O_3 = CH2O; cN2_3 = CN2;
Ae_3 = Ae;     Be_3 = Be;     Ce_3 = Ce;     De_3 = De;     E_3 = E;
WX_3 = WX;     WYG_3 = WYG;   WYL_3 = WYL;
iO2_3 = iO2;   iCO2_3 = iCO2; iNH3_3 = iNH3; iNO2_3 = iNO2;
iSub_3 = iSub; iXNs_3 = iXNs; iXNb_3 = iXNb; iXag_3 = iXag;
KlNs_3 = KlNs; KlNb_3 = KlNb; KmNs_3 = KmNs; KmNb_3 = KmNb;
mumax_3=mumax; maint_3=maint;
Yx_3 = Yx;     Yx1_3 = Yx1;   Ym1_3 = Ym1;
```

```
indG_3 = indG; indL_3 = indL;
T_3 = T;           dG_3 = dG;     dL_3 = dL;
MSG_3=MSG;     MSL_3=MSL;     MSS_3=MSS;
Vl_3 = Vl;     alpha_3=alpha; KLa_3=KLa;

if arret
% break
end
disp(' *** Nitri - End of initialization ***')
```