ESTEC/CONTRACT ECT/FG/CB/95.205
PRF 141315

Modelling of the MELiSSA artificial ecosystem

Compartment II:
stoichiometries and experimental data analysis

- Description of carbon and energy metabolism of the Purple Non Sulfur Bacteria
- Biomass composition and building
- Stoichiometry building by using a metabolic matrix
- Correlations for the biomass composition as a function of growth conditions
- Comparison of stoichiometries and experimental data for different growth conditions

TECHNICAL NOTE 23.3
Version 1 - Issue 0

L. Poughon
Laboratoire de Génie Chimique Biologique, 63177 AUBIERE Cedex, FRANCE

September 1995

CONTENTS

T.N. 23.3: Modelling of the MELiSSA artificial ecosystem
COMPARTMENT II: STOICHIOMETRIES AND EXPERIMENTAL DATA ANALYSIS

L. Poughon
Laboratoire de Génie Chimique Biologique
63177 AUBIERE Cedex. France.

INTRODUCTION

This note deals with the second compartment of the MELiSSA loop. Its aim is to present an updating of the stoichiometric approach of the compartment for the simulations, taking into account metabolic informations from litterature and the experimental results obtained by the MELiSSA partners.

The TN is divided in 3 parts.

First, an overview of the metabolic abilities of the two strains considered in the phototrophs anoxygenic compartment (*Rs. rubrum* and *Rb. capsulatus*), has been made. Special care has been taken in the study of carbon metabolism (assimilation and storage) and of energetic metabolism (photosystem and electron transport). Closing this part, the biomass compositions found in the literature and those extrapolated from the stoichiometries building are presented.

In a second part, the methodology of the stoichiometries building is presented. The main metabolic assumptions used to build the stoichiometries are described here.

The third part proposes an analysis and an integration in the stoichiometries of some experimental results about the biomass composition, obtained at Estec laboratory and reported by Albiol (1994[b]). A comparison of the CO_2 production and of the biomass chemical composition is then made between the experiments and the stoichiometries established.

I The purple non sulphur bacteria (PNSB) of compartment II

In order to establish the stoichiometries for the micro-organisms used in compartment II, it is necessary to determine the characteristics of their metabolism *i.e.* their metabolic pathway for the anabolic reactions, the catabolic reactions and the energy generation.

Thus, the general and specific metabolic characteristics of the strains used in the compartment II of the MELiSSA loop must be first described. Such description associated to the building of the stoichiometries for the micro-organisms of compartment II were reported in TN 9.3 (Dussap, 1991), but since this TN the studies were continued and thus the results presented in TN 9.3 , which have been the basis for the further simulations of the loop, must be brought up again.

1.1 - Description and main characteristics of the strains

The two strains chosen to colonize the anoxygenic phototrophs compartment (compartment II) are two genus of the *Rhodospirillaceae* (or PNSB):

- one strain of the genus *Rhodobacter*. The species chosen is *Rb. capsulatus*, and the present strain used in the experimental studies at ESTEC was obtained from the American Type Culture Collection : *Rb. capsulatus*, ATCC 23782. (Albiol, 1994[b]).

- one strain of the genus *Rhodospirillum* . The species chosen is *Rs. rubrum*, and the present strain used in the experimental studies at ESTEC was obtained from the American Type Culture Collection : *Rs. rubrum*, ATCC 25903.(Albiol, 1994[b]).

The *Rhodospirillaceae* are phototroph gram negative bacteria. The photosynthetic pigments, located in the cytoplasmic membrane and the internal membrane, are bacteriochlorophyll a or b and various types of carotenoids. In most species, the formation of pigments and of the internal membrane systems are repressed under aerobic conditions but become derepresseed at oxygen tension below a certain level.

Under anaerobic conditions in the light, the species grow as photoheterotrophs with various organic substrates or as photoautotrophs with either molecular hydrogen (H_2) or in some species, sulphide, thiosulfate or elemental sulphur as electron donor and CO_2 as sole carbon source (Imhoff et al., 1989). However, it must be noted that one or more vitamins are generally required as growth factors (most commonly required are biotin, thiamine and niacin).
Under microaerobic to aerobic conditions in the dark, many strains can grow as chemoheterotrophs and some as chemoautotrophs.

PNSB are, by far, the most diverse group of phototrophic purple bacteria. This diversity is reflected in greatly varying morphology, internal membrane structure, carotenoids composition, utilization of carbon sources and electron donors.

Table 1: General properties of *Rs. rubrum* and *Rb. capsulatus* (Imhoff et al., 1989)

	Rs. rubrum	*Rb. capsulatus*
Cell shape width x length (μm)	Spiral 0.8-1.0 x 7-10	Rod/Sphere 0.5-1.2 x 2-2.5
Type of intracytoplasmic membrane system	Vesicles	Vesicles
Slime formed	-	-
Motile by flagella	+	+
Mode of multiplication	Binary fission	Binary fission
Colour of cell under anaerobic growth conditions	Red	Purple-red
Bacteriochlorophyll	a	a
Predominant carotenoids	Spirillixanthin	Spirillixanthin, Spheroidenone
Growth factor required for the majority of strains	Biotin	Thiamine
Aerobic and microaerophilic growth ability in the dark	Aerobic	Aerobic
DNA base ratio (%mol G+C)	63.8-65.8	65.5-66.8

The two next sections will deal with the characteristics of the two strains *Rs. rubrum* and *Rb. capsulatus* , and especially with their metabolic abilities. The description of the metabolic pathways involved in synthesis of cellular material and production of energy will be the purpose of further sections.

1.1.1 - *Rb. capsulatus*

The cells are ovoid to rod-shaped, 0.5-1.2 µm in diameter and 2.0-2.5 µm in long, and are motiles by means of a polar flagella.

The photosynthetic pigments are bacteriochlorophyll a (esterified with phytol) and carotenoids of the spheroidene series.

Photoautotrophic growth is possible with sulphide (oxidized to elemental sulphur only) or molecular hydrogen as electron source. Such growth with molecular hydrogen is excellent.

Under photoheterotrophic anaerobic growth conditions in the light, a number of organic carbon compounds are used as carbon and electron source.

Anaerobic dark growth occurs with sugars and in presence of DMSO (dimethyl-sulfate-oxyde) or trimetylamine-N-oxide as oxidant. Some strains may use nitrate as electron acceptor under similar conditions, reducing it to nitrite.

Aerobic growth in the dark is possible as a chemoheterotroph, or with molecular hydrogen as an autotroph at the full oxygen tension of air.

Rb. capsulatus is unable to grow with mannose, tartrate, gluconate, glycerol and ethanol (Sojka, 1978).

Good growth occurs with ammonia, dinitrogen and a number of amino acids as nitrogen sources. It can be noted that thiosulfate and elemental sulphur are not used and that thiamine is required as growth factor.

The mol G+C% of the DNA is 65.5-66.8.

These general abilities of the species (Imhoff et al.,1989) are summarised in table 1. The carbon sources and electron donors of *Rb. capsulatus* are listed in table 2 (Pfennig and Trüper, 1971).

In *Rb. capsulatus* glucose is metabolized via the Entner-Doudoroff pathway, fructose via the Embden-Meyerhoff pathway and CO_2 fixation during autotrophic growth occurs mainly via the Calvin cycle (Willison, 1988 - Ohman, 1979)

The studies of pyruvate and acetic acid metabolism in *Rb. capsulatus* (Willison, 1988) have demonstrated the importance of the anaplerotic pathway of pyruvate carboxylase for the growth on pyruvate, lactate or other compounds metabolised via pyruvate. It was suggested too that acetic acid was mainly assimilated via the glyoxylate cycle in *Rb. capsulatus* . The results have been incorporated in a global scheme (figure 1) which represents the central metabolic pathway of carbon metabolism in *Rb. capsulatus* .

The metabolic behaviour of *Rb. capsulatus* ATCC 23782 has been the subject of several studies (Suhaimi et al., 1987 - Gicqueau, 1993 - Albiol, 1994[a-b]), especially its ability to grow on different C and N sources, and its behaviour for different C/N ratios and light illumination conditions. The main results of these studies are reported in tables 3 and 4.

Suhaimi et al. (1987) studied the effect of the C/N ratio and of light on the growth efficiency and the N-NH_4^+ assimilation with lactate as carbon source. They concluded that a C/N ratio of 5, with a light intensity of 120 $\mu E/m^2/s$ and an initial condition of N-NH_4^+ in the range of 0.05-0.4 g/l, were sufficient to provide rapid substrate assimilation and to maintain dominance of the bacteria under non axenic conditions. Partial results presented by Suhaimi et al. (1987) and reported in table 3 show the different limiting situations (N limiting for C/N>5, C limiting for C/N <5 and light limiting for light intensity <120 $\mu E/m^2.s$).

Similar studies have been conducted by Gicqueau (1993) and Albiol (1994[a-b]) at ESTEC laboratory. Their results on the ability of the strain to grow on different carbon and nitrogen sources are combined in table 4. The qualitative results (mainly the biomass composition changes as a function of substrates concentration and light illumination conditions) will be detailed in a further section.

Table 3: Growth of *Rb. capsulatus* ATCC 23782 in different Lactate-C, NH_4^+-N and light illumination conditions. Partial results taken from Suhaimi et al.(1987).

Initial substrate level (g/l)			%NH_4^+-N assimilated				Cell biomass-C (g/l)			
			Light intensity ($\mu E/m^2/s$)				Light intensity ($\mu E/m^2/s$)			
NH_4^+-N	Lactate-C	C/N	12	40	120	320	12	40	120	320
0.05	0.25	5	-	91.49±1.80	98.87±0.06	95.90±0.00	-	0.25±0.03	0.23±0.01	0.20±0.01
0.05	0.50	10	-	85.81±0.84	100.00±0.00	98.57±0.69	-	0.37±0.01	0.35±0.01	0.34±0.06
0.05	1.00	20	-	92.16±0.00	100.00±0.00	98.63±0.60	-	0.33±0.05	0.31±0.05	0.45±0.15
0.20	1.00	5	88.78±2.89	94.59±0.28	96.61±3.10	98.09±0.57	0.84±0.05	0.88±0.02	0.82±0.02	0.94±0.01
0.20	2.00	10	-	92.59±0.57	86.66±3.49	99.45±0.26	-	0.86±0.01	0.72±0.04	1.02±0.09
0.20	4.00	20	-	85.30±0.28	76.86±2.83	98.37±1.29	-	0.81±0.03	0.68±0.03	0.97±0.09
0.40	1.00	2.5	37.97±0.48	51.11±0.21	59.63±0.68	-	0.44±0.02	0.76±0.04	0.95±0.06	-
1.00	1.00	1	17.78±0.78	20.30±0.11	24.46±0.53	-	0.84±0.17	0.77±0.09	0.92±0.01	-
0.40	2.00	5	78.09±5.48	77.80±0.30	98.29±0.13	-	1.10±0.15	1.24±0.02	1.72±0.05	-
0.80	2.00	2.5	28.09±1.25	42.21±0.76	51.42±0.35	-	0.81±0.02	1.33±0.06	1.55±0.25	-
2.00	2.00	1.00	2.80±0.41	15.85±0.00	18.62±0.86	-	0.31±0.14	1.58±0.12	1.43±0.34	-
0.40	2.00	5			99.07±0.01				1.80±0.09	
0.8	4.00	5			71.22±0.86				2.39±0.09	
1.20	6.00	5			48.59±3.55				2.20±0.26	

1.1.2 - *Rs. rubrum*

Cells are vibroid-shaped to spiral, 0.8-1.0 μm wide. The photosynthetic pigments are bacteriochlorophyll a (esterified with geranyl geraniol or phytol) and carotenoids of the spirilloxanthin series with spirilloxanthin as the predominant component.

Photoautotrophic growth is possible with molecular hydrogen as electron donor. Microaerobic to aerobic growth in the dark is possible. Fermentative metabolism with pyruvate under anaerobic dark conditions and "oxidant dependant" anaerobic dark metabolism are also possible.

Cells preferably grow photoheterophically under anaerobic conditions in the light with various organic compounds as carbon and electron sources.

It must be kept in mind that the glyoxylate shunt is inactive in *Rs. rubrum* , and thus the acetic acid assimilation pathway is still lacking (Sojka, 1978). Instead ethanol can not be the sole carbon source, *Rs. rubrum* is possessed of an inductible alcohol dehydrogenase (Albers and Gottshalk, 1976) and can then use ethanol (table 4).

Ammonia, dinitrogen, several amino acid and by some strains, nitrate, adenine, guanine, xanthin and uric acid may be used as nitrogen source. It can be noted that small amount of yeast extract may be favourable and that biotin is required as growth factor.

The mol G+C% of the DNA is 63.8-65.8.

These general abilities of the species (Imhoff and Trüper,1989) are summarized in the table 1. The carbon sources and electron donors of *Rs. rubrum* are listed in table 2 (Pfennig and Trüper, 1971).

Table 2: Photosynthetic electron donor and carbon sources (Pfennig and Trüper, 1971)

Donor/source for...	*Rs. rubrum*	*Rb. capsulatus*
Formate	+	
Acetic acid	+	+
Propionate	+	+
Butyric acid	+	+
Valerate	+	+
Caproate	+	+
Caprylate	+	+
Pelargonate	±	+
Pyruvate	+	+
Lactate	+	+
Malate	+	+
Succinate	+	+
Fumarate	+	+
Tartrate	-	-
Citrate	±	-
Aspartate	±	+
Arginine	+	
Glutamate	+	+
Benzoate	-	
Gluconate	-	-
Glucose	+	+
Fructose	+	+
Mannose	-	
Sorbitol	±	
Glycerol	-	-
Methanol	-	
Ethanol	-	-
Propanol	+	
Hygrogen	+	+
Sulphide	+	
Thiosulfate	-	-
Sulphur	-	

Studies of *Rs. rubrum* ATCC 25903 were performed by Viprey (1994) and Albiol (1994[a-b]) at ESTEC laboratory. Their results on the ability of the strain to grow on different carbon and nitrogen sources are combined in table 4.

The qualitative results (mainly the biomass composition changes as a function of substrates concentration and light illumination conditions) will be detailed in a further section.

Table 4: Summary of results obtained in carbon and nitrogen tests (Albiol, 1994[b])

	NH_4^+		Urea	
	Rs. rubrum ATCC 25903	*Rb. capsulatus* ATCC 23782	*Rs. rubrum* ATCC 25903	*Rb. capsulatus* ATCC 23782
Acetic acid	+	+	+	-
Lactic acid	+	+	+	+
Isobutyric acid	+	+		
Propionic acid	+	+		
Butyric acid	+	+		
Valeric acid	+	+		
Isovaleric acid	+	+		
Caproic acid	+ <15mM	+ <15mM		
Isocaproic acid	+ <15mM	+ <15mM		
Ethanol	+	-	+	-
Lactic acid + ethanol			+	+
Acetic acid + ethanol			+	-

1.2 - Carbon metabolism

1.2.1 - Autotrophic carbon dioxide assimilation

Glover et al.(1952) working with *Rs. rubrum*, first established that PGA was the earliest product of CO_2 fixation indicating that the reductive pentose phosphate cycle (Calvin cycle) represents the major mechanism of carbon dioxide assimilation. Further investigations done by other research groups confirmed these early results and gave convincing evidence for the operation of the Calvin cycle (figure 1) in different genera and strains of Chromatiaceae and Rhodospirillaceae.

The data now available do indicate that in purple bacteria, some auxiliary carboxylation reactions are involved in the process of CO_2 assimilation, at least under strong reducing conditions, but they do not question the dominant role of the Calvin cycle as the main route (Ohman, 1979).

The Calvin-Benson cycle can be represented by the following group of stoichiometric equations:

$$\text{Ribulose} - 5P + ATP \longrightarrow \text{Ribulose} - 1,5diP + ADP$$

$$\text{Ribulose} - 1,5diP + H_2O + CO_2 \longrightarrow 2,3 - \text{Phosphoglycerate}$$

$$3 - \text{Phosphoglycerate} + ATP \longrightarrow 1,3 - \text{diPhosphoglycerate} + ADP$$

$$1,3 - \text{diPhosphoglycerate} + NADPH,H^+ \longrightarrow 3 - \text{Phosphoglyceraldehyde} + Pi + NADP^+$$

$$3 - \text{Phosphoglyceraldehyde} \longrightarrow \text{Dihydroxyacetone} - P$$

$$3 - \text{Phosphoglyceraldehyde} + \text{Dihydroxyacetone} - P \longrightarrow \text{Fructose } 1,6 - diP$$

$$\text{Fructose } 1,6 - diP + ADP \longrightarrow \text{Fructose } 6 - P + ATP$$

$$\text{Fructose } 6 - P + 3 - \text{Phosphoglyceraldehyde} \longrightarrow \text{Ribulose } 5 - P + \text{Erythrose } 4 - P$$

$$\text{Erythrose } 4 - P + \text{Fructose } 6 - P \longrightarrow 2 \text{ Ribulose } 5 - P$$

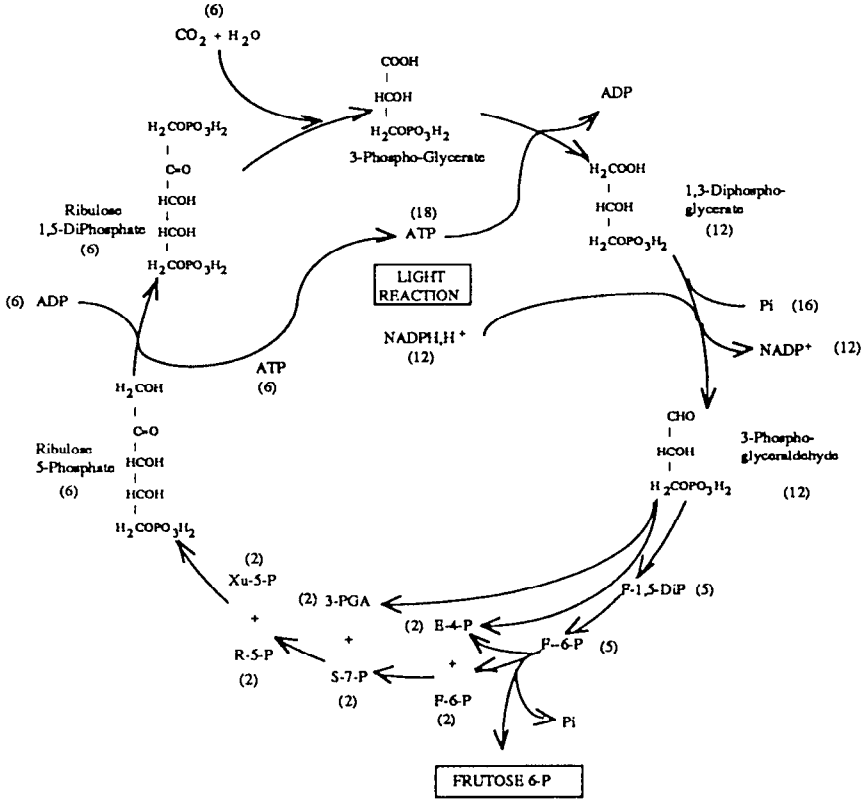


Figure 1 ; Calvin-Benson cycle

In Athiorhodaceae, the activity of the Calvin cycle is high when they live as photolithoautotrophs and apply H_2 as reductant. Characteristically RuBP carboxylase and the capabilities of CO_2 fixation are reduced drastically in the presence of organic substrates, especially when the bacteria live aerobically by respiration in the dark.

Some Ferredoxin-linked carboxylation reactions have been presented by Buchanan et al. (1967) for photoautotrophic carbon fixation:

- a pyruvate synthase (antagonist of the pyruvate dehydrogenase, PDH) has been found in *Rs rubrum*. It is possible that at least some organisms may use one enzyme for pyruvate synthesis and one for pyruvate breakdown (PDH).

$$\text{Acetyl} - \text{CoA} + CO_2 + \text{Fd}_{red} \xrightarrow{\text{Pyruvate synthase}} \text{Pyruvate} + \text{CoA} - \text{SH} + \text{Fd}_{ox}$$

- a phenylpyruvate synthase which allows phenylalanine synthesis via phenyl pyruvate from condensation of phenyl acetic acid and CO_2, has been found in *Rs rubrum*. The enzyme appears to function in the synthesis of aromatic amino-acids via a pathway that is independent of the shikimate pathway established for aerobic cells. It is not know whether such cells also utilize the shikimate pathway for the synthesis of aromatic amino-acids.

$$\text{Phenylacetyl} - \text{CoA} + CO_2 + \text{Fd}_{red} \xrightarrow{\text{Phenylpyr. Synth.}} \text{Phenylpyruvate} + \text{CoA} - \text{SH} + \text{Fd}_{ox}$$

Buchanan et al. (1967) have speculated the contribution of a reductive carboxylic cycle in the photoautotrophic fixation of CO_2, but this cycle has been contested by Ohman (1979).

The photoautrophic fixation of CO_2 seems mainly under the control of energy charge and availability of reducing power The regulation of the balance between autotrophic and heterotrophic aspect of the metabolism of Athiorhodaceae involves combinations of the regulation of the key enzyme of their main carbon metabolic pathway (figure 2) (Ohman, 1979).

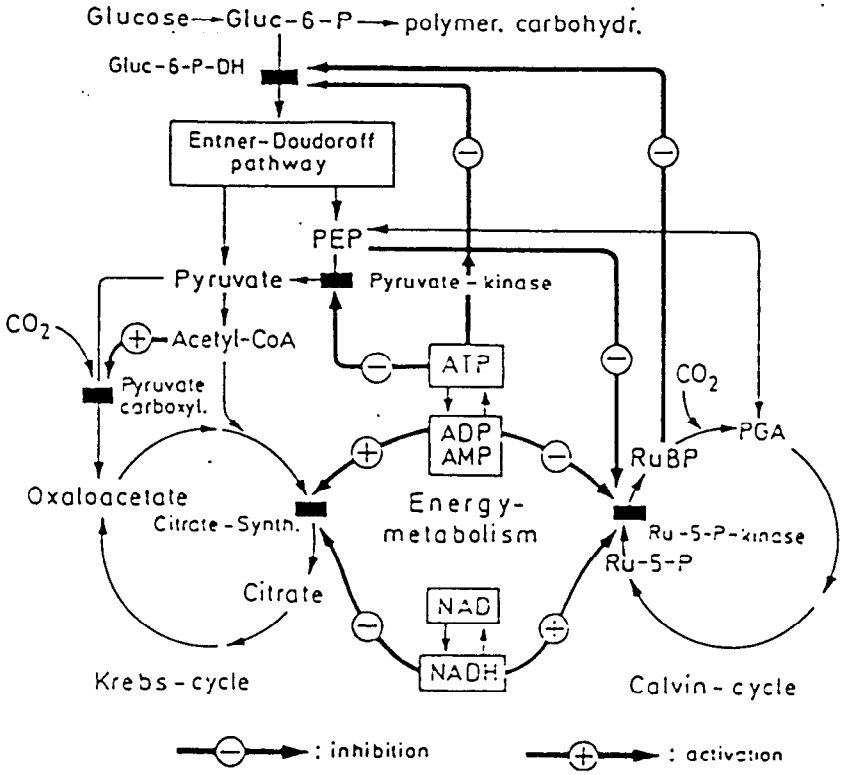


Figure 2 : Schematic presentation of the regulatory interaction of carbon dioxide assimilation and heterotrophic breakdown processes in some Athiorhodaceae (*Rs. spheroides*, *Rs. rubrum*, *Rb. capsulatus*). Taken from Ohman (1979).

It must be kept in mind that this regulation aspect is difficult to take into account in the stoichiometric description of the metabolism. In fact the presence or the absence of a pathway (*i.e.* the functioning or not of an enzyme) is the only way to account for it.

1.2.2. Photometabolism of organic compounds

Purple bacteria can grow when illuminated under anaerobic conditions in synthetic medium containing NH_4^+ as N-source and an organic compound as C-source. It has been observed that the CO_2 yield (produced or assimilated) depends on the organic compound for sulphur bacteria (table 5). Similar results have been obtained for PNSB (Ormerod and Gest, 1962).

The CO_2 production by *Rs rubrum* growing on acetic acid in different C/N conditions has been studied by Albiol (1994[b]) and will be discussed in section 3.3.1.

In presence of light as energy source and substrates more reduced than CO_2, it may be assumed that the major function of various metabolic cycles in photosynthetic bacteria is the synthesis of new cellular material rather than the production of energy (Doelle,1975).

Table 5: Over-all carbon dioxide metabolism in growing cultures of purple sulphur bacteria (taken from Ormerod and Gest, 1962)

Substrate	Moles of CO_2 produced per mole of substrate consumed
Acetic acid	+0.17
Lactate	+0.29
Succinate	+0.70
Malate	+1.22
Butyric acid	-0.74

Willison (1988), studying the pyruvate metabolism in *Rb. capsulatus*, described the central pathways of carbon metabolism in this organism (figure 3). This scheme of the central pathway of carbon metabolism is very useful because it shows the entry points in the central carbon pathway (TCA and Entner Meyerhoff pathway) of the main carbon compounds supposed to be present in compartment II (VFA, lactate, CO_2).

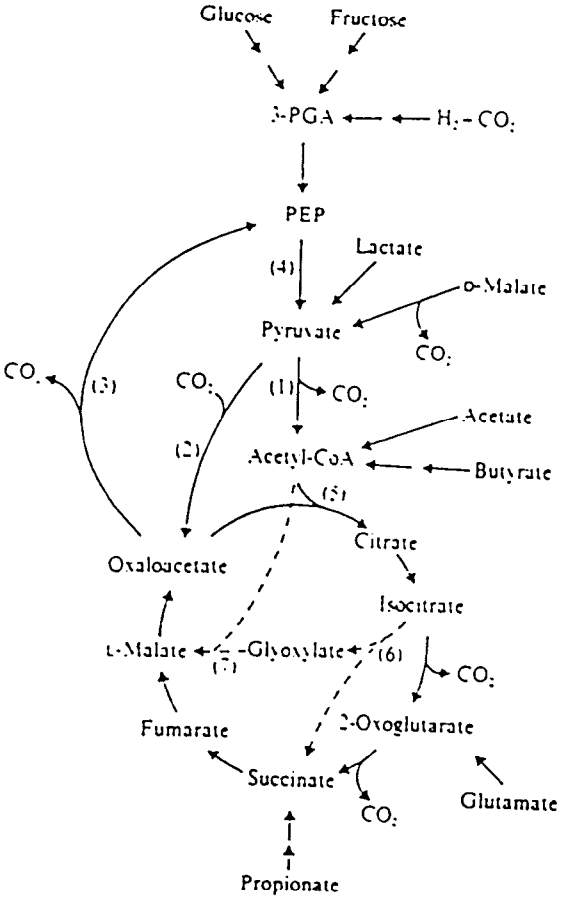


Figure 3 : Central pathway of carbon metabolism in *R capsulatus*. (1) pyruvate dehydrogenase (PDH); (2) pyruvate carboxylase (PC); (3) phosphoenolpyruvate carboxykinase (PCK); (4) pyruvate kinase (PK); (5) citrate synthase (CS); (6) isocitrate liase (ICL); (7) malate synthase (MS). PC is the major anaplerotic enzyme for the grow on pyruvate. Taken from Willison, 1988.

The functioning (regulation, stoichiometries) of the central pathway of carbon metabolism (TCA, Entner-Doudoroff and Embden-Meyerhoff pathways) are well-known, and the stoichiometric equations that describe these pathways are reported in Appendix. The stoichiometric description of the reactions from the organic compound (VFA, lactate ...) to the central carbon pathway is the purpose of the following lines.

Acetic acid

Rs. rubrum is able to assimilate acetic acid directly in the absence of CO_2. Acetic acid is converted to acetyl-CoA by an acetic acid activating enzyme:

$$\text{Acetic acid} + \text{ATP} + \text{CoA} - \text{SH} \longrightarrow \text{Acetyl} - \text{CoA} + \text{AMP} + \text{PPi}$$

Without CO_2, the main storage compound product formed is poly-β-hydroxybutyric acid (see section 1.4). Because this synthesis is reductive, some acetic acid must be oxidized to provide the necessary reducing power via the TCA (Doelle, 1975).

The formation of poly-β-hydroxybutyric acid depends on the amount of CO_2 present, because CO_2 shifts the synthesis toward polysaccharide (glycogen) formation. The influence of the type of substrate on the type of storage material (glycogen or PHB) will be discussed in section 1.4.

Butyric acid

As acetic acid, it can be assumed that butyric acid is assimilated to poly-β-hydroxybutyric acid (Doelle, 1975). It is supposed that butyric acid is converted to acetyl-CoA, as acetic acid, via the β-oxidation of fatty acid pathway (TN 9.3).

$$\text{Butyric acid} + 2\ \text{CoA} - \text{SH} + \text{NAD}^+ + \text{ATP} + \text{FAD}^+ + \text{H}_2\text{O}$$
$$\downarrow$$
$$2\ \text{Acetyl} - \text{CoA} + \text{FADH,H}^+ + \text{NADH,H}^+ + \text{PPi} + \text{AMP}$$

Propionic acid

The classical pathway for the assimilation and the catabolism of propionic acid lead to succinic acid (figure 3). The stoichiometric description of this pathway is:

$$\text{Propionic acid} + \text{ATP} + \text{CoA} - \text{SH} \longrightarrow \text{Propionyl} - \text{CoA} + \text{AMP} + \text{PPi}$$
$$\text{Propionyl} - \text{CoA} + \text{OAA} \longrightarrow \text{Pyruvate} + \text{Succinate} + \text{CoA} - \text{SH}$$

It must be noted that propionyl-CoA is an intermediate in alanine synthesis.

Other VFA

It can be assumed that the other VFA (excepted branched VFA, see below), lead, via the β-oxidation of fatty acid pathway, to acetyl-CoA and propionyl-CoA.

Branched VFA

The catabolism of branched fatty acids (*i.e.* isobutyric acid, isovalerate, isocaproate which have been considered in the studies of compartment II), is different from the non branched ones.

In the case of isobutyric acid, two pathways can occur:

- the classical path leading to succinate:

$$\text{Isobutyric acid} + \text{ATP} + \text{FAD}^+ + \text{NAD}^+ + 2\ \text{H}_2\text{O} \downarrow \text{Succinic acid} + \text{AMP} + \text{PPi} + \text{FADH,H}^+ + \text{NADH,H}^+$$

- or if it exists an aldehyde dehydrogenase:

$$\text{Isobutyric acid} + \text{ATP} + \text{FAD}^+ + 2\ \text{NAD}^+ + 2\ \text{H}_2\text{O} + \text{CoA} - \text{SH} \downarrow \text{Propionyl} - \text{CoA} + \text{CO}_2 + 2\ \text{NADH,H}^+ + \text{FADH,H}^+ + \text{AMP} + \text{PPi}$$

Lactate

The first intermediate of lactic acid assimilation is pyruvate, which plays a central role in the carbon metabolism (figure 3). This assimilation can be represented by the following stoichiometry:

$$\text{Lactic acid} + \text{NAD}^+ \longrightarrow \text{Pyruvate} + \text{NADH,H}^+$$

Ethanol

The assimilation of ethanol by *Rs. rubrum* involves an alcohol dehydrogenase. The functioning of the enzyme leads to acetic acid, of which assimilation has been described.

$$\text{Ethanol} + \text{H}_2\text{O} + 2\ \text{NAD}^+ \longrightarrow \text{Acetic acid} + 2\ \text{NADH,H}^+$$

1.3 - Nitrogen metabolism

The Rhodospirillaceae are able to grow with NH_4^+ as N-source whatever is the C-source. Although NH_4^+ is the main and the preferential N-source, other inorganic and organic N-compounds can be used as N-source (Gicqueau, 1993; Viprey, 1994).

Under specific growth conditions, especially the absence of NH_4^+, N_2, NO_3^- and NO_2^- can be used as inorganic N-source. Amino acids can be used both as Carbon (table 2) and Nitrogen source.

It must be noted that H_2 production is closely linked to the Nitrogen metabolism (Ormerod and Gest, 1962; Viprey, 1994), but this production seems not to occur when NH_4^+ or other N-source producing NH_4^+ are present.

The production of NH_4^+ , when amino-acids are supplied to the growth medium, seems to depend on the C/N ratio of the medium (low C/N -> repression of the nitrogenase -> NH_4^+ production) (Moreno-Vivian et al., 1989). Whatever, the production of NH_4^+ from N-organic compounds is dependent of the inhibitory level of the nitrogenase. This aspect, as well as the H_2 production, will not be considered here.

For simplicity the sole N-sources considered in our stoichiometric description are NH_4^+ (metabolism of which is reported in figure 4) and urea. It must be noted that when the two N-sources are present, NH_4^+ is better assimilated than urea (Christiansen, 1990).

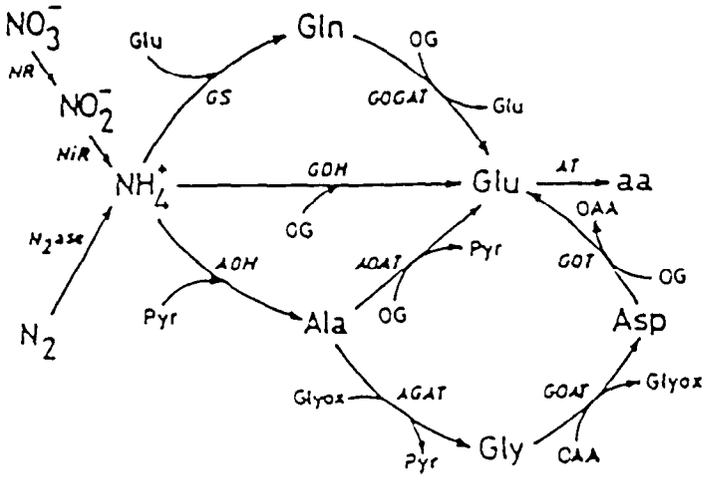


Figure 4 : NH_4^+ metabolism of Rhodospirillaceae (Cardenas et al., 1987). (aa) amino acid; (ADH) alanine dehydrogenase; (AGAT) alanine glyoxylate aminotransferase; (AOAT) alanine oxoglutarate aminotransferase; (GDH) glutamate dehydrogenase; (GOAT glycine oxaloacetic acid aminotransferase; (GOGAT) glutamate synthase; (GOT) glutamate oxaloacetic acid transaminase; (GS) glutamine synthase; (NiR) nitrite reductase; (NR) nitrate reductase; (N_2ase) nitrogenase.

The assimilation of NH_4^+ is then described by the stoichiometric description of the amino-acid synthesis. The different pathways for the biosynthesis of amino-acids was described in TN 9.3 (Dussap, 1991).

The catabolism of urea involves an urease activity and leads to NH_4^+ (NH_3) :

$$\text{Urea} + H_2O \longrightarrow CO_2 + 2\ NH_3$$

1.4 - Reserves metabolism

Two forms of carbon storage can occur (Ormerod and Gest, 1983): glycogen and poly-β-hydroxybutyric acid (PHB). The synthesis of reserve material depends mainly on the carbon

source and on the carbon excess (*i.e.* N, S or P limitation) (Clayton et al.,1978). That means that the C/N ratio in the medium could probably be linked with the reserve material synthesis.

Glycogen

The main C-sources leading to glycogen are CO_2, pyruvate, malate, succinate, lactate. The glycogen metabolism involves both synthesis and breakdown of the molecule and can be represented by the scheme reported in figure 5 .

The synthesis can be represented by the following equation:

$$ATP + Glucose - 1P + (\alpha - 1,4 - glucosyl)_n \longleftrightarrow ADP + PPi + (\alpha - 1,4 - glucosyl)_{n+1}$$

where $(\alpha$-1,4-glucosyl$)_n$ can be represented by the formula $[C_6H_{11}O_5]_n[H_2O]$.

The breakdown can be described by the following equation:

$$(Glucosyl)_{n+1} + Pi \longrightarrow (Glucosyl)_n + Glucose - 1P$$

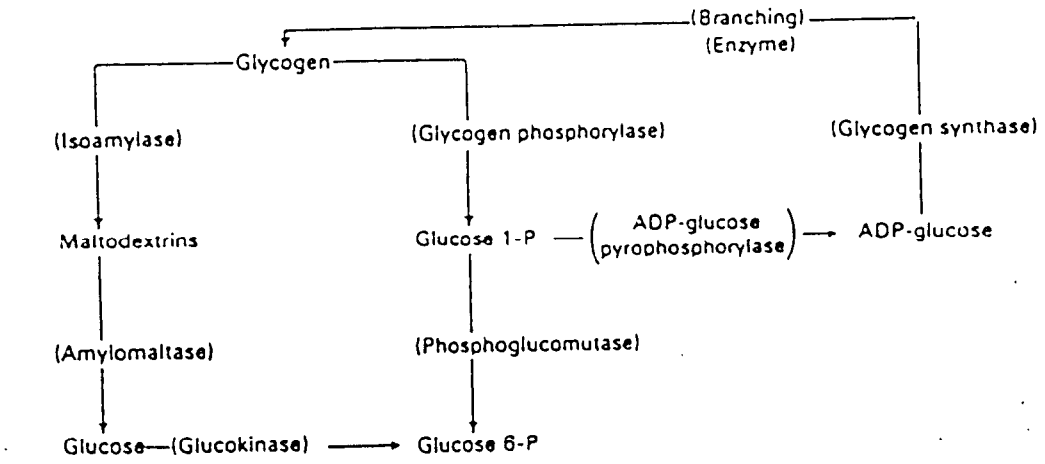


Figure 5 : Glycogen metabolism by bacteria (taken from Merrick, 1978)

Poly-β-hydroxybutyric acid (PHB)

The main C-sources leading to poly-β-hydroxybutyric acid are acetic acid, butyric acid and generally all VFA and fatty acids. The poly-β-hydroxybutyric acid synthesis and breakdown (figure 6) involve pathways of the fatty acid synthesis and oxidation.

The stoichiometry of the PHB synthesis is:

$$D(-) - \beta - Hydroxybutyryl - CoA + PHB_n \longrightarrow CoA - SH + PHB_{n+1}$$

where PHB_n is $[C_4H_6O_2]_n[H_2O]$

The synthesis of D(-)-β-hydroxybutyric acid from acetic acid can be described by (Merrick, 1978):

$$2\,\text{Acetyl} - \text{CoA} + \text{NADH},\text{H}^+ \longrightarrow \text{D}(-) - \beta - \text{Hydroxybutyryl} - \text{CoA} + \text{NAD}^+ + \text{CoA} - \text{SH}$$

The synthesis of D(-)-β-hydroxybutyric acid from butyric acid can be described by:

$$\text{Butyryl} - \text{CoA} + \text{H}_2\text{O} + \text{FAD}^+ \longrightarrow \text{D}(-) - \beta - \text{hydroxybutyryl} - \text{CoA} + \text{FADH},\text{H}^+$$

The breakdown of PHB can be described by the following equation:

$$\text{PHB}_n + \text{H}_2\text{O} + \text{Succinyl} - \text{CoA} + \text{NAD}^+ \downarrow \text{PHB}_{n-1} + \text{NADH},\text{H}^+ + \text{Succinic acid} + 2\ \text{Acetyl} - \text{CoA}$$

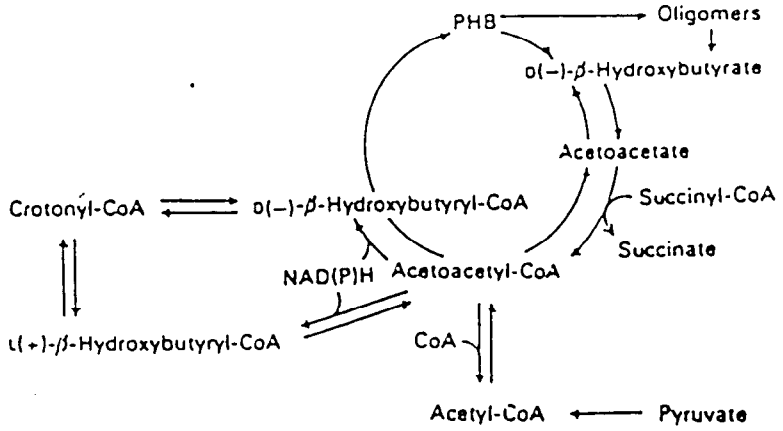


Figure 6 : Synthesis and degradation of PHB (taken from Merrick, 1978)

While the quantity (*i.e.* mass percentage in the biomass) is under the control of the C-source excess, the choice of the storage material (glycogen or PHB), depends on growth conditions (see section 1.3), and is probably under the control of an energetic balance ($\frac{\text{ATP}}{\text{reduced power}}$). As shown by the previous stoichiometric equations, the storage of Carbon under a PHB form is associated to a consumption of reduced cofactors. It can then be assumed that the more the assimilation of a substrate leads to the synthesis of reduced cofactors, the more important will be the synthesis of PHB versus the synthesis of glycogen. On the other hand, if the substrate leads to ATP synthesis, rather than reduced cofactors synthesis, the carbon storage will be oriented to a glycogen synthesis rather than a PHB synthesis.

Table 6 presents the results in term of XTP (*i.e.* ATP, GTP...) and reduced cofactors of the complete degradation of a substrate to CO_2 via the central metabolic pathways (see appendix). Taking acetate as the main substrate leading to PHB and glucose as the main substrate leading

to glycogen, from energy balance of the degradation of others substrates it can be guessed to which storage form they led.

Table 6: Substrate, energy balance and storage

Substrate (number of Carbon)	XTP produced by Carbon of substrate	Reduced factors produced by Carbon of substrate	Storage oriented to
Glucose (6)	1.66	2	Glycogen
Acetic acid (2)	0	2	PHB
Butyric acid (4)	0	2.5	PHB
Lactic acid (3)	0.33	2	Glycogen-PHB
Pyruvic acid (3)	0.33	1.66	Glycogen

1.5 - Energy metabolism

The non sulphur bacteria contain bacteriochlorophylls a and b, and are primarily dedicated to the metabolism of organic compounds They are capable of growing anaerobically when exposed to light and are also able to grow in the dark when exposed to oxygen. The organisms therefore can switch from photophosphorylation to oxidative phosphorylation and both electron transport systems are present in the various organisms (Dussap, 1991). Assuming the anoxygenic and illuminated conditions of compartment II, the photosystem is the sole electron transport system that is considered.

At the contrary of plants and algae, photosynthetic bacteria have only one photosystem, similar to the PS I of plants. A representation of the structure of such bacterial photosystem is given in figure 7.

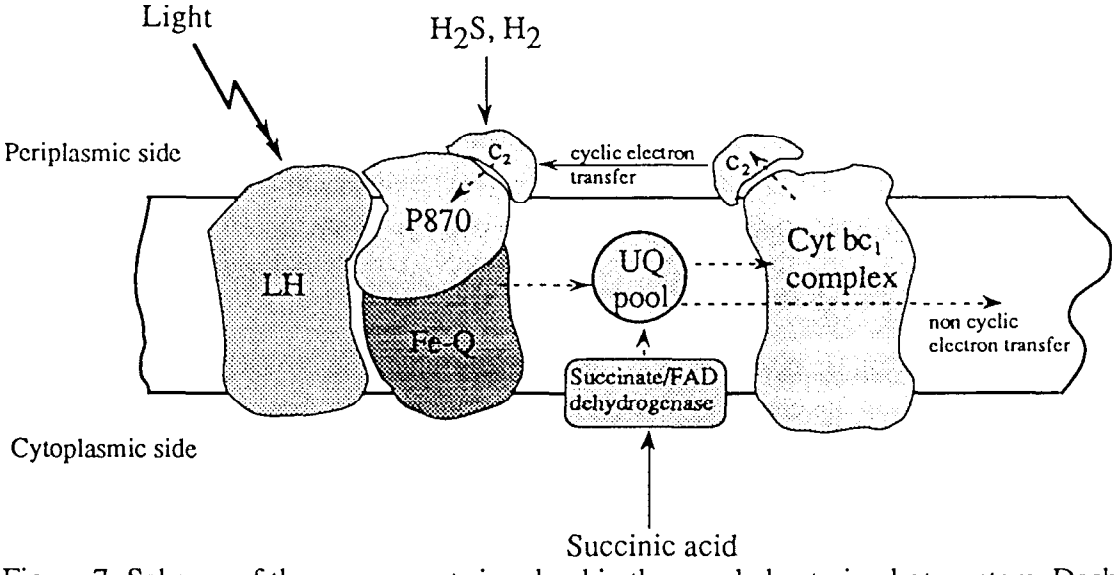


Figure 7: Scheme of the components involved in the purple bacteria photosystem. Dashed lines are for the electron transfer.

This structure contains several components (Gottschalk, 1986):

- the Light Harvesting centers (LH). They consist of bacteriochlorophylls (more than 90% of the total bacteriochlorophylls of the cell are in LH), carotenoids and several proteins.

- the photosynthetic Reaction Center, call P870 in purple bacteria, associated to an iron Ubiquinone (Fe-Q) complex.
- an Ubiquinone pool.
- the membrane integrated cytochrome bc_1 complex (similar to that described in TN 23.2). It consist of cytochromes b_L, b_H, c_1 and of an iron sulphur protein (Rieske protein).
- a cytochrome c_2 (electron carrier).

In PNSB, the H-donors (electron donors for the photosystem) are mainly H_2 and organic compound (Gottschalk, 1986). Two electron pathways are involved in electron transfer: cyclic electron transfer and non cyclic electron transfer (figures 8).

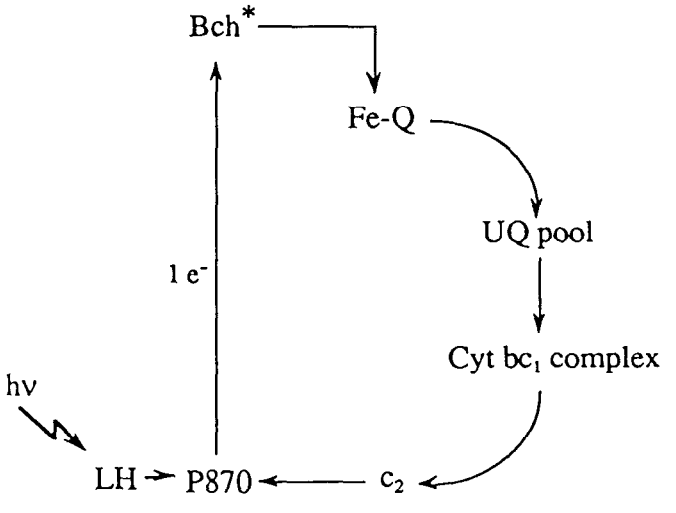


Figure 8a: Cyclic electron transfer

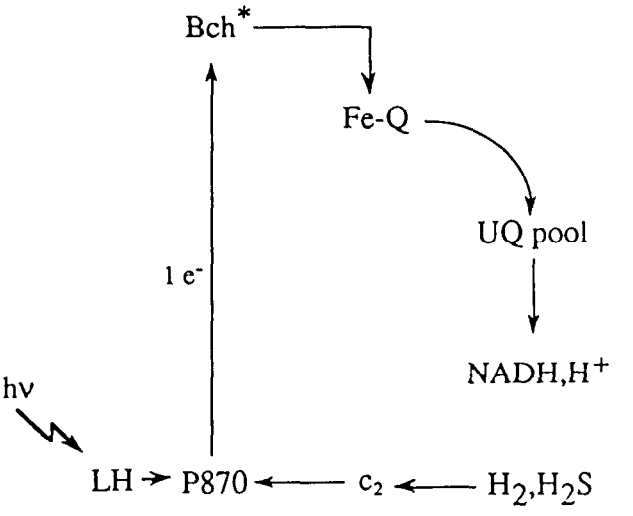


Figure 8b: Non cyclic electron transfer

The cyclic electron transport is associated to proton (H^+) translocating across the membrane and then generates a Δp. This Δp is latter used (by the non cyclic electron transport) to drive a reverse electron transfer from the Ubiquinone pool to a NADH dehydrogenase (Gottschalk, 1986). Such a reverse electron flow as been described in TN 23.2.

The non cyclic electron transport leads to the regeneration of the $NADH,H^+$ pool, from the H-donor and using the Δp generated by the cyclic electron transfer. The span Fe-Q -> cyt bc_1 complex is the only part of the electron transfer system that is associated to a proton translocation (with a stoichiometry of 4 H^+ per 2 e^-), involving probably the same mechanism than this described for mitochondria in TN 23.2.

The cyclic and the non cyclic electron transfer can be combined in a three stages system:

- stage 1: initiation of the electron transfer from H_2 or from succinate
- stage 2: propagation of the electron, involving or not the cyclic electron transfer
- stage 3: termination of the electron transfer by feeding the final acceptor NADH dehydrogenase.

The stoichiometric description of such a system is:

Initiation:

$$H_2 + UQ \xrightarrow{2hv} 2\ H^+_{periplasmic} + UQH_2$$

or, from succinate

$$\text{Succinic acid} + UQ \longrightarrow UQH_2 + \text{fumaric acid}$$

Cyclic propagation

$$c.\left[4\ H^+_{cytoplasmic} \xrightarrow{2hv} 4\ H^+_{periplasmic}\right]$$

where c is the number of cycles.

Termination

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

In order to complete the energy metabolism description of purple bacteria, the ATP synthesis, involving the proton depending ATP synthase, must be taken into account. The stoichiometric description of the functioning of ATP synthase (TN 23.2) can be represented by the following equation:

$$ADP + Pi + 3\ H^+_{periplasmic} \longrightarrow ATP + H_2O + 3\ H^+_{cytoplasmic}$$

1.6 - Biomass composition and building

1.6.1 Biomass chemical and biochemical composition

The biomass composition can be expressed in two forms:
- a chemical composition (CHON composition)
- a general components composition (proteins, fats, DNA ....)

Some chemical biomass composition of *Rb. capsulatus* and *Rs. rubrum* are reported in table 7 for different growth conditions. The biochemical composition (table 8) of the biomass, as well as the chemical composition changes with the growth conditions.

From experimental results, Albiol (1994[b]) observed that the change in the biomass chemical and biochemical composition can be linked to the change of C/N ratio of the medium and of the light availability. The influence of these growth conditions will be discussed in the third section of this TN.

Table 7: Some chemical composition of *Rb. capsulatus* and *Rs. rubrum*

	Rb. capsulatus % dry mass	*Rb. capsulatus* ATCC 23782 % dry mass	*Rb. capsulatus* ATCC 23782 % dry mass	*Rs. rubrum* ATCC 25903 % dry mass	*Rs. rubrum* ATCC 25903 % dry mass
C		48.6 ± 0.1	48.5 ± 0.2	49.5 ± 0.02	46 ± 2 (5.5)
H		6.7 ± 0.2	6.6 ± 0.03	6.7 ± 0.1	6.8 ± 0.2 (3.5)
O		23.8 ± 0.2	25.3 ± 0.01	25.3 ± 0.1	27 ± 1 (5.2)
N	9.75	10.3 ± 0.05	9.7 ± 0.1	11 ± 0.04	9.4 ± 1 (11)
P	2.49	ND	ND	1.55 ± 0.01	1.4 ± 0.9 (60)
S		ND	ND	0.68 ± 0.15	0.5 ± 0.1 (28)
Growth conditions	NH_4^+ ? Propionate ?	NH_4^+ Lactic acid DR = 0.12 h^{-1} 180 W/m^2	NH_4^+ Acetic acid DR = 0.12 h^{-1} 180 W/m^2	NH_4^+ Lactic acid DR = 0.09 h^{-1} 442 W/m^2	Average from different growth condition (% variation)
Ref.	Kobayashi, 1978	Albiol, 1994[a]	Albiol, 1994[a]	Albiol, 1994[a]	Albiol, 1994[b]

Table 8: Biochemical composition of *Rb. capsulatus* and *Rs. rubrum* (% of dry weight). It must be outlined that no data giving a complete biochemical composition were found.

	Rb. capsulatus				*Rs. rubrum*		Bacteria (general)
	Kobayashi, 1978	Vrati, 1984	Aiking, 1979 (mean values)	Albiol, 1994[a] (2)	Vrati, 1984	Albiol, 1994[b] (3)	Albiol, 1994[b]
Proteins	60.95	61.25	54.8	62	46.25	48.± 1	55
Total carbohydrates	20.83 (1)	-	7.98 (1)	6.2 (1)	-	-	3% lipopolysaccharide 5% peptidoglycans
Total fats	9.91	-	-	-	-	-	9
Fibre	2.92	-	-	-	-	-	-
Ash	5.39	-	-	-	-	5.17	-
Total	*100*	-	-	-	-	-	-
RNA	4.9	-	10.56	-	-	-	15
DNA	1	-	5.02	-	-	-	3
Glycogen	-	-	-	-	-	1.3 ± 0.4	2
PHB	-	-	-	-	-	5.0 ± 0.8	-
Chlorophylls	5.61	-	1.26	-	-	9.4 nmol	-
Carotenoids	4.17	-	0.39	-	-	0.02	-
Pigments (tot)	(9.78)	-	(1.65)	-	-	-	-
Total N	9.75	11.1	-	-	8.2	-	-
Dry weight	-	17.52%	-	-	17.29%	-	-

(1) Kobayashi defined the soluble carbohydrates, while Aiking and Albiol defined the data for glucid. It may be possible that in the data of Kobayashi, RNA and DNA are assumed to be carbohydrates.

(2) 180W/m^2; DR=0.12h^{-1}; Lactic acid + Ammonium

(3) 133 W/m^2; DR=0.034h^{-1}; acetic acid + Ammonium (C/N=5)

As a consequence of the variability of the biochemical composition of *Rb. capsulatus* and *Rs. rubrum* with the growth conditions, the determination of a basic composition for the biomass of the two organisms is difficult. However from the experimental data a basic composition of *Rb. capsulatus* has been extrapolated (table 9). The biochemical composition of *Rs. rubrum* and its evolution as a function of the C/N ratio will be studied the third section of the TN.

Table 9: Biochemical composition of *Rb. capsulatus* for the stoichiometries building

	Global composition	Active biomass composition	Composition used in previous descriptions
Proteins	60	64,86	65
Fats	10,5	11,35	16.9
DNA	2,5	2,70	1
RNA	5	5,40	4.9
Glycogen	6,5	-	-
PHB	1	-	-
Carbohydrates	7	7,57	12,2
Ash	7,5	8,11	-

1.6.2 The biomass macrocomponents composition

Proteins

The amino acid composition of proteins has been studied by several authors (table 10). It can be noted that Driessen et al. (1987) found that the proteins, the amino acids levels and the pigment contents were not significantly different in continuous and batch culture.

According to these experimental data a mean amino acid composition of proteins can be computed. This composition is reported in table 11 and will be the reference composition used in the building of proteins in the stoichiometries of biomass biosynthesis.

Vrati (1984) presented a comparison of some amino acid protein composition (Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophane and Valine) between *Rb. capsulatus* and *Rs. rubrum*. The amino acid protein composition of the two organisms seems enough closed to assume that the proteins composition is the same for these organisms.

Table 10: Amino acids composition (*Rb. capsulatus*)

	Kobayashi, 1978 g/.100g DW	g/100g prot. (1)	Driessen, 1987 % mol prot.	Suhaimi, 1987 g/100g prot.	Vrati, 1984 % mol prot.
Lysine	2.86	4.69	4.52	4.31	5.41
Histidine	1.25	2.05	-	-	2.82
Arginine	3.34	5.48	-	-	-
Aspartic acid	4.56	7.48	-	-	-
Threonine	2.70	4.43	-	4.72	5.12
Serine	1.68	2.76	-	-	-
Glutamic acid	5.34	8.76	-	-	-
Proline	2.80	4.59	-	-	-
Glycine	2.41	3.95	-	-	-
Alanine	4.65	7.63	-	-	-
Valine	3.51	5.76	7.66	5.51	7.20
Methionine	1.58	2.59	1.61	2.91	3.23
Isoleucine	2.64	4.33	5.13	4.25	5.24
Leucine	4.50	7.38	9.89	7.59	8.02
Tyrosine	1.71	2.81	-	-	-
Phenylalanine	2.60	4.27	7.13	4.22	5.23
Tryptophan	1.09	1.79	-	-	-
Asparagine	-	-	-	-	-
Glutamine	-	-	-	-	-
Cysteine	-	-	-	0.37	-

(1) calculated assuming proteins represent 60.95 % of dry weight

Table 11: Theoretical mean amino acid composition

	g/100g prot	%mol/mol prot
Lysine	4.69	4.81
Histidine	2.05	1.98
Arginine	5.48	6.21
Aspartic acid	7.48	8.42
Threonine	4.43	5.57
Serine	2.75	3.93
Glutamic acid	8.75	8.92
Proline	4.59	5.98
Glycine	3.95	7.89
Alanine	7.62	12.82
Valine	5.75	7.36
Methionine	2.59	2.60
Isoleucine	4.33	4.94
Leucine	7.38	8.43
Tyrosine	2.80	2.32
Phenylalanine	4.26	3.87
Tryptophan	1.79	1.31
Asparagine	1.00	1.13
Glutamine	1.00	1.03
Cysteine	0.40	0.49

It can be remarked that using this composition, mainly deduced from Kobayashi data, gives a N content of about 10.3% of the dry matter of the biomass considering only proteins which represents 60.95% of the biomass. But the N content determined by Kobayashi is 9.75%.

DNA

DNA can be considered to be a polymer of dCTP, dATP, dGTP and dTTP. From general knowledge, the amount of dATP and dGTP are respectively equal to dTTP and dCTP.

Assuming a G+C% of 66 (mean value from table 1) for *Rb. capsulatus*, the stoichiometry for DNA synthesis is then:

$$0.33\ dCTP + 0.33\ dGTP + 0.17\ dATP + 0.17\ dTTP \longrightarrow DNA + PPi$$

Assuming a G+C% of 64.8 (mean value from table 1) for *Rs. rubrum*, the stoichiometry for DNA synthesis is then:

$$0.324\ dCTP + 0.324\ dGTP + 0.176\ dATP + 0.176\ dTTP \longrightarrow DNA + PPi$$

RNA

No data are available for RNA synthesis of Rhodobacteraceae. However a stoichiometry for ARN synthesis has been supposed (Pons et al., 1995):

$$0.200\ CTP + 0.322\ GTP + 0.262\ ATP + 0.216\ UTP \longrightarrow RNA + PPi$$

Carbohydrates

No data are available for the glucidic composition of Rhodobacteraceae. However, assuming the classical bacterial composition (table 8), it can be supposed that carbohydrates are composed of 62.5% of peptidoglycans and 37.5% of lipopolysaccharides.

Whatever, at the present time, only a stoichiometry for the peptidoglycan biosynthesis is available, thus carbohydrates are assumed to be equivalent to peptidoglycan as a first approximation.

Fats

Excepted the presence of lipid A in the membrane (Imhoff and Trüper, 1989), no data have been found for the fats composition of Rhodobacteraceae. An hypothesis is then made for the average phosphatidic acid composition: 43.9% palmitic acid, 33.9% palmitoleic acid and 22.2% vaccenic acid. From this average phosphatidic acid the lipid fraction of the biomass was built (see Appendix) (Pons et al., 1995).

1.6.3 Chemical biomass composition deduced from the biochemical description

From the biochemical description of *Rb. capsulatus* (table 9) and from the composition of the macrocomponents of this biomass (see above), a chemical composition of the biomass can be deduced.

C	H	O	N	S	P	Ash
1	1.6023	0.3644	0.2177	0.0042	0.0103	7.5% mass
45.76%	6.11%	22.23%	11.62%	0.51%	1.21%	7.5%

These results can be compared to those reported in table 7 for *Rb. capsulatus* .

From this biomass composition and from the metabolic pathways a stoichiometric description of the biomass synthesis can be made involving the building and the resolution of a metabolic matrix.

II Stoichiometries: building and resolution of the metabolic matrix

2.1 - Principles

A biochemical process catalyzed by micro-organisms is composed of a large number of chemical coumpounds and elements. For mathematical applications this number must be reduced to the number of relevant components sufficient for an adequate description of the process.

Two types of macroscopic system description can be encountered (Noorman, 1991): the black box description and the metabolic description

In the black box description, the process is considered as a black box, exchanging only heat and compounds (products and substrates) with the environment, and dissipating Gibbs energy. Linear relations are obtained from conservation principles and Gibbs energy balance. In the black box description, only the chemistry of the conversion process is considered and accordingly, the micro-organism is only specified by its elementary composition.

In the metabolic description, the relevant compounds are involved in various metabolic reactions. In addition to the exchangeable compounds already defined in the black box description, non exchangeable compounds which are only active inside the cell, such as metabolic intermediates, reduction equivalents and ATP, may also be considered. The metabolic approach thus uses the available biochemical knowledge on metabolic reaction in addition to the chemical characteristics.

Although the metabolic description is more difficult to establish than the black box description, this approach as been chosen for the stoichiometries building because it presents several advantages compared to the black box description:

- first, in the metabolic description compounds like macroelements (proteins, fats...) can be included, what is an important aspect for the description of the biomass synthesis in the MELiSSA studies;
- the different aspects of the metabolism can be introduced (anabolism, catabolism, energetic metabolism, maintenance). This allows to determine the relative importance of the different metabolisms (case of the maintenance for nitrifying organisms - TN 23.2), or the quantities of quanta needed for the growth of phototrophic organisms. The metabolic description appeared as a useful tool for the calculation of metabolic flux distribution in an organism from measured net conversion rates (Vallino and Stephanopoulos, 1992, Vallino and Stephanopoulos, 1990).
- the addition of new Carbon or Nitrogen sources is easier in a metabolic description when the general metabolic network , involving, the main pathway of the organism (TCA cycle, pentose pathway...) has been already established.

The metabolic reaction can be depicted in term of matrices and vectors algebra. The bioreaction network equation (Vallino and Stephanopoulos, 1990) represents a metabolite balance and is constructed by determining the time rate of production of change of each metabolite in the network ($r_{metabolite}$) as a function of all the unknown flow (J_i for the i^{th} reaction in the network).

Example of a bioreaction network equation

$A + 2B \longrightarrow C + D$ (J_1)

$B + C \longrightarrow E$ (J_2)

$F + G \longrightarrow B$ (J_3)

$r_B = J_3 - 2.J_1 - J_2$

The accumulation rate of a metabolite in a metabolic network is then given by the summation of all reactions producing that metabolite minus the reactions consuming that metabolite:

$$r_i(t) = \sum_l \alpha_l J_l(t) - \sum_k \alpha_k J_k(t)$$

where α is a stoichiometric coefficient.

The set of equations formed from such balances constructed for each metabolite in the network is represented in matrix notation by

$$A.J(t) = r(t)$$

where A is an (m, n) matrix of stoichiometric coefficients (m metabolic reactions, n metabolites), J(t) the m dimensional flux vector and r(t) the n-dimensional vector of the accumulations rate of the metabolites.

2.2 - Building of the metabolic network: the metabolic matrix

The metabolic reactions involved in the description of the growth of the two organisms studied can be classified in several items:

Anabolism
- General carbon metabolism (Appendix)
  - TCA cycle
  - Embden-Meyerhof pathway
  - Pentose Phosphate pathway
  - Calvin benson cycle (section 1.)
- Amino acids biosynthesis pathways
- Lipids biosynthesis pathway
- Carbohydrates biosynthesis pathway
- DNA and RNA biosynthesis
- Biomass synthesis (*i.e.* its biochemical composition)

Reserve metabolism (section 1)

Energetic metabolism (section 1)

Maintenance

The maintenance can be represented as an ATP hydrolysis

Catabolism

This aspect appears in heterotrophic growth conditions with the breakdown of organic compounds (section 1).

The matrix built from these reactions is a 104 x 114 matrix

2.3 Resolution of the metabolic network and stoichiometries

Exchangeable and non exchangeable compounds notion

The compounds involved in the metabolic network can be classified in two categories: the exchangeable and the non exchangeable compounds.

The non exchangeable compounds are only involved inside the cell. A realist pseudo steady state assumption (Vallino and Stephanopoulos, 1992) is generally made for these compounds *i.e.* their accumulation rate can be set to 0.

The exchangeable compounds are exchanged with the growth medium. On a macroscopic point of view these compounds are the products and the substrates involved in the growth. Their accumulation rates are linked to the stoichiometric yields of the growth.

Principles of matrix resolution and stoichiometry building

The metabolic matrix (M) can be represented by two sub-matrices M_{AC} and M_{NC}:

$$M = \left[\frac{M_{AC}}{M_{NC}}\right]$$

where M_{NC} is the stoichiometric matrix associated to
- the compounds which are implicitly dependent to some other compounds through a chemical or a biochemical balance (as NAD^+ and $NADH,H^+$);
- the compounds for which the accumulation rate, $r_i(t)$, is not know.

The set of metabolic equations can then be represented by :

$$M.J(t) = r(t)$$

$$\begin{cases} M_{AC}.J(t) = r_{AC}(t) \\ M_{NC}.J(t) = r_{NC}(t) \end{cases}$$

In order to establish a stoichiometry, our aim is to calculate the accumulation rate vector $r_{NC}(t)$ for the exchangeable compounds involved in this stoichiometry by solving the following relation:

$$r_{NC}(t) = M_{NC}.J(t)$$

This implies to know the metabolic flux vector J(t). The flux vector J(t) can be calculated only if M_{AC} is an inversible matrix and if $r_{AC}(t)$ is a known accumulation rate vector, by:

$$J(t) = M_{AC}^{-1}.r_{AC}(t)$$

The building of the stoichiometry for the growth and the calculation of the metabolic flux from a set of metabolic equations can then be reduced to the determination of an inversible matrix M_{AC} and of its associated accumulation rate vector $r_{AC}(t)$.

The building of an inversible M_{AC} matrix is made through different steps.

First, the data available for the resolution of the metabolic network are determined. From this knowledge, the M_{NC} matrix, including the exchangeable compounds for which the accumulation rate is not determined, can be built and from the knowledge of the metabolic matrix (M), the M_{AC} matrix can then be deduced. The M_{AC} deduced from M and M_{NC} is generally not a square matrix (and thus not an inversible matrix).

The second step consists in the analysis of this M_{AC} matrix and especially the determination of the number of degrees of freedom of the system. In a third step assumptions were made in order to complete the M_{AC} matrix and then built an inversible matrix.

Finally, the relation presented above can be solved and a stoichiometry can be established.

2.3.1 Autotrophic growth (*Rb. capsulatus*)

Building of M_{AC} and r_{AC} for autotrophic growth (*Rb. capsulatus*)

Step one: determination of known data

Exchangeable compounds: CO_2, sole carbon source
H_2, electron donor for photosynthesis
NH_3, sole N-source
H_2SO_4, sole S-source
H_3PO_4, sole P-source
biomass, as the result of growth
H_2O

Exchangeable compounds accumulation rate: the only known accumulation rate is the carbon substrate rate (CO_2) and it is set to -1 (consumption) as a reference for the calculation of the others rates.

The biomass biochemical composition and the macrocomponents biochemical composition are supposed to be known (see section 2.1).

Step two: description and analysis of M_{AC} and r_{AC}

The M_{AC} matrix is a 101 x 97 matrix. From the 97 compounds involved in its description, CO_2 is the only exchangeable compound. As a consequence, the accumulation rate vector, r_{AC}, is composed of zero values excepted the value of the accumulation of CO_2, set to -1.

The analysis of M_{AC} indicates that there are 4 degrees of freedom.

Step three: completion of M_{AC} (building of an inversible matrix)

Four relations are then necessary in order to obtain an inversible matrix. These relations are established from the following assumptions:

1- maintenance assumption: 50% of ATP produced by ATPase is used by maintenance phenomena

2- the presence of α-ketoglutarate dehydrogenase is not compatible with the carbon fixation via the Calvin-Benson pathway (see TN 21.2)

3- the presence of the PEP carboxylase is not compatible with the carbon fixation via the Calvin-Benson pathway

4- the path glucose-gluconate-ribulose is a parallel pathway of CO_2 fixation by Calvin and of $NADPH,H^+$ regeneration. This path is therefore deleted in the matrix description.

Results

Assuming the biochemical composition proposed in table 9 for *Rb. capsulatus*, the following stoichiometry is calculated from the assumptions presented above

$$CO_2 + 0.0103\ Pi + 0.0042\ H_2SO_4 + 0.2177\ NH_3 + 2.1484\ H_2 \downarrow$$
$$CH_{1.6023}O_{0.3644}N_{0.2177}S_{0.0041}P_{0.0103} + 1.6933\ H_2O$$

The calculation of the metabolic fluxes indicates that:

- a sum of 16.4 quanta are needed for the synthesis of 1 C-mol of biomass. But it must be kept in mind that it is supposed that 1 quantum is sufficient to allow the reaction

$$1\,e^- + Bch \longrightarrow Bch^*$$

- the electron transport involves 2.7 cyclic transfer for each non cyclic transport
- the ratio $\frac{ATP}{\text{reduced power}}$ calculated for the biosynthesis of 1 C-mol of biomass is equal to 2.47.

- the thermodynamic efficiency η_{th} is calculated as defined by Roels (1983)

$$\eta_{th} = \frac{\text{Free enthalpy entering the system}}{\text{Free enthalpy leaving the system}}$$

The value calculated from the equation is 12.5% and can be compared with those determined by Cornet (1994) for Spirulina (between 5% and 15%, the 15% value corresponding to a maximum quantum yield in the photoreactor)

- the real maintenance ratio ($\frac{\text{ATP for maintenance}}{\text{Total ATP synthetized}}$) is 42%, and 3.25 ATP are then needed for the maintenance of 1C-mol of biomass.

2.3.2 Heterotrophic growth (*Rs. rubrum*)

Building of M_{AC} and r_{AC} for heterotrophic growth (*Rs. rubrum*)

Step one: determination of known data

Exchangeable compounds: CO_2, substrate or product
an organic carbon source
NH_3, sole N-source
H_2SO_4, sole S-source
H_3PO_4, sole P-source
biomass, as the result of growth
H_2O

Exchangeable compounds accumulation rate: the only known accumulation rate is the organic carbon substrate uptake rate; it is set to -1 (consumption) as a reference for the calculation of the other rates.

The biomass biochemical composition and the macrocomponents biochemical composition are supposed to be known (see section 2.1). It must be kept in mind that the composition of the biomass changes with the growth conditions. This aspect and its consequences on the stoichiometries will be discussed in section 3.

Step two: description and analysis of M_{AC} and r_{AC}

The M_{AC} matrix is a 102 x 98 matrix. From the 98 compounds involved in its description, the organic carbon substrate is the only exchangeable compound. As a consequence, the accumulation rate vector, r_{AC}, is composed of zero values excepted the value of the accumulation of the organic carbon source, sets to -1.

The analysis of M_{AC}, indicates that there are 4 degrees of freedom.

Step three: completion of M_{AC} (building of an inversible matrix)

Four relations are then necessary in order to obtain an inversible matrix. These relations are established from the following assumptions:

1- maintenance assumption: 3.25 ATP are used for the maintenance of 1 C-mol of biomass (result deduced from the autotrophic growth equation)
2- there is no carbon dioxide fixation via the Calvin-Benson path
3- The shunt of glyoxylate is supposed to be inactive (see section 1.1.2)
4- the number of cycles is fixed to 3.

Results

The changes in the biochemical composition of the biomass reported by Albiol (1994b) for different growth conditions (C/N and light limitations, carbon source) were integrated in the stoichiometries building. The results and the analysis of these integrations are described in the following section. The proposed stoichiometries of the growth of *R. rubrum* in the different C/N conditions studied by Albiol are reported in section 3.3.2.

III Analysis and integration of ESTEC laboratory data

The purpose of this section is to analyse and integrate the experimental data obtained at ESTEC MELiSSA laboratory for the heterotrophic growth of *Rs. rubrum* on acetic acid for different C/N growth conditions.

First, the changes in the biomass biochemical composition are analysed and explained as a function of the limiting growth factor (C, N, light). From an extrapolated complete biochemical composition and from assumptions for the metabolic matrix resolution (see section 2.3.2), stoichiometric equations can then be established for the different growth conditions on acetic acid.

In a second step, some results deduced from stoichiometries (CO_2 production and chemical biomass composition) are compared with the experimental ones.

3.1 Heterotrophic growth conditions and biochemical composition of *Rs. rubrum*

3.1.1 Effects of limitations on biochemical composition

Albiol (1994[b]) presents the protein and the reserves (PHB and glycogen) content of the biomass as function of C/N (figures 9a and 9b).

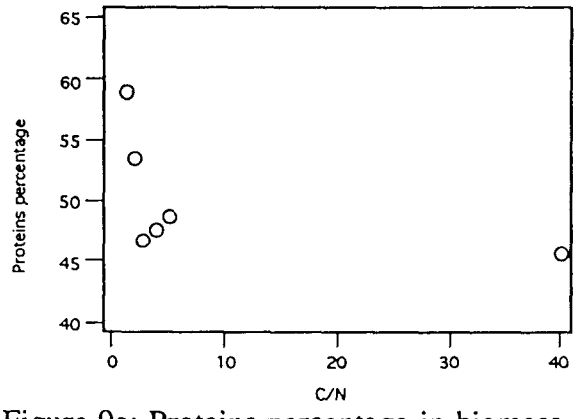


Figure 9a: Proteins percentage in biomass as a function of C/N. Fo=133W/m^2. D=0.034h-1. From Albiol 1994[b].

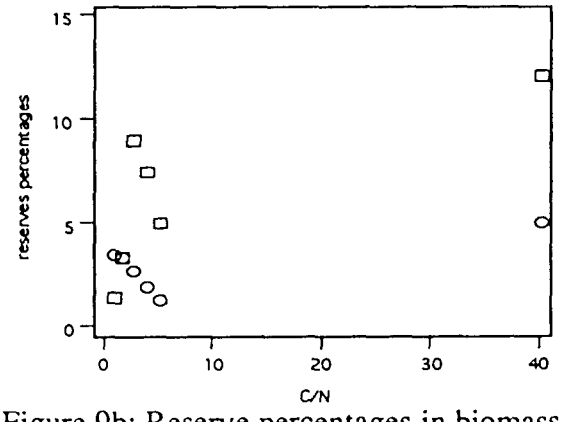


Figure 9b: Reserve percentages in biomass as a function of C/N. Glycogen: O. PHB: □. Fo=133W/m^2. D=0.034h-1. From Albiol 1994[b].

Figure 9a indicates that the protein content increases with the N availability of the medium (low C/N). The profile of the reserves content supposes that an other factor (independent of the C/N ratio) plays an important role in the reserve metabolism. The most probable factor is the light availability.

The experiments (continuous cultures) were made using the same dilution rate and the same illumination conditions (Fo=133 W/m2), but the biomass concentration is not the same resulting of the different acetic acid concentrations feeding the reactor. It can be assumed that for fixed light conditions, the increase in the biomass concentration decreases the light avaibility in the reactor (similar observations can be made in table 3). Then, in such situation the biomass concentration can be used as an indicator of the light availability.

Figures 10 where proteins and reserves are plotted as function of the biomass concentration show the differents effects of C/N and light limitation. For the illuminations conditions used in the experiments, it seems that the light-limiting begins at a biomass concentration of 0.4-0.5 g/l.

A dual limitation can then be assumed: C/N limitation and a light limitation.

However, it must be kept in mind that the C/N limitation can only be considered as a qualitative limitation. Considering a Ks (saturation constant) value of *Rh. capsulatus* for $NH4^+$ of 0.97 mg/l (reported by Albiol ,1994[b]), and considering the concentrations of $NH4^+$ used in these experiments (0.2 g-N/l or 0.02 g-N/l for C/N=40), it can be assumed there is no N-limitation in these experiments. But the Ks value of 0.118 g/l for acetate calculated for batch cultures of *Rs. rubrum* by Albiol (1994[b]), supposed a possible quantitative limitation by acetate for the low values of C/N (0.2 g/l acetic acid for C/N=1). As a consequence, the correlations (see sections 3.1.3) established with these values must be carefully considered.

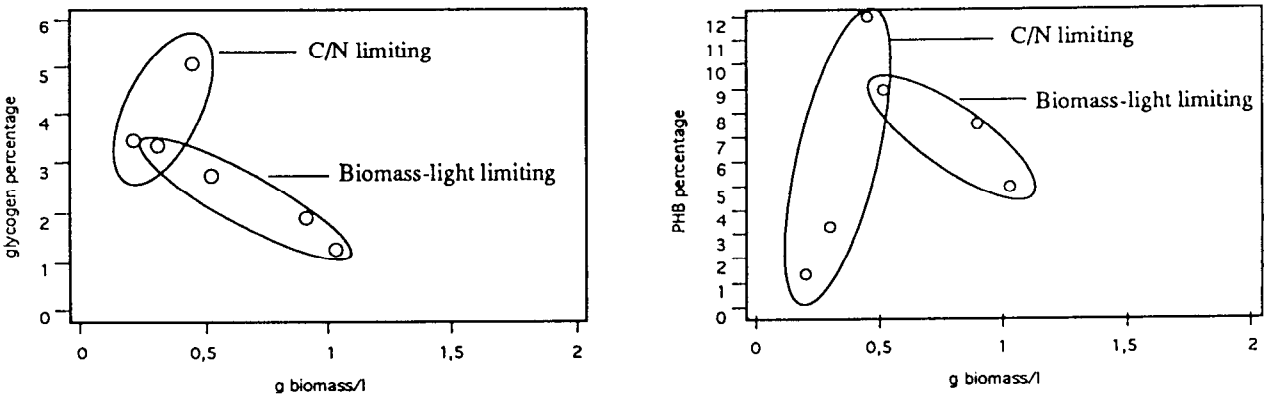


Figure 10a;10b: Reserves percentages plotted as function of the biomass concentration (D=0.034 h^{-1}; Fo=133 W/m^2). The combinated effects of C/N and light (biomass) are shown.

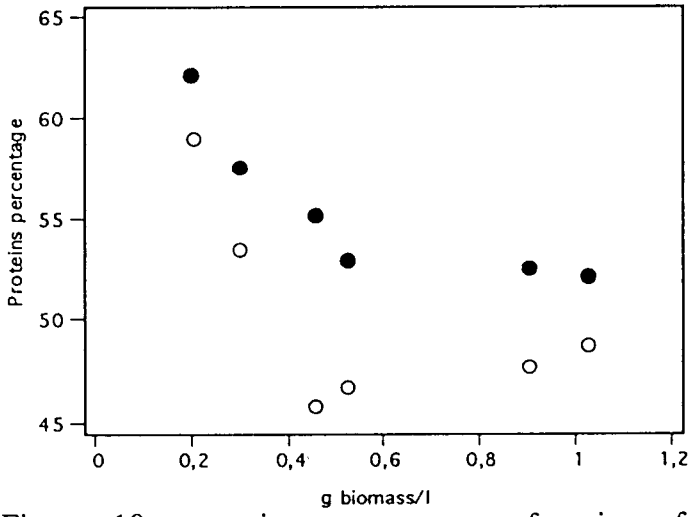


Figure 10c: proteins percentage as function of biomass concentration. ● active biomass. ○ total biomass.

3.1.2 Effect of the carbon source on the reserves forms

The two main carbon reserves forms are glycogen and PHB. The biosynthesis of these reserve material is under the control of the carbon excess or limitation and under the control of the balance free energy (ATP)/reduced power (NAD) of the cell (see section 1.4). For a low ATP/NADH,H+ ratio, the carbon storage is oriented to PHB synthesis, while for a high ratio the carbon storage is oriented to glycogen.

As a consequence, the organic compound of the catabolism of which leads to ATP production [NADH,H+ production], will drive the storage to glycogen [PHB].

For this reason, the ATP/NADH,H+ ratio is indicated for each stoichiometry established.

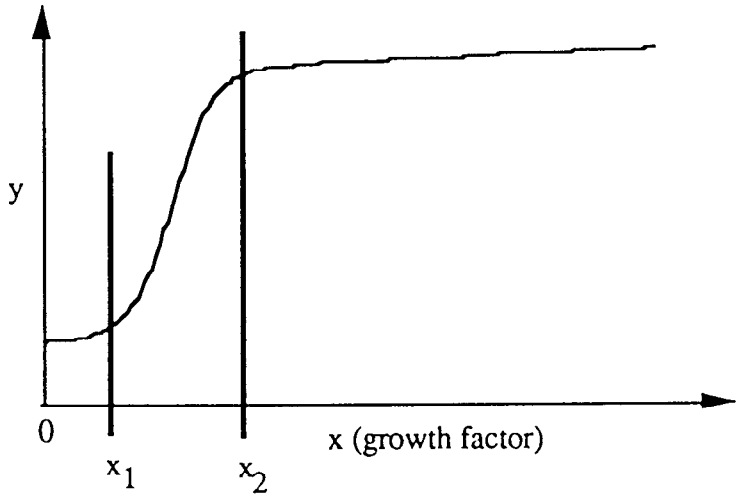
3.1.3 Proposed correlations between biochemical composition and limiting factors

It is presently difficult to link the biochemical composition to the different metabolic behaviours of the cell. Nevertheless correlations for the protein or reserve composition and an expression as a function of the limiting factors (C/N and light) can be established.

C/N limitation

From experimental plots, the following profile can be extrapolated, where y is a macrocomponent of the biomass (reserve, protein) and x a growth parameter (C/N, Biomass-light). This representation is based upon the following assumptions:

- the growth factor has a limited effect until a value x_1
- in the range x_1-x_2, the growth factor has an important effect on y
- obove the limit value x_2, the growth factor has again a limited effect on y.



Such a curve can be drawn using a relation involving both a linear form and a sigmoid form:

$$y = a.x + b + \frac{c.e^{d.x}}{1 + f.e^{d.x}}$$

This relation involves 5 parameters but some of them can be arbitrary set to a specified value in the study. For example in the case of the evolution of glycogen as a function of C/N, c can be set to 0 (the sigmoid form is not observed).

Biomass (light) limitation

The influence of biomass can be introduced as a corrective factor on the biomass composition. This factor can be described by using a relation similar to the one proposed for the C/N correlations:

$$y = \frac{a'.e^{b'.x}}{1 + c'.e^{b'.x}} + d'$$

Identification of the correlation parameters

Because of the very few data used, the parameters must be considered with caution.The * indicates values that have been fixed and not identified. For proteins the correlations have been determined on the mass percentage of proteins in the active biomass

Table 12: fitted parameters of the correlations

	Protein (active biomass)		Glycogen		PHB	
	f(C/N)	f(biomass)	f(C/N)	f(biomass)	f(C/N)	f(biomass)
a	0 *	-0.000014	0.041	12.61	0.051	236.55
b	63 *	16.662	3.459	-3.10	0 *	-5,17
c	-0.026	0.00025	0 *	12.61	0.024	236.55
d	3.739	1 *	0 *	0 *	3.084	0 *
f	0.0033		0 *		0.0024	

Comparison between experimental and correlated values

From the previous correlation, the biomass composition can be calculated from the C/N and the biomass concentrations (for Fo=133W/m2), by the relation biomass%=f(C/N).f(biomass). The results are reported in the following table.

Table 13: comparison experimental and calculated biomass composition in dual light and C/N limiting growth.

		% Proteins (total biomass)		% Glycogen		% PHB	
Biomass g/l	C/N	exp.	correlation	exp.	correlation	exp.	correlation
0.2	1	59	58.98	3.5	3.55	1.4	0.70
0.3	1.75	53.6	53.49	3.4	3.43	3.5	3.81
0.52	2.5	46.8	47.25	2.8	2.97	9	7.95
0.9	3.75	47.7	47.19	1.9	1.84	7.5	6.91
1.02	5	48.9	48.80	1.3	1.49	5	5.53
0.45	40	45.8	44.99	5.1	4.60	12	11.53

3.1.4 Estimation of the complete biomass composition

Despite the protein and the reserve content of the biomass has been measured in ESTEC experiment, the complete biochemical composition is still not known.

In order to defined a complete biomass composition, a mean composition of an active biomass is supposed:

Protein	52%
Carbohydrates	13%
Lipids	14,5%
DNA	4%
RNA	9%
Ash	7.5%

Assuming the changes in the protein content with the growth conditions, the composition of the other compounds is calculated from the mean composition in order to conserve the same ratio between these compounds. The estimated biomass composition as a function of C/N and biomass concentration calculated from the experimental data reported by Albiol are presented in table 14. It can be noted that the computed values are in the experimental ranges reported in table 8.

Table 14: Biochemical composition of *Rs. rubrum*. Growth conditions: acetic acid, $D=0.034h^{-1}$, $Fo=133W/m^2$. * indicates data taken from Albiol 1994[b].

C/N *	biomass (g/l) *	Protein *	Carbohydrates	Lipids	DNA	RNA	Glycogen *	PHB *	Ash
1	0.2	59	9.78	10.91	3.01	6.77	3.50	1.40	5.64
1.75	0.3	53.6	10.70	11.93	3.29	7.41	3.40	3.50	6.17
2.5	0.52	46.8	11.21	12.51	3.45	7.76	2.80	9.00	6.47
3.75	0.9	47.7	11.62	12.96	3.58	8.04	1.90	7.50	6.70
5	1.02	48.9	12.13	13.53	3.73	8.40	1.30	5.00	7.00
40	0.45	45.8	10.05	11.21	3.09	6.96	5.10	12.00	5.80

3.2 Stoichiometries

3.2.1 Stoichiometries for the different growth conditions on acetic acid

From the different biochemical compositions reported in table 14, the metabolic matrix for heterotrophic growth can be established and resolved considering the hypotheses presented in section 2.3.2.

The following stoichiometries are then obtained for the different continuous growth conditions on acetic acid ($D=0.034h^{-1}$ and $Fo=133\ w/m^2$).

C/N=1

$$\text{Acetic acid} + 0.4177\ NH_3 + 0.0227\ H_3PO_4 + 0.0075\ H_2SO_4 \downarrow$$

$$1.8681\ CH_{1.5938}O_{0.3627}N_{0.2236}S_{0.0040}P_{0.0121} + 0.1319\ CO_2 + 1.1794\ H_2O$$

The metabolic fluxes indicate that:

- a sum of 13.38 quanta are involved in the reaction;
- the ratio $\frac{ATP}{\text{reduced power}}$ calculated for this biosynthesis is equal to 0.904;
- the thermodynamic efficiency η_{th} calculated is equal to 24.05%
- the maintenance represents 53.7% of the total ATP synthesised.

C/N=1.75

$$\text{Acetic acid} + 0.3985\ NH_3 + 0.0248\ H_3PO_4 + 0.0068\ H_2SO_4 \downarrow$$

$$1.8607\ CH_{1.5945}O_{0.3707}N_{0.2142}S_{0.0037}P_{0.0134} + 0.1393\ CO_2 + 1.1584\ H_2O$$

The metabolic fluxes indicate that:

- a sum of 13.21 quanta are involved in the reaction;
- the ratio $\frac{\text{ATP}}{\text{reduced power}}$ calculated for this biosynthesis is equal to 0.890;
- the thermodynamic efficiency η_{th} calculated is equal to 24.28%
- the maintenance represents 53.0% of the total ATP synthesised.

C/N=2.5

$$\text{Acetic acid} + 0.3650\ NH_3 + 0.0259\ H_3PO_4 + 0.0059\ H_2SO_4 \downarrow$$

$$1.8497\ CH_{1.5913}O_{0.3818}N_{0.1973}S_{0.0032}P_{0.0140} + 0.1503\ CO_2 + 1.1205\ H_2O$$

The metabolic fluxes indicate that:

- a sum of 12.93 quanta are involved in the reaction;
- the ratio $\frac{\text{ATP}}{\text{reduced power}}$ calculated for this biosynthesis is equal to 0.900;
- the thermodynamic efficiency η_{th} calculated is equal to 24.72%
- the maintenance represents 53.6% of the total ATP synthesised.

C/N=3.75

$$\text{Acetic acid} + 0.3745\ NH_3 + 0.0269\ H_3PO_4 + 0.0061\ H_2SO_4 \downarrow$$

$$1.8495\ CH_{1.5926}O_{0.3760}N_{0.2025}S_{0.0033}P_{0.0145} + 0.1505\ CO_2 + 1.1353\ H_2O$$

The metabolic fluxes indicate that:

- a sum of 12.98 quanta are involved in the reaction;
- the ratio $\frac{\text{ATP}}{\text{reduced power}}$ calculated for this biosynthesis is equal to 0.90;
- the thermodynamic efficiency η_{th} calculated is equal to 24.63%
- the maintenance represents 53.6% of the total ATP synthesised.

C/N=5

$$\text{Acetic acid} + 0.3876\ NH_3 + 0.0282\ H_3PO_4 + 0.0062\ H_2SO_4 \downarrow$$
$$1.8505\ CH_{1.5951}O_{0.3699}N_{0.2094}S_{0.0034}P_{0.0152} + 0.1495\ CO_2 + 1.1540\ H_2O$$

The metabolic fluxes indicate that:

- a sum of 13.06 quanta are involved in the reaction;
- the ratio $\frac{\text{ATP}}{\text{reduced power}}$ calculated for this biosynthesis is equal to 0.905;
- the thermodynamic efficiency η_{th} calculated is equal to 24.50%
- the maintenance represents 53.1% of the total ATP synthesised.

C/N=40

$$\text{Acetic acid} + 0.3467\ NH_3 + 0.0231\ H_3PO_4 + 0.0058\ H_2SO_4 \downarrow$$
$$1.8521\ CH_{1.5884}O_{0.3943}N_{0.1872}S_{0.0031}P_{0.0125} + 0.1479\ CO_2 + 1.0896\ H_2O$$

The metabolic fluxes indicate that:

- a sum of 12.86 quanta are involved in the reaction;
- the ratio $\frac{\text{ATP}}{\text{reduced power}}$ calculated for this biosynthesis is equal to 0.910;
- the thermodynamic efficiency η_{th} calculated is equal to 24.88%
- the maintenance represents 54.0% of the total ATP synthesised.

3.2.2 Stoichiometries for different carbon sources

Rs. rubrum is able to growth on different carbon sources (tables 2 and 4).

The data obtained on acetic acid have been compared to those obtained on lactic acid by Viprey (1994). From this comparison, Albiol (1994[b]) deduced that the C/N of the medium governs the C/N of the biomass, and that changes in the carbon source have not a strong influence on the biomass composition.

As a first assumption, it can be then supposed that for the same growth conditions (C/N, light, dilution rate...) on different carbon sources, the biomass composition remains constant. But it must be kept in mind that in fact, as reported in section 1.4, the type of the carbon source will probably change the ratio PHB/glycogen.

The following stoichiometries have been established for the biomass composition calculated for the growth on acetic acid with a C/N of 5.

Propionate as carbon source:

$$\text{Propionic acid} + 0.6782\ NH_3 + 0.0493\ H_3PO_4 + 0.0102\ H_2SO_4 + 0.2383\ CO_2 \downarrow$$
$$3.2383\ CH_{1.5951}O_{0.3699}N_{0.2094}S_{0.0034}P_{0.0152} + 2.5195\ H_2O$$

The metabolic fluxes indicate that:

- a sum of 22.54 quanta are involved in the reaction;
- the ratio $\frac{ATP}{\text{reduced power}}$ calculated for this biosynthesis is equal to 0.97;
- the maintenance represents 58.44% of the total ATP synthesised.

Butyric acid as carbon source:

$$\text{Butyric acid} + 0.9689\ NH_3 + 0.0704\ H_3PO_4 + 0.0156\ H_2SO_4 + 0.6261\ CO_2 \downarrow$$
$$4.6261\ CH_{1.5951}O_{0.3699}N_{0.2094}S_{0.0034}P_{0.0152} + 1.8850\ H_2O$$

The metabolic fluxes indicate that:

- a sum of 30.73 quanta are involved in the reaction;
- the ratio $\frac{ATP}{\text{reduced power}}$ calculated for this biosynthesis is equal to 0.59;
- the maintenance represents 30.71% of the total ATP synthesised.

Lactate as carbon source:

$$\text{Lactic acid} + 0.5813\ NH_3 + 0.0422\ H_3PO_4 + 0.0093\ H_2SO_4 \downarrow$$
$$2.7757\ CH_{1.5951}O_{0.3699}N_{0.2094}S_{0.0034}P_{0.0152} + 1.7310\ H_2O + 0.2243\ CO_2$$

The metabolic fluxes indicate that:

- a sum of 15.10 quanta are involved in the reaction;
- the ratio $\frac{ATP}{\text{reduced power}}$ calculated for this biosynthesis is equal to 1.00;
- the maintenance represents 65.70% of the total ATP synthesised.

Malate as carbon source:

$$\text{Malic acid} + 0.5813\ NH_3 + 0.0422\ H_3PO_4 + 0.0093\ H_2SO_4 \downarrow$$
$$2.7757\ CH_{1.5951}O_{0.3699}N_{0.2094}S_{0.0034}P_{0.0152} + 1.7310\ H_2O + 1.2243\ CO_2$$

The metabolic fluxes indicate that:

- a sum of 13.81 quanta are involved in the reaction;
- the ratio $\frac{ATP}{\text{reduced power}}$ calculated for this biosynthesis is equal to 1.08;
- the maintenance represents 66.74% of the total ATP synthesised.

3.3 Comparison stoichiometries - experimental measurements

The conversion yields calculated from the stoichiometries can be compared to the different experimental measurement. The comparison stoichiometries-experimental measurements is here focused on two aspects: the CO_2 production for different growth conditions and the chemical biomass composition.

3.3.1 CO_2 production

From the measurement of the biomass produced (and biomass composition) and from the measurement of the carbon consumed, Albiol (1994[b]) estimated the quantity of CO_2 produced for different growth conditions on acetic acid, assuming that the only form of carbon assimilation are the biomass and CO_2. The results of these estimations are reported in table 15.

Table 15: Estimation of CO_2 production

Acetic acid (g/l)	Acetic acid consumed (mol/mol BM)	CO_2 (mol/mol BM)	ratio (mol CO_2/mol acet.)
0.2	1.0	1.0	1.0
0.35	1.2	1.4	1.2
0.5	0.92	0.85	0.9
0.75	0.72	0.44	0.6
1	0.79	0.59	0.7

The estimated ratio CO_2/acetic acid is higher than that reported by Ormerod and Gest (1962) (table 5). The ratio of 0.17 reported by Ormerod can be compared to the stoichiometric ratios calculated from the equations of the previous section and which are in the range of 0.13-0.15.

Moreover the comparison of the ratio CO_2/carbon source for different substrates (table 16) improves the validity of the different stoichiometries established.

Table 16: Comparison of the ratio CO_2/carbon source

Substrate	Ormerod and Gest (1962)	Stoichiometries
Acetic acid	0.17	0.15
Butyric acid	-0.74	-0.62
Malic acid	1.22	1.22
Lactic acid	0.29	0.22

According to Albiol, the excess of the estimated CO_2 production can be due to the production of, at least, one extracellular carbon compound. Albiol notes that the strain studied produces proteins, especially a carbonic anhydrase.

By calculating a C and N balance (table 17), the hypothesis of the production of C+N compounds (proteins, amino acids) is confirmed.

Table 17: Comparison between N and C consumed and N and C assimilated in the biomass and CO_2 (an average of 0.15 CO_2/acet. is supposed). Experimental data are taken from Albiol (1994b).

C g/l	N g/l	C consumed g/l	N consumed g/l	N assimilated (bio) g/l	N ass/N cons	C assimilated (bio+CO2) g/l	C ass/ C cons
0.2	0.2	0.2	0.035	0.021	**0.610**	0.099	**0.497**
0.35	0.2	0.35	0.072	0.030	**0.414**	0.147	**0.419**
0.5	0.2	0.475	0.11	0.044	**0.402**	0.260	**0.547**
0.75	0.2	0.63	0.15	0.083	**0.554**	0.450	**0.714**
1	0.2	0.825	0.155	0.100	**0.645**	0.545	**0.661**

3.3.2 Comparison between observed and stoichiometric chemical compositions

The chemical composition of the biomass calculated for the stoichiometries is reported in table 18 for the different growth conditions on acetic acid. These chemical compositions can be compared with those determined for the same growth conditions by Albiol (1994b).

Table 18: chemical biomass composition. Stoichiometries (st) vs. experiments (exp)

C/N medium	1		1.75		2.5		3.75		5		40	
	st	exp	st	exp	st	exp	st	exp	st	exp	st	exp
C	1	1	1	1	1	1	1	1	1	1	1	1
H	1.5938	1.797	1.5945	1.84	1.5913	1.80	1.5926	1.828	1.5951	1.75	1.5884	1.75
O	0.3627	0.452	0.3707	0.409	0.3818	0.454	0.3760	0.431	0.3699	0.410	0.3943	0.445
N	0.2236	0.197	0.2142	0.187	0.1973	0.158	0.2025	0.171	0.2094	0.170	0.1872	0.159
S	0.0040	0.0037	0.0037	0.0029	0.0032	0.0026	0.0033	0.0029	0.0034	0.0021	0.031	0.0018
P	0.0121	0.0232	0.0134	0.025	0.0140	0.0111	0.0145	0.0079	0.0152	0.0084	0.0125	0.0301
Ash %	5.64	4.89	6.17	9.48	6.47	8.8	6.70	9.6	7.00	5.17	5.8	5.9
C/N biomass	3.83	4.35	4.00	4.58	4.34	5.42	4.23	5.01	4.08	5.04	4.5	5.39

From experimental measurements the N biomass content in stoichiometries seems overestimated, while the H and O content are underestimated. That can be due to the assumption on the carbohydrates content of the biomass and of the composition of carbohydrates (it is presently assumed that carbohydrates are equivalent to peptidoglycan, the formula of which is $CH_{1.5404}O_{0.5135}N_{0.1892}$).

But the bad estimations on carbohydrates are not sufficient to explain the totality of the difference between stoichiometic composition and experimental measurements.

As an example, for a C/N in the medium of 1, if it was supposed that carbohydrates are only a polymer of glucose, the C/N of the biomass would be 4.10 (instead of 3.83).

CONCLUSION

The method developed to establish the stoichiometries of compartment II (phototrophs) and of the compartment III (nitrifying - see TN 23.2) has been presented. Besides the stoichiometric yields of the reaction, this approach allows the calculation of some metabolic characteristics of the cell that usually did not appear in a stoichiometry, such as the maintenance, the energy balance or the thermodynamic efficiency. By this method, stoichiometries for different growth conditions (C/N) of *Rs. rubrum* on acetic acid have been established.

In order to establish the stoichiometries, a biochemical composition of the biomass was needed. For *Rs. rubrum*, only a part of this composition was known, then the unknown part (lipids, carbohydrate, RNA and DNA) was estimated. Moreover, the composition of these macrocomponents, mainly lipids, carbohydrate and RNA, was supposed. The sum of these assumptions leads to an overestimation of the N content in the biomass (underestimation of the C/N ratio), but at the present time no data were found to correct this error. Nevertheless, it has been noted that the mean amino-acid composition of proteins chosen to built the stoichiometries (table 11) , gives a N content of about 10.3% (considering only proteins) and of about 11.2% (considering all N-macrocomponents) of the dry matter of the biomass. But the total N content determined by Kobayashi (from which this mean amino acid composition is taken) is 9.75%. It can then be assumed that the amino acid composition is the main factor of deviation between experimental and calculated N composition of the biomass.

The carbon and nitrogen balances calculated from the experiments reported by Albiol (1994[b]) show that it is necessary to consider that unknown C-N compounds are synthesised. However, it seems surprising that the mean C/N ratio of the not measured C-N compounds is close to 1.

Despite the differences between the experimental and the stoichiometric biomass, the yield of the CO_2 production for different carbon sources is in agreement with experimental values.

Correlations giving the biomass composition of *Rs. rubrum* for the growth conditions presented by Albiol have been proposed. These correlations must be improved by further experiments and, as previously suggested, must be considered carefully. For studying the

qualitative effect of C/N on biomass composition, experiments needs to be leaded in absence of other limitations (light, quantitative limitation by a substrate). Similar studies can be leaded for the effects of light.

The storage material (PHB, glycogen) appears to be under the control of two factors (wich can themself depend on the different growth condition):

- the type of substrate;
- the internal energetic balance of the cell. This balance depends on the type of the substrate, but depends too on the growth conditions.

REFERENCES

Aiking H. and Sojka G.(1979) "Response of *Rhodopseudomonas capsulatus* to illumination and growth rate in a light-limited continuous culture" J. of Bacteriology. Vol. 139. N°2. pp 530-536.

Albers H. and Gottshalk G. (1976) "Acetate metabolism in Rhodopseudomonas gelatinosa and several other Rhodospirillaceae" Arch. Microbiol. 111. p45

Albiol J (1994b) "Study of the MELiSSA photoheterotrophic compartment: kinetics effects of C limitation. (ESA/YCL/2148.JAS) ESA-EWP-1808.

Albiol J., Gicqueau B. and Lasseur C. (1994[a]) "Preliminary studies on the performance and behaviour of the MELiSSA photoeterotrophic compartment" Proceeding of the 24th. ICES. Friedrishshafen. Germany.

Buchanan B. B., Evans M. C. W. and Arnon D. I. (1967). "Ferredoxin-linked carbon assimilation in *Rhodospirillum rubrum*" Arch.für Mikrobiol. 59. pp 32-40

Cardenas J., Caballero F. J., Moreno-vivian C. and Castillo F. (1987) "Enzymology of ammonia assimilation in nonsulfur bacteria" Enzymes of ammonium metabolism. 25. pp 148-153.

Christiansens H. (1991) " The growth of pure cultures of Rhodospirillum rubrum and Rhodobacter capsulata on the supernatant of the liquefying compartment" TN 7.1. MELiSSA Contract 8125/NL/FG.

Cornet J. F. Dussap C. G. and Gros J. B. (1994) "Conversion of radian light energy in photobioreactors" AIChE journal, Vol. 40, N° 6. pp 1055-1066.

Doelle H. W. (1975) Bacterial metabolism second edition. Academic press.

Driessens K., Liessen J., Masduki S., Verstraete W., Nelis H and De Leenheer A. (1987) "Production of *Rhobacter capsulatus* ATCC 23782 with short residence time in a continuous flow photobioreactor". Process Biochemistery. pp 160-164

Dussap C. G.(1991) "Study of the metabolism of Clostridia, Rhodospirillum and Rhodobacter" TN 9.3. MELiSSA Contract 8125/NL/FG.

Gicqueau B. (1993). "MELiSSA: culture continue de *Rhodobacter capsulatus*. Etude de sources carbonées et influence de la lumière". ESA-953. YCL 1775.BG.

Glover J., Kamen M. D. and Van Genderen H. (1951) "Studies on the metabolism of photosynthetic bacteria: XII Comparative light and dark metabolism of acetate and carbonate by *Rs. rubrum*" Arch. Biochem. Biophys. 35. pp. 384-408.

Gottshalk G. (1986) Bacterial metabolism 2nd Ed. pp. 302-307.

Imhoff J. F. and Trüper H. G. (1989) "Purple non sulfur bacteria" in Bergey's manual of systematic bacteriology . 9th edition. Vol. 3. pp 1658-1673.

Kobayashi M. and Kurata S. (1978) " Mass culture and cell utilization of photosynthetic bacteria" Process Biochem. 13. pp. 27-30

Merrick J. M (1978). "Metabolism of reserve material" in The photosynthetic bacteria. Clayton R. K. and Sistrom W. R editors. Plenum Press. pp 199-219.

Moreno-Vivian C., Caballero J., Cardenas J. Castillo F. (1989) "Effects of the C/N balance on the regulation of nitrogen fixation in *Rhodobacter capsulatus* E1F1". Biochemica et Biophysica Acta. Vol. 977. pp. 297-300.

Noorman H. (1991) Methodology on monitoring and modelling of microbial metabolism. Thesis of the Delft university of technology. Netherlands.

Ohman E. (1979) Encyclopedia of plant physiology. Springer Verlag. New-York. Vol. 6. pp 54-67.

Ormerod J. G. and Gest H. (1962) "Hydrogen photosynthesis and alternative metabolic pathway in photosynthetic bacteria" in Symposium on metabolism of inorganic compounds. Metabolic pathway in photosynthetic bacteria Vol 26 pp 51-62.

Pfennig N.and Trüper H. G. (1971) "The Rhodospirillales (phototrophic or photosynthetic bacteria)" in Handbook of microbiology, Vol 1. pp. 17-27.

Pons A. Dussap C. G. Péquinot C. and Gros J. B. (1995) "Metabolic flux distribution in *Corynebacteium melassecola* ATCC17965 for various carbon sources" Submitted to Biotech. and Bioeng.

Roels J. A. (1983) "Energetics and kinetics in biotechnology" Elsevier Biomedical Press

Sojka G. A. (1978) "Metabolism of non aromatic organic compounds" in The photosynthetic bacteria. Clayton R. K. and Sistrom W. R editors. Plenum Press.. pp 708-717.

Suhaimi M., Liessen J. and Verstraete W. (1987) "NH_4^+-N assimilation by *Rhodobacter capsulatus* ATCC 23782 grown axenically and non-axenically in N and C rich media" J. App. Bacteriol. 62. pp 53-64

Vallino J. J. and Stephanopoulos G. (1990) "Flux determination in cellular bioreaction networks: applications to lysine fermentation" Frontiers in Bioprocessing. Sikdar S. K., Bier M. and Todd P. editors. CRC press. pp 206-219.

Vallino J. J. and Stephanopoulos G. (1992) "Metabolic flux distribution in *Corynebacterium glutamicum* during growth and lysine overproduction" Biotech. and Bioeng. Vol 41. pp 633-646.

Viprey E. (1994) "MELiSSA: *Rhodospirillum rubrum*. Etude de la limitation azotée". ESA-X-1102.

Vrati S. (1984) "Single cell protein production by Photosynthetic bacteria grown on the clarified effluent of biogas plant" Appl. Microbiol. Biotechnol. 19. pp 199-202.

Willison J. C. (1988) "Pyruvate and acetate metabolism in the photosynthetic bacterium *Rhodobacter capsulatus*" J. Gen. Microbiol. 134. pp 2429-2439.

APPENDIX A

Stoichiometric description of the metabolic network

Carbon source

CO_2 (calvin cycle)
Organic compound

Central Metabolic Pathways.

Emden-Meheyrhof (glycolysis)

glucose-6-P → fructose-6-P
fructose-6-P + ATP → fructose-1,6-diP + ADP
fructose-1,6-diP → glyceraldehyde-3-P + dihydroxyacetone-P
dihydroxyacetone-P → glyceraldehyde-3-P
glyceraldehyde-3-P + NAD^+ + ADP + Pi → 3-P-glycerate + $NADH,H^+$ + ATP
3-P-glycerate → P-enolpyruvate + H_2O
P-enolpyruvate + ADP → pyruvate + ATP
pyruvate + NAD^+ + CoASH → acetyl-CoA + CO_2 + $NADH,H^+$

Tri-Carboxylic acids cycle

acetyl-CoA + AMP + PPi → acetate + ATP + CoASH
OAA + acetyl-CoA + H_2O → isocitrate + CoASH
isocitrate + NAD^+ → a-ketoglutarate + CO_2 + $NADPH,H^+$
α-ketoglutarate + NAD^+ + CoASH → succinyl-CoA + CO_2 + $NADH,H^+$
succinyl-CoA + ADP + Pi → succinate + ATP + CoASH
succinate + FAD → fumarate + $FADH_2$
fumarate + H_2O → malate
malate + NAD^+ → oxaloacetate (OAA) + $NADH,H^+$

Pentose pathway

glucose-6-P + $2NADP^+$ + H_2O → ribose-5-P + 2 $NADPH,H^+$ + CO_2
2 ribose-5-P → fructose-6-P + erythrose-4-P
ribose-5-P + erythose-4-P → fructose-6-P + glyceraldehyde-3-P

Anaplerotic Reaction

Glyoxylate shunt

acetyl-CoA + isocitrate + H_2O → succinate + malate + CoASH

PEP carboxylase pathway

P-enolpyruvate + CO_2 + H_2O → oxaloacetate + Pi

Anabolism network reactions

Amino Acids synthesis

α–ketoglutarate + NH_3 + $NADPH,H^+$ → **glutamate** + $NADP^+$ + H_2O

glutamate + NH_3 + ATP → **glutamine** + ADP + Pi

glutamate + ATP + $NADPH,H^+$ + $NADH,H^+$ → **proline** +ADP +Pi + $NADP^+$ +NAD^+.+ H_2O

glutamate + glutamine + aspartate + acetyl-CoA + CO_2 + 4ATP + $NADH,H^+$ + $3H_2O$
→ **arginine** + a-ketoglutarate + acetate + NAD^+ + fumarate + AMP + PPi + 3ADP + 3Pi +CoASH

oxaloacetate + glutamate → **aspartate** + a-ketoglutarate

aspartate + NH_3 + ATP → **asparagine** + ADP + Pi

aspartate + succinylCoA + pyruvate + glutamate + ATP + $NADPH,H^+$ + $NADH,H^+$
→ diaminopimelate + succinate + a-ketoglutarate + CoASH + ADP + Pi + $NADP^+$ + NAD^+

diaminopimelate → **lysine** + CO_2

aspartate + cysteine + succinyl-CoA + CH_3-THF + 2 $NADH,H^+$ + ATP + H_2O
→ **methionine** + succinate + pyruvate + NH_3 + ADP + Pi + 2 NAD^+ + THF + CoASH

aspartate + 2 $NADH,H^+$ + 2 ATP + H_2O → **threonine** + 2 ADP + 2 Pi + 2 NAD^+

threonine + glutamate + pyruvate + $NADPH,H^+$
→ **isoleucine** + a-ketoglutarate + NH_3 + CO_2 + H_2O + $NADP^+$

pyruvate + glutamate → **alanine** + a-ketoglutarate

pyruvate + glutamate + $NADPH,H^+$ → **valine** + a-ketoglutarate + $NADP^+$ + CO_2 + H_2O

2pyruvate + glutamate + acetyl-CoA + $NADPH,H^+$ + NAD^+
→ **leucine** + a-ketoglutarate + $2CO_2$ + CoASH + $NADP^+$ + $NADH,H^+$

3Pglycerate + glutamate + NAD^+ + H_2O → **serine**+ a-ketoglutarate + $NADH,H^+$ + Pi

serine + THF → **glycine** + CH_2-THF + H_2O

serine + acetyl - CoA + H_2SO_4 + 2ATP + 4 $NADPH,H^+$
→ **cysteine** + acetate + CoASH + $2H_2O$ + 4 $NADP^+$ + ADP + Pi + AMP + PPi

ribose-5-P + ATP → 5'-Pribosyl-1PP + AMP

5'-Pribosyl-1PP + erythrose-4P + glutamine + serine + 2 P-enolpyruvate + ATP + $NADPH,H^+$
→ **tryptophan** + glutamate + pyruvate + glyceraldehyde-3P + ADP + PPi + 4Pi + $NADP^+$ + CO_2 + $2H_2O$

erythrose-4P + glutamate + 2 P-enolpyruvate + ATP + $NADPH,H^+$
→ **phenylalanine** + a-ketoglutarate + $NADP^+$ + ADP + 4Pi + CO_2 + H_2O

erythrose-4P + glutamate + 2 P-enolpyruvate + ATP + NAD^+ + $NADPH,H^+$
→ **tyrosine** + a-ketoglutarate + $NADP^+$ + ADP + 4Pi + CO_2 + $NADH,H^+$

5'-Pribosyl-1PP + glutamine + ATP + 2 NAD^+ + 3 H_2O
→ **histidine** + a-ketoglutarate + ACR + Pi + 2 PPi + 2 $NADH,H^+$

Nucleotides synthesis

5'-Pribosyl-1PP + aspartate + glutamine + 2ATP + H_2O + NAD^+
→ UMP + glutamate + 2ADP + 2Pi + PPi + $NADH,H^+$

UMP + ATP → UDP + ADP

UDP + ATP → UTP + ADP

$UTP + NH_3 + ATP \rightarrow CTP + ADP + Pi$

$CMP + ATP \rightarrow CTP + AMP$

$CTP + H_2O \rightarrow CDP + Pi$

5'-Pribosyl-1PP + 2 glutamine + aspartate + glycine + 4 ATP + CO_2 + 2 H_2O + CHO-THF
→ ACR + 2 glutamate + fumarate + 4 ADP + 4 Pi + PPi + THF

ACR + aspartate + GTP + CHO-THF → AMP + fumarate + GDP + Pi + H_2O + THF

ACR + NH_3 + 2ATP + NAD^+ + CHO-THF → GDP + ADP + AMP + PPi + $NADH,H^+$ +THF

$GDP + ATP \rightarrow GTP + ADP$

UDP + 2 ATP + CH_2-THF + $NADPH,H^+$ → dTTP + 2 ADP + Pi + DHF + $NADP^+$

CDP + ATP + $NADPH,H^+$ → dCTP + ADP + $NADP^+$ + H_2O

ATP + $NADPH,H^+$ → dATP + $NADP^+$ + H_2O

GDP + ATP + $NADPH,H^+$ → dGTP + ADP + $NADP^+$ + H_2O

Peptidoglycan's bricks synthesis

fructose-6-P + glutamine + acetyl-CoA + UTP
→ UDP-Nacetylglucosamine + glutamate + CoASH + PPi

UDP-Nacetylglucosamine + glutamate + 3 alanine + P-enolpyruvate + $NADPH,H^+$
+ diaminopimelate + 4 ATP → UDP-Nacetylpentapeptide + $NADP^+$ + 4 ADP + 5 Pi

Average phosphatidic acid synthesis

dihydroxyacetoneP + $NADPH,H^+$ → glycerol-3-P + $NADP^+$

8 acetyl-CoA + 14 $NADPH,H^+$ + 7 ATP + ACPH
→ palmitic-ACP + 14 $NADP^+$ + 7 ADP + 7 Pi + 8 CoASH

palmitic-ACP + $NADP^+$ → palmitoleic-ACP + $NADPH,H^+$

palmitoleic-ACP + 2 $NADPH,H^+$ + ATP + acetyl-CoA
→ vaccenic-ACP + 2 $NADP^+$ + ADP + Pi + CoASH

glycerol-3-P + 0.678 palmitoleic-ACP + 0.878 palmitic-ACP + 0.444 vaccenic-ACP
→ **Phosphatidic $acid_{av}$ (P)** + 2 ACPH

Phosphatidic $acid_{av}$ + serine + CTP → **P**-ethanolamine + CO_2 + CMP + PPi

Phosphatidic $acid_{av}$ + glycerol-3P + CTP → **P**-glycerol + CMP + PPi + Pi

2 Phosphatidic $acid_{av}$ + glycerol-3P + 2 CTP → di**P**-glycerol + 2 CMP + 2 PPi

Cofators regeneration

THF + CO_2 + NADH, H^+ → CHO-THF + NAD^+ + H_2O

CHO-THF → CH_2=THF + H_2O

CH_2=THF + $NADPH,H^+$ → CH_2-THF + $NADP^+$

CH_2-THF + $NADH,H^+$ → CH_3-THF + NAD^+

DHF + $NADPH,H^+$ → THF + $NADP^+$

Energetic metabolism reactions

maintenance

$ATP + H_2O \rightarrow ADP + Pi$

Cofactors regeneration

$AMP + ATP \rightarrow 2\ ADP$

$PPi + H_2O \rightarrow 2\ Pi$

Photosynthesis (see section 1.6)

transhydrogenase

$NADH,H^+ + NADP^+ \rightarrow NAD^+ + NADPH,H^+$

Synthesis of biomass macromolecules

RNA

$0.262\ ATP + 0.216\ UTP + 0.322\ GTP + 0.2\ CTP \rightarrow RNA_{av} + PPi$

Lipids

0.7778 P-ethanolamine + 0.201 P-glycerol + 0.0212 diP-glycerol $\rightarrow Phospholipid_{av}$

Peptidoglycan

UDP-Nacetylglucosamine + UDP-Nacetylpentapeptide

$\rightarrow$ $Peptydoglycan_{av}$ + alanine + UMP + UDP + Pi

Reserves synthesis

See section 1.6

APPENDIX B

Profiles of the percentage of PHB in biomass computed by using the correlation developed in section 3.1.3