MELISSA

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Technical note 9

Table of Contents P.

TECHNICAL NOTE 9.1

MODELLING MELISSA COMPARTMENTS

General framework

Mathematical modelling of the MELISSA loop will be performed by a modular approach. This means that each compartment will be first considered apart with its own inputs and outputs ; in a second step, the different compartments will be connected in order to check the mass balances feasability and to define additional inputs and outputs to the system.

Such a study requires a basic knowledge of the metabolism features of the strains which are used in the process and this in turn calls for a quantative and a basic understanding of stoichiometry, energetics and kinetics of biological transformations.

The aim of the present note is to establish a hierachy between the different kinds of models which may be involved to describe the behavior of the microorganisms.

Unstructured models

A so-called classical approach consists in formulating kinetics expressions for the consumption or the production of the main substrates and products of the microorganisms. The resulting system of equations (the mathematical model) does not insure the conservation of carbon, hydrogen, oxygen and nitrogen elements.

The simplest kinetic relations which may be used for biomass growth and metabolites production are summarized in tables 1 and 2. Three main metabolic activities have been considered :

- synthesis of biomass (growth) ;
- metabolites production ;
- maintenance reactions

These models use constant yields and maintenance coefficients. The great disadvantages of such models are their inability for taking into account the mass and the elementary balances. Therefore this approach does not seem to be sufficient for the MELISSA analysis which will be devoted to mass balances even for the preliminary studies.

Stoichiometric models

A more refined technique for modelling biological processes consists to separate stoichiometry and kinetics as it is usually done for chemical reactor design. Each main metabolic reaction is characterized by an overall stoichiometric equation (which uses constant coefficients) and by a kinetic equation which expresses the reaction rate as a function of the concentrations of the different dissolved species. The overall production or consumption rates are calculated by summating overall reactions.

This approach has two main advantages :

- the mass conservation balances are fulfilled *per se* ;
- the model may lead to variable transformation yields during culture time for a batch process or with dilution rate for a continuous process.

Furthermore, this kind of model may be easily simplified assuming that the conversion yields reach unity (total reactions) which suppresses a detailed description of the reactions kinetics. This kind of hypothesis will be extensively used for preliminary studies of MELISSA compartments ; in a preliminary analysis, the culture conditions (choice of microorganisms, design of the reactors) will be supposed to lead to ideal (maximal) conversion yields of the susbstrates into products.

Biochemically structured models

The robustness of the previous models are generally not sufficient to describe the behavior of the microorganisms over a large range of culture conditions. A more complete description of cellular machinery is thus necessary including the specific production and consumption rates of several important intracellular components.

The complexity of the model is clearly related to the number of biotic fluxes which are considered for the description. With more than 5 intracellular components, the model becomes acceptable from a biological point of view but its complexity increases and experimental verification becomes more difficult.

At the lowest level, only ATP and cofactors can be considered ensuring a description of energy balances. In a global way, three types of reaction patterns can be distinguished :

- catabolic pathways, in whose reactions energy-rich susbtrates are converted into products which are less energy-rich. Part of the energy contained in the substrate is converted into energy carriers such as ATP and GTP either directly by substrate phosphorylation, or indirecly via hydrogen carrier like cofactors ;

- anabolic pathways which construct the building blocks for new biomass (amino acids, carbohydrates, fatty acids, nucleic acids) from the substrates available and polymerization reactions where the monomers are converted into cellular macromolecules ; in these processes energy carriers and reducing equivalents are generally consumed ;

- maintenance metabolism ; it is generally assumed that a constant supply of energy carriers is needed to keep the cellular machinery operative.

These functional characteristics must be completed for aerobic and photosynthetic microorganisms. In the presence of oxygen ATP can be derived from the oxidation of hydrogen-carrying intermediates (reduced cofactors). For photosynthetic organisms, ATP and oxidized cofactors are generated by the chloroplasts. Oxidative phosphorylation and photosynthesis do not obey fixed and constant stoichiometries. The theory of the linear energy converters in terms of thermodynamics of irreversible processes may be a useful tool to treat such processes.

This kind of analysis is generally applicable provided that the main metabolic features of the microorganisms are known ; it enables a detailed description of mass and energy balances (enthalpic and entropic balances) which could be very important to characterize the isolated system MELISSA.

Conclusion

In an effort to develop an understanding and a mathematical description of the compartments of MELISSA system, it is necessary to build stoichiometric representations of the functionning microorganisms ; these equations must be established assuming simplifications of the complete metabolism. Introducing the energy carriers balances will lead to biochemically structured models which can be particularly useful to analyse the behavior of the compartments under variable conditions such as nutrients, oxygen or light limitations.

Table 1 : Models for growth

Model	:	Specifications
classical model	:	3 constant coefficients
$r_X = \mu_m X S / (K_S + S)$		μ_m Y_{SX} , K_S
$r_S = - r_X / Y_{SX}$	:	
Pirt model : exogeneous maintenance		
$r_X = \mu_m X S / (K_S + S)$	:	4 constant coefficients
$r_S = - r_X / Y_{XS} - m_S X$		μ_m, Y_{SX}, K_S, m_S
Herbet model : endogeneous maintenance		
$r_S = - \mu_m Y_{XS} XS / (K_S + S)$	:	4 constant coefficients
$r_X = r_S / Y_{XS} - m_S X$	:	

Table 2 : Model for the production of metabolites

Models	:	Specifications
Luedeking Piret model		
$r_p = Ar_x + BX$	:	4 constant coefficients
$r_s = - r_p / Y_{sp} - r_x / Y_{sx}$	:	A, B , Y_{SP} , Y_{SX}
Luedeking Pirt - maintenance model		
$r_p = Ar_x + BX$	:	5 constant coefficients
$r_s = - r_p / Y_{sp} - r_x / Y_{sx} - m_s X$		

TECHNICAL NOTE 9.2

MAIN METABOLIC FEATURES OF CLOSTRIDIA SPECIES. MASS BALANCES ON THE LIQUEFYING COMPARTMENT

Objective

The aim of the present note is to establish the mass balances on the liquefying compartment and to state the main hypothesis on metabolism. This includes the characterization of the inputs, the description of the main metabolic features of *clostridia* species cultivated anaerobically and the determination of the products which are released to the autotrophic compartment.

Inputs in the liquefying compartment

In the first compartment, *clostridia* are used to transform organic polymers into compounds that can be assimilated by the phototrophic bacteria. The organic material (solid wastes) mainly contains proteins, amino acids, urea, NH_4^+ ions and carbohydrates such as cellulose which have been produced by the consumer's compartment. The macromolecules (proteins, cellulose) cannot be metabolized by the phototrophs of the second compartment. These might be decomposed into smaller molecules such as volatile fatty acids and ammonium by *clostridia*

In this approach, three hypothesis have been stated :

- the nitrogen content of wastes is considered as protein nitrogen ;
- the carbohydrates are assumed to be represented by cellulose ;
- the protein content of wastes represents 73% w/w of the total dry matter of the input ; the remaining 27% are carbohydrates.

If the first and the second hypothesis are not fulfilled, the assimilation of smaller molecules would be easy in the second compartment.

Mean formula for proteins and carbohydrates have been established (PONS ; 1987). Assuming 16% w/w nitrogen content for protein and that the general formula for amino acids is $C_{n+2}\ H_{2n+5}\ O_2\ N$, it follows that proteins can be represented by the C-mole formula $C\ H_{1.760}\ O_{0.239}\ N_{0.239}$; the average formula for a mixture of amino acids is then $C\ H_{2.24}\ O_{0.478}\ N_{0.239}$.

The carbohydrates are assumed to be represented by cellulose i.e. by an unbranched chain of D-glucose subunits , the general formula $C\ H_{1.667}\ O_{0.833}$ is established straightforwards. The elemental composition of wastes produced by a crew of three persons per day is summarized in table 1.

Carbohydrates metabolism

The *clostridia* compartment must liquefy the wastes in order to produce small molecules (CO_2, volatile fatty acids, ammonium ...) which can be utilized in the other compartments.

The hydrolysis of carbohydrates to fermentable sugars can be achieved using *clostridium* species some of which are usefully thermophilic. The production of butanol, acetone and isopropanol is a classical degradation route of

glucose for *clostridia*. However, there is a number of variations (DOELLE ; 1975 ; BULLOCK and KRISTIANSEN, 1987) ; some *clostridia* produce butyrate, acetate CO_2 and H_2 whilst others produce mainly acetone rather than isopropanol. The proportions of end-products varies according to the chosen species and strain and according to the cultural conditions. Ethanol and acetone are normally minor products (DOELLE, 1975). All strains produce butyric acid and acetic acid together with carbon dioxide and hydrogen. According to the general sheme proposed in figure 1, the relative amounts of end-products clearly depend on the NAD^+/ NADH, H^+ flux i.e. on the energy metabolism.

As soon as a strain has been chosen, it might be possible to select a metabolic route from glucose to end products and to estimate conversion yields.

In order to establish a preliminary mass balance on the degradation of carbohydrates by *clostridia* species, we suppose an equimolar production of CO_2 and H_2. This is justified by the fact that CO_2 and H_2 are only produced equimolarly between pyruvate and acetyl-Co A (figure 1). Furthermore, the only organic end products are supposed to be acetic and butyric acids. Ensuring the conservation of C, H, O elements, the following stoichiometric equation for glucose degratation is deduced :

$$C_6H_{12}O_6 \rightarrow 1.6\,CO_2 + 1.6\,H_2 + 0.8\,\underset{\text{butyric acid}}{C_4H_8O_2} + 0.6\,\underset{\text{acetic acid}}{C_2H_4O_2}$$

This equation represents fairly well glucose degradation by *C. butyricum* and *C. lactoacidophilum* despites strong discrepancies for other species may be noted. Few is known about *C. thermocellum* and *C. thermosaccharolyticum* routes of glucose degradation (MINTON and CLARKE, 1979).

The hydrolysis of polymers to glucose might not be problematic since the cellulolitic properties of *clostridia* have been extensively proved so that we can write

$$C\,H_{1.667}\,O_{0.833} + 1/6\,H_2O \rightarrow C\,H_2\,O$$

Proteins metabolism

Many *clostridia* growing on protein hydrolysates or amino acids mixtures appear to obtain most of their energy by a coupled oxidation-reduction reaction between suitable amino acids. The coupled decomposition of amino acids is commonly referred to as the "Stickland reaction" (DOELLE, 1975). Bacteria using this Stickland reaction are mainly the proteolytic *clostridia* such as *C. acetobutylicum* , *C. sporogenes* *C. butyricum* It must be mentionned that *C. thermocellum* and *C. thermosaccharolyticum* are not generally classified in proteolytic *clostridia*

The overall mechanism of the decomposition of amino acids is classically established as follows :

$$R - \underset{\displaystyle NH_3^+}{\underset{|}{CH}} - COO^- + 2\,R' - \underset{\displaystyle NH_3^+}{\underset{|}{CH}} - COO^- + 2\,H_2O$$

$$\rightarrow \quad R - COOH + CO_2 + 2\,R' - CH_2 - COOH + 3NH_3$$

The present evidence suggests that the Stickland reaction is composed of a number of steps where hydrogen carriers (NAD^+, FAD ...) are directly involved.

The decomposition rate of amino acids by *clostridia* is thus coupled to the energy metabolism and cannot be studied independently of glucose and carbohydrates degradation.

Despites, different degradation pathways have been proposed for amino acids degradation, the previous stoichiometric equation can be rewritten using the general formula $C\ H_{2.240}\ O_{0.478}\ N_{0.239}$ for an average mixture of amino acids (see above) ; using a N-mole basis for an amino acid (i.e. multiplying by 1/0.239) it follows :

$$3\ C_{4.184}\ H_{9.372}\ O_2\ N + 2\ H_2O \rightarrow 3\ NH_3 + CO_2 + 11.552\ C\ H_{2.00}\ O_{0.520}$$

The product $11.552\ C\ H_{2.00}\ O_{0.520}$ represents the mixture of volatile fatty acids resulting from the decomposition of the mixture of amino acids ; it cannot be determined without defining the exact composition of the amino acids mixture and without stating a metabolic sheme for the decomposition of each amino acid. Such a sheme does depend on the strain that will be used. Therefore, the above stoichiometric equation will be supposed valid for mass balances purposes. The hydrolysis of proteins to amino acids is catalysed by protease enzymes which might be operative for *clostridia* genus ; the following stoichiometric relation may be admitted :

$$\underset{\text{proteins}}{C\ H_{1.76}\ O_{0.239}\ N_{0.239}} + 0.239\ H_2O \rightarrow \underset{\text{amino acids mixture}}{C\ H_{2.240}\ O_{0.478}\ N_{0.239}}$$

Mass balances over the liquefying compartment

Protein degradation

According to the previous stoichiometric equations, the resulting mass balances give for 100 g dry proteins :

- 20.56 g H_2O consumed for protein hydrolysis
- 13.71 g H_2O consumed for amino acids degradation
- 16.75 g CO_2 produced
- 19.41 g NH_3 produced
- 98.11 g volatile fatty acids produced

Carbohydrates degradation

According to the hypothesis already discussed for the carbohydrates metabolism of *clostridia*, the mass balance for 100 g dry carbohydrates consumed gives :

- 11.11 g H_2O consumed for hydrolysis
- 43.46 g CO_2 produced
- 43.46 g butyric acid produced
- 22.22 g acetic acid produced
- 1.97 g H_2 produced

The total degradation of wastes which has been defined in table 1, leads to :

- 74.05 g H_2O consumed
- 63.16 g CO_2 produced
- 37.48 NH_3 produced

- 189.45 g volatile fatty acids produced
- 30.81 g butyric acid produced
- 15.75 g acetic acid produced
- 1.40 g H_2 produced

Several comments must be done :

. The produced CO_2 leaves the liquefying compartment both in a gas in the liquid phases. The ratio of gazeous to dissolved CO_2 depends on the temperature and on the composition of the liquid phase and especially of the pH.
The Henry constant must be known or estimated in order to calculate the flow rates of the gas and liquid phases.

. The same comments must be done for hydrogen ; it must be noted that the calculation of dissolved hydrogen tension in the liquid phase and of the partial pressure of hydrogen over the compartment should be of a primary importance to get an insight in the regulation mechanisms of carbon metabolism. Indeed, it is well known that dissolved hydrogen tension has a marked effect on conversion yields and on the relative proportions of end products of anaerobic glucose degradation by *clostridia*

. As the pH will remain below 9, the NH_3 production will be essentially NH_4^+ ions.

. The volatile fatty acids formed by proteins degradation cannot be strictly separated from butyric acid production. It must be noted that the general C-mole formula for fatty acid ($C\ H_{2.00}\ O_{0.520}$) is quite similar to the C-mole butyric acid formula ($C\ H_2\ O_{0.50}$).

Concluding remarks

Although several hypothesis have been formulated about the composition of wastes and about the metabolism of *clostridia*, the present approach enables to propose mass balances on the liquefying compartment satisfying to elements conservation rules

Two major problems remain to be solved :

- the choice of a *clostridium* strain capable of assimilation of proteins (proteolytic activity) growing at high temperatures (≈ 60°C) in order to prevent the development of methanogenes species ;

- the determination of the supplementary input of cellulose material (papers ...) which can be assimilated in the compartment.

The present evidence suggests that these two problems are strongly related ; the hydrogen carriers (NAD^+, FAD) and ATP /ADP balances might be particularly useful to investigate the relative proportions of carbohydrates and proteins which are necessary to ensure the maintenance of the bacteria in the compartment.

Once a specific strain has been chosen, a structured model of metabolism will be therefore a powerful tool to analyse the behavior of the cells.

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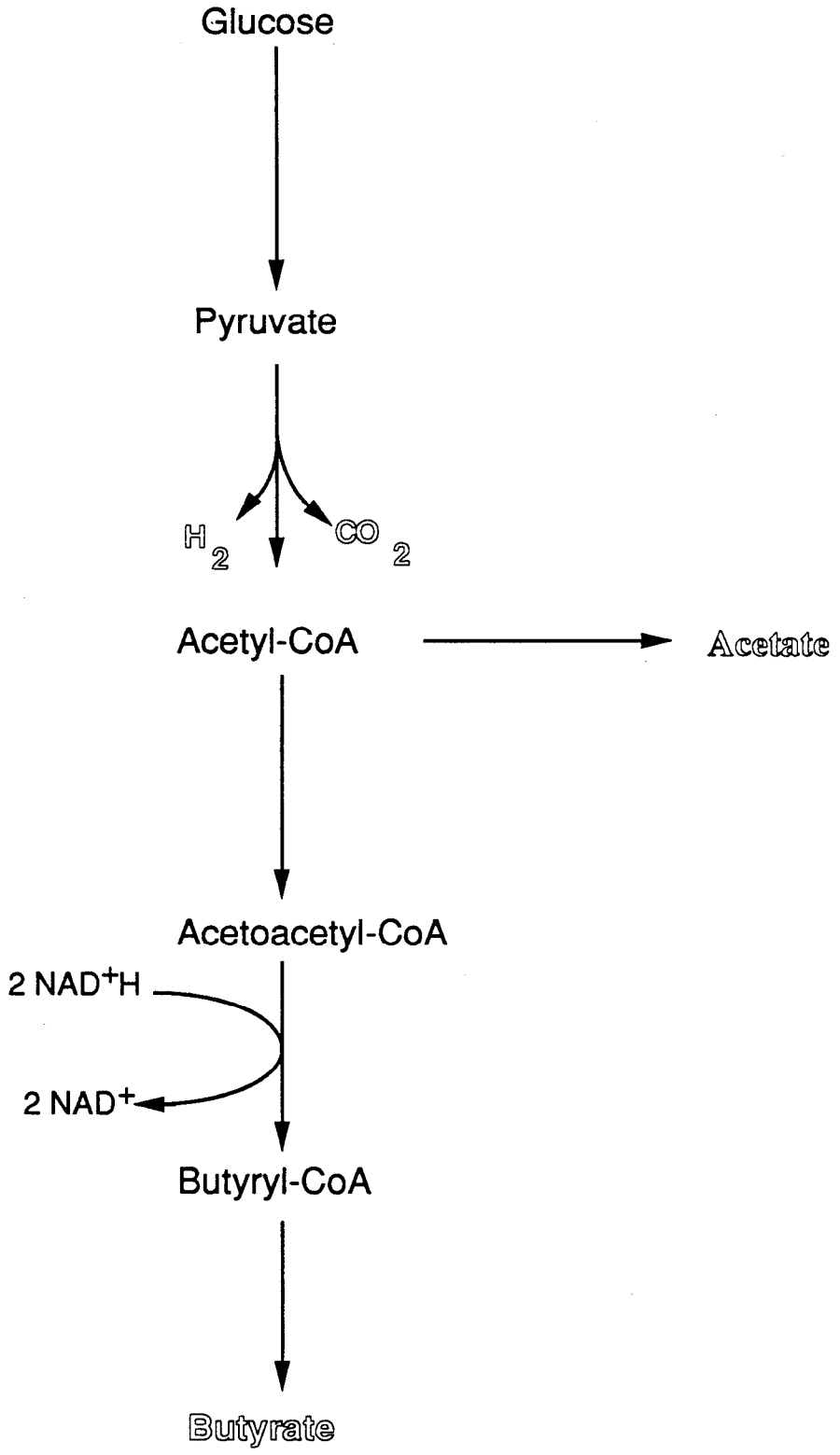

Figure 1 : Main metabolic features for carbohydrates degradation by *Clostridia*

table 1

Elemental composition of wastes. Mass fluxes are calculated for three persons per day.

	C (g)	H (g)	O (g)	N (g)	C-mole	M (g)
Dry protein $CH_{1.760}O_{0.239}N_{0.239}$	110.7	16.2	35.3	30.9	9.228	193.1
Dry carbohydrates $CH_{1.667}O_{0.833}$	31.5	4.4	35.0	-	2.625	70.9
Total	142.2	20.6	70.3	30.9	11.853	264

TECHNICAL NOTE 9.3

Scope

Waste liquefied by Clostridia transmit to the compartment which should recycle the solubilized waste products by transforming acids, acetate, H_2 and H_2S evolved from the liquefying compartment into biomass readily available for the consumers and minerals (mainly NH_4^+) which will be directed to the nitrifying compartment. Photoheterotrophic bacteria as Rhodobacter are particularly well designed to accomplish these recycling (MERGEAY et al, 1988).

The bacteria which have been chosen are Rhodopseudodomonas capsulatus and Rhodospirullum rubrum. Both organisms are capable of utilizing acetate and butyrate which are the fatty acids expected from the liquefying compartment.

In addition Rp. capsulatus can grow photoautotrophically with H_2 as an electron donor and CO_2 reduction. It was therefore suggested to split the second compartment into two compartments. The first one will be designed for photoheterotrophic growth ; the second will handle the gas phase from compartment 1 and a minimum of soluble effluent (WIEDERHOLD, 1990).

The aim of this note is to collect informations about the metabolism of the strains which are involved in these two compartments in order to establish the relevant balances. More experimental and bibliographic data are available for the culture conditions of the strains than for the first compartment ; a structured model of metabolism, including ATP and cofactors balances, may be considered. This preliminary study concerns the metabolism of the two strains in both heterotrophic and autotrophic growth conditions. Special attention will be drawn to nitrogen and amino acids metabolism.

I - Photometabolism of Rhodobacter species : electron transport systems

The nonsulfur bacteria, including the genus Rhodobacter contain bacteriochlorophylls a and b; they are primarily dedicated to the metabolism of organic compounds. They are capable of growth anaerobically when exposed to light and are also able to grow in the dark when exposed to air. The microorganisms therefore can switch from photophosphorylation to oxidative phosphorylation and both electron transport systems are present in the various organisms (DOELLE, 1975).

From a general point, photosynthesis is mainly devoted to the production of ATP and to the formation of a reductant that is able to reduce high-energy compounds into cellular material. As the calculation of the ratio between ATP formation rate versus cofactors reduction rate will

be of primary importance to build a structured model of metabolism, a general presentation of cyclic and noncyclic photophosphorylation is required.

1-1 Electron transport and phosphorylation (DOELLE, 1975 ; BAILEY and OLLIS, 1986)

Two different pathways are involved in electron transfer : cyclic phosphorylation and noncyclic phosphorylation.

Cyclic phosphorylation

All photosynthetic organisms have one common denominator : ATP generation by the absorption of visible light. This involves cyclic phosphorylation. A light-absorbing molecule (bacteriochlorophyll) becomes excited and acquires a tendency to expel an electron. The expelled electron is raised to a high energy potential and is transfered to ferredoxin which has an unusual negative potential which is - 0,432 V. From ferredoxin an electron pair travels through several redox systems and finally returns to chlorophyll (figure 1). Two sites for ATP generation in the electron transport chain are generally admitted though different stoichiometries have been proposed. Formally the cyclic phosphorylation mechanism can be summarized by the following stoichiometry :

$$2\ ADP + 2\ Pi \xrightarrow[h\upsilon]{Fd} 2\ ATP + 2\ H_2O \qquad (ES - 1)$$

Noncyclic phosphorylation

For the operation of different anabolic reactions, reducing power is required ; the generation of reduced cofactors (NAD(P)H, H^+) is performed by noncyclic photophosphorylation.

The illuminated chromophore reacts not directly with $NADP^+$ but first with ferredoxin as previously explained for cyclic photophosphorylation. The reoxidation of the ferredoxin occurs with a flavoprotein enzyme which is reoxidized by $NADP^+$. Electrons flow unidirectionally via a portion of the cyclic chain through a cytochrome so that a phosphorylation site will be induced (figure 2). As long electron are produced by an external hydrogen donor, cyclic photophorylation cannot proceed in such a way that these organisms are able to regulate ATP and reducing power production. Cyclic phosphorylations mainly operate when reducing power is devoted to ATP production whereas noncyclic photophosphorylation operates when reducing power is required with the help of an exogenous hydrogen donor. Formally the non-cyclic phosphorylation mechanism by photosynthetic bacteria such as Rhodobacter species can be summarized as follows :

$$R\text{-}H_2 + NADP^+ + ADP + Pi \xrightarrow[h\upsilon]{Fd} R + NADPH, H^+ + ATP + H_2O \quad (ES - 2)$$

$R\text{-}H_2$ denotes for the reductant which is oxidized to the radical R. The assimilation of different reductants is specific of species. Rhodospirullum rubrum and Rhodopseudomonas capsulatus are capable to oxidize organic acids (acetate, butyrate), alcohols (isopropanol), molecular hydrogen and minerals.

More generally species of the family Rhodospirillaceae are characterized by the ability to grow on a wide array of different organic compounds such as fatty acids, amino acids, alcohols, TCA cycle intermediates, sugars, acetate and pyruvate (SOJKA, 1978).

The schemes we have presented on cyclic and noncyclic photophosphorylation (figures 1 and 2) are certainly oversimplified. However they should demonstrate why one could regard both as separate pathways although neither of them could function alone in photosynthesis. It must be emphasized that the systems operate simultaneously with two different sites of ATP formation : one site is located prior to the point of entry of electrons from the outside donor and is common to the two pathways while the other is exclusive for the cyclic system.

II - Carbon metabolism

The non-sulfur bacteria often inhabit anaerobic niches where fermentation products of other organisms may accumulate. Many studies (KOBAYASHI AND TCHAN, 1973 ; VRATI, 1984 ; SAWADA and ROGERS, 1977) concern the use of photosynthetic bacteria such as Rp capsulata in the treatment of high BOD effluents in batch and continuous cultures. Unfortunately, the results are often expressed in terms of chemical oxygen demand (COD) reduction which prevents to establish a carbon balance. The main problem in establishing stoichiometric equations is to determine in what proportions the organic substrates are incorporated into cellular material or converted to CO_2. In order to give an insight into the diversity of the metabolic features which are achieved by Rhodobacter species, the following examples can be cited from bibliographic informations :

- CO_2 is either evolved or consumed depending on the oxidation level of the organic substrates (SOJKA ; 1978) ;

- organic substrates are metabolized to acetyl subunits (acetate and butyrate) which can yield to poly-β–hydroxybutyrate (PHB) ;

- substrates which are metabolized to pyruvate (succinate, malate, propionate) contribute primarily to the formation of polysaccharide reserve material ;

- the primary function of organic substrates in the photosynthesis is to serve as a source of reducing power for CO_2 fixation ;

- the carbon skeletons of the organic substrates can be incorporated into the synthesis of amino acids and therefore into proteins.

Some of these informations are contradictory ; furthermore, it is difficult to list exhaustively the metabolic differences which exist between Rhodospirullum rubrum and Rhodopseudomonas capsulatus both microorganisms being used in coculture in the photoautotrophic and the photoheterotrophic compartments. The main difference which have been reported (SOJKA, 1978) concerns the existence of the glyoxylate cycle of Rs. rubrum.

Rs. rubrum can incorporate carbon from acetate into cell material. With added CO_2, acetate carbon is found in polysaccharide whereas without CO_2 the acetate carbon is predominantly converted to poly-β-hydroxybutyrate. Unlike many other organims that utilize acetate as sole carbon source, Rs. rubrum does not possess an active glyoxylate cycle (ALBERS, GOTTSCHALK, 1976) and the mechanism by which Rs. rubrum is able to grow photosynthetically on acetate as a sole carbon source in the absence of a functional glyoxylate cycle, remains unsolved (SOJKA, 1978). In contrast to Rs. rubrum it has been reported that Rp. capsulata formed significant levels of isocitrate lyase, the enzyme which is essential for the operation of glyoxylate cycle (WILLISON, 1978). However this enzyme has been shown to be inducible by the presence of acetate in the culture broth.

Some other main features can be listed as follows :

- Rs rubrum has been shown to possess an inducible alcohol deshydrogenase which is NAD- dependent ;

- Rs rubrum can grow anaerobically in the light on glutamate ; more generally most amino acids can be metabolized by all strains (see below) ;

- it has been shown that Rs rubrum was able to grow an methanol provided pH was higher than for other conditions. SIEFERT and PFENNIG (1979) have demonstrated that some strains of Rp capsulata were able to grow in a mineral medium in the dark chemotrophically on methanol leading to 0.23 g cells/g methanol. CH_4, formate and CO can be slightly used by some strains but apparently not by Rp capsulata : these substrates are first oxidized to CO_2 before being fixed via the reductive carboxylic acid cycle ;

- WIEDERHOLD (1990) has shown that ethanol could not be used as sole carbon source by either microorganisms in a medium containing yeast extract ; when Ca-lactate is also added, growth occurs but the results do not clearly indicate if ethanol is effectively consumed ; further discussion and a treatment of the results in terms of carbon balance would be necessary ;

- more generally Ca- lactate can be used by both organisms as a sole carbon source ; it might be considered as a good promotor for the assimilation of complex nitrogen sources ;

- malate is a popular carbon source for culture of Rhodospirillaceae at the laboratory indicating that it is easily metabolized by all strains. Rp. capsulata can grow on D or L-malate under photosynthetic and heterotrophic conditions , malate is probably used via the TCA cycle trough the action of the malic deshydrogenase.

The informations clearly indicate that the species Rs rubrum and Rp capsulata can metabolize the carboxylic acids which are produced by the liquefying compartment ; in addition the strains possess the ability to eliminate other by-products such as ethanol, amino acids .. wich may appear in the influent. All the carbon skeletons are incorporated into cellular material.

However, it is not clear why both microorganisms are necessary and **more information is required on the reason why the two species are planned to be used in coculture**.

From a stoichiometric point of view, all the influent carbon in the photoheterotrophic compartment will be supposed to be acetate, butyrate and CO_2. We must point out again that it is not possible to state other hypothesis on the liquefying compartment at this moment. It may be considered that if a more precise composition of the products of the liquefying compartment could be established, the use of both species Rp. capsulatat and Rs. rubrum should perhaps be justified.

In that event, the metabolism of acetate, butyrate and CO_2 will be considered only. Thus the differences between the metabolisms of the two strains will not be taken into account.

It must be emphasized that the results obtained by WIEDERHOLD (1990) cannot be discussed in terms of stoichiometric equations ; the COD reduction of the influent represents a too global parameter to be used to establish carbon and oxygen balances.

In other words, the problem which remains to be solved concerns the establishment of stoichiometric equations which relate the synthesis of cellular material (proteins, polysaccharides, lipids, nucleic acids) from acetate, butyrate and CO_2 in the photoheterotrophic compartment and from CO_2 in the photoautrophic compartment.

III - Nitrogen metabolism

We summarize the headlines of nitrogen metabolism of Rs rubrum and Rp. capsulata as follows :

- all strains are capable to incorporate NH_4^+ by a reductive

amination of α-cetoglutarate to glutamate. The synthesis of the other amino acids is achieved classically by transaminations ;

- in the absence of NH_4^+ in the medium Rs rubrum and Rp capsulata are capable to fix N_2 under anaerobic conditions in the light ; N_2 fixation is inhibited by O_2 firstly because the nitrogenase enzyme is inhibited by O_2 and secundly because the strong reductant (reduced ferredoxin) is easily oxidized by O_2 (YOCH, 1978). The mechanism which is generally proposed involves a reduction of N_2 to NH_4^+ followed by a reductive amination of α-cetoglutarate to glutamate ; NH_4^+ is known to be an inhibitor of N_2 fixation and continuous cultures of Rs rubrum supplied with limiting concentrations of NH_4^+ are able to fix nitrogen : organic acids not only serve as a source of electrons for the reduction of N_2 but also furnish the suitable carbon skeletons to accept NH_4^+ and therefore prevent inhibition by accumulation of excess NH_4^+ ; this inhibitory effect is specific of N_2 fixation system because Rs rubrum grows quite well on high concentrations of NH_4^+ (SUHAIMI et al, 1987) ;

It must be noted that most bibliographic data are related to N_2 fixation ; few informations concern NH_4^+ assimilation ; no specific nutritional study on the requirements of the strains for different amino acids is available in litterature : it can be stated that they can be metabolized, some further informations about their transport inside the cell being necessary.

WIEDERHOLD (1990) has realized a preliminary study about the assimilation of different nitrogen sources.The results which have been interpreted in terms of nitrogen balances indicate that (detailed calculations are not available in the publication) :

- Rp capsulata and Rs rubrum are capable to incorporate NH_4^+ nitrogen into cell proteins whatever carbon source is used ; despites no kinetic equations can be established, Ca-lactate is proved to be the more efficient carbon source which leads to the exhaustion of nitrogen after 6.5 days cultivation.

- Rp capsulata can metabolize betaïne and ureum with Ca-lactate as carbon source ; the conversion yields of ureum and betaine after 6.5 days cultivation time reach 70% and 13% respectively

- Rs rubrum is capable of assimilation of ureum whatever the carbon source (lactate, acetate, butyrate) ; the conversion yields remain however below 30% ;

- glutamate is metabolized by both strains with acetate as carbon source for Rs rubrum and with lactate for Rp capsulata ; the conversion

yields are respectively 15 and 80%. It may be concluded from the study that :

- NH_4^+ can be always assimilated by both strains with an efficiency of 100%.

- exhaustion of ureum in the presence of NH_4^+ will be difficult to achieve as both strains have a higher affinity for NH_4^+.

- although both strains present significant differences for their nitrogen metabolism, it is difficult to treat separately the two metabolisms (for stoichiometric or kinetic studies) the data being too scarce.

For MELISSA simulation it will be necessary to perform experiments on the exhaustion of organic acids in the presence of an excess NH_4^+. It must be emphasized that the role of the photoheterotrophic compartment is directed towards the exhaustion of the carbon sources the output NH_4^+ being oxidized in the nitrifying compartment and further converted to digestible biomass by the spiruline compartment.

In this preliminary study, the nitrogen sources will thus be considered to be exclusively NH_4^+ ; it will be further supposed that the organic acids could be exhausted, excess NH_4^+ remaining in the medium ; in such conditions (carbon depletion) it might be correct to suppose that ureum could be exhausted before NH_4^+ despites no experimental evidence is presently available.

IV - Hydrogen and sulfur metabolism

Rhodospirillaceae such as Rs rubrum and Rp capsulata can grow autotrophically using a suitable reductant such as H_2 ; it has been reported that Rp capsulata can tolerate sulfide concentrations to the same extent as observed in Chromatium vinosium (TRÜPER ; 1978).

Presently, it is not clear if H_2S produced by the first compartment could not be entirely processed in the Rhodobacter compartment.

H_2 can be either consumed or produced by photosynthetic bacteria. A number of factors, including cultural conditions have been shown to affect photoevolution of H_2. In fact the two behaviors (production and consumption of H_2) must be considered for MELISSA studies : H_2 may be produced by the heterotrophic compartment and must be consumed by the autotrophic compartment for CO_2 reduction.

Most studies are concerned with H_2 production (HILLMER and GEST, 1977 a,b ; SEGERS and VERSTRAETE, 1983). The photoevolution of H_2 by photosynthetic bacteria can be accounted for by the dissimulation

of organic acids. There is a close relationship between light-dependent H_2 evolution and N_2 fixation. It has been proposed that both H_2 production and N_2 fixation in Rs rubrum are catalysed by the same enzyme complex namely nitrogenase. On the basis of mass and energy balances considerations, N_2 fixation and H_2 evolution require reductants and ATP. It may be concluded that if Rs rubrum or Rp capsulata are cultivated on a medium containing TCA cycle intermediates (malate, fumarate, pyruvate or lactate) as carbon sources and glutamate, aspartate or N_2 as nitrogen sources, CO_2 and H_2 are extensively produced. The photoevolution of H_2 is completely inhibited by NH_4^+, yeast extract, alamine and serine which are decomposed to release NH_4^+. It appears that NH_4^+ represses the enzymes required for light-dependent H_2 production (YOCH, 1978). Conversely ammonium limitation activates the nitrogenase enzyme and energy losses via H_2 production can occur (WIEDERHOLD, 1990). The experimental studies which have been performed by WIEDERHOLD (1990) do not indicate any H_2 evolution.

In that event, H_2 production in the photoheterotrophic compartment will not be considered assuming that the culture will not be NH_4^+ limited.

Much less information is available as far as H_2 consumption is concerned. MADIGAN and GEST (1979) have reported that Rp capsulata was capable of growing chemoautotrophically under aerobic conditions with H_2 as a source of reducing power and O_2 as the terminal electron acceptor for energy transduction and CO_2 as a sole carbon source with a high generation time of 6 h. It has also been reported (YOCH, 1978; PFENNIG and TRÜPER, 1989) that Rp capsulata and Rs rubrum were able to grow photoautotrophically with H_2 as electron donor and CO_2 reduction. The detailed mechanism of H_2 oxidation has not been extensively studied. It must be kept in mind that H_2 is an accessory donor ; consequently, H_2 assimilation is completely inhibited by organic acids ; this fact has been used to justify the division of the two Rhodobacter compartments the first one being responsible for the exhaustion of organic compounds , the second one directed towards H_2 assimilation, CO_2 and ammonium being partially consumed . We remark here that, when the system will operate in continuous culture, the residence times will be chosen in such a way that the organic acids concentrations in the photoheterotrophic compartment will be negligible which may enable H_2 consumption in the same compartment.

Experimental results for H_2 consumption are scarce ; the preliminary results obtained by WIEDERHOLD (1990) are only available. These experiments clearly show the feasability of the process indicating that :

- the growth was much more rapid for Rp capsulata than for Rs rubrum
- CO_2 and H_2 are significantly consumed and incorporated into cell constituants

Furthermore, it appears that higher biomass production is achieved under autotrophic conditions than under heterotrophic conditions.

The results cannot be presently treated in terms of CO_2 and H_2 balances : the precise gas balances require specific and expensive investments.

V - Towards a stoichiometric model of growth of Rhodospirillaceae

Using the informations which have been previously listed, the aim of this section is to write one or several stoichiometric equations which may be used for MELISSA loop simulations.

5-1 Main metabolic pathways

Rs rubrum and Rp capsulata appear to possess very similar enzymatic equipment ; the metabolic pathways will be considered identical. Their ability to grow in at least five different physiological growth modes suggests that they are among the most metabolically versatile procaryotes known (MADIGAN and GEST, 1979 ; PFENNIG and TRUPER, 1989 ; BEATTY and GEST, 1981).

5-1-1 Catabolism

Rs rubrum and Rp capsulata metabolize glucose via the Entner-Doudoroff pathway (OHMANN, 1979) (figure 3). The Krebs cycle (TCA cycle) furnishes reducing power and the intermediates which are used for growth.

The microorganisms always present all the enzymes of the TCA cycle despites some of them remain inhibited (BLASCO and al, 1989).

The glyoxylate shunt is operative for Rs rubrum (YOCH, 1978) (figure 4).

5-1-2 Anabolism

In order to establish a stoichiometric equation for biomass growth, it is necessary to characterize first the synthesis of the procursors i.e of the amino acids.

No specific information is available for the biosynthetic pathways of Rhodospirillaceae ; they will be considered to be identical to Escherichia coli pathways. General reference texts (LEHNINGER, 1985 ; DOELLE, 1975 ; GOTTSCHALK, 1988) separate 5 families of amino acids synthetized from different carbon skeletons (Table 1). The general pathways are summarized in figure 5.

5-1-3 CO_2 assimilation

Rs rubrum and Rp capsulata assimilate CO_2 mainly via the Calvin-Benson cycle (OHMANN, 1979) (figure 6). Formally this enables to satisfy the electron balance resulting from the assimilation of reduced substrates such as organic acids.

Rs rubrum should possess a second pathway (Hatch-Slak cycle) which enables to metabolize directly carboxylic acids and CO_2. This reducing carboxylic acid cycle corresponds to the reverse reactions of the Krebs cycle (BUCHANAN and al, 1967) and leads to the synthesis of one acetyl-Co-A from two molecules of CO_2 (figure 7).

This enable to obtain the precursors of the synthesis of amino acids (figure 8) and of carbohydrates (figure 9).

5-1-4 Synthesis of reserve carbohydrates

Poly-β-hydroxybutyrate (PHB) is accumulated inside the cells of Rp capsulata and can further be metabolized furnishing a fatty acid pool inert from an osmotic point of view. The synthesis of PHB occurs when carbon sources are present in excess and/or when the growth is limited by an other nutriment.
Excess acetate and butyrate are used via acetyl-Co-A.

Glycogen can be also synthetized by both Rs rubrum and Rp capsulata. The nature of the substrate directs either PHB or glycogen synthesis. It may considered that lactate, succinate, CO_2 and pyruvate induce glycogen synthesis whereas acetate and butyrate lead to PHB.

5-2 Establishment of stoichiometric equations for growth

5-2-1 Principle of the calculations

In order to establish stoichiometric equations for growth, the biomass composition must be known.

From the work of KOBAYASHI and KURATA (1978) and VRATI (1984) it may be considered that an average composition of 65% proteins, 5.9% nucleic acids, 17% lipids and carbohydrates and 12% of other components is representative. Furthermore, the amino acids composition of proteins has been determined : the results are given in table 2.

Stoichiometric equations for the synthesis and the polymerizations will thus be established for the different fractions of cells. The photoautotrophic and the photoheterotrophic compartments must be considered separately.

5-2-2 Photoautotrophic compartment

Stoichiometric equations have been established in different steps.

Step 1 : CO_2 fixation by Calvin-Benson cycle (figure 6)

The conversion of CO_2 to hexoses can be written as follows :

$$6\,CO_2 + 18\,ATP + 12\,H_2O + 12\,NADPH,\,H^+ \rightarrow C_6H_{12}O_6 + 18\,ADP + 18\,Pi + 12\,NADP^+ \quad \text{(ES-3)}$$

Step 2 : degradation of hexoses to pyruvate

$$C_6H_{12}O_6 + ADP + Pi + 2\,NADP^+ \rightarrow 2\,Pyr + ATP + H_2O + NADPH,\,H^+ \quad \text{(ES-4)}$$

Step 3 : formation of the precursors of amino acids (table 1)

$$Pyr + 2\,ATP \rightarrow 3\,PG + 2\,ADP + Pi \quad \text{(ES-5)}$$

$$Pyr + 2\,ATP \rightarrow PEP + 2\,ADP + Pi \quad \text{(ES-6)}$$

$$Pyr + CO_2 + 5\,ATP + 3\,NADPH,\,H^+ + 3\,H_2O \rightarrow E4P + 5\,ADP + 4\,Pi + 3\,NADP^+ \quad \text{(ES-7)}$$

$$3\,Pyr + CO_2 + 9\,ATP + 5\,NADPH,\,H^+ + 7\,H_2O \rightarrow 2R5P + 9\,ADP + 7\,Pi + 5\,NADP^+ \quad \text{(ES-8)}$$

$$Pyr + CoASH + NADP^+ \rightarrow Ac\,Co\,A + CO_2 + NADPH,\,H^+ \quad \text{(ES-9)}$$

$$Pyr + CoASH + NADP^+ \rightarrow Ac\,Co\,A + CO_2 + NADPH,\,H^+ \quad \text{(ES-10)}$$

$$3\ \text{Ac Co A} + 4\ NADP^+ + 4\ H_2O$$
$$\rightarrow \alpha\ \text{KG} + CO_2 + 3\ \text{Co ASH} + 4\ NADPH,\ H^+ \qquad \text{(ES-11)}$$

$$2\ \text{Ac Co A} + 7\ NADP^+ + 5\ H_2O + ADP + Pi$$
$$\rightarrow OAA + 2\ CO_2 + 3\ \text{Co ASH} + 7\ NADPH,\ H^+ + ATP \qquad \text{(ES-12)}$$

Step 4 : synthesis of amino acids from precursors.

These equations have been established considering the pathways of synthesis were not significantly different from these of E. coli.
The results of the calculations, which are heavy are presented in table 3.

Step 5 : synthesis of amino acids from CO_2

The use of (ES-3) to (ES-12) and of the stoichiometric equations which are presented in table 3 leads to the results presented in table 4. It must be pointed out that NH_3 and H_2S consumptions are predicted.

Step 6 : weighted summations using the data of table 2

The preceeding equations are added using the molar composition of amino acids in proteins. This leads to :

$$1.7986\ CO_2 + 0.4866\ NH_3 + 0.0095\ H_2S + 3.656\ NADPH,\ H^+ + 9.2829\ ATP + 6.2518\ H_2O$$
$$\rightarrow 0.329\ \text{"pool amino acid"} + 3.656\ NADP^+ + 9.2828\ ADP + 9.2829\ Pi \qquad \text{(ES-13)}$$

Step 7 : polymerisations of amino acids into proteins

Considering that a peptide bond requires two ATP it is established that :

$$1\ \text{"pool amino acid"} + 4\ ATP + H_2O \rightarrow 1\ \text{mol proteins} + 4\ ADP + 4\ Pi \qquad \text{(ES-14)}$$

In this equation 1 mol protein corresponds to the elemental formula

$$C_{1.7986}\ H_{2.7286}\ O_{0.566}\ N_{0.4866}\ S_{0.0095}$$

The overall stoichiometric equation for the synthesis of Rhodospirillaceae protein is thus :

$1.7986\ CO_2 + 0.4866\ NH_3 + 0.0095\ H_2S + 3.656\ NADPH, H^+ + 10.029\ ATP + 6.9898\ H_2O$

$\rightarrow$ 1 mol proteins + $3,656\ NADP^+ + 10.029\ ADP + 10.029\ Pi$ (ES-15)

The carbohydrates, nucleic acids and lipids synthesis can treated in a similar fashion.

Carbohydrates : CH_2O

$$CO_2 + 2\ H_2O + 3\ ATP + 2\ NADPH, H^+$$
$$\rightarrow CH_2O + 3\ ADP + 3\ Pi + 2\ NADP^+ \quad \text{(ES-16)}$$

Nucleic acids

$$CO_2 + 0.393\ NH_3 + 2.382\ H_2O + 3.776\ ATP + 1.337\ NADPH, H^+$$
$$\rightarrow CH_{1.273}O_{0.720}N_{0.393}P_{0.104} + 3.776\ ADP + 3.672\ Pi + 1.337\ NADPH, H^+ \quad \text{(ES-17)}$$

Lipids

$$CO_2 + 0.0028\ H_2S + 1.214\ H_2O + 3.0127\ ATP + 2.6502\ NADPH, H^+$$
$$\rightarrow CH_{1.714}O_{0.204}S_{0.0028}P_{0.0027} + 3.0127\ ADP + 3.01\ Pi + 2.6802\ NADP^+ \quad \text{(ES-18)}$$

Finally the stoichiometric equation for the formation of cell components can be deduced :

$$CO_2 + 0.2264\ NH_3 + 0.0041\ H_2S + 3.3668\ H_2O + 4.9546\ ATP + 2.0241\ NADPH, H^+$$
$$\rightarrow CH_{1.575}O_{0.42}N_{0.226}S_{0.0041}P_{0.0072} + 4.9546\ ADP + 4.9474\ Pi + 2.0241\ NADP^+ \quad \text{(ES-19)}$$

It must be noted that the use of (ES-1) and (ES-2) could theoritically lead to the prediction of H_2, CO_2 yields and of the light-energy consumption. However the conversion yields of reducing power to ATP are generally far to be unity and it is necessary at this step to fit this conversion yield (which in turns is similar to the $P/2e^-$ ratio) to experimental results.

5-2-3 Photoheterotrophic compartment

The techniques of the calculations are quite similar as those previously given.

As the exact composition of acetate and butyrate in the effluent of the liquefying compartment is not known, a molar ratio of 1/1 has been postulated. Futhermore it has been supposed that acetate and butyrate are converted to acetyl Co A via :

$$\text{Acétate} + \text{Co ASH} + \text{ATP} \rightarrow \text{Ac Co A} + \text{ADP} + \text{Pi} \qquad \text{(ES-20)}$$

$$\text{Butyrate} + 2\ \text{Co ASH} + 2\ \text{NADP}^+ \rightarrow 2\ \text{Ac Co A} + 2\ \text{NADPH, H}^+ \qquad \text{(ES-21)}$$

and that pyruvate is synthetized via CO_2 fixation by the Calvin-Benson cycle which is supposed to remain operative when carboxylic acids are present in the medium. This enables to predict CO_2 assimilation into cell components.

Detailed calculations are not given here. The result for proteins synthesis is expressed as follows :

$$0.1949\ \text{Acetate} + 0.1949\ \text{Butyrate} + 0.6295\ CO_2 + 0.4866\ NH_3 + 0.0095\ H_2S + 3.776\ H_2O + 5.2483\ \text{ATP} + 0.9281\ \text{NADPH, H}^+$$

$$\rightarrow 1\ \text{mol proteins} + 5.2483\ \text{ADP} + 5.2483\ \text{Pi} + 0.928\ \text{NADP}^+$$

The stoichiometric equation for biomass synthesis is further established considering that carbohydrates nucleic acids and lipids are synthetized from CO_2 (ES-16 to ES-18)

The same comment as for the photoautotrophic compartment must be done at this point : a precise comparison with experimental results might lead to the calculation of conversion yields.

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Table 1 : Main precursors for amino acids synthesis (E. Coli "hypothesis").

Precursor	Amino acid
Pyruvate	Ala-Val-Leu
Oxaloacetate	Asp-Asn-Met-Lys-Thr-Ile
α–cetoglutarate and pyruvate	Glu-Gln-Arg-Pro
*PEP and erythrose 4-P	Phe-Tyr-Trp
*PRPP and ATP	His

*PEP : phophoenol pyruvate
*PRPP : phosphoribosylpyrophosphate

Table 2 : Amino acids composition of proteins of Rp capsulata (KOBAYASHI and KORATA, 1978) : 19 amino acids are considered here (cystein composition has not been measured). Total protein composition of dry biomass reaches 65 % w. These discrepancies must be attributed to nitrogen-components such as ornithine, citrulline, biotin, diaminopinélate... Asparagine and glutamine have been lumped in the measurements. We consider further that the molar ratio is 1/1.

Proteins	65.00 %
Vitamins	0.06 %
Mineral	2.38 %
Nucleic acids	5.90 %
Bacteriochlorophy	5.61 %
Carotenoïds	4.17 %
	83.00 %

Acide aminé	Formule brute	Masse molaire (g/mol)	g/100 g PS	Moles
Isoleucine	$C_6H_{13}O_2N$	149	2.64	0.0177
Leucine	$C_6H_{13}O_2N$	149	4.50	0.0302
Lysine	$C_6H_{15}O_2N_2$	165	2.86	0.0173
Méthionine	$C_5H_{11}O_2NS$	167	1.58	0.0095
Phénylalanine	$C_9H_{11}O_2N$	183	2.60	0.0142
Tyrosine	$C_9H_{11}O_3N$	199	1.71	0.0086
Thréonine	$C_4H_9O_3N$	137	2.70	0.0197
Tryptophane	$C_{11}H_{12}O_2N_2$	222	1.09	0.0049
Valine	$C_5H_{11}O_2N$	135	3.51	0.0260
Arginine	$C_6H_{15}O_2N_4$	193	3.34	0.0173
Histidine	$C_6H_{10}O_2N_3$	174	1.25	0.0072
Alanine	$C_3H_7O_2N$	107	4.65	0.0435
Aspartate	$C_4H_6O_4N$	150	4.56	0.0304
Glutamate	$C_5H_8O_4N$	164	5.34	0.0326
Glycine	$C_2H_5O_2N$	93	2.41	0.0259
Proline	$C_5H_8O_2N$	132	2.80	0.0212
Sérine	$C_3H_7O_3N$	123	1.68	0.0137
Asparagine / Glutamine	$C_{4,5}H_8O_3N_2$	138	4.01	0.0291
			53.23	**0.3690**

Table 3 : Amino acids synthesis from NH_3, glycolysis and TCA cycle intermediates

Glutamate

$$\alpha KG + NH_3 + NADPH, H^+ \rightarrow Glu + NADP^+$$

Glutamine

$$\alpha KG + 2\ NH_3 + ATP + NADPH, H^+ \rightarrow Gln + ADP + Pi + NADP^+$$

Proline

$$\alpha KG + NH_3 + ATP + 3\ NADPH, H^+ \rightarrow Pro + ADP + Pi + 3\ NADP^+ + H_2O$$

Arginine

$$\alpha KG + 3\ NH_3 + 8\ ATP + 3\ NADPH, H^+ + CO_2 + 3\ H_2O \rightarrow Arg + 8\ ADP + 8\ Pi + 3\ NADP^+$$

Aspartate

$$OAA + NH_3 + NADPH, H^+ \rightarrow Asp + NADP^+$$

Asparagine

$$OAA + 2\ NH_3 + ATP + NADPH, H^+ \rightarrow Asp + ADP + Pi + NADP^+$$

Threonine

$$OAA + NH_3 + 2\ ATP + 3\ NADPH, H^+ + H_2O \rightarrow Thr + 2\ ADP + 2\ Pi + 3\ NADP^+$$

Isoleucine

$$OAA + pyr + NH_3 + 2\ ATP + 5\ NADPH, H^+ \rightarrow Ile + CO_2 + 2\ ADP + 2\ Pi + 5\ NADP^+$$

Lysine

$$OAA + pyr + 2\ NH_3 + 2\ ATP + 2\ H_2O + 4\ NADH, H^+ \rightarrow Lys + 4\ CO_2 + 2\ ADP + 2\ Pi + 4\ NAD^+$$

Table 3 : (continued)

Valine

$$2\ pyr + 2\ NADPH,\ H^+ + NH_3 \rightarrow Val + CO_2 + 2\ NADP^+$$

Leucine

$$3\ pyr + NH_3 + ATP + NADPH,\ H^+ \rightarrow Leu + 3\ CO_2 + ADP + Pi + NADP^+$$

Alanine

$$Pyr + NH_3 + NADPH,\ H^+ \rightarrow Ala + NADP^+$$

Serine

$$3\ PG + NH_3 \rightarrow Ser$$

Glycine

$$3\ PG + NH_3 + THF \rightarrow Gly + CH_2\ THF$$

Cysteine

$$3\ PG + NH_3 + ATP + H_2S + H_2O \rightarrow Cys + ADP + Pi$$

Methionine

$$3\ PG + OAA + NH_3 + 4\ ATP + NADP^+ + H_2S + 4\ H_2O + CH_3 - THF \rightarrow Met + 3\ CO_2 + 4\ ADP + 5\ Pi + NADPH,\ H^+ + THF$$

Phenylalanine

$$E4P + 2\ PEP + NH_3 + ATP + 2\ NADPH,\ H^+ \rightarrow Phe + CO_2 + ADP + 2\ Pi + 2\ NADP^+ + H_2O$$

Tyrosine

$$E4P + 2\ PEP + NH_3 + ATP + NADPH,\ H^+ \rightarrow Tyr + CO_2 + ADP + 2\ Pi + NADP^+$$

Table 3 : (continued)

Tryptophane

$$E4P + PEP + R5P + 2\ NH_3 + 4\ ATP \rightarrow Trp + CO_2 + 4\ ADP + 8\ Pi$$

Histidine

$$R5P + 3\ H_2O + NADP^+ + 3\ ATP + 2\ NH_3 + CHO\text{-}THF \rightarrow His + 3\ ADP + 3\ Pi + NADPH, H^+ + THF$$

Table 4 : Synthesis of amino acids from CO_2 and NH_3

Glutamate

$$5\ CO_2 + NH_3 + 25{,}5\ ATP + 9\ NADPH,\ H^+ + 20{,}5\ H_2O \rightarrow Glu + 25{,}5\ ADP + 25{,}5\ Pi + 9\ NADP^+$$

Glutamine

$$5\ CO_2 + 2\ NH_3 + 26{,}5\ ATP + 9\ NADPH,\ H^+ + 20{,}5\ H_2O \rightarrow Gln + 26{,}5\ ADP + 26{,}5\ Pi + 9\ NADP^+$$

Proline

$$5\ CO_2 + NH_3 + 26{,}5\ ATP + 11\ NADPH,\ H^+ + 19{,}5\ H_2O \rightarrow Pro + 26{,}5\ ADP + 26{,}5\ Pi + 11\ NADP^+$$

Arginine

$$6\ CO_2 + 3\ NH_3 + 33{,}5\ ATP + 11\ NADPH,\ H^+ + 23{,}5\ H_2O \rightarrow Arg + 33{,}5\ ADP + 33{,}5\ Pi + 11\ NADP^+$$

Aspartate

$$4\ CO_2 + NH_3 + 24{,}5\ ATP + 6\ NADPH,\ H^+ + 21{,}5\ H_2O \rightarrow Asp + 24{,}5\ ADP + 24{,}5\ Pi + 6\ NADP^+$$

Asparagine

$$4\ CO_2 + 2\ NH_3 + 25{,}5\ ATP + 6\ NADPH,\ H^+ + 21{,}5\ H_2O \rightarrow Asn + 25{,}5\ ADP + 25{,}5\ Pi + 6\ NADP^+$$

Threonine

$$4\ CO_2 + NH_3 + 26{,}5\ ATP + 8\ NADPH,\ H^+ + 22{,}5\ H_2O \rightarrow Thr + 26{,}5\ ADP + 26{,}5\ Pi + 8\ NADP^+$$

Isoleucine

$$6\ CO_2 + NH_3 + 35\ ATP + 15\ NADPH,\ H^+ + 27\ H_2O \rightarrow Ile + 35\ ADP + 35\ Pi + 15\ NADP^+$$

Table 4 : (continued)

Lysine

$$6\,CO_2 + 2\,NH_3 + 43{,}5\,ATP + 15\,NADPH,\,H^+ + 34{,}5\,H_2O$$
$$\rightarrow Ile + 43{,}5\,ADP + 43{,}5\,Pi + 15\,NADP^+$$

Valine

$$5\,CO_2 + NH_3 + 17\,ATP + 12\,NADPH,\,H^+ + 9\,H_2O$$
$$\rightarrow Val + 17\,ADP + 17\,Pi + 12\,NADP^+$$

Alanine

$$3\,CO_2 + NH_3 + 9{,}5\,ATP + 6\,NADPH,\,H^+ + 5{,}5\,H_2O$$
$$\rightarrow Ala + 9{,}5\,ADP + 9{,}5\,Pi + 6\,NADP^+$$

Leucine

$$6\,CO_2 + NH_3 + 26{,}5\,ATP + 16\,NADPH,\,H^+ + 16{,}5\,H_2O$$
$$\rightarrow Leu + 26{,}5\,ADP + 26{,}5\,Pi + 16\,NADP^+$$

Serine

$$3\,CO_2 + NH_3 + 10{,}5\,ATP + 5\,NADPH,\,H^+ + 8{,}5\,H_2O$$
$$\rightarrow Ser + 10{,}5\,ADP + 10{,}5\,Pi + 5\,NADP^+$$

Glycine

$$3\,CO_2 + NH_3 + 11{,}5\,ATP + 5\,NADPH,\,H^+ + 7{,}5\,H_2O + THF$$
$$\rightarrow Gly + 11{,}5\,ADP + 11{,}5\,Pi + 5\,NADP^+ + CH_2\text{-}THF$$

Cysteine

$$3\,CO_2 + NH_3 + 11{,}5\,ATP + 5\,NADPH,\,H^+ + H_2S + 8{,}5\,H_2O$$
$$\rightarrow Cys + 11{,}5\,ADP + 11{,}5\,Pi + 5\,NADP^+$$

Methionine

$$4\,CO_2 + NH_3 + 39\,ATP + 9\,NADPH,\,H^+ + H_2S + 33\,H_2O + CH_3\text{-}THF$$
$$\rightarrow Met + 39\,ADP + 39\,Pi + 9\,NADP^+ + THF$$

Table 4 : (continued)

Phenylalanine

$$9\,CO_2 + NH_3 + 35{,}5\,ATP + 20\,NADPH,\,H^+ + 20{,}5\,H_2O \rightarrow Phe + 35{,}5\,ADP + 35{,}5\,Pi + 20\,NADP^+$$

Tyrosine

$$9\,CO_2 + NH_3 + 35{,}5\,ATP + 19\,NADPH,\,H^+ + 21{,}5\,H_2O \rightarrow Tyr + 35{,}5\,ADP + 36{,}5\,Pi + 19\,NADP^+$$

Tryptophane

$$11\,CO_2 + 2\,NH_3 + 45{,}25\,ATP + 23\,NADPH,\,H^+ + 25{,}25\,H_2O \rightarrow Trp + 45{,}25\,ADP + 45{,}25\,Pi + 23\,NADP^+$$

Histidine

$$5\,CO_2 + 3\,NH_3 + 23{,}25\,ATP + 9\,NADPH,\,H^+ + 14{,}25\,H_2O + CHO\text{-}THF \rightarrow His + 23{,}25\,ADP + 23{,}25\,Pi + 9\,NADP^+ + THF$$

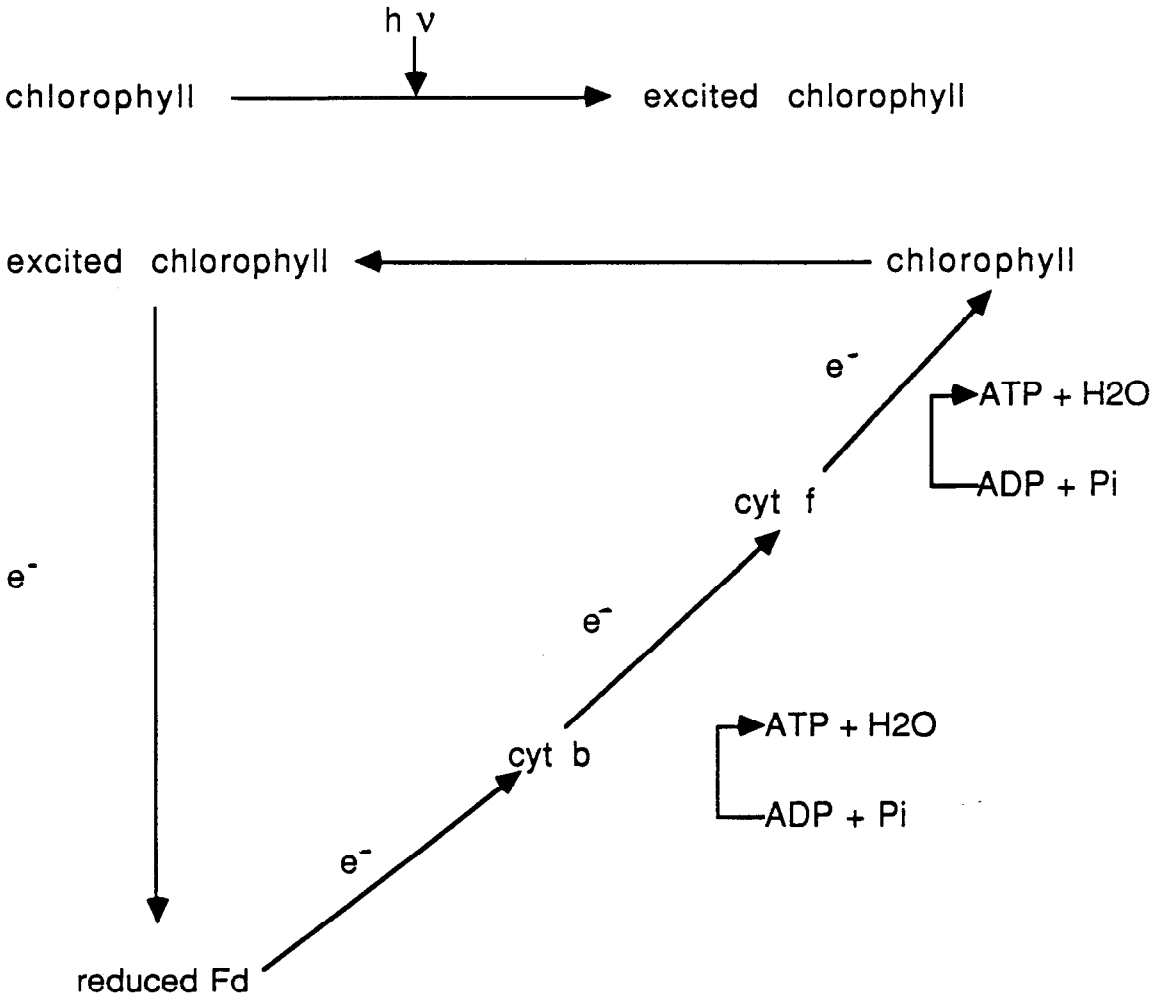

Figure 1: Cyclic phosphorylation pathway.

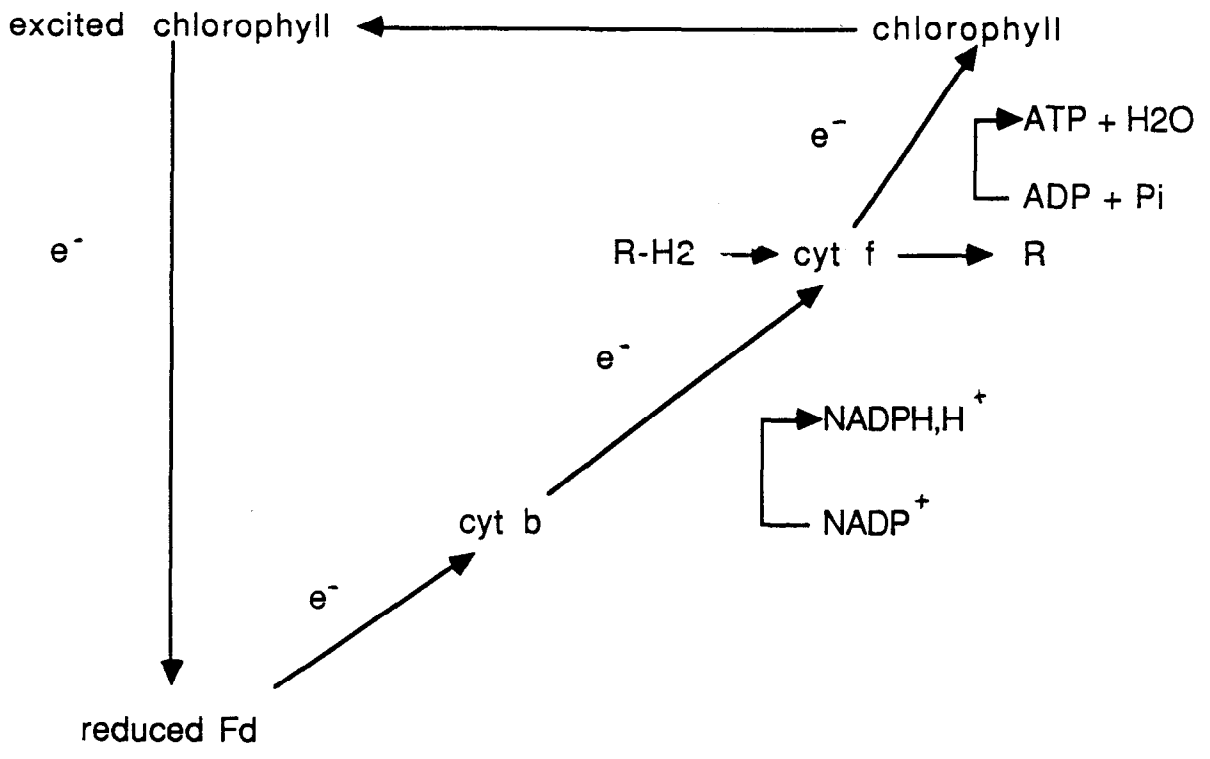

Figure 2: Non cyclic phosphorylation pathway.

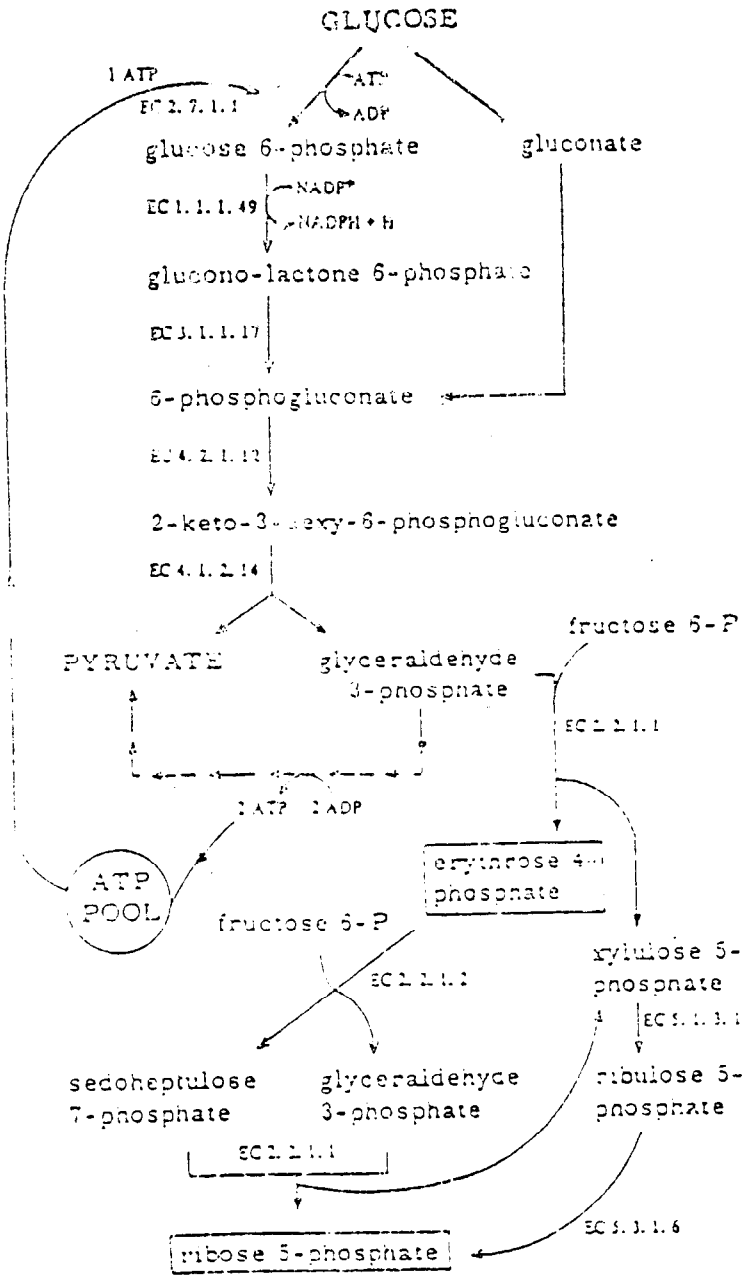

FIGURE 3 : Entner-Doudoroff pathway

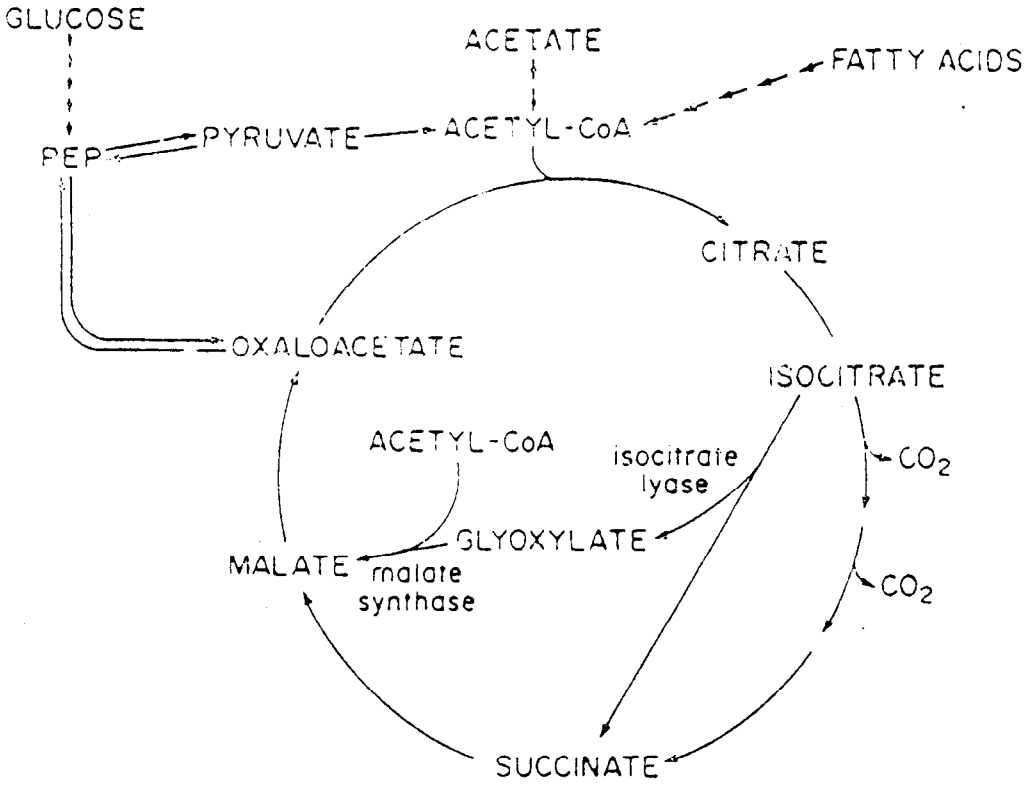

FIGURE 4 : glyoxylate shunt

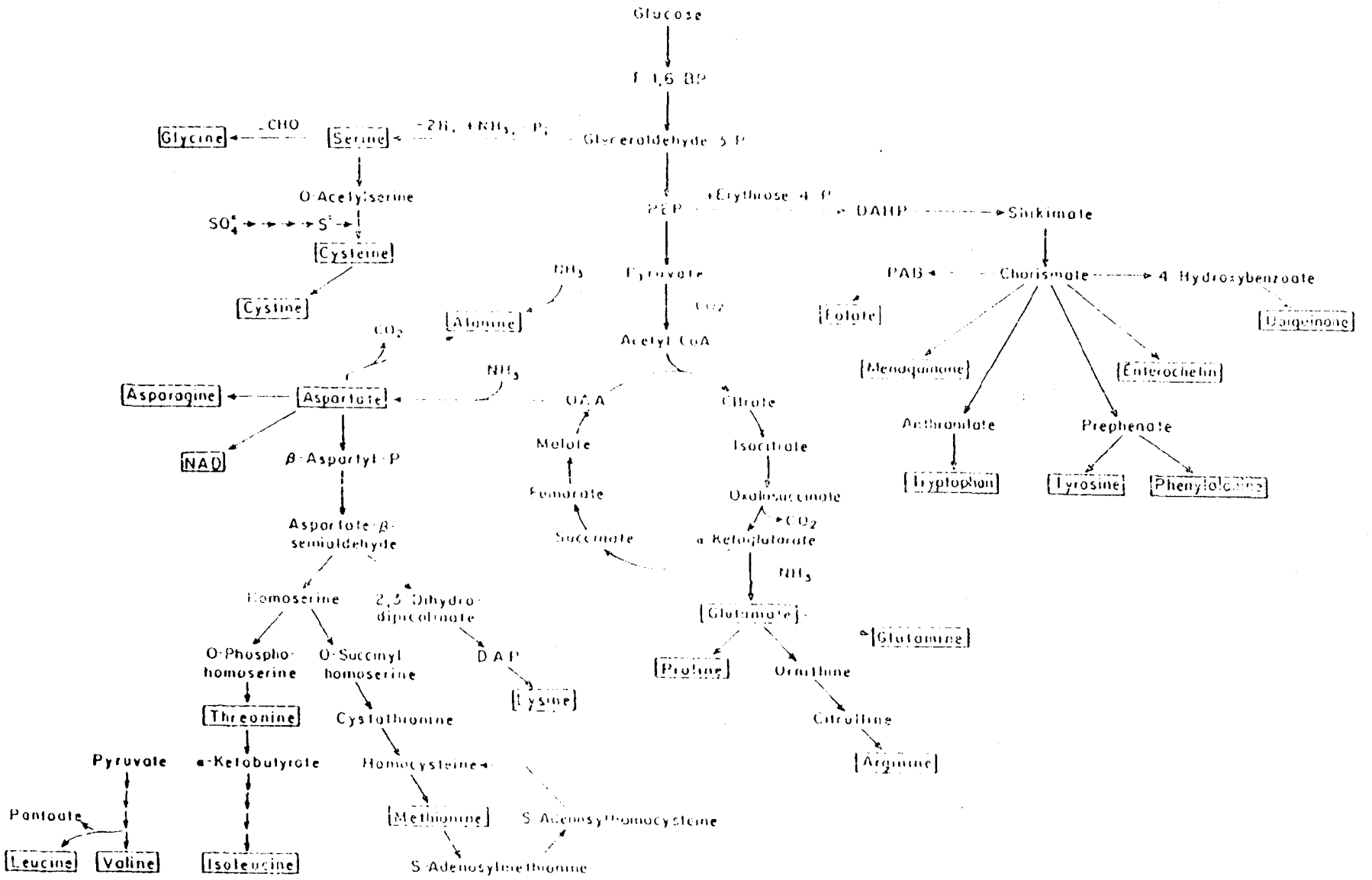

FIGURE 5 : General schemes for amino acids synthesis pathways (E. Coli "hypothesis")

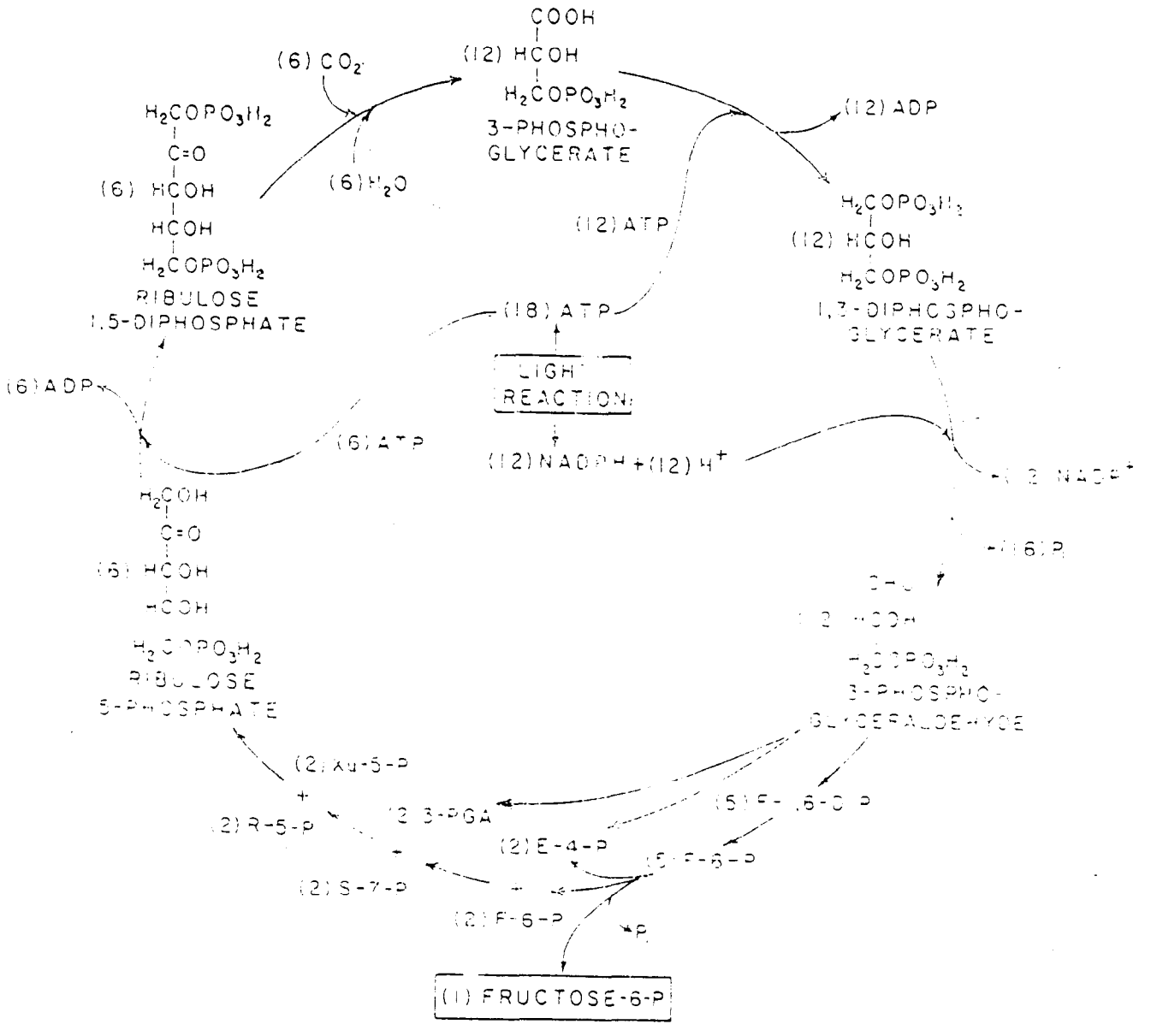

FIGURE 6 : Calvin-Benson cycle

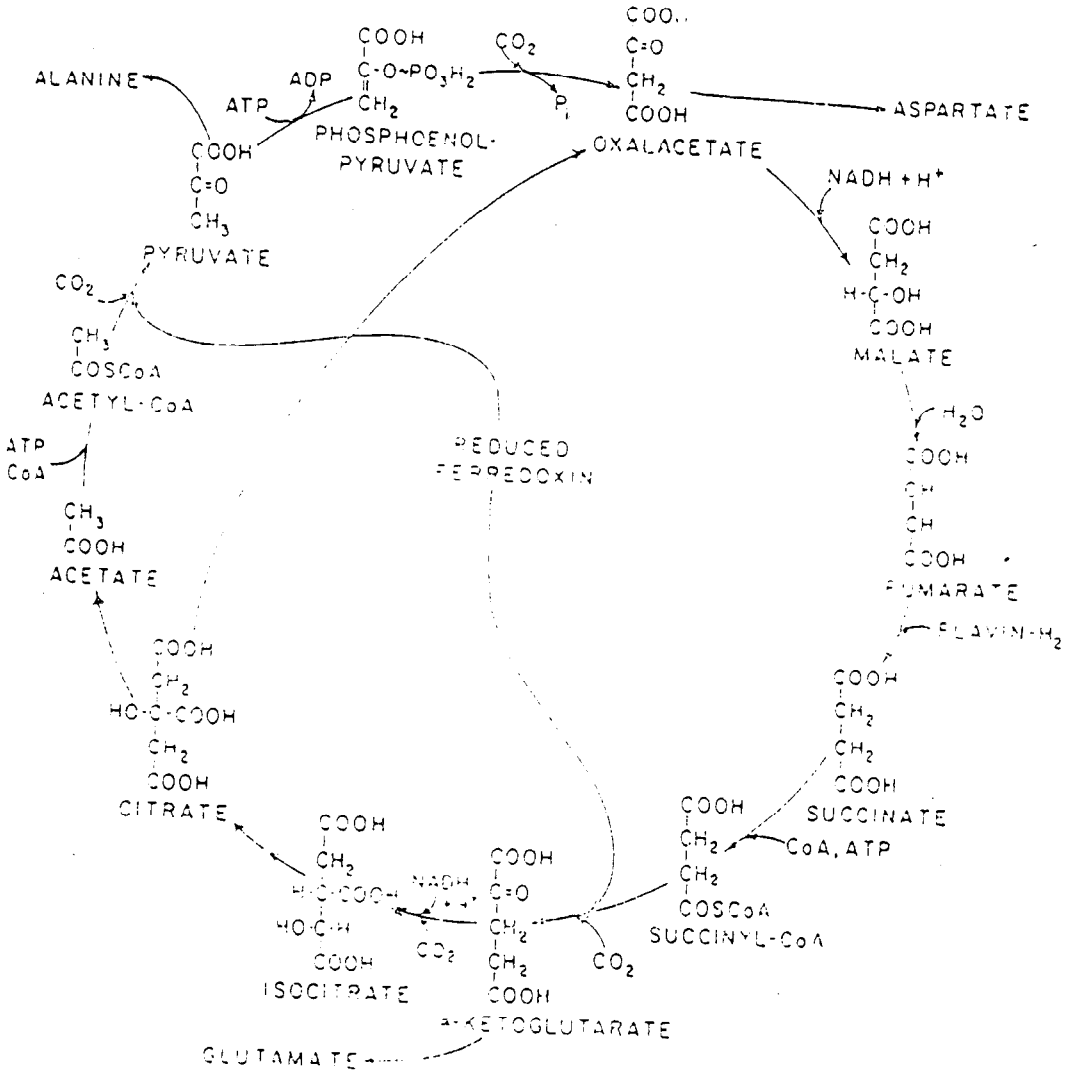

FIGURE 7 : Hatch-Slack cycle

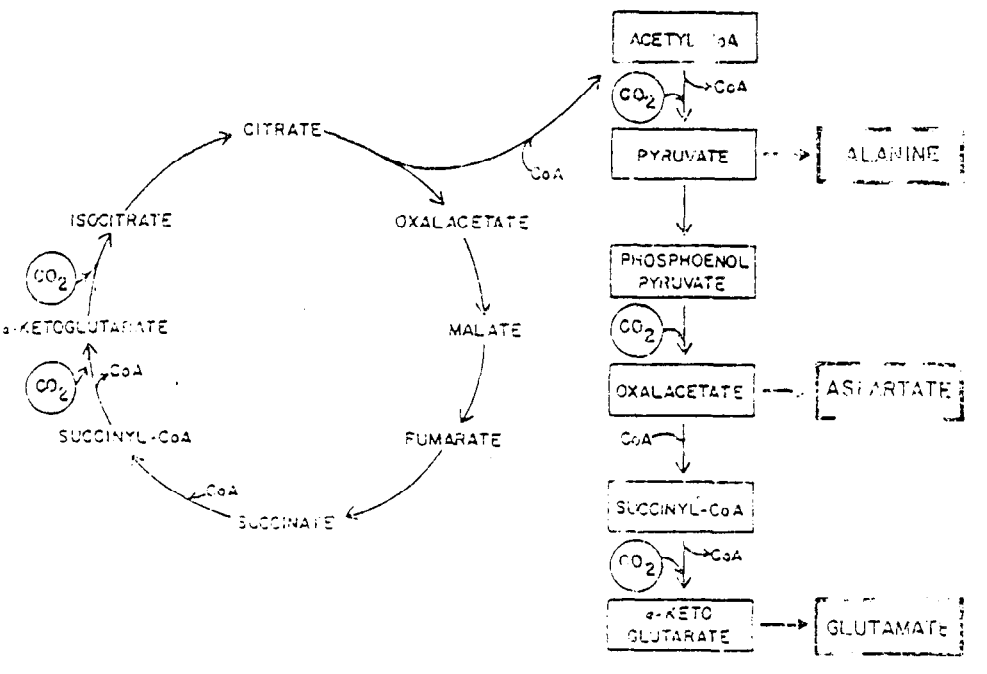

FIGURE 8 : Amino acids synthesis via Hatch-Slack cycle

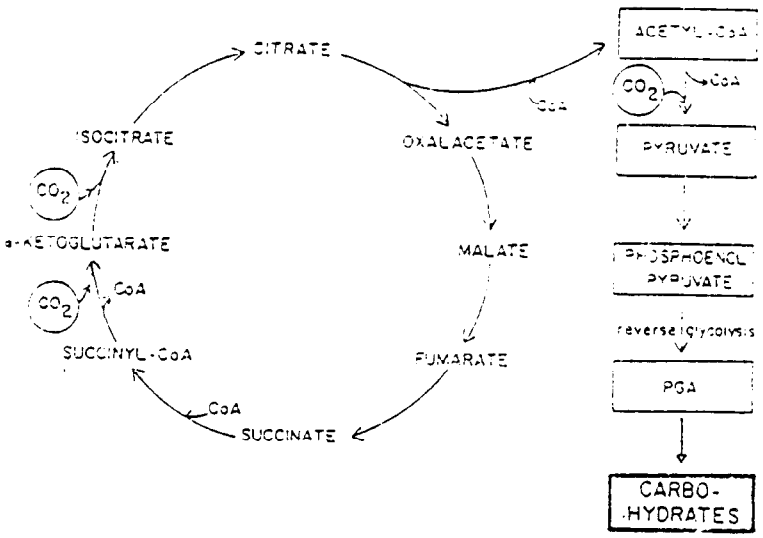

FIGURE 9 : Carbohydrate synthesis via Hatch-Slack cycle