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CCN4

MELiSSA SIMULATION AND MODELLING
Spirulina modelling

- Variable global stoichiometric equation of *Spirulina platensis* in different light conditions
- Effects of light on diet composition
- Best conditions to insure maximal mass recycling

TECHNICAL NOTE 17.3

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T.N 17.3: MELiSSA simulation and modelling *Spirulina* modelling

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INTRODUCTION

The cyanobacterium *Spirulina platensis* in comparment IV and the *Rhodobacteraceae* in compartment II are cultivated in photobioreactors. Thus, light is a growth limiting factor for these microorganisms.

For *Rhodobacter capsulatus*, preliminary studies (1) showed that the light acts upon the growth rate, but seems at the present time to have small effects on the biomass composition. Other studies shown that the nitrogenase of *Rhodobacter capsulatus* is inhibited at low light energy input (7) and that the quantity of bacteriochlorophylls depend on the light (8); this suggest an influence of the light on the microorganism metabolism and by the way on the microorganism biomass composition. At the present time, not enough data are available to consider the influence of light on the *Rhodobacteraceae* stoichiometric model.

In the case of *Spirulina platensis*, both the growth rate and the biomass composition varied with the radiant light energy available in the photoreactor. Preliminary results have shown that the percentage of exopolysaccharide varies under physical limitation by light in the photobioreactor. In the Technical Note 19.2, Cornet (2) proposed theoretical methods to obtain independently the volumetric rate of active biomass formation and of exopolysaccharide synthesis. Considering the elemental formula of active biomass and exopolysaccharide, the global formula composition of *Spirulina platensis* was obtained, and a good agreement was observed between the theoretical composition and the experimental data under different light limiting conditions.

In the present simulations of the MELiSSA loop, the kinetic aspects are not considered; thus the model developped by Cornet cannot be used just as it is. To introduce the influence of light on the biomass composition, a non kinetic approach must be considered.

The purpose of this note is to establish a stoichiometric model for *Spirulina platensis* depending on a light parameter and providing a good accuracy in the determination of the biomass composition under different light conditions. Simulation of the MELiSSA loop will be then performed under different light conditions on the *Spirulina* compartment.

1- A STOICHIOMETRIC MODEL FOR *Spirulina platensis*

The aim is to introduce the effect of light in the stoichiometric description of *Spirulina platensis*. A correlation between the experimental effects observed on the Spirulina composition in different light limiting conditions and the stoichiometric description of *Spirulina* is then searched.

1.1- Results on continuous cultures of *Spirulina platensis* in photobioreactor under different incident radiant light fluxes

Continuous cultures in a 5 L cylindrical photobioreactor have been performed under different incident light fluxes and different dilution rates in CNRS of Gif sur Yvette (for all details on this study, see final report of Centre National d'Etudes Spatiales, France, n° 91/CNES/0411).

The main results on the biomass composition obtained under different light limiting conditions are reported in table 1.

Table 1: Composition of *Spirulina platensis* in continuous cultures under different light limiting conditions.

Mean radiant incident flux Fo (W/m^2)	Volumetric rate of radiant energy energy absorbed <A> (W/m^3)	Overall composition of biomass (%)
10	80	Proteins: 65 Fat: 10 Carbohydrates: 15 Exopolysaccharide: 10
100	720	Proteins: 56 Fat: 10 Carbohydrates: 14 Exopolysaccharide: 20
160	1200	Proteins: 48 Fat: 10 Carbohydrates: 15 Exopolysaccharide: 27
230	1600	Proteins: 42 Fat: 10 Carbohydrates: 14 Exopolysaccharide: 34

Table 1 shows the great changes in proteins and exopolysaccharide percentages in the biomass composition with the increase of the incident and/or absorbed radiant energy, while carbohydrates and fat percentages seem not affected. Thus, the global elemental formula of the biomass changes with the different light illuminating conditions of the reactor.

1.2-Spirulina biomass and stoichiometric equations

The biomass is composed of a biotic and of an abiotic phase the description of which, proposed by Cornet in TN 19.2 (2), is reported in table 2.

Table 2: definition of the biotic and abiotic phases.

Biotic phase

<table>
<tr><td colspan="6">Active biomass</td><td rowspan="3">Glycogen</td><td rowspan="4">Exopoly-saccharide</td></tr>
<tr><td colspan="2">Proteins</td><td rowspan="2">Chlorophylls</td><td colspan="3">Biomass</td></tr>
<tr><td>Phyco-cyanins</td><td>Other proteins</td><td>Carbohydrates</td><td>Fat</td><td>Nucleic acids</td></tr>
<tr><td colspan="7">Vegetative biomass</td></tr>
<tr><td colspan="8">Total biomass</td></tr>
</table>

Abiotic phase

Nitrates	Sulfates

The biosynthesis of each macroelement of the biotic phase can be described by a stoichiometric equation. Considering the percentage of this macroelement in biomass and its corresponding equation, a global stoichiometric equation can be deduced. This percentage depends on culture conditions.

The intracellular glycogen included in the vegetative biomass is a form of energy storage by the microorganism. It appears during mineral limitations (nitrate, sulfate). Because these limitations are not considered in the present MELiSSA simulations, it is supposed that the total biomass is free of glycogen.

The C.H.O.N. formula of phycocyanins ($CH_{1.525}O_{0.306}N_{0.250}$) and their biosynthesis equation are quite similar with those of the total proteins ($CH_{1.526}O_{0.327}N_{0.250}$) (3). This justifies the use of fixed formula and equation for total proteins; the changes in the ratio of phycocyanins and other proteins with the culture conditions are not considered; thus these conditions affects only the global quantity of proteins in the biomass and not their quality.

The chlorophylls which represent less than 1% of the total biomass are neglected in the stoichiometric model for Spirulina.

The above assumption, lead to consider five macroelements: proteins, carbohydrates, fat , nucleic acids and exopolysaccharide. The stoichiometric equations have been established for these macroelements by Cornet (3) but there were modified in order to consider only the four elements C.H.O.N. and to satisfy the constraints on mass balances imposed by ProSim.

Carbohydrates:

$$CO_2 + 2.041\ H_2O + 3.33\ ATP + 2.1245\ NADPHH^+ \Downarrow CH_{1.670}O_{0.711} + 3.33\ Pi + 3.33\ ADP + 2.1245\ NADP^+ \quad (1)$$

Fat:

$$CO_2 + 1.2167\ H_2O + 3.0127\ ATP + 2.653\ NADPHH^+ \Downarrow CH_{1.714}O_{0.204} + 3.0127\ Pi + 3.0127\ ADP + 2.653\ NADP^+ \quad (2)$$

Proteins:

$$CO_2 + 1.2799\ H_2O + 0{,}2496\ HNO_3 + 3.702\ ATP + 3.0604\ NADPHH^+ \Downarrow CH_{1.526}O_{0.327}N_{0.2496} + 3.702\ Pi + 3.702\ ADP + 3.0604\ NADP^+ \quad (3)$$

Nucleic acids:

$$CO_2 + 1.307\ H_2O + 0{,}393\ HNO_3 + 3.776\ ATP + 2.909\ NADPHH^+ \Downarrow CH_{1.273}O_{0.710}N_{0.393} + 3.776\ Pi + 3.776\ ADP + 2.909\ NADP^+ \quad (4)$$

Exopolysaccharide:

$$CO_2 + 2.28\ H_2O + 3.33\ ATP + 1.875\ NADPHH^+ \Downarrow CH_{1.650}O_{0.950} + 3.33\ Pi + 3.33\ ADP + 1.875\ NADP^+ \quad (5)$$

The biosynthesis of these macroelements involve ATP and reduced cofactors (note that these cofactors are considered as equivalent to $NADP^+$). A sixth equation can be established for the maintenance of the microorganism:

$$ATP + H_2O \Rightarrow ADP + Pi \quad (6)$$

The regeneration of cofactor and the production of ATP are associated to the photosynthesis, and can be described by the two following equation:

$$ADP + Pi \Rightarrow ATP + H_2O \quad (7)$$

$$NADP^+ + H_2O \Rightarrow NADPHH^+ + \frac{1}{2} O_2 \quad (8)$$

Assuming a pseudo-steady state in the cell for ATP and reduced cofactors, equations (7) and (8) are summed to the six others, and non-structured classical stoichiometric equations for each macroelement are obtained (note that because the kinetic aspect of the equations is not considered, equation (6) will not appear in the stoichiometric model of *Spirulina* biosynthesis). The five remaining equations are the following:

Carbohydrates:

$$CO_2 + 0.8355\ H_2O \Downarrow CH_{1.670}O_{0.711} + 1.06225\ O_2 \quad (9)$$

Fat:

$$CO_2 + 0.8570\ H_2O \Downarrow CH_{1.714}O_{0.204} + 1.3265\ O_2 \quad (10)$$

Proteins:

$$CO_2 + 0.6383\ H_2O + 0{,}2496\ HNO_3 \Downarrow CH_{1.526}O_{0.327}N_{0.2496} + 1.5302\ O_2 \quad (12)$$

Nucleic acids:

$$CO_2 + 0.440\ H_2O + 0{,}393\ HNO_3 \Downarrow CH_{1.273}O_{0.710}N_{0.393} + 1.4545\ O_2 \quad (13)$$

Exopolysaccharide:

$$CO_2 + 0.825\ H_2O \Downarrow CH_{1.650}O_{0.950} + 0.9375\ O_2 \quad (14)$$

1.3- Correlations between light and stoichiometric equations

Each macroelement represents a given percentage of the total biomass; thus in order to establish a global stoichiometric equation for the biomass biosynthesis, the equations of macroelements, affected with the percentage of the macroelement in biomass, are summed. The purpose of this part is to express these percentages as a function of the light energy availability.

From table 1 the percentages of macroelements vary with the light limiting conditions. Correlations between the macroelements percentages (note that these percentages are mass percentages) and the mean light radiant incident flux Fo (in W/m^2) can be calculated using a linear regression approach; these correlations are presented in table 3.

Table 3: correlations between macroelements percentages and Fo (deduced from table1)

Macroelement (M)	Correlation $\%^mM$ (macroelement mass percentage) Fo (W/m^2)	Standard deviation
Proteins	$\%^mP = -0.106705\ Fo + 66.088123$	+/- 0.265%
Fat	$\%^mF = 10$	-
Carbohydrates	$\%^mC = 100 - \%P + \%F + \%E$	+/- 0.268%
Exopolysaccharide	$\%^mE = 0.109770\ Fo + 9.028736$	+/- 0.041%

Note that the percentage of carbohydrates in biomass is calculated here in order to have 100% when all macroelements presented in table 3 are summed.

The nucleic acids are not considered in table 1, *i.e.* no correlation between their percentage in biomass ($\%^mAN$)and light illuminating conditions can be calculated. In order to consider the nucleic acids in the biomass composition from table 2, the correlations established in table 3 have to be corrected. It is supposed that the nucleic acids represent 4% (weight/weight) of the active biomass (3), *i.e.* 4% of the biomass composed of proteins, carbohydrates, fat and nucleic acids. This assumption leads to the following corrections upon the previous correlations:

$$\%^mP_c = \%^mP \,.\, 0.96$$
$$\%^mF_c = \%^mF \,.\, 0.96$$
$$\%^mC_c = \%^mC \,.\, 0.96$$
$$\%^mAN_c = 0.04\ (100 - \%^mE)$$
$$\%^mE_c = \%^mE$$

1.4- Final stoichiometric equation for *Spirulina* biomass

From the macroelements mass percentages ($\%^mMc$), the molar percentages in biomass (%M) can be calculated. This molar percentages are used to affect the stoichiometric equations of macroelements, which are then summed.

Example 1 for Fo equal to 10 W/m^2 (*i.e.* <A>=80 W/m^3 in the Gif reactor).

The global equation for the total biomass is:

$$CO_2 + 0.7012\ H_2O + 0.1694\ HNO_3$$
$$\Downarrow$$
$$CH_{1.5717}O_{0.4208}N_{0.1694} + 1.3944\ O_2$$

The P/$2e^-$ ratio for this biosynthesis is then 1.2686

Biomass composition

	Mass percentage	Molar weight (g/Cmol)	Molar percentage (%M)	Mass composition of active biomass
Proteins	62.42	22.2771	63.60	69.45
Fat	9.6	17.0026	12.82	10.68
Carbohydrates	14.26	25.0699	12.91	15.87
Nucleic acids	3.59	30.1584	2.71	4
Exopolysaccharide	10.13	28.8736	7.96	-
Total biomass	100	22.7001	100	-

Example 2 for Fo equal to 230 W/m^2 (*i.e.* <A>=1600 W/m^3 in the Gif reactor).

Global equation for the total biomass:

$$CO_2 + 0.7425\ H_2O + 0.1153\ HNO_3$$
$$\Downarrow$$
$$CH_{1.6002}O_{0.5456}N_{0.1153} + 1.2715\ O_2$$

The P/2e$^-$ ratio for this biosynthesis is then 1.3591

Biomass composition

	Mass percentage	Molar weight (g/Cmol)	Molar percentage (%M)	Mass composition of active biomass
Proteins	39.88	22.2771	42.91	60.68
Fat	9.6	17.0026	13.53	14.61
Carbohydrates	13.61	25.0699	13.02	20.71
Nucleic acids	2.63	30.1584	2.09	4
Exopolysaccharide	34.28	28.8736	28.45	-
Total biomass	100	23.9683	100	-

Considering the results obtained under these two extreme light illuminating conditions, two main observations can be made:

1- the ratio CO_2/O_2 increases with the raise of the light radiant energy input (+8.8%);

2- the changes with light in the active biomass composition are less than in the total biomass composition and for low values of Fo (example 1) this composition is in great agreement with the classical composition of *Spirulina i.e.* 70% proteins, 10% fat, 16% carbohydrates and 4% nucleic acids (3).

1.5- Global scheme of the Spirulina compartment for simulations

The following figure 1 represents the scheme of the different operations computed by the simulation program and leading to the final stoichiometric equation of formation of *Spirulina* biomass.

Note that the light radiant energy input Fo is a parameter of compartment IV as temperature, pH ,pressure or fractional conversion of the key substrate.

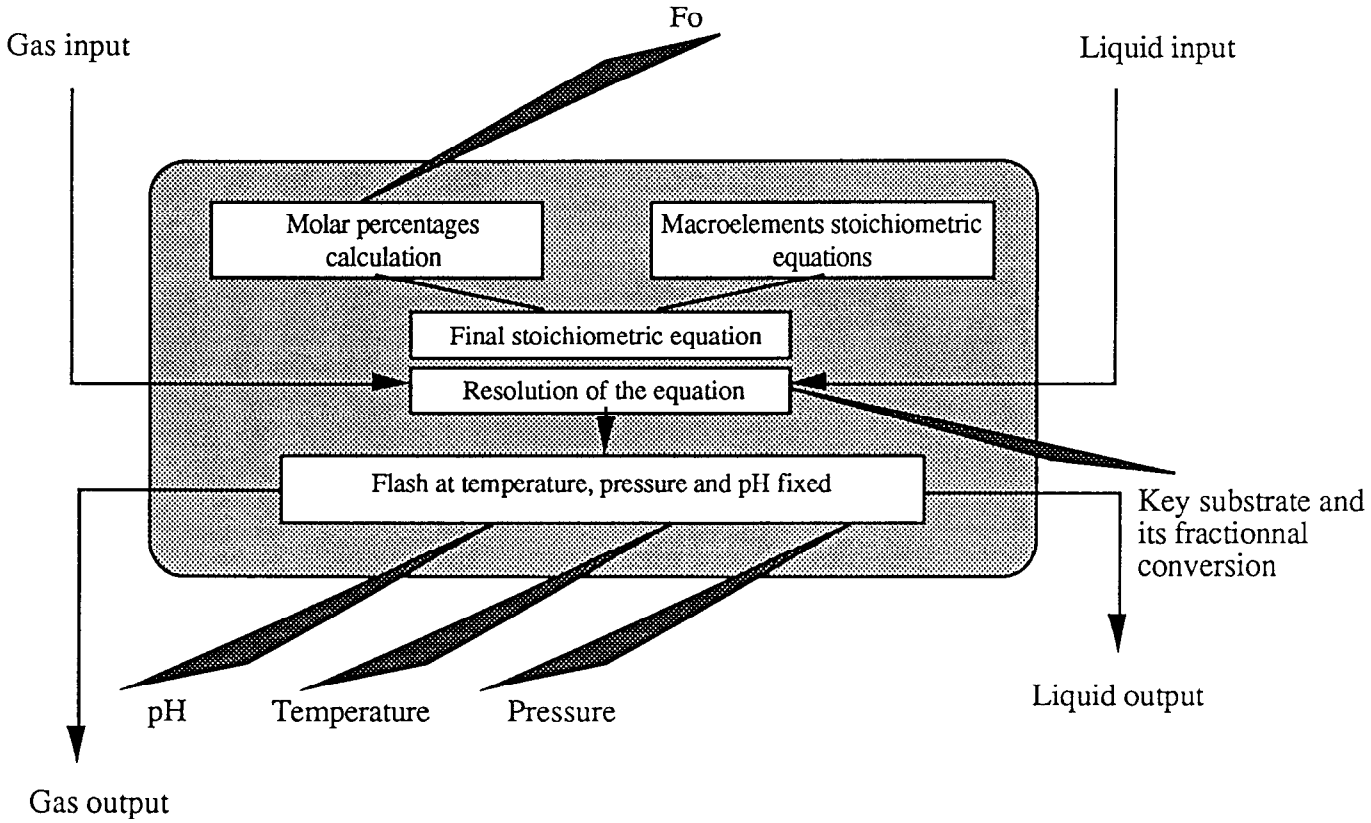

Figure 1: Scheme of the *Spirulina* compartment

A stoichiometric model for *Spirulina platensis* has been established. It is based upon the 5 equations of the macroelements which compose biomass, affected with their percentage in biomass. This percentage is a function of the light radiant energy input Fo. The photosynthetic compartment is then described for simulations (TN 17.1) by temperature, pressure, pH, Fo and the five equations.

2- SIMULATIONS UNDER DIFFERENT LIGHT CONDITIONS

2.1- Parameters of the simulations

The simulation flowsheet is the flowsheet presented in TN 17.1 (4) (the global scheme of the flowsheet is reported in appendix 1). As mentioned in this TN the conditions under which the simulation is performed (working conditions of the compartments, values of the constraints and of the degrees of freedom for the simulation) must be described before presenting the results.

Description of the compartments

The stoichiometric equations which describe the behaviour of the different compartments are reported in appendix 2.

- Consumer -

Pressure = 1 atm.
Temperature = 293 K
Faeces composition fixed: $CH_{1.649}O_{0.15}N_{0.1055}$

Oxygen need considered :

$$O2=0.2975\ ED - 0.2$$

where ED is the Energy Demand in kcal/p.d.,
thus, for ED = 3000 kcal/p.d.

$$O2=892.3\ mol/p.d.$$

It is supposed that 1500 g of water/p.d. is vaporized by perspiration and respiration.

The key substrate is the food (glucose, palmitic acid and biomass), with a fractional conversion equal to 1.

- Compartment I (Liquefying) -

Pressure = 1 atm.
Temperature = 330 K
pH = 5

The key substrate for the first equation is the faeces, and for the second the urea, both with a fractional conversion equal to 1.

- Compartment II (Phototrophs) -

Pressure = 1 atm.
Temperature = 303 K
pH = 7

The key substrate for the photoautotrophs is the hydrogen with a fractional conversion equal to 1. The key substrates for the photoheterotrophs are the volatile fatty acids (respectively acetate and butyrate) with fractional conversions equal to 1.

- Compartment III (Nitrifying) -

Pressure = 1 atm.
Temperature = 303 K
pH = 8

The key substrate is the ammonia with a fractional conversion equal to 1.

-Compartment IV (*Spirulina*)

Pressure = 1 atm.
Temperature = 309 K
pH =9.5
Light radiant incident flux (Fo) = variable

The key substrate is the nitrate with a fractional conversion equal to 1.

- Gas separator and condenser -

The operations on the gas fluxes are supposed to be ideal (*i.e.* complete separation of the different compounds)

Constraints and degrees of freedom

The values for the three degrees of freedom are fixed on the following values :

100% for Y (the totality of the biomass produced by the *Rhodobacter* is consumed);

100% for Z (the totality of the biomas produced by the *Spirulina* is consumed);

Fo varied in the range of 10 to 250 W/m^2.

The constraints applied on the flowsheet of the MELiSSA loop are the following:

-the diet constraints are those determined for an energy demand by the consumers of 3000 kcal/p.d. The protein needs are supposed to be provided only by the consumption of biomass (for details report on TN 17.1);

-the gas composition in the consumer compartment is maintained to 20% of oxygen, less than 0.5% of carbon dioxide and a relative humidity of 55%. The gas input in the compartement is fixed to 390 kg/h;

-the dinitrogen/oxygen flux to the oxygenic compartment (III) is fixed to 100 mol/h.

2.2- Results

2.2.1- Influence of Fo on the recycling performances of the MELiSSA loop

Considering the previous results (TN 14.1 and 14.2) (5,6), the simulation was first performed in order to have a maximal N recycling percentage (Y and Z equal to 100%, *i.e.* the totality of the edible biomass is used in the diet). The recycling performances of the MELiSSA are observed considering four recycling percentages defined as following:

the N element recycling percentage

$$RN=\left(1-\frac{\text{flow rate of N introduced in the system}}{\text{maximal flow rate of N in the system}}\right).100$$

the C element recycling percentage

$$RC=\left(1-\frac{\text{flow rate of C introduced in the system}}{\text{maximal flow rate of C in the system}}\right).100$$

the CO_2 recycling percentage

$$RO_2=\left(1-\frac{\text{flow rate of } O_2 \text{ entering the system}}{O_2 \text{ consumed in the system}}\right).100$$

the O_2 recycling percentage

$$RCO_2=\left(1-\frac{\text{flow rate of } CO_2 \text{ leaving the system}}{CO_2 \text{ produced in the system}}\right).100$$

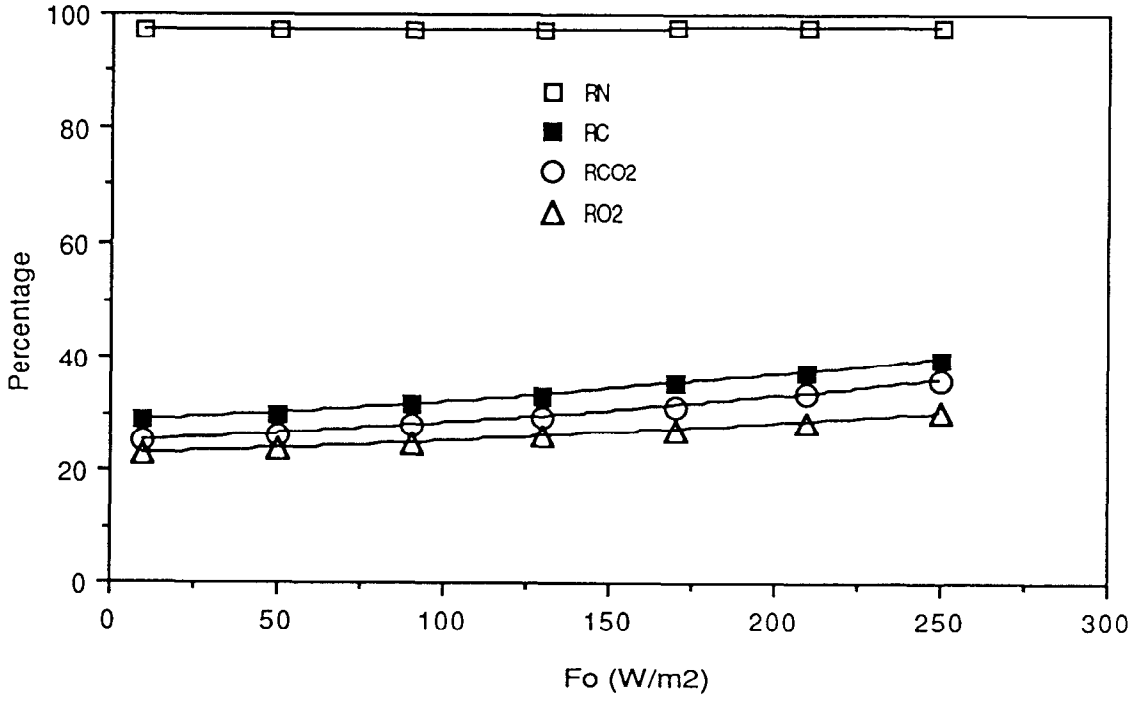

Figure 2: recycling percentages as a function of the light radiant energy input. Y=100%. Z=100%

Figure 2 shows the variations of these percentages for Fo in the range of 10 to 150 W/m^2. Several comments can be made:

1- the N recycling percentage is not affected by Fo and remains equal to 98%; thus RN does not depend on the light conditions in the photosynthetic compartment;

2- the three other percentages (RC, RCO_2, RO_2) increase with Fo, but remain lower than RN (in the range of 20 to 40%);

3- although of RC remain higher than RCO_2, the two percentages follow the same evolution;

4- the difference between RCO_2 and RO_2 increases with Fo; this can be linked with the observation of the changes in the CO_2/O_2 ratio with Fo in the global stoichiometric equation of the *Spirulina* biomass.

Whatever the value of Fo, the MELiSSA loop have the same behaviour: a good N element recycling and a low atmosphere regeneration. Thus Fo seems not to affect the global behaviour of the ecosystem.

This is confirmed in figure 3, where the parameters Y and Z are respectively 100% and 50%. As expected, with new values of Y and Z these recycling percentages RO2 and RCO2 increase, while RN decrease and RC is not affected. Except their values the curves are similar to curves in figure 2.

The difference between RC and RCO2 can be directly attributed to the loss in wasted biomass (edible and non edible).

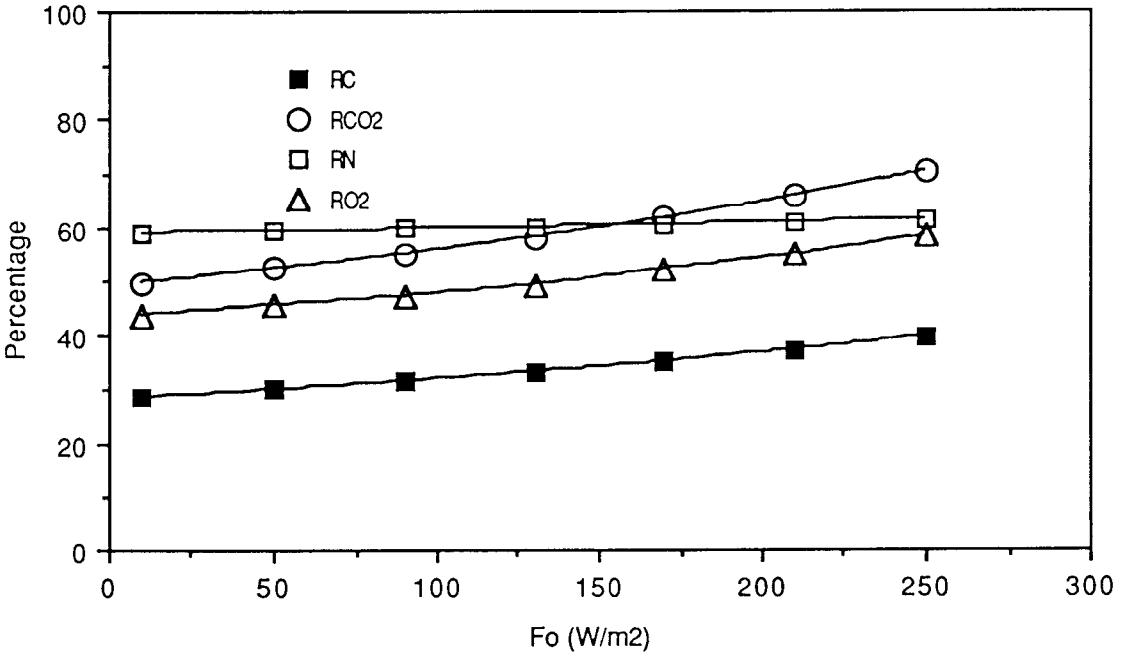

Figure 3: recycling percentages as a function of the light radiant energy input. Y=100%. Z=50%

2.2.2- Influence of Fo on the diet composition

The percentage of biomass in the diet (among 595.5 g dry food/d.p.) increases with Fo (figure 4). With increasing Fo, the protein content of *Spirulina* decreases (table 1), and to

preserve the quantity of proteins in the diet, the quantity of *Spirulina* has to increase. Thus, the quantity of exopolysaccharide in the diet increases (because of increasing the quantity of biomass and of the rise of exopolysaccharide in the *Spirulina* composition); therefore external inputs (glucose, palmitic acid) decrease.

This explains the rise in the percentage of carbon recycled.

To provide more *Spirulina*, the work of the photosynthetic compartment increases, and more oxygen and carbon dioxide are recycled, which explains the changes in the recycling percentages (figure 2 and 3).

Figure 4 shows the diet composition under different light conditions *Spirulina* represents 70% of the biomass, whereas in the previous simulation design *Spirulina* represented only 14%. This is the main consequence of the new approach of the consumer and liquefying compartments (this approach will be presented in a further technical note). The biomass represents between 29 to 41% of the dry weight of the diet. These values can be considered as acceptable, because rats have been fed with a diet composed of 40% of *Spirulina* with no apparent toxic effects or no apparent nutritional problems.

Note that results are obtained for Y and Z equal to 100%, thus by modifying these two parameters the diet composition will be modified too. In every case, the percentage of biomass in diet depends mainly on the parameter Fo, and the percentages of the microorganisms which composed the biomass depend on the parameters Y and Z.

Finally, it could be noticed that nucleic acids represent 1.81% to 1.96% in mass of the total food, *i.e.* 10.77g/p.d. to 11.67g/d.p.

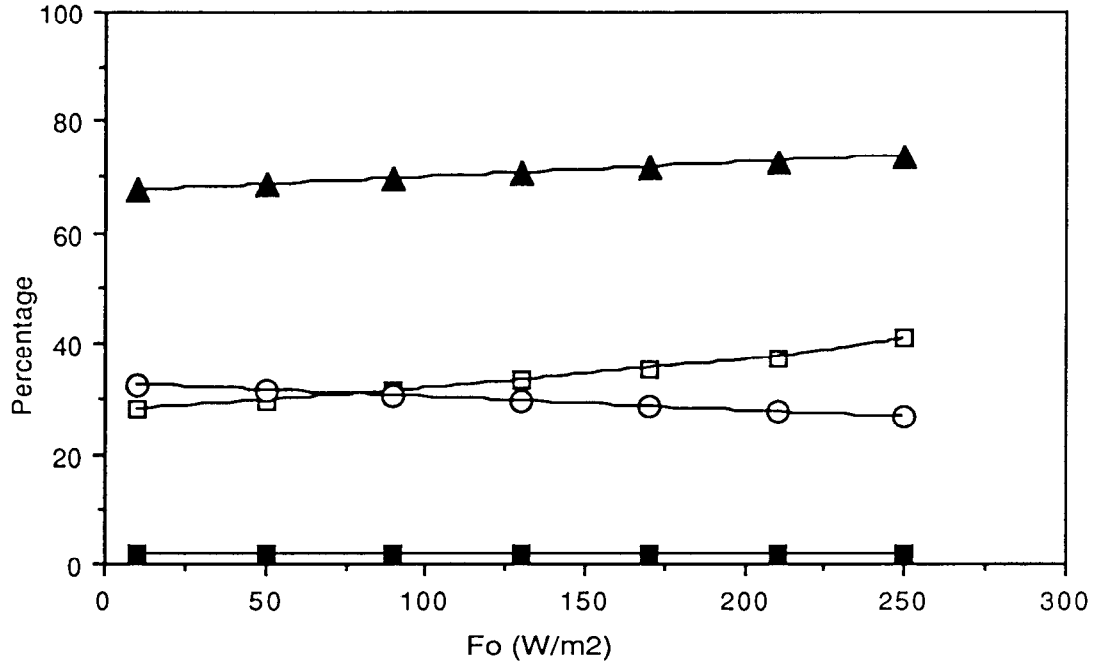

Figure 4: Diet composition as a function of the ligth radiant energy input (Y=100%, Z=100%); ▲ % Spirulina in biomass; ○ % Rhodobacter in biomass; ❑ % biomass in the diet; ■ % nucleic acids in the diet.

The general effect of increasing the incident light energy from 10 W/m^2 to 250W/m^2 is to increase the overall carbon recycling by almost 12% (RC ranges between 28% to 40%) by the decrease in external carbon inputs (glucose and palmitic acid) modulating the diet composition to match the fixed energy

requirements of the consumer (3000 kcal/d.p.). Despite the results reported in figure 3 obtained for maximal edible biomass recycling (Y=Z=100%), it is stressed that carbon recycling depends only on the composition of the diet irrespective of the values of Y and Z. The losses of carbon occur mainly by CO_2, or by both CO_2 and wasted edible and non-edible biomass when Y and Z are less than 100%.

3- BEST CONDITIONS TO INSURE MAXIMAL MASS RECYCLING

From the previous simulation results (4) and results presented in part 2- of this T.N., the range of the recycling performance of the MELiSSA loop and the influence of the parameters Y, Z and Fo can be determined.

The carbon recycling percentage (RC) ranges from 29 to 40%. The greatest changes are obtained by varying the light radiant energy input Fo. The parameters Y and Z have a low influence on RC.

The nitrogen recycling percentage (RN)ranges from 36 to 98%. At the opposite of RC, the greatest changes are obtained by varying the parameter(s) z or (and) Y, and Fo has a low influence on RN.

The oxygen (RO2) and carbon dioxide (RCO2) recycling percentages are respectively in the ranges of 24-85% and 26-100%. The main influence of Fo is to increase the difference between RO2 and RCO2 (figure 2). The maximal recycling percentages are obtained for the minimal value of Y and Z, at the opposite of RN.

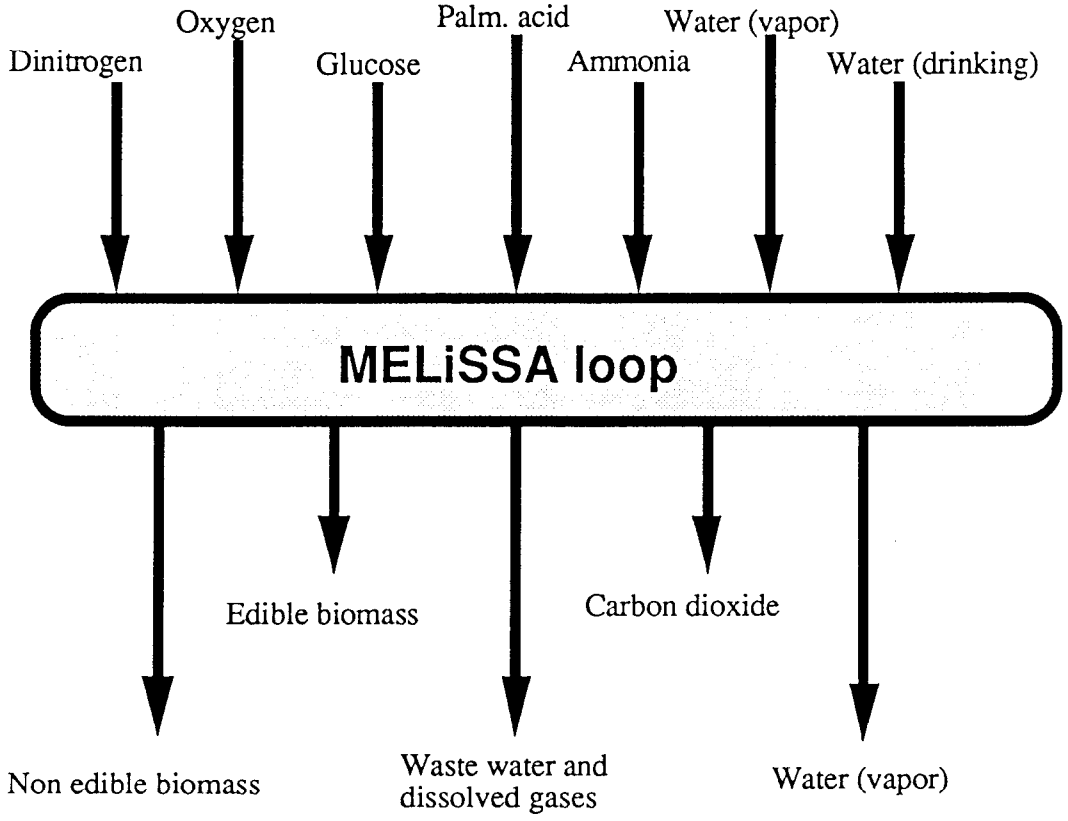

Figure 5: inputs and outputs on the MELiSSA loop

MELiSSA is not a closed system, it involves several inputs and outputs as shown in figure 5. A criterium was defined to minimize these inputs (and consequently the outputs), *i.e.* minimize the total mass of the components to introduce in the MELiSSA loop and by the way increasing the recycling performances of the loop.

The mass inputs for various conditions of simulation (various Y, Z and Fo parameters) are reported in table 4.

Table 4: Inputs mass in g/h.3p. and recycling performances of the MELiSSA loop in different cases (simulations from the flowsheet described in part 2). Case 1: Z=100%, Y=100%, Fo=250. Case 2: Z=100%, Y=100%, Fo=150. Case 3: Z=30%, Y=100%, Fo=150. Water (a) as atmospheric input and water (d) as drinking input. Total mass (a) considering the water inputs and (b) without water inputs.

Case	ammonia	oxygen	glucose	palm. acid	dinitrogen	water (d)	water (a)	Total mass	Recycling performances
1	0.071	81.568	29.419	14.626	0.0027	330	3245.93	3701.167 (a) 125.687 (b)	RN=97.40% RC=39.81% RCO2=36.02% RO2=30.22%
2	0.074	85.898	34.013	14.934	0.0025	330	3246.79	3711.712 (a) 134.922 (b)	RN=97.40% RC=34.16% RCO2=30.41% RO2=26.59%
3	4.185	27.1648	34.013	14.934	0.0039	330	3247.35	3657.65 (a) 80.3 (b)	RN=39.95% RC=34.16% RCO2=91.91% RO2=79.24%

Because of the water recycling is not considered in the present MELiSSA loop design, it is here more interessing to consider the total mass without the water inputs (b).

The total mass of the input components decreases with Y, Z and with the rise of Fo. The first idea to minimize these inputs is then to use minimal values for Y and Z, and maximal value for Fo. But surprisingly by minimizing the inputs, the nitrogen recycling percentage decreases. Moreover, it is for the highest values of Fo that the difference between the oxygen and the carbon dioxide recycling is the greatest. As shown in table 4, to minimize the total mass input is quite equivalent to minimize the oxygen input in the loop.

Considering both the mass and the recycling performances constraints, intermediate values for the three parameters Y, Z and Fo must be considered.
These values can be:

Z=50%
Y=100%
Fo=150 W/m^2

which gives the following results:

ammonia	oxygen	glucose	palm. acid	dinitrogen	water (d)	water (a)	Total mass	Recycling performances
1.835	60.499	34.013	14.934	0.0027	330	3244.55	3685.84 (a) 111.29 (b)	RN=60.28% RC=34.16% RCO2=50.40% RO2=60.65%

CONCLUSION

A stoichiometric description of the photosynthetic compartment has been established considering the light factor. This model, which provides is in accordance with the experimental data, introduces a new degree of freedom in the MELiSSA flowsheet: the light radiant energy input Fo.

The value of Fo has no influence on the nitrogen recycling in the loop, but acts upon the difference between oxygen and carbon dioxide recycling percentage, and mainly upon the carbon recyling and the food composition for the crew.

The studies of the best conditions to insure maximal mass recovery in the MELiSSA loop show the incompatibity of this maximal recovery and of good nitrogen recycling in the loop. Thus, both the maximal mass recycling and the maximal recycling for each element (N,C, CO2, O2) must be take into account in order to determine optimal conditions for the loop.

At the present time we can only estimate these conditions, but the use of an algorythm to optimize the working conditions of the loop is on study.

The photosynthetic compartment is not the solely compartment of MELiSSA functionning with light. The light is a growth limiting factor for the photroph compartment. The effect of light in the stoichiometric model of this compartment will be the purpose of further studies.

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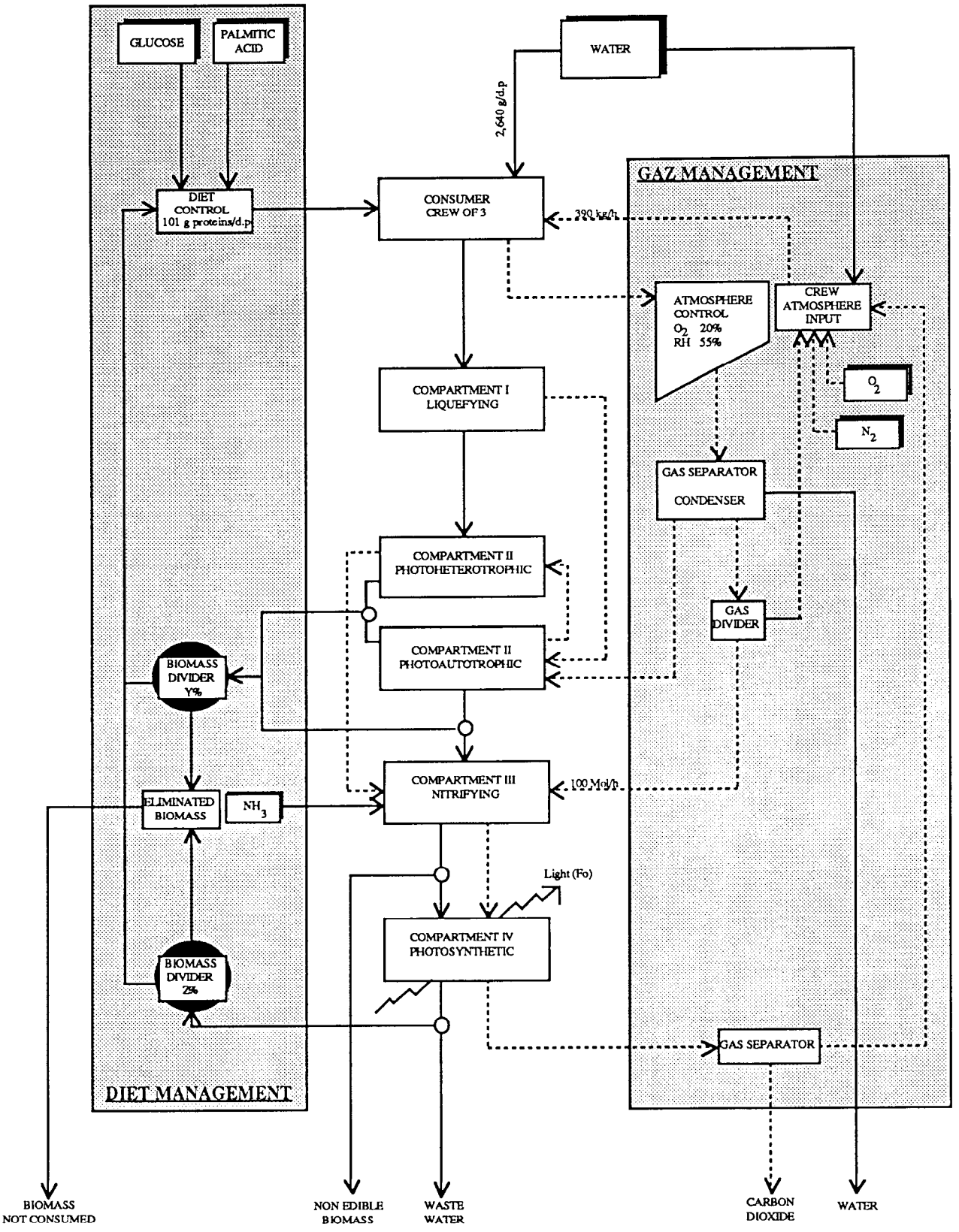
APPENDICES

APPENDIX I



Simplified representation of the simulation design of the MELISSA loop

O : biomass extraction ; ❐ external input

-------- Gas ——— Liquid / solid

APPENDIX II

Stoichiometric equations describing the compartments of the MELiSSA loop.

Consumer compartment	
Diet control	glucose + palmitic acid + biomass $\Rightarrow$ $[C.H.O.N.]_{FOOD}$
Consumption of food	$\underline{\underline{[C.H.O.N.]_{FOOD}}} + \alpha\, O_2 \Downarrow \beta\,[C.H.O.N.]_{FAECES} + \delta\, CO_2 + \varepsilon\, CH_4ON_2 + \zeta\, H_2O$ α=0.2975 ED - 0.2 Energy Demand (kcal/d.p.) $[C.H.O.N.]_{FAECES} = CH_{1.649}O_{0.15}N_{0.1055}$
Liquefying compartment Faeces fermentation (mainly proteins, carbohydrates and microorganisms)	$\underline{\underline{[C.H.O.N.]_{FAECES}}} + 0.975\, H_2O \Downarrow 0.25\, CO_2 + 0.89125\, H_2 + 0.25\, C_2H_4O_2 + 0.0625\, C_4H_8O_2 + 0.1055\, NH_3$
Urea degradation	$\underline{\underline{CH_4ON_2}} + H_2O \Rightarrow CO_2 + 2\, NH_3$
Phototroph compartment	
Biosynthesis of *Rh. capsulatus* (photoautotroph)	$CO_2 + 0.2022\, NH_3 + 2.1042\, \underline{\underline{H_2}} \Downarrow CH_{1.6511}O_{0.4156}N_{0.2022} + 1.5826\, H_2O$
Biosynthesis of *Rs. rubrum* from acetate	$0.5266\, \underline{\underline{C_2H_4O_2}} + 0.2022\, NH_3 \Downarrow CH_{1.6511}O_{0.4156}N_{0.2022} + 0.5301\, H_2O + 0.0533\, CO_2$
Biosynthesis of *Rs. rubrum* from butyrate	$0.3063\, C_2H_4O_2 + 0.0881\, \underline{\underline{C_4H_8O_2}} + 0.2022\, NH_3 + 0.0342\, CO_2 \Downarrow CH_{1.6511}O_{0.4156}N_{0.2022} + 0.4418\, H_2O$
Nitrifying compartment	$CO_2 + 4.8\, \underline{\underline{NH_3}} + 8.1435\, O_2 \Downarrow CH_{1.6511}O_{0.4156}N_{0.2022} + 4.0763\, H_2O + 4.5978\, HNO_3$
Spirulina compartment Proteins biosynthesis (P)	$CO_2 + 0.6383\, H_2O + 0.2496\, \underline{\underline{HNO_3} \Rightarrow CH_{1.526}O_{0.327}N_{0.2496}} + 1.5302\, O_2$
Carbohydrates biosynthesis (C)	$CO_2 + 0.8355\, H_2O \Rightarrow CH_{1.670}O_{0.711} + 1.06225\, O_2$
Lipids biosynthesis (L)	$CO_2 + 0.8570\, H_2O \Rightarrow CH_{1.714}O_{0.204} + 1.3265\, O_2$
Exopolysaccharides biosynthesis (E)	$CO_2 + 0.825\, H_2O \Rightarrow CH_{1.65}O_{0.950} + 0.9375\, O_2$
Nucleic acids biosynthesis (N)	$CO_2 + 0.44\, H_2O + 0.393\, \underline{\underline{HNO_3} \Rightarrow CH_{1.273}O_{0.710}N_{0.393}} + 1.4545\, O_2$
Biomass composition	Biomass = % P + % C + % L + % E + % N