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Stoichiometric analysis of *Rs. rubrum* growth for transient and short residence time in a dark operative zone

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T.N. 49.1 : STOICHIOMETRIC ANALYSIS OF *Rs. rubrum* GROWTH IN A DARK OPERATIVE ZONE

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1. Introduction

This technical note completes T.N. 45.4 (Favier et al., 1999) and deals with the second compartment (anoxygenic phototrophs) of the MELiSSA loop. Our previous work proposed a stoichiometric biochemical structured model for the anaerobic photoheterotrophic growth of *Rs. rubrum* only valid in a working illuminated volume of the photobioreactor.

The purpose of this work is to **develop a stoichiometric model for *Rs. rubrum* growth on different carbon substrates under anaerobic transient photoheterotrophic conditions by short residence times of cells in a dark zone of a photobioreactor, where the metabolic activity is supposed to continue**. This zone will be referred as a dark operative zone or a dark efficient zone (Cornet et al., 1999). Its existence is a priori supported by experimental evidence : from the results already obtained in UAB (Lenguaza et al., 1998). It has been concluded that the yields were changing when varying illuminations conditions indicating that the metabolism was also functionning in the dark part of the photobioreactor.

The strain studied here was the one used at ESTEC and at UAB, obtained from the American Type Culture Collection, namely *Rs. rubrum* ATCC 25903. The studied carbon

substrates (acetate, propionate, butyrate, valerate, caproate, isobutyrate, isovalerate and isocaproate) were those detected at the exit of the liquefying compartment of the MELiSSA loop. We are interested to explain the experimental results obtained in UAB.

As previously (Favier et al., 1999) for the analysis of the photometabolism of *Rs. rubrum* the stoichiometric model will be established. It will characterise **only a transient metabolic state of the cells during a short residence time in darkness**. It must be noted that each metabolism behaviour can be described by a specific set of stoichiometric equations and that, generally, changes in the growth conditions lead to changes in metabolism and then, in the stoichiometric description of the growth.

The metabolic network used in this study is similar to the previous one proposed by Favier et al. (1999). Several supplementary metabolic assumptions are formulated in order to characterise the metabolic activity of cells in the dark operative zone.

The flux values are obtained considering the pseudo-steady state assumption of the metabolic intermediates inside the cells and using analytical information on cell composition including macromolecules, intracellular metabolites, substrate consumption, biomass and major byproduct synthesis.

This T.N. includes three parts :

- in the first part a brief review of the kinetic model of *Rs. rubrum* and several metabolic considerations during the growth of cells in the dark operative zone, are presented.
- in the second part the main metabolic assumptions used to derive the stoichiometric equations in the dark operative zone are described and discussed.
- in the third part, we present the stoichiometric model for *Rs. rubrum* growth in the dark operative zone, the global stoichiometric equations obtained on different carbon substrates and the calculated mass yields for the biomass synthesis.

This stoichiometric model is only a prerequisite step in building a knowledge model for the growth of *Rs. rubrum* under anaerobic photoheterotrophic conditions.

2. The kinetic model of *Rs. rubrum*

For the photoautotrophic bacterium *Spirulina platensis***,** a simple monodimensional mathematical model coupling the light transfer and growth kinetics in a photobioreactor (Cornet et al., 1992 ; Cornet et al., 1995 ; Cornet, 1998) was proposed and validated. In this model the authors considered a dark zone with no metabolic activity of this autotrophic microorganism and an illuminated zone in which growth exclusively occurs. This approach allowed to use constant kinetic parameters over a wide range of incident fluxes providing a fully predictive model (Cornet et al., 1992 ; Cornet et al., 1995).

From preliminary experimental results (Lenguaza et al., 1998) in batch cultures performed in rectangular photobioreactors under different illumination conditions (45 and 420 W/m^2), it appeared that this assumption was invalid for *Rs. rubrum* in photoheterotrophic growth conditions (Cornet et al., 1999). It was assumed that for this bacteria, growth occured even in a dark zone, in which the residence time of the cells was sufficiently short. For higher residence time of cells at obscurity, all metabolic activity was stopped if no proper electron acceptor was available. Therefore a dark intermediate zone with some metabolic activity was defined and introduced (Cornet et al., 1999).

In order to establish the kinetic rate model for *Rs. rubrum* growth under photoheterotrophic conditions in a photobioreactor, Cornet et al. (1999) proposed a zone model which took into account the local metabolism of cells. Consequently, they considered that it was necessary to divide the total volume of the reactor into three different metabolic zones (Figure 1) : **an illuminated zone, a dark zone in which the residence time of cells remains sufficiently short such as metabolic activity to continue (called''dark operative zone'' or ''dark efficient zone'') and a dark zone where no significant biomass growth takes place (called ''dark inoperative zone'')**.

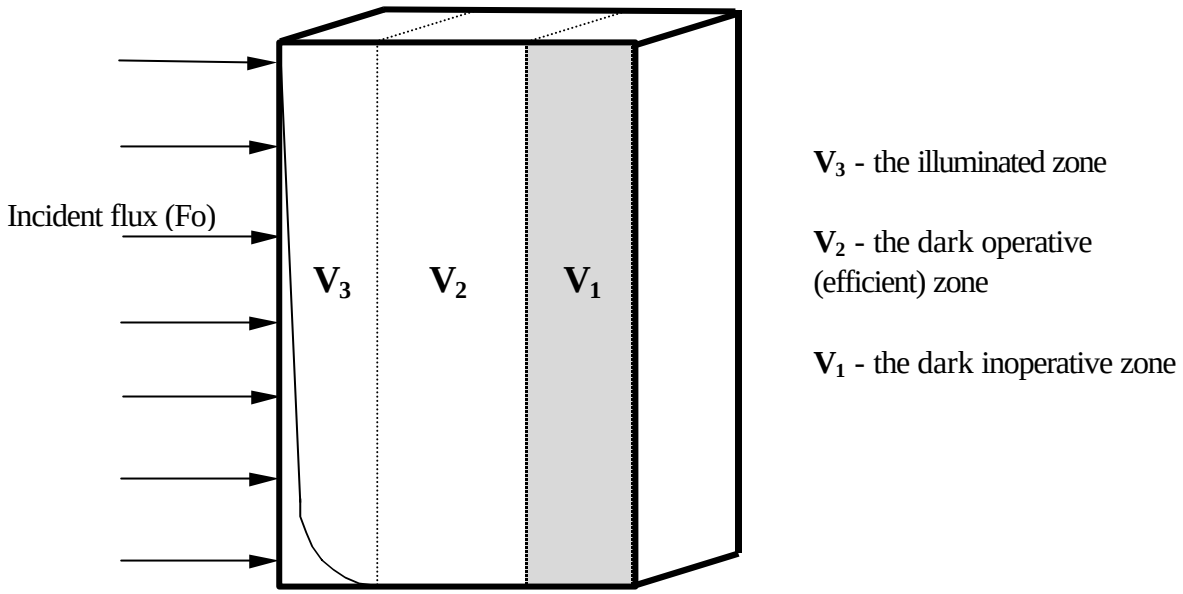

Figure 1: Definition of the three zones with different metabolic activity in modeling photo-heterotrophic growth kinetics in rectangular photobioreactors illuminated from one side (incident flux F_0).

3. Specific considerations on *Rs. rubrum* metabolism in the dark operative zone

Usually growth of an organism is characterised by its energetic metabolism and its carbon metabolism. An heterotrophic growth is relevant of a growth where both carbon and energy source are organic.

3.1. Carbon metabolism

It should be noted that the phototrophic purple non-sulphur bacterium *Rs. rubrum* is known to produce intracellular energy and carbon storage products which have been generally described as being poly-β-hydroxybutyrate, (PHB). This particular polymer belongs to the family of poly-β-hydroxyalkanoates (PHA), which are formed as intracellular inclusions under unbalanced or stressed

growth conditions, i.e. in the presence of sufficient carbon or energy source and a limiting nutriment or growth factor (Brandl et al., 1989). The choice of storage material (glycogen or PHB), depends on growth conditions and is probably under the control of an energetic balance (ATP/reduced power). Its accumulation represents an alternative for expending reducing power. For instance, nitrogen limitation leads to PHB accumulation in *B. megaterium* (Macrae and Wilkinson, 1958) and in *Rs. rubrum* (Doudoroff, 1966). The nature of the exogenous carbon source can also affect PHB production. *Rs. rubrum* can assimilate substrates such as lactate, pyruvate, malate, succinate or CO_2 mainly to glycogen (Stanier et al., 1959). Substrates such as acetate, β-hydroxybutyrate, or butyrate that are metabolised to acetyl-CoA without the intermediate formation of pyruvate lead to PHB accumulation (Merrick, 1983). With acetate a wild-type of *Rhodobacter sphaeroides* can store PHB up to 70% of cell dry weight (Hustede et al., 1993).

As preliminary hypothesis of this work it was assumed that in the dark efficient zone of the reactor *Rs. rubrum* is able to assimilate acetate or another VFA (propionate, butyrate, caproate, valerate, isobutyrate, isocaproate, isovalerate) and to convert these substrates as PHB intracellular inclusions only which are considered as cell material.

The considered pathway for the synthesis and degradation of PHB was described by Poughon in T.N. 23.3 (1995) and in our previous work (Favier et al., 1999). It had been well established that PHB is synthesised from acetyl coenzyme A. The PHB C-molar formula is : $C H_{1.505} O_{0.5}$.

Concerning the *Rs. rubrum* carbon metabolism it was supposed that in the dark operative zone of the photoreactor the active biomass production (proteins, fats, carbohydrates, DNA, RNA) is completely repressed because in this metabolic zone the ATP and reduced cofactors synthesis do not occur.

3.2. Energetic metabolism

In order to characterise the *Rs. rubrum* metabolism in the dark operative zone, a special interest must be paid also to the energetic metabolism. It is considered that the main metabolic modifications between dark and illuminated zones affect primarily the energetic metabolism. Some general aspects of the energetic metabolism of this bacteria are presented below.

It is already known that the bacteria are able to switch from the photo-phosphorylation (anaerobic and light conditions) to the oxidative phosphorylation (aerobic and dark conditions) (Sawada and Rogers, 1977), which supposes the presence of 2 systems (photosystem and chain of oxydoreduction).

Assuming that compartment II of the MELiSSA loop is in anoxygeny and in illuminated conditions, the photosystem (PS) is the sole electron transport system that is considered. In this case the donor of electron for the photosystem is the organic carbon. At the contrary of plants and algae the photosynthetic bacteria have only one photosystem similar to the PSI of plants. Two electrons pathways are involved in the electron transfer : a cyclic electron transfer and a noncyclic electron transfer (Gottschalk, 1986) (see Figure 2a and 2b).

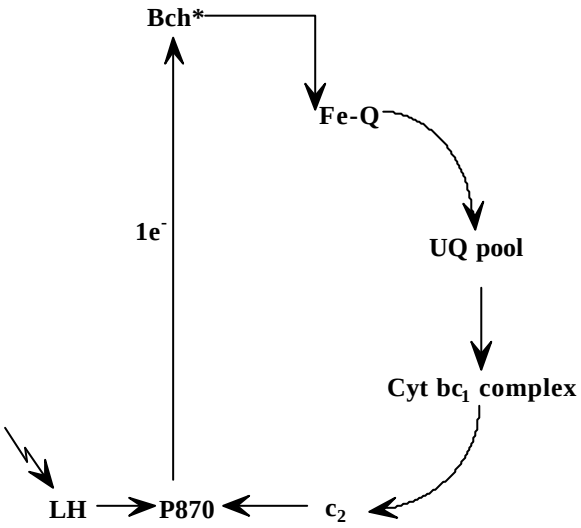

Figure 2a : Cyclic electron transfer

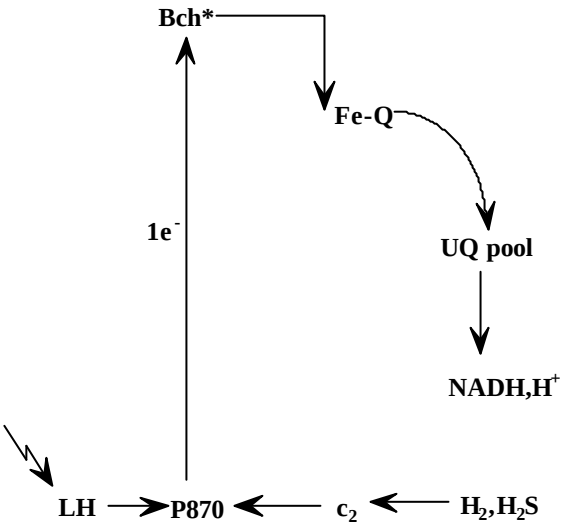

Figure 2b : Non cyclic electron transfer

The cyclic and the noncyclic electron transfer can be combined in a three step system :

1) Initiation of the electron transfer from FAD or from succinate :

$$FADH_2 + UQ \rightarrow FAD + UQH_2 \qquad \text{(Eq. 1)}$$

2) Propagation of the electron, involving the cyclic electron transfer :

$$c\ [4H^+_{cytoplasmic} \xrightarrow{2hn} 4H^+_{periplasmic}] \qquad \text{(Eq. 2)}$$

where c is the number of cycles

3) Termination of the electron transfer by feeding the final acceptor NADH deshydrogenase :

$$UQH_2 + NAD^+ + 4H^+_{periplasmic} \rightarrow UQ + NADH,H^+ + 4H^+_{cytoplasmic} \qquad \text{(Eq.3)}$$

As it was proposed by Poughon (1995), the ATP synthesis, involving the proton depending ATP syntase must be added in order to complete the energy metabolism :

$$ADP + Pi + 3\ H^+_{periplasmic} \rightarrow ATP + H_2O + 3\ H^+_{cytoplasmic} \qquad \text{(Eq. 4)}$$

Taking into account these main aspects of the energetic metabolism it became clear that during the short and transient residence time of cells at obscurity the cyclic propagation reaction (Eq. 2) do not occur, which implies that no proton gradient can be generated. A proton gradient can be generated only at theNADH-deshydrogenase level (Eq. 3), if there is an oxydation of $NADH,H^+$ instead of a reduction, which corresponds to a classical functioning of the complex I (NADH-deshydrogenase). In such a case , the acceptor of electrons must be FAD. In the dark, the electron transport chain associated to proton gradient generation is reduced to :

Initiation :

$$NADH,H^+\ UQ + 4H^+_{cytoplasmic} \rightarrow NAD^+ + UQH_2 + 4H^+_{periplasmic}$$

Termination :

$$FAD + UQH_2 \rightarrow FADH_2 + UQ$$

Concerning the *Rs. rubrum* energetic metabolism during the short residence time of cells at obscurity we assumed that the cyclic propagation reaction do not occur in this zone of the photobioreactor. Therefore in this case the electron transfer is combined only to a two step system.

4. Structured stoichiometric equations for *Rs. rubrum* in the dark during short residence time

4.1. Metabolic matrix

The mass balance technique was applied to *Rs. rubrum* in order to quantify metabolic fluxes and to establish the stoichiometric equations for the anaerobic photoheterotrophic growth in the dark during the short residence time of cells.

The principle of this technique is to formulate a flux model, i.e. a mass balance model for the metabolism of cells in which the important cellular reactions are described (Holms, 1986 ; Vallino and Stephanopoulos, 1990). All the calculated fluxes are required to completely understand any given physiological state of a micro-organism. The general and the specific metabolic characteristics of *Rs. rubrum* and the principle of calculation of the metabolic fluxes were extensively presented by Poughon (1995) and Favier et al. (1999) in their previous studies (T.N. 23.3 and 45.4).

In this study it was considered that the C/N ratio of the culture media for all of the carbon substrates was maintained constant at C/N=5, and then the active biomass composition remains also fairly constant for all the carbon substrates tested. The active biomass formula were the same as the one used in our previous work (Favier et al., 1999) : $C\ H_{1.6004}\ O_{0.3621}\ N_{0.2218}\ S_{0.0036}\ P_{0.0161}$.

The previous structured stoichiometries established for the *Rs. rubrum* growth under anaerobic photoheterotrophic conditions in the light involved a metabolic matrix of 125 compounds and 117 metabolic reactions. The metabolic matrix used for this study was described in our previous work T.N. 45.4. It is a 125×117 matrix. An analysis of the metabolites of the metabolic network indicated that there are 104 intermediates and 21 exchangeable compounds.

This separation of metabolites into exchangeable and nonexchangeable compounds is an important step in the calculation of the number of degrees of freedom. The obtained system presents 19 degrees of freedom. This must be reduced to zero. Therefore, 19 supplementary informations must be added to the system for a complete specification and resolution. The solution of the metabolic network required to fix 11 variables and 8 metabolic fluxes (report in section 4.2.). By taking into account the operating conditions, i.e. anaerobic photoheterotrophic in a dark operative zone, the following 11 exchangeables were fixed : oxygen, hydrogen, acetate, propionate, butyrate, valerate, caproate, isobutyrate, isovalerate, isocaproate, PHB.

A complete reaction list for the metabolic network discussed above is included in appendix.

4.2. Resolution of the metabolic matrix equation for the dark operative zone of a photobioreactor

In order to solve the metabolic network and to establish the anaerobic photoheterotrophic stoichiometry in the **light** on different carbon substrates the following assumptions were used :

- there is no maintenance $\rightarrow$ $\mathbf{J_m = 0}$ (E1)
- the PEP carboxylase which catalyses the transformation of phospho-enolpyruvate into oxaloacetate is inactive $\rightarrow$ $\mathbf{J_{21} = 0}$ (E2)
- the pyruvate carboxylase which catalyses the transformation of pyruvate into oxaloacetate is inactive $\rightarrow$ $\mathbf{J_{21'} = 0}$ (E3)
- the glyoxylate shunt is absent $\rightarrow$ $\mathbf{J_{25} = 0}$ (E4)
- the phosphogluconate deshydrogenase is absent $\rightarrow$ $\mathbf{J_{17} = 0}$ (E5)
- the pyruvate deshydrogenase or the pyruvate kinase are inactive $\rightarrow$ $\mathbf{J_9 = 0}$ or $\mathbf{J_8}$ (E6)

The stoichiometric equations of these reactions are presented in the appendix.

According to the metabolic considerations reported in paragraph 3 the metabolic assumptions used for the light growth conditions of *Rs. rubrum* were slightly modified.

For *Rs. rubrum* growth during the **transient short residence time in obscurity**, it was obvious that, all metabolic reactions which involved the photosystem did not occur. Therefore the reaction of cyclic propagation could not occur (**flux Jnrj 4 = 0**) (E7). It was also assumed that in obscurity the biomass production was completely repressed (**flux Biomass = 0**) (E8) and that in these conditions, only a PHB accumulation (**flux of $JPHB_c \geq 0$**) occured.

A stoichiometic reaction is balanced in mass, electric charge and degree of reduction.

Thus a stoichiometry which only include the accumulated PHB, one of the tested VFA, the CO_2 and the H_2O would be unbalanced with respect to the degree of reduction. Consequently to establish a stoichiomeric equation which characterizes the PHB accumulation from organic carbon substrate in the dark operative zone, it was necessary to consider a supplementary compound with a degree of reduction lower than poly-β-hydroxybutyrate (PHB). The degree of reduction of PHB, of the differents VFA (Roels, 1983) and of other metabolic compounds are indicated in Table1.

Compound	***Formula***	***Degree of reduction γ (-)***
Acetic acid	$C_2H_4O_2$	4
Propionic acid	$C_3H_6O_2$	4.67
Butyric acid	$C_4H_8O_2$	5
Valeric acid	$C_5H_{10}O_2$	5.2
Caproic acid	$C_6H_{12}O_2$	5.34
Poly-β-hydroxybutyrate	$\mathbf{C_{400}H_{602}O_{201}}$	**4.5**
Succinic acid	$C_4H_6O_4$	3.5
3-Pglyceric acid	$C_3H_7O_7P$	3.32
$NADH_2$		2
$FADH_2$		2

Table 1 : Degree of reduction of different VFA, of PHB and of other metabolic compounds.

Several metabolic intermediates, **P- glycerate** and **succinate** were first considered. It is known that in the Calvin cycle **P-glycerate** serves as primary product of the CO_2 fixation and that **succinate** is an important intermediate in the tricarboxylic acid cycle and an electron donor. **Succinate** is generally a metabolic intermediate, but at times it can also serve as a metabolic product.

Preliminary tests were done with this two intermediates. For each simulation test, the obtained fluxes values were analysed. For both compounds, **succinate** and **P-glycerate** a violation of the thermodynamic constraints was observed in the fluxes analysis. In the case of succinate for example, these inconsistencies in the metabolic network are characterised by negative flows in the Calvin Benson cycle and in citrate synthase reaction which are known to be irreversible reactions. A negative flow in the Calvin Benson cycle implied that there was a CO_2 production via this metabolic pathway, which is obviously thermodynamically infeasible. A succinate consumption was also obtained.

To establish a stoichiometric equation for *Rs. rubrum* growth during the transient short residence time of cells at obscurity, other metabolic compounds were also tested. They were **the reduced cofactors, FAD and NAD,** which are generally considered as nonexchangeable compounds. Generally these metabolic compounds serve as electron donors or acceptors for the respiratory chain.

At the contrary of this classical hypothesis, it has been assumed that these metabolic intermediates are accumulated in the dark transient zone of the photobioreactor. This assumption seems adequate because the residence time of cells in this zone is relatively short and their accumulation rates are limited. **This suppose that FAD or NAD are immediately consumed when conventional metabolism is restored in working illuminated volume**. It is based on the evidence that this metabolic intermediates (accumulated in the dark operative zone) are preferentially consumed with respect to other reduced cofactors, which comes from the assimilation or of degradation of the carbon substrate.

Metabolic fluxes computation using FAD as intermediate gave consistent values for the fluxes. Then, instead of **succinate** and **P-glycerate**, **FAD** will be used as a temporary compound between the dark and the light zones.

5. Results and discussion

Solving the metabolic network a **predicted value for the PHB assimilation yield** could be calculated.

Using the built metabolic network and the constraint relations necessary to break down the metabolic cycles (E1 to E6) it appears that one yield remain to be assumed for a complete resolution of the system of relations. This indicate that the computation has to be done using one degree of freedom (one experimental or a assumed yield) which lead to calculate all the other conversion yields and the intracellular metabolic fluxes. In order to characterise the transient metabolism in darkness, we have chosen to express all the results as a function of the molar production yield PHB/substrate.

5.1. Metabolic yields on acetate

For *Rs. rubrum* growth on **acetate,** the possible assimilation yields of PHB range from 0 to 0.857 (mol/mol). For superior yield values the thermodynamic constraints are violated for Calvin Benson cycle (i.e. negative flows in irreversible reactions).

Conversely, if it is assumed that Calvin Benson cycle is not operative in darkness this enables to fix the lacking degree of freedom for flux calculation and consequently the production yield value of PHB/acetate becomes a predicted value equal to 0.857. This assumption is consistent with the observed results because it leads to consider that this limit conditions (inoperative Calvin Benson cycle) correspond to a maximum PHB synthesis and a minimum $FADH_2$ pool production.

Figure 3 and 4 shows the stoichiometric yields evolution of $FADH_2$/acetate and of CO_2/acetate ratio as a function of PHB/acetate using the flux calculation method.

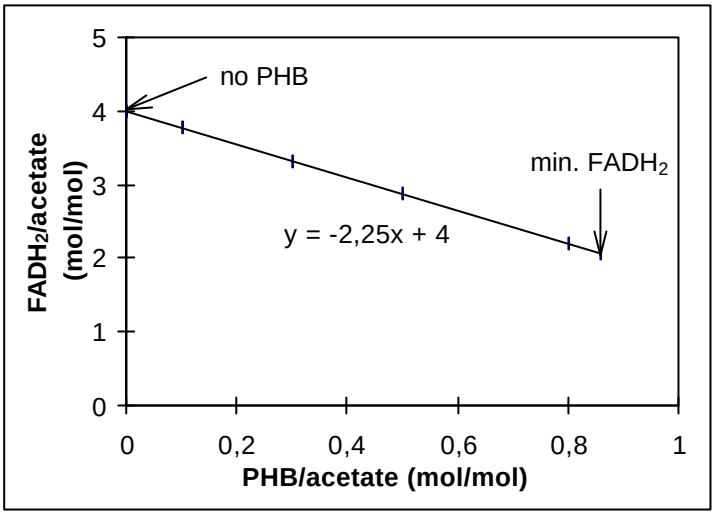

Figure 3 : Evolution of the stoichiometric yields of PHB/acetate as a function of $FADH_2$/acetate.

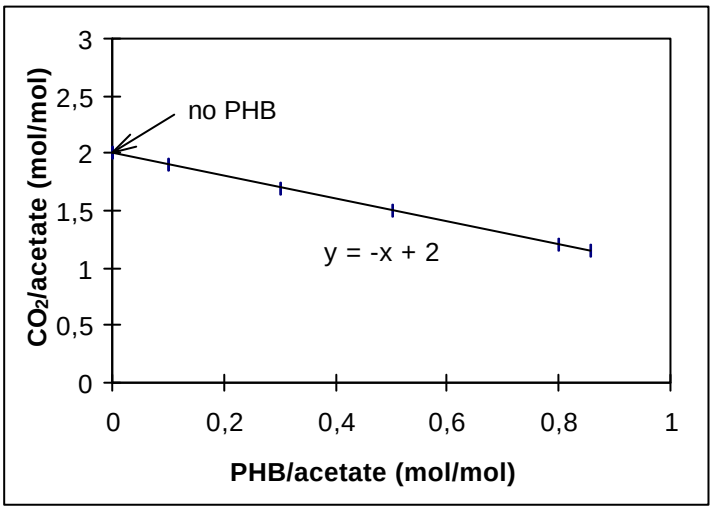

Figure 4 : Evolution of the stoichiometric yields of PHB/acetate as a function of CO_2/acetate.

5.2. Metabolic yields on propionate

For **propionate,** the calculated stoichiometric yield ranges from 0 to 2 (mol/mol). A PHB/propionate yield of 0 means that no PHB is produced, i.e. propionate is completely oxidized to CO_2, while the upper value corresponds to the predicted yield, i.e. minimun $FADH_2$. It must be noted that for higher yield values the thermodynamic constraints are violated for Calvin Benson cycle (i.e. negative flows in irreversible reactions).

The stoichiometric yields of PHB/propionate as a function of $FADH_2$/propionate and of CO_2/propionate are presented in figue 5 and 6.

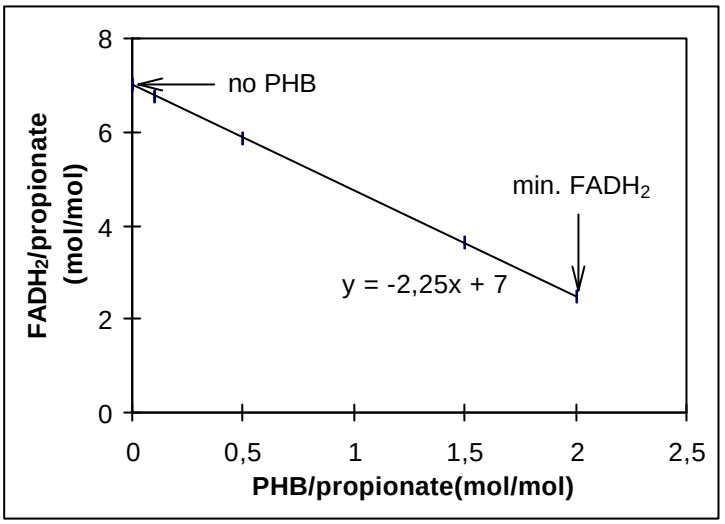

Figure 5 : Evolution of the stoichiometric yields of PHB/propionate as a function of $FADH_2$/propionate.

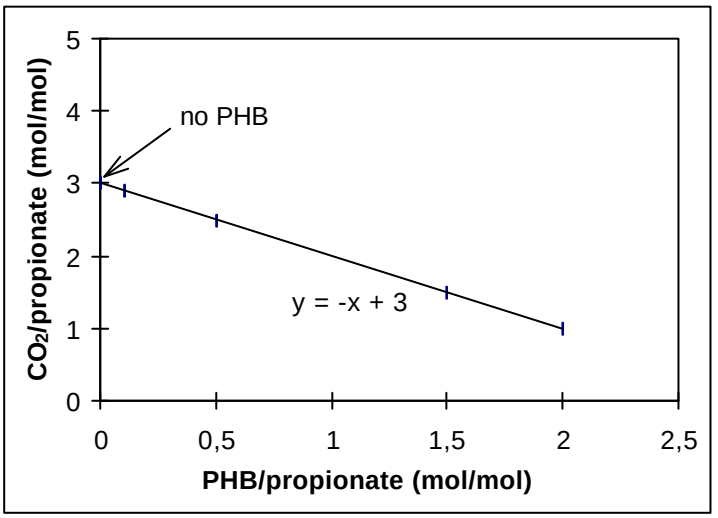

Figure 6 : Evolution of the stoichiometric yield of PHB/propionate as a function of CO_2/propionate.

5.3. Metabolic yields on butyrate

In the case of **butyrate** the stoichiometric yields range from 0 to 3.143. A PHB/butyrate yield of 0 means that no PHB is produced, i.e. butyrate is completely oxidized to CO_2 while the upper value corresponds to the predicted yield, i.e. minimun $FADH_2$. Inconsistencies in the metabolic network (i.e. Calvin Benson cycle) were obtained for stoichiometric yields higher than 3.143. Figures 7 and 8 show the assimilation yield of butyrate into PHB as a function of $FADH_2$/butyrate and the CO_2/butyrate. Same results were obtained with **isobutyrate** as carbon substrate.

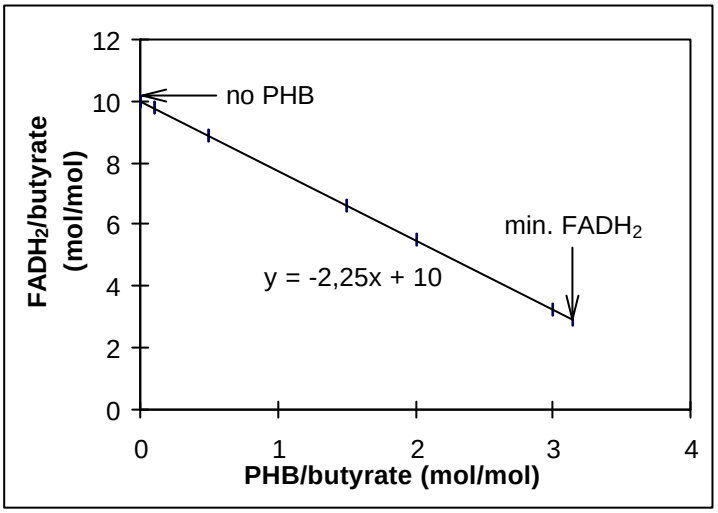

Figure 7 : Evolution of the stoichiometric yields of PHB/butyrate as a function of $FADH_2$/butyrate.

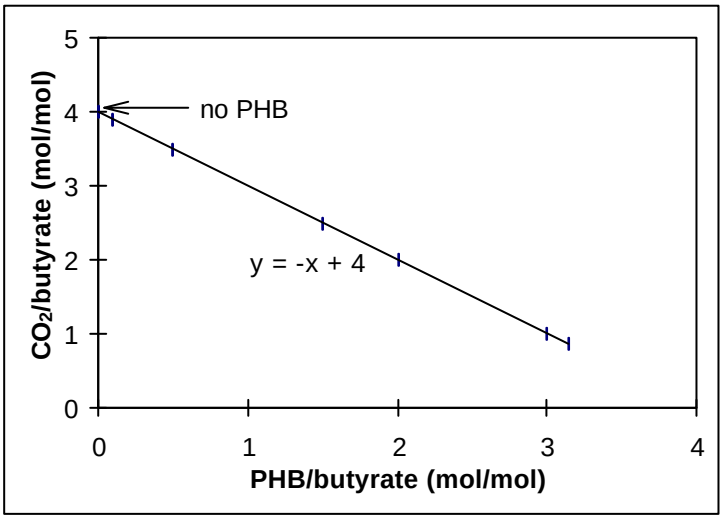

Figure 8 : Evolution of stoichiometric yields of PHB/butyrate as a function of CO_2/butyrate.

5.4. Metabolic yields on caproate and valerate

The calculated stoichiometric yields range from 0 to 5.429 for **caproate** and between 0 and 3.429 for **valerate**. A PHB/valerate (or caproate) yield of 0 means that no PHB is produced, i.e. caproate and valerate are completely oxidized to CO_2, while the upper value corresponds to the predicted yield, i.e. minimun $FADH_2$. Figure 9, 10, 11, 12 show their evolution as a function of $FADH_2$ and CO_2. Similar result were obtained for the branched fatty acids : **isocaproate** and **isovalerate**.

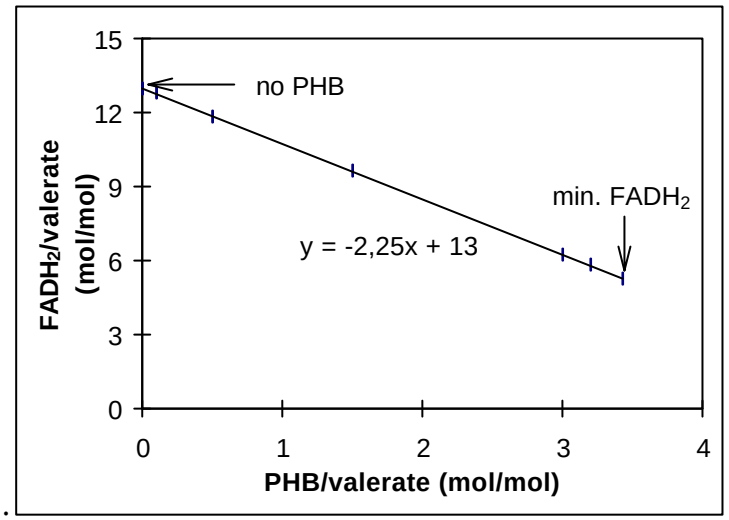

Figure 9 : Evolution of the stoichiometric yields of PHB/valerate as a function of $FADH_2$/valerate.

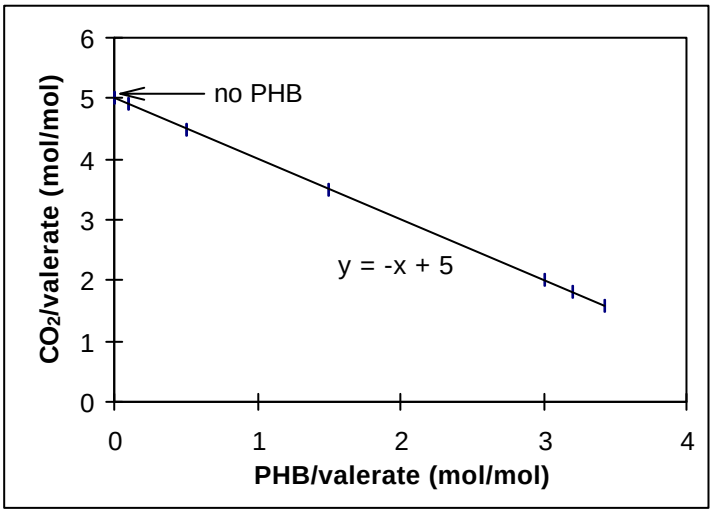

Figure 10 : Evolution of the stoichiometric yields of PHB/valerate as a function of CO_2/valerate.

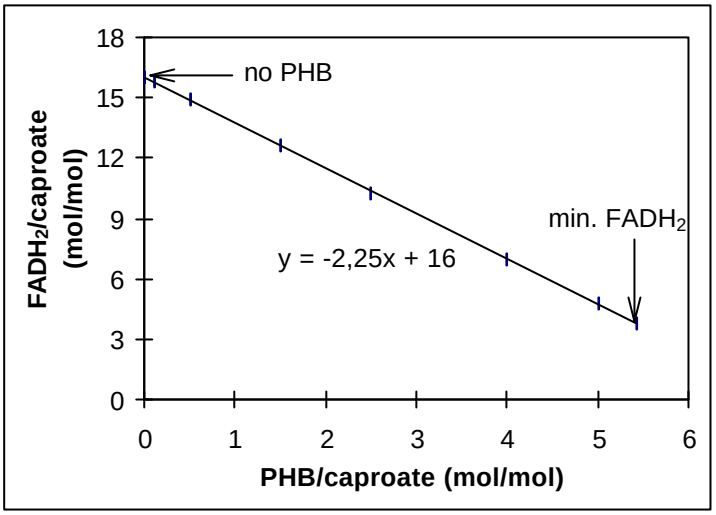

Figure 11 : Evolution of the stoichiometric yields of PHB/caproate as a function of $FADH_2$/caproate.

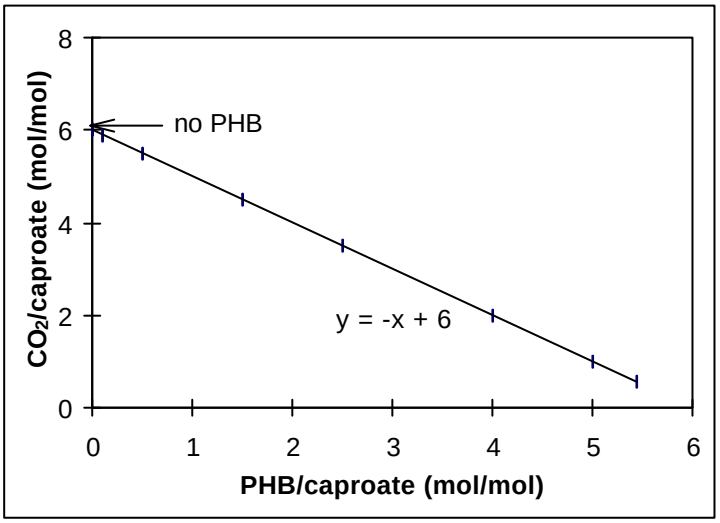

Figure 12 : Evolution of the stoichiometric yields of PHB/caproate as a function of CO_2/caproate.

5.5. Rs. rubrum stoichiometries in the dark operative zone

It can be outlined that the predicted value corresponds to the minimum of $FADH_2$ production, which is in accordance with the previous assumption of a limited accumulation of this intermediate. These values were used to establish stoichiometric equations for the anaerobic photoheterotrophic growth of *Rs. rubrum* on different carbon substrates in the dark operative zone of the photobioreactor (Table 2).

Acetic acid
$C_2H_4O_2 + 2.0713\ FAD + 0.7164\ H_2O \rightarrow 0.8571\ PHB + 1.1428\ CO_2 + 2.0713\ FADH_2$ **(S 1)**
Propionic acid
$C_3H_6O_2 + 2.5\ FAD + 1.005\ H_2O \rightarrow 2\ PHB + CO_2 + 2.5\ FADH_2$ **(S 2)**
Butyric acid
$C_4H_8O_2 + 2.9238\ FAD + 1.2935\ H_2O \rightarrow 3.1427\ PHB + 0.857\ CO_2 + 2.9238\ FADH_2$ **(S 3)**
Valeric acid
$C_5H_{10}O_2 + 5.2852\ FAD + 2.8653\ H_2O \rightarrow 3.4282\ PHB + 1.5711\ CO_2 + 5.2852\ FADH_2$ **(S 4)**
Caproic acid
$C_6H_{12}O_2 + 3.7861\ FAD + 1.8708\ H_2O \rightarrow 5.4289\ PHB + 0.0517\ CO_2 + 3.7861\ FADH_2$ **(S 5)**

Table 2 : Stoichiometric equations for the anaerobic photoheterotrophic growth of *Rs. rubrum* in the dark operative zone.

5.6. Stoichiometric model of $FADH_2$ consumption in the light

Consequentely, it is necessary to establish a stoichiometric equation for $FADH_2$ consumption in light, in order to ensure the nonaccumulation hypothesis of this compound in the cells.

As presented previously the $FADH_2$ is accumulated during the short residence time of cells at obscurity. Therefore the established stoichiometric model for *Rs. rubrum* growth under anaerobic photoheterotrophic in the light must include this supplementary reaction of FAD consumption. The $FADH_2$ consumption stoichiometry have been established by using the flux calculation method and is the same for all of tested carbon substrates. In this case any thermodynamic inconsistences were not detected. The proposed stoichiometric equation is the following :

(S 6) :

$$CO_2 + 2.1564\ FADH_2 + 0.0036\ H_2SO_4 + 0.0161\ H_3PO_4 + 0.2218\ NH_3 \downarrow$$

$$C\ H_{1.6004}\ O_{0.3621}N_{0.2218}S_{0.0036}P_{0.0161} + 1.7167\ H_2O + 2.1564\ FAD$$

5.7. Global stoichiometric equations

As an overall result of the existence of a dark efficient zone, the equations obtained for $FADH_2$ (and PHB) production in darkness and $FADH_2$ consumption in light have to be summed up in order to achieve a global intracellular balanced stoichiometry which is representative for the metabolic activity of cells in obscurity.

The global stoichiometric equations established on different carbon substrates (acetate, propionate, butyrate, caproate, valerate) are presented below . It must be noted that acetic acid is partly converted into carbon dioxide when acetate is the carbon source. For the others tested VFA there is a carbon dioxide consumption.

Acetic acid : (S 1) + (S 6)

$$C_2H_4O_2 + 0.0035\ H_2SO_4 + 0.0155\ H_3PO_4 + 0.2130\ NH_3$$

$$0.9605\ CH_{1.6004}O_{0.3621}N_{0.2218}S_{0.0036}P_{0.0161} + 0.8571\ PHB + 0.1823\ CO_2 + 0.9326\ H_2O$$

Propionic acid : (S 2) + (S 6)

$$C_3H_6O_2 + 0.1593\ CO_2 + 0.0042\ H2SO_4 + 0.0187\ H_3PO_4 + 0.2571\ NH_3$$

$$1.1593\ CH_{1.6004}O_{0.3621}N_{0.2218}S_{0.0036}P_{0.0161} + 2\ PHB + 0.9852\ H_2O$$

Butyric acid : (S 3) + (S 6)

$$C_4H_8O_2 + 0.5010\ CO_2 + 0.0049\ H_2SO_4 + 0.0219\ H_3PO_4 + 0.3012\ NH_3$$

$$1.3580\ CH_{1.6004}O_{0.3621}N_{0.2218}S_{0.0036}P_{0.0161} + 3.1427 + 1.0377\ H_2O$$

Valeric acid : (S 4) + (S 6)

$$\mathbf{C_5H_{10}O_2 + 0.8798\ CO_2 + 0.0088\ H_2SO_4 + 0.0395\ H_3PO_4 + 0.5436\ NH_3}$$

$$\mathbf{2.4509\ CH_{1.6004}O_{0.3621}N_{0.2218}S_{0.0036}P_{0.0161} + 3.4282\ PHB + 1.3422\ H_2O}$$

Caproic acid : (S 5) + (S 6)

$$\mathbf{C_6H_{12}O_2 + 1.7041\ CO_2 + 0.0063\ H_2SO_4 + 0.0283\ H_3PO_4 + 0.3894\ NH_3}$$

$$\mathbf{1.7558\ CH_{1.6004}O_{0.3621}N_{0.2218}S_{0.0036}P_{0.0161} + 5.4289\ PHB + 1.1433\ H_2O}$$

5.8. Characterization of biomass composition obtained in darkness as a function of the carbon substrates

The computed values for the global biomass formula including the PHB content of *Rs. rubrum* growth on different carbon substrates in a dark operative zone are presented in Table 3. These values are obtained from the previous stoichiometric equations.

VFA	Global biomass formula						% PHB total biomass
	C	H	O	N	S	P	
Acetic acid	1	1.5554	0.4271	0.1172	0.0019	0.0085	45 %
Propionic acid	1	1.5400	0.4494	0.0814	0.0013	0.0059	58 %
Butyric acid (isobutyric acid)	1	1.5338	0.4584	0.0669	0.0011	0.0049	41 %
Valeric acid (isovaleric acid)	1	1.5448	0.4425	0.0925	0.0015	0.0067	56 %
Caproic acid (isocaproic acid)	1	1.5283	0.4663	0.0542	0.0009	0.0039	74 %

Table 3 : The global biomass formula for *Rs. rubrum* growth in a dark operative zone.

5.9. Global yields

The calculated global yields of biomass synthesis for the tested VFA are presented in Table 4. These yields were calculated by taking into account the total biomass produced with respect to the total VFA consumed. They were calculated from the proposed global stoichiometries. Therefore average values between the illuminated zone and the dark operative zone have to be calculated. In any case it appears that the mass yields are slightly greater in the dark efficient zone that in the illuminated zone, both the yields being within 10 % and strongly depending on the substrate. Neverthless it must be noticed that the biomass composition is very different in the two zones, the biomass synthesis containing c.a. 50 % PHB (see Table 3). However, the mass yields of the calculated values are lower than the experimental ones of Lenguaza et al. (1998) obtained at 45 W/m^2 of incident light whatever the considered substrate. This difference is probably due to the fact that the carbon balances are not satisfied in these experiments (Table 4). This fact could result from OD_{700} measurement for dry weight assessment. Theoretical calculations from the Lorentz-Mie theory showed indeed that the scattering coefficient of light at 700 nm is dramatically modified by PHB inclusions (it increases 3 times when PHB content varies from 10 to 50%). In this case we can conclude that this correlation was improperly used and must be restricted only to batch cultures at high fluxes (low PHB content) or continuous cultures with a proper calibration.

Corrected values for this experimental yields are proposed by imposing that the Carbon Recovery Percentage (CRP) is 1 (see in Table 4). Corrections are made according to the formula :

$$CRP = (C_{\text{final biomass}} - C_{\text{initial biomass}})/C_{\text{substrate}} \quad [gC/gC]$$

$$(Y_{X/S})_{\text{corrected}} = (Y_{X/S})_{\text{experimental}} / CRP$$

For this study it was considered that the final biomass and the initial biomass had the same carbon content (about 50%). This is an reasonable assumption, whatever is the PHB content of the total biomass, as both active biomass and PHB contain about 50% carbon. It must be noted that, with the previous assumption corrected yields are close to the calculated

ones and in satisfactory agreement in any case.

VFA	$Y_{X/S}$ (calculated) in in the illuminated zone (g/g)	$Y_{X/S}$ (calculated) in a dark efficient zone (g/g)	$Y_{X/S}$(experimental) (g/g)		
			UAB measurement (Lenguaza et al.,1998)	Corrected values	
				CRP	$\mathbf{Y_{X/S}}$
acetic acid	0.71	0.68	1.2	1.5	**0.8**
propionic acid	1.03	0.94	1.6	2	**0.8**
butyric acid (isobutyric acid)	1.22	1.12	2.6	2	**1.3**
valeric acid (isovaleric acid)	1.36	1.28	2.4	2	**1.2**
caproic acid (isocaproic acid)	1.48	1.36	-	-	-

Table 4 : Mass yields of biomass synthesis during the anaerobic photoheterotrophic growth of *Rs. rubrum* in the dark operative zone of photobioreactor.

It can also be noted that the calculated values of the mass yields obtained in the dark operative zone are quite similar to the ones obtained in the illuminated zone (Table 5). This implies that the stoichiometric model could not be validated on the mass yields. Therefore complementary experiments in view to determine the PHB content and the CO_2 molar consumption rate will be necessary in order to validate the proposed stoichiometric model
(Table 6).

VFA	$Y_{X/S}$ (calculated) for the illuminated zone	$Y_{X/S}$ (calculated) for the dark efficient zone
acetic acid	0.71	0.68
propionic acid	1.03	0.94
butyric acid	1.22	1.12
valeric acid	1.36	1.28
caproic acid	1.48	1.36

Table 5: Mass yields of biomass synthesis during the anaerobic photoheterotrophic growth of *Rs. rubrum* in the illuminated zone and the dark operative zone of photobioreactor.

VFA	ILLUMINATED ZONE		DARK EFFICIENT ZONE	
	$CO_2/C_{mol\ X}$	$PHB/C_{mol\ X}$	$CO_{2\ cons}/C_{mol\ X}$	$PHB/C_{mol\ X}$
Acetic acid	0.0782	0	-0.1898	0.8923
Propionic acid	0.0758	0	0.1374	1.7252
Butyric acid (Isobutyric acid)	0.1374	0	0.3689	2.3178
Valeric acid (Isovaleric acid)	0.1706	0	0.3590	1.3988
Caproic acid (Isocaproic acid)	0.1914	0	0.9706	3.0920

Table 6: The CO_2 molar consumption and PHB content during the anerobic photoheterotrophic growth of *Rs. rubrum* in the illuminated and in the dark operative zone of photobioreactor.

6. Conclusions

The method developed to establish a stoichiometric model for *Rs. rubrum* growth on different carbon substrates under anaerobic photoheterotrophic conditions during the short residence time of cells in the dark efficient zone of a photobioreactor was presented. It is based on the zone model for *Rs. rubrum* growth in photobioreactors proposed by Cornet et al. (1999).

To obtain a consistent stoichiometric model in dark operative zone of the photobioreactor, the modeling approach proposed for the working illuminated volume (Favier et al., 1999) was slightly modified. In the dark efficient zone, it was assumed that :

- *Rs. rubrum* is able to assimilate acetate or another VFA as cell material only into PHB ;
- the biomass production is completely repressed ;
- the cyclic propagation reaction does not occur during the short and transient residence time of cells at obscurity.

In this study we have showed that it was necessary to consider FAD (compound with a degree of reduction lower than poly-β-hydroxybutyrate) as a supplementary compound in order to establish a stoichiometric equation which can characterize the PHB accumulation from different organic carbon substrates. It was assumed that this metabolic intermediate is accumulated in the dark operative zone and then is immediately consumed by cells into the working illuminated volume. This hypothesis seems adequate because the residence time of cells at obscurity is relatively short and the accumulation rate of FAD are limited.

At present time this model indicates that, for each carbon substrate two stoichiometric equations are necessary. The first one is only valid in the dark operative zone and corresponds to a storage of PHB in the cell. The second stoichiometry, established for the $FADH_2$ consumption, is only valid in the working illuminated volume of the photobioreactor. This implies that in the illuminated zone two stoichiometric reactions (the stoichiometric equation proposed in our previous work, T.N. 45.4 and the one proposed for the $FADH_2$ consumption) occurs simultaneously.

The fact that the stoichiometric analysis shows that it is necessary to have two linked and consecutive reactions (one valid in darkness and one in light) is a first theoretical proof of the kinetic

hypothesis previously made (Cornet et al., 1999) on the equality of the mean rates in the light and in the dark zone.

The predicted values for the PHB assimilation yield for different carbon substrates were computed and then used to establish the stoichiometry during the anaerobic photoheterotrophic growth of *Rs. rubrum* in a dark operative zone. It was shown that this calculated values corresponds to a minimum $FADH_2$ production. Moreover global stoichiometries on acetate, propionate, butyrate, caproate and valerate were proposed.

As presented in section 5.8. the calculated global yields of biomass synthesis for the tested VFA are lower than the experimental ones of Lenguaza et al. (1998) obtained at 45 W/m^2 of incident light. This discrepancy probably came from a non verified carbon balances on the photobioreactor resulting in a missleading utilisation of a OD_{700} measurement for dry weight assessment. In reality the scattering coefficient of light at 700 nm is dramatically modified by the PHB inclusions. Therefore this correlation in the case of the experiments of Lenguaza et al. (1998) was improperly used. Corrected values for these experimental results are then proposed (table4).

Generally the predicted mass biomass/substrate yields seem to be very close in the illuminated or dark zones. This implies that the stoichiometric model can not be validated from mass yields. Therefore further studies are necessary in order to validate the PHB content, and the measurement of the CO_2 consumption/production rate is desirable in order to validate the proposed stoichiometric model.

It must be pointed out that the flux calculation method has been used here to characterise a transient metabolic state of cells, whereas this technique is based on quasi-steady-state assumption for the metabolic intermediates. <u>This approch in building a knowledge kinetic model for photoheterotrophic growth and consisting in defining a volume in the reactor in which a transient metabolism exists is proposed, in our acquaintance, for the first time !</u>

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ANNEXE

Synthesis of biomass

Biomasse : 0 .0077 ARN_{av} + 0.0034 $peptidoglycan_{av}$ + 0.005 $lipid_{av}$ + 0.1153 $protein_{av}$ + + 0.0033 ADN_{av} + 0.0000204 Glycogene $\rightarrow$ $biomasse_{av}$

Synthesis of biomass macromolecules

Lipides : 0.7778 P-ethanolamine + 0.201 P-glycerol + 0.0212 diP-glycerol $\rightarrow$ $lipid_{av}$

Peptydoglycan : UDP-N- acetylglucosamine + UDP-N- acetylpentapeptide $\rightarrow$ alanine + UMP + UDP + peptydoglycan $_{av}$ + Pi

Proteines : 0.0892Glu + 0.0103 Gln + 0.0598 Pro + 0.0621 Arg + 0.0842 Asp + 0.0113 Asn + 0.0557 Thr + 0.0494 Ile + 0.0481 Lys + 0.026 Met + 0.0736 Val + 0.0843 Leu + 0.1282 Ala + 0.0393 Ser + 0.0789 Gly + 0.0049 Cys + 0.0387 Phe + 0.0232 Tyr + 0.0131 Trp + 0.0198 His + ATP + 2 GTP + + 2 H2O
$\rightarrow$ AMP + PPi +2 GDP + $Protein_{av}$ + 2 Pi

ARN : 0.262 ATP + 0.216 UTP + 0.2 CTP + 0.322 GTP $\rightarrow$ PPi + ARN

ADN : 0.33 dCTP + 0.33 dGTP + 0.17 dTTP + 0.17 dATP $\rightarrow$ PPi + ADN

Energetic metabolism reactions

Jm : ATP + H_2O $\rightarrow$ ADP + Pi

Jnrj 1 : UQ + $NADH_2$ + 4Hintra $\rightarrow$ 4Hextra + NAD + UQH_2

Jnrj 2 : UQH_2 + 6 Hintra + 0.5 O_2 $\rightarrow$ 6 Hextra + UQ + H_2O

Jnrj 3 : UQ + $2H_2$ $\rightarrow$ 2Hextr + UQH_2

Jnrj 4 : 4Hintra $\rightarrow$ 4Hextra

JFAD : UQ + $FADH_2$ $\rightarrow$ FAD + UQH_2

Jatp : 3Hextra + ADP + Pi $\rightarrow$ ATP + 3Hintra + H_2O

Cofactors regeneration

Jpp : PPi + H_2O $\rightarrow$ 2Pi

Jamp : ATP + AMP $\rightarrow$ 2ADP

Transhydrogenase

JNADP : NAD + NADPH + 2Hintra $\longrightarrow$ 2Hextra + NADP + $NADH_2$

Substrates catabolism

J23 : acetyl-CoA + AMP + PPi $\longrightarrow$ CoASH + ATP + acetic acid

Jlactate : NAD + lactate $\longrightarrow$ pyruvate + $NADH_2$

Jpropionate 1 : CoASH + ATP + propionic acid $\longrightarrow$ propiony l-CoA + AMP + PPi

Jpropionate 2 : propionyl-CoA + OAA + H_2O $\longrightarrow$ pyruvate + succinate + CoASH

Jbutyrate : 2CoASH + FAD + NAD + ATP + H_2O + butyric acid $\longrightarrow$ 2 acetyl-CoA + AMP + PPi + + $NADH_2$ + $FADH_2$

Jisobutyrate : CoASH + FAD + 2NAD + ATP + $2H_2O$ + isobutyric acid $\longrightarrow$ propionyl-CoA + + AMP + PPi + $2NADH_2$ + $FADH_2$ + CO_2

Jvalerate : 2CoASH + FAD + NAD + ATP + H_2O + valeric acid $\longrightarrow$ acetyl-CoA + propinyl-CoA + AMP + PPi + $NADH_2$ + $FADH_2$

Jcaproate : 3CoASH + 2FAD + 2NAD + ATP + $2H_2O$ + caproic acid $\longrightarrow$ 3acetyl-CoA + AMP + PPi + $2NADH_2$ + $2FADH_2$

Jisovalerate : CoASH + FAD + 2ATP + CO_2 + $2H_2O$ + isovaleric acid $\longrightarrow$ acetoacetate + +acetyl-CoA + ADP + AMP + PPi + $FADH_2$ + Pi

Jisocaproproate : CoASH + FAD + 2ATP + CO_2 + $2H_2O$ + isocaproic acid $\longrightarrow$ acetoacetate + + propionyl-CoA + ADP + AMP + PPi + $FADH_2$ + Pi

Jacetoacetate : acetoacetate + succinyl-CoA + CoASH $\longrightarrow$ 2acetyl-CoA + succinate

Reserve metabolism

Jglycogen : 100glucose6P + 98 ATP $\longrightarrow$ 98ADP + 99 PPi + Glycogen

JPHB : 200 acetyl-CoA + 100 $NADH_2$ + H_2O $\longrightarrow$ 200 CoASH + 100 NAD + PHB

Calvin Benson cycle

Jcalben2 : ribose-5P + ATP $\longrightarrow$ Ribose1-5DP + ADP

Jcalben1 : Ribose1-5DP + CO_2 + H_2O $\longrightarrow$ 2 P-glycerate

Central metabolic pathways

Embden Meyerhoff pathways (glycolysis)

J2 : glucose-6-P $\longrightarrow$ fructose-6-P

J3 : fructose-6-P + ATP $\longrightarrow$ fructose-1,6-diP + ADP

J4 : fructose-1,6-diP $\longrightarrow$ dihydroxyacetone-P + glyceraldehyde-3-P

J5 : dihydroxyacetone-P + NADPH $\longrightarrow$ glycerol-3-P + NADP

J6 : glyceraldehyde-3-P + NAD + ADP + Pi $\rightarrow$ 3-P-glycerate + ATP + $NADH_2$
J7 : 3-P-glycerate $\rightarrow$ P-enolpyruvate + H_2O

J8 : P-enolpyruvate + ADP $\rightarrow$ pyruvate + ATP

J9 : pyruvate + CoASH + NAD $\rightarrow$ acetyl-CoA + $NADH_2$ + CO_2

Tri-carboxylic acids cycle

J10 : acetyl-CoA + oxaloacetate + H_2O $\rightarrow$ isocitrate + CoASH

J11 : isoci trate + NADP $\rightarrow$ α-ketoglutarate + NADPH + CO_2

J12 : α-ketoglutarate + CoASH + NAD $\rightarrow$ succinyl-CoA + $NADH_2$ + CO_2

J13 : succinyl-CoA + ADP + Pi $\rightarrow$ succinate + CoASH + ATP

J14 : succinate + FAD $\rightarrow$ fumarate + $FADH_2$

J15 : fumarate + H_2O $\rightarrow$ malate

J16 : malate + NAD $\rightarrow$ oxaloacetate + $NADH_2$

Penthose pathway

J17 : glucose-6-P + 2NADP + H_2O $\rightarrow$ ribose-5-P + 2NADPH + CO_2

J18 : ribose-5-P + ATP $\rightarrow$ 5'-P-rybosyl-1PP + AMP

J19 : 2 ribose-5P $\rightarrow$ glyceraldehyde-3-P + sedoheptulose-7-P

J20 : glyceraldehyde-3-P + sedoheptulose-7-P $\rightarrow$ fructose-6-P + erythrose-4-P

J24 : ribose-5-P + erythrose-4-P $\rightarrow$ fructose-6-P + glyceraldehyde-3-P

Anaplerotic reactions

PEP carboxylase pathway
J21 : P-enolpyruvate + CO_2 + H_2O $\rightarrow$ oxaloacetate + Pi

Pyruvate carboxylase pathway
J21' : pyruvate + ATP + CO_2 + H_2O $\rightarrow$ oxaloacetate + ADP + Pi

PEP carboxykinase pathway
J21" : oxaloacetate + ATP $\rightarrow$ P-enolpyruvate + ADP + CO_2

Glyoxylate shunt
J25 : acetyl-CoA + isocitrate + H_2O $\rightarrow$ succinate + malate + CoASH

Anabolism network reactions

J26 : α-ketoglutarate + NADPH + NH_3 $\rightarrow$ glutamate + NADP + H_2O

J27 : glutamate + ATP + NH_3 $\rightarrow$ glutamine + ADP + Pi

J28 : glutamate + ATP + NADPH + $NADH_2$ → proline + NAD + NADP + ADP + H_2O + Pi

J29 : 2glutamate + carbamine + aspartate + acetyl-CoA + 2ATP + NADH2 + H_2O → α-ketoglutarate + fumarate + arginine + CoASH + NAD + ADP + AMP + PPi + 2Pi + acetate

J30 : oxaloacetate + glutamate → α-ketoglutarate + aspartate

J31 : aspartate + ATP + NH_3 → asparagine + ADP + Pi

J32 : aspartate + ATP + $NADH_2$ → asp-semialdehide + NAD + ADP + Pi

J33 : pyruvate + succinyl-CoA + glutamate + asp-semialdhide + NADPH → α-ketoglutarate + succinate + CoASH + NADP + diaminopimelate

J34 : diaminopimelate → lysine + CO_2

J35 : asp-semialdehide + NADH2 → NAD + homoserine

J36 : succinyl-CoA + CH_3-THF + cysteine + homoserine + H_2O → pyruvate + succinate + methionine + THF + CoASH + NH_3

J37 : homoserine + ATP + H_2O → threonine + ADP + Pi

J38 : pyruvate + glutamate + threonine + NADPH → α-ketoglutarate + isoleucine + NADP + CO_2 + NH_3 + H_2O

J39 : pyruvate + glutamate → α-ketoglutarate + alanine

J40 : 2pyruvate + NADPH → NADP + oxoisovalerate + CO_2 + H_2O

J41 : glutamate + oxoisovalerate → α-ketoglutarate + valine

J42 : acetyl-CoA + glutamate + NAD + oxoisisovalerate + H_2O → α-ketoglutarate + leucine + CoASH + $NADH_2$ + CO_2

J43 : 3-P-glycerate + glutamate + NAD + H_2O → α-ketoglutarate + serine + $NADH_2$ + Pi

J44 : serine + THF → CH_2-THF + glycine + H_2O

J45 : acetyl-CoA + serine + 2ATP + 4NADPH + H_2SO_4 → AMP-3P + cysteine + CoASH + 4NADP + ADP + PPi + $3H_2O$ + acetate

J46 : 2P-enolpyruvate + erythrose-4-P + ATP + NADPH → NADP + chorismate + ADP + 4Pi

J47 : 5'-P-ribosyl-1PP + glutamine + serine+ chorismate → glyceraldehyde-3-P + pyruvate + glutamate + tryptophane + PPi + CO_2 + $2H_2O$

J48 : chorismate → prephenate

J49 : glutamate + prephenate → α-ketoglutarate + phenylalanine + CO_2 + H_2O

J50 : glutamate + NAD + prephenate → α-ketoglutarate + tyrosine + $NADH_2$ + CO_2

J51 : 5'-P-ribosyl-1PP + glutamine + 2NAD + ATP + $3H_2O$ → α-ketoglutarate + ACR + histidine

+ 2PPi + 2$NADH_2$ + Pi

J52 : 5’-P-ribosyl-1PP + carbamyl-P + aspartate + NAD $\longrightarrow$ PPi + UMP + $NADH_2$ + CO_2 + H_2O + Pi

J53 : ATP + UMP $\longrightarrow$ ADP + UDP

J54 : CH_2-THF + 2ATP + UDP + NADPH $\longrightarrow$ DHF + NADP + 2ADP + dTTP + Pi

J55 : ATP + UDP $\longrightarrow$ ADP + UTP

J56 : ATP + UTP + NH_3 $\longrightarrow$ ADP + CTP + Pi

J57 : ATP + CMP $\longrightarrow$ AMP + CTP

J58 : CTP + H_2O $\longrightarrow$ CDP + Pi

J59 : ATP + CDP + NADPH $\longrightarrow$ NADP + ADP + dCTP + H_2O

J60 : 5'-P-rybosyl-1PP + CHO-THF + 2glutamine + aspartate + glycine + 4ATP + CO_2 + $2H_2O$ $\longrightarrow$ fumarate + ACR + 2glutamate + THF + 4ADP + PPi + 4Pi

J61 : ACR + CHO-THF $\longrightarrow$ THF + IMP + H_2O

J62 : aspartate + IMP + GTP $\longrightarrow$ fumarate + AMP + GDP + Pi

J63 : ATP + NADPH $\longrightarrow$ NADP + dATP + H_2O

J64 : NAD + ATP + IMP + NH_3 + H_2O $\longrightarrow$ AMP + PPi + GMP + $NADH_2$

J65 : ATP + GMP $\longrightarrow$ ADP + GDP

J66 : ATP + GDP $\longrightarrow$ ADP + GTP

J67 : ATP + GDP + NADPH $\longrightarrow$ NADP + ADP + dGTP + H_2O

J68 : fructose-6-P + acetyl-CoA + glutamine + UTP $\longrightarrow$ glutamate + CoASH + UDP-N-agn + PPi

J69 : P-enolpyruvate + UDP-N-agn + NADPH $\longrightarrow$ NADP + UDP-Namu + Pi

J70 : glutamate + 3alanine + diaminopimelate + UDP-Namu + 4ATP $\longrightarrow$ UDP-Na5A + 4ADP + 4Pi

J71 : acetyl-CoA + ACPH $\longrightarrow$ CoASH + acetyl-ACP

J72 : acetyl-CoA + ACPH + ATP + CO_2 + H_2O $\longrightarrow$ CoASH + malonyl-ACP + ADP + Pi

J73 : 7malonyl-ACP + acetyl-ACP + 14NADPH $\longrightarrow$ 7ACPH + 14NADP + palmitic-ACP + 7CO_2 + 7H_2O

J74 : NADP + palmitic-ACP $\longrightarrow$ palmitoleic-ACP + NADPH

J75 : malonyl-ACP + palmitoleic-ACP + 2NADPH $\longrightarrow$ ACPH + 2NADP + vaccenic-ACP + CO_2 + H_2O

J76 : glycerol-3P + 0.878 palmitic-ACP + 0.678 palmitoleic-ACP + 0.444 vaccenic-ACP $\longrightarrow$ 2 ACPH + + 1 phosphatidic_{av} acid

J77 : serine + phosphatidic$_{av}$ acid + CTP $\rightarrow$ P-ethanolamine + PPi + CMP + CO_2

J78 : glycerol-3P + phosphatidic$_{av}$ acid + CTP $\rightarrow$ P-glycerol + PPi + CMP + Pi
J79 : glycerol-3P + 2ac.phosphatidic$_{av}$ + 2CTP $\rightarrow$ diP-glycerol + 2PPi + 2CMP + Pi

J80 : glutamine + 2ATP + CO_2 + 2H2O $\rightarrow$ carbamyl-P + glutamate + 2ADP + Pi
J81 : AMP-3P + H_2O $\rightarrow$ AMP + Pi

J82 : THF + $NADH_2$ + CO_2 $\rightarrow$ CHO-THF + NAD + H_2O

J83 : CHO-THF $\rightarrow$ CH_2=THF + H_2O

J84 : CH_2=THF + NADPH $\rightarrow$ CH_2-THF + NADP

J85 : CH_2-THF + $NADH_2$ $\rightarrow$ CH3-THF + NAD

J86 : DHF + NADPH $\rightarrow$ THF + NADP

J87 : dihydroxyacetone-P $\rightarrow$ glyceraldehyde-3-P