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1 INTRODUCTION

The MELISSA Pilot Plant laboratory has been devoted during the last five years, to the development of the individual compartments of the loop. To this purpose a systematic approach has been followed in a wide range of tasks, including strains selection, study of their growth kinetics at different conditions, design and set-up of the corresponding bioreactors and associated instrumentation, development of the corresponding mathematical models, characterisation of the continuous operation of the compartments and test of control laws. Research done has been mainly focused on compartments II, III and IV. As a main result, the interconnected operation of this compartments at two different sizes, bench scale and pilot scale, has been achieved for long periods of time ranging from weeks to months.

In order to complete the objectives envisaged since the conception of the project, and in order to demonstrate the validity of MELISSA as a model system for advanced life support systems, the closure of the loop of compartments in the Pilot Plant has to be completed. This goal will be reached in a gradual way following a step by step approach during the following years.

Attainment of this goal is a very complex task that requires a careful design, combining all the information and conclusions generated during the previous years of research, including the different MELISSA brainstorming sessions and design meetings, together with a thorough preparation, scheduling and meticulous implementation.

This technical note is devoted to the analysis of the preliminary engineering design of the final loop of the MELISSA Pilot Plant laboratory and its main target is to identify the different requirements that the final demonstration of MELISSA will need to incorporate.

2 GENERAL OBJECTIVES AND PREMISES

For the implementation of the Pilot Plant MELISSA Demonstration Loop, an 'scenario' of operation has been decided which in turn provides the general design constraints. The global objective is to demonstrate that an interconnected loop of all the foreseen compartments can be built, operated and maintained in stable operational conditions, for reasonably long periods of time. To this purpose, an operational scenario has been chosen which, after thorough discussion among the MELISSA partners, will become the reference point for the new set up of the MELISSA Pilot Plant.

2.1 General description of the Pilot Plant loop operation scenario.

As an experimental set up, and in order to simplify safety rules, legal requirements and experimental design, the loop will be designed to use experimental animals, more specifically rats, as the 'crew' to be maintained alive. These animals will breathe air generated/recycled completely inside the MELISSA loop. The animals diet will consist on edible compounds mainly generated inside the loop. However its diet can vary depending on the degree of closure assayed in each test and could range from a mixture of external food with the MELISSA generated edible biomass, most probably *Spirulina*, to a complete MELISSA diet made with a mixture of higher plants biomass and *Spirulina*.

Due to the fact that this loop is designed to be a human life support system test bed, some of its components, mainly the first compartment, have to be adapted to this purpose. More specifically the strains used in the first compartment are adapted to the degradation of human wastes. Therefore the organic waste (faeces and urine) produced by the rats will not be recycled through the MELISSA loop. In consequence, the first compartment will have to be feed with human wastes. To obtain them, a waste collection program, using voluntary people, will be engaged and carried on under medical supervision. A human waste collection unit will also be required.

This implies that the demonstration loop will not be completely closed until humans are used as the 'test crew'. However, in terms of the amount of recycled mass, the solid waste compounds are a small part of the total mass to recycle per 'unit of crew member' basis. Thus the majority of the mass passing through the 'rat crew' compartment (gases+food) will be recycled inside the loop because the percentage of

the input mass on this compartment going to the rats organic waste (urine +faces) will be small. Furthermore part of the loop generated plant biomass could be consumed by volunteers. Therefore the use of this partially closed approach of the MELISSA loop as a test bed for a closed biological system is considered a valid one.

The definitive methodology for recycling of the non-edible and the edible but not consumed parts of the higher plant compartment still have to be decided, nevertheless the recycling of the plant biomass through the first compartment of the MELISSSA loop has already been tested and offers a similar efficiency as it is obtained with the human wastes (Hermans & Demey 2000) and, therefore, should be the preferred choice. Nevertheless it can be estimated *a priory* that, in the Pilot Plant, the higher plants will produce much more biomass and O_2 than the amount that will be consumed by the rats. They will also fix more CO_2 than the amount produced by the rodents. The main reason for this resides on the fact that if this compartment was to be sized only to feed those rats, it will probably be too small for the results to be of any significance. Sizing the first compartment to recycle all the higher plants biomass produced in the Pilot Plant would result in big size bioreactors, which at present time is considered unnecessary for the demonstration purposes. For this preliminary sizing it will be assumed that the extra biomass is recycled only partly through the first compartment and the rest assumed to be recycled by a technology yet to be defined and that could be among biological (mushrooms, hiperthermophilic bacteria,..) or physicochemical treatments. Recycling of human waste and biological gases inside the loop will be done by means of the use of different biological compartments or bioreactors. Those will be interconnected trough the appropriated interfaces allowing to assure the correct control (sensors, actuators, buffer tanks, automatic control systems), phase separation (centrifugation and filtering steps, gas liquid separation), and biological safety (biological barriers separating the crew and the biological compartments, sterility barriers among compartments, monitoring and control of any generated toxic compounds).

In previous MELISSA meetings (Toulouse meeting July 2000) tentative design constraints were proposed such as:

- Higher plant compartment production should supply around 20% of the dietary requirements of one man.

- The inedible part of the higher plant compartment, the faeces produced by one man per day, together with the total *Spirulina* and *R.rubrum* produced inside the loop should be recycled using the first compartment
- The aim is to balance the gas loop between the 'crew' (composed of 3 rats) and compartments 3 (Nitrifying, O_2 and CO_2 consumer), 4a (algae, CO_2 consumer, O_2 producer) and 4b (HPC, CO_2 consumer, O_2 producer).
- As compartments 1 (Liquefying) and 2 (*R. rubrum*) are still in development, they are let apart from the gas loop.
- The water loop has to be as closed as possible: a physico-chemical process (filtration) will allow to regenerate waste water into pure water.
- The solid loop will not be balanced due to the choice of producing only 20% of the diet by means of the HPC.

Although those were the main guidelines guiding the beginning of this study, this preliminary approach reflects only the initial considerations adopted for calculations but differs slightly from the finally adopted concept for the MELISSA integration loop at the Pilot Plant. **Therefore the actual final procedure will follow the decisions taken at the MELISSA brainstorming session during the MELISSA yearly meeting held in May 2001 and summarized in chapter four.**

3 COMPARTMENT SIZING

For the sizing of all the compartments of this MELISSA Pilot Loop, all the previously generated scientific data, together with the previously described operational scenario are considered. In the following, a more detailed description of the preliminary sizing of the required MELISSA compartments is presented.

3.1 Compartment I

As explained in the 'general objectives' the feed composition of compartment I for this study will be considered as mixture of the diluted human faeces produced by one man in one day, the non edible biomass produced by compartment IVb (in a first approach sized to provide 20% of the daily food requirements for one man), as well as all the *Spirulina* and *R.rubrum* biomass produced in the loop.

In previous MELISSA experimental determinations a degradation of a 58% of the human faeces (1/10 diluted) was reached, using two different reactors and an enzymatic treatment (Hermans and Demey, 1999). The first reactor had a volume of 1.6L and reached a total degradation efficiency of 40%, being the protein degradation efficiency 60% and the fibre efficiency 29%, when the residence time was 21 days. The composition of the effluent of this compartment is given in table 1. This outlet was centrifuged, treated with enzymes and used for the second compartment. The cake was diluted up to the same volume that had before centrifuging, and later with an equal volume of an enzymatic solution in order to improve the fibre biodegradation (the use of other pre-treatment like Fenton's reagent, Laccase or their combinations, modify the dilution volumes).

	Reactor I		Reactor 2	
	Input average(g/L)	Output average(g/L) [range]	Input average(g/L)	Output average(g/L) [range]
pH	6,9	[6,5-6,9]	6,8	[7,3-8,5]
Dry Matter	23	18 [7.54-26.5]	5,99	4,9 [4.3-15]
Ash	3,7	3 [1.7-5.1]	0,76	1,7 [1.5-3.2]
total-N	1,241	1,25 [0.75-1.97]	0,227	0,3 [0.17-1.7]
N-NH_3	0,1	0,7 [0.55-1.4]	0,043	0,09 [0.07-1.1]
total-VFA	0,85	2,4 [0.59-3.26]	0,21	0,3 [0.048-1.4]
acetic	0,354	0,4 [0-1.8]	0,047	0,1 [0-0.63]
Propionic	0,218	0,85 [0.31-1.2]	0,066	0,2 [0.047-1.02]
Iso Butyric	0,029	0,24 [0-0.28]	0,024	0 [0-0.055]
Butyric	0,167	0,26 [0-0.35]	0,03	0 [0-0.082]
Iso Valeric	0,046	0,51 [0-0.6]	0,043	0 [0-0.071]
Valeric	0,033	0,03 [0-0.08]	0	0 [0-0.017]
Caproic	0,02	0,015 [0-0.032]	0	0 [0-0.013]
Iso Caproic	0	0	0	0
CO_2 (g/L-reactor)	0	0,59 [0,5-1,95]	0	-
CH_4 (molar %)	0	0		73 [51-79]

pH	6,5	8
temp	55	55
vol	1,6	0,9
feed rate (mL/2days)	150	150
gas flush sec/6h	10	each feed
HRT	21	18

Table 1: Composition and operational conditions on the compartment I MELISSA demonstration reactors (TN 45.3).

The second reactor had a volume of 0.9 L and reached a total degradation efficiency of 38%, being the protein degradation efficiency 40% and the fibre efficiency 37%, when the residence time was 18 days. The composition at the outlet of this compartment can also be found in table 1. The global degradation efficiency reached between both compartments is about 53%, which could be increased using improved separation techniques (Hermans and Demey, 1999). The bioreactors were operated semicontinuously and each bioreactor was fed with 150 mL every two days. For calculation purposes, the equivalent flow-rate per hour (3.1 mL/h) will be taken.

The improvement obtained in the degradation efficiency by including a second bioreactor in compartment I is considered insufficient at this time to justify its inclusion in the calculations and as a consequence in this preliminary design, only one bioreactor will be considered.

The composition of human faeces can present some variations. However, from the previous different experiments performed in this compartment, as those described in the above paragraph, using samples of human faeces diluted to 1/10, its average composition has been analysed. The ranges and the average results of those analyses, recorded in TN 45.3 (Poughon 2000), can be seen in table 1. This averaged composition is the one that will be used in the following calculations. The output obtained by the degradation of these compounds can be also found in table 1.

The no edible part of the higher plants biomass produced in compartment IVb, will be considered as being degraded similarly as the human wastes, according to TN 51.2. However, volatile fatty acids are not part of their composition, as they are of human faeces. Therefore, conversion factors for biomass degradation, will be obtained from table 1, but the inputs of volatile fatty acids, will be discounted from the outputs.

At present time there is no experimental data to account for the degradation in the first compartment of the microbial biomass produced in the loop. Therefore to calculate the outputs obtained from their degradation, the following will be considered:

The degradation efficiency of bacterial biomass will be considered of 60% similarly to the protein degradation. Of the biomass completely degraded, the ammonium nitrogen obtained will be stoichiometrically calculated from the elemental biomass composition. Volatile fatty acid composition obtained from the degraded biomass, is considered to be produced at the same carbon molar ratios as those obtained experimentally and shown in table 1.

As will be explained in the corresponding section, different biomass compositions and productivities are being considered (Poughon 1997 (TN 32.3) and Cloutier 2001 TN 46.2) which lead to slightly different results. In this technical note both options will be considered. Also a third option is proposed taking into account the same proportions considered in options I and II but for a total biomass of 23 gDW/day which is equivalent to the amount of dried faecal material produced by one man.

Option		Inputs (Biomass gDW/day)				Outputs (gDW/day)			
	Vol (L)	C-IVb	C-IVa	Faeces	C-II	VFAs	CO_2	DM	$N\text{-}NH_3$
I	220	103	36	23.5	75.8	65	25	41	11
II	330	202	36	23.5	94.5	81	31	27	15
III	21.4	13	2.3	1.5	6.1	5.3	2.	1.7	1.

Table 2: Summary of inputs and outputs from compartment I according to the different options. Option I: comp IVb prod according to TN 46.2. Option II: comp IVb prod according to TN 32.3. Option III assuming total dry weight equivalent to the human faeces produced by one man in one day.

Once the total biomass to process in compartment I is determined, the volume is calculated, considering that this biomass is diluted to the same dry weight concentration as is done in table 1 and processed in a bioreactor with a residence time of 21 days.

As shown in table 2, the compartment I volumes, calculated for the 3 different options, are 330, 220 and 21.4 litres respectively.

3.2 Compartment II

In this compartment, The VFA coming from the first compartment are degraded and transformed to biomass.

Although different VFA appear in the output of compartment I only kinetic data for *R. rubrum* in acetic acid is available at the moment in a continuous mode. Preliminary data indicates that growth rate is different in this VFA than on the other ones (Cabello, 1998). Thus, until kinetic data is obtained for the other VFA, the data for growth on acetic acid will be used.

In previous experiments, a 0.724 mL/min (43.4 mL/h) flow rate containing 5 g acetic acid/L (0.22g/h) were treated in a continuous 2.4 L fermentor reaching a complete conversion (0.092 g/hLr). The residential time was 2.3 days (dil. $0.018h^{-1}$), the incident light intensity was 135 W/m^2 and the illuminated surface was 0.08 m^2 (Creus et al., 1999). It can also be calculated that 0.132 moles of CO_2 are produced by each VFA mol consumed (Poughon, 1995). Other experiments using 1gVFA/L at a dilution rate of $0.04h^{-1}$ (liquid flow 0.32 L/h) in the 8 litres bioreactor presently in operation, consumed 0.32gVFA/h and of $0.032gN\text{-}NH_4^+/h$.

Option		Inputs (Biomass gDW/day)		Outputs (gDW/day)		
	Vol (L)	VFAs	$N\text{-}NH_3$	biomass	$N\text{-}NH_3$	VFAs
I	50.6	65	11	75.8	0.95	0
II	63.12	81	15.2	94.7	2.46	0
III	4.1	5.3	1	6.13	0.16	0

Table 3:Summary of inputs and outputs from compartment II according to the different options. Option I: comp IVb prod according to TN 46.2. Option II: comp IVb prod according to TN 32.3. Option III assuming total dry weight equivalent to the human faeces produced by one man in one day.

Assuming that the biomass composition is $C\ H_{1.6}\ O_{0.36}\ N_{0.22}\ S_{0.0036}\ P_{\ 0.016}$, and taking into account previously calculated stoichiometries for each VFA (Favier-Teodorescu 1999) the total biomass that has to be produced in the II compartment and the $N\text{-}NH_3$ consumed can be calculated. The biomass produced in the second compartment is totally dependent on the local available light intensity in the bioreactor, which in turn depends on the bioreactor geometry and biomass concentration. As a compromise, it has been taken that the new bioreactor will be able to supply similar local light intensity as one of the smaller bioreactors previously used. Therefore, a reference maximum biomass concentration of 1.5 g/L and a maximum productivity of (0.092 g/hLr) has been considered. This allows to calculate the bioreactor volume for the three different scenarios presented in the description of compartment I as presented in table 3.

3.3 Compartment III

Biomass production in compartments IVa and IVb will require a continuous supply of nitrogen source, that in the present MELISSA configuration should come from the III compartment. Therefore this compartment has to supply the required amount of nitrogen source.

It has to be mentioned however that, once the higher plant compartment will be in operation, this compartment could also supply, at least partially, the nitrogen source for those plants, if nitrogen fixing organisms (ex. *Rizobium*) are used as cohabitants in that higher plant compartment, which here are considered not present.

Options	Comp. IVa ($gN\text{-}NO_3/day$)	Comp. Ivb ($gN\text{-}NO_3/day$)	**Comp. III ($gN\text{-}NO_3/day$)**
I	-3.21	-4.58	**7.79**
II	-3.21	-8.17	**11.4**
III	-0.21	-0.32	**0.53**

Table 4: Nitrate requirements in compartments and IVa and IVb leading to the nitrate requirement of compartment III. Negative values means consumption.

For the present calculations it will be assumed that the nitrate required to be generated by this compartment is in the range calculated for the nitrogen source requirements of the cyanobacteria *Spirulina* in compartment IVa and the nitrate required in compartment IVb. In table 4 the nitrate that should be provided by compartment III is presented.

In previous experiments a maximum productivity of about 11.1 $N\text{-}NO_3^-$ g/day (0.46 $N\text{-}NO_3^-$ g/h) was reached using a packed bed column of 3.82 L (liquid volume) (Pérez, 1999). Taking into account this result, a column with this size and configuration can be appropriate for all these MELISSA scenarios, and the bioreactor presently used for this compartment in the Pilot plant can be used.

After working during a period of one year, the pilot plant column has produced 13 g biomass/L_{bed} (Pérez, 2000). Taking into account that the bed volume was 6 L the biomass production was 6.5 g/month. If the biomass composition is considered to be a general averaged one such as $CH_{1.75}N_{0.2}O_{0.43}$ (Blanch and Clark, 1998), and in order to achieve this biomass production, the carbon and nitrogen requirements are of 3.32 gC/month and 0.78 gN/month, which will have to be added to the previous mentioned figures as requirements for this compartment.

As seen when comparing tables 3 and 4 nitrogen has to be added in the three scenarios, the ammonia outlet of compartment II is not enough to supply the nitrate demand of compartments IVa and IVb. Nevertheless it is expected that urine can also be used as a complementary nitrogen source, which can be possibly added in different compartments allowing a more flexible operation.

	URINE (excreted/ day)
Water	**1.5 L**
Proteins	**0.1 g**
Sodium (Na^+)	**4.6 g**
Chloride (Cl^-)	**6.3 g**
Bicarbonate (HCO_3^-)	**0**
Glucose	**0**
Urea	**25 g**
Potassium (K^+)	**2.0 g**
Uric acid	**0.8 g**
Creatinine	**1.6 g**

Table 5: Amount and composition of human excreted urine per day (Tortora and Grabowski, 1992).

The composition of urine taken for the calculations can be found in table 5 (Tortora and Grabowski, 1992). According to this data, and assuming that for each mol of urea two moles of ammonia and one mol of carbon dioxide are produced, and for the uric acid 4 mols of ammonia and 5 of carbon dioxide, the complete decomposition of the urine excreted by a man will generate 12.7g N-NH_4^+/day and 18 g CO_2 /day. Thus, with the urine excreted by one man the three scenarios would have enough ammonia for the whole loop. The procedures for urine decomposition and the best compartment to incorporate it will be the subject of future studies.

3.4 Compartment IVa

Following the decision to use rats as a 'test crew' an amount of 3 rats, of about 400g in weight, is considered as an adequate number for the first approach, taking into account that three rats are equivalent, in terms to oxygen demand, to a human being. From this starting point, and to size the rest of the compartments, an evaluation of the required amounts of oxygen consumption, carbon dioxide production and food ingestion is necessary. As a reference point the previous experimental data (Tranquille *et al*. 1994, de Chambure 1992) obtained, using rats feed with 25g of a diet enriched with different percentages of *Spirulina*, can be taken. The previous tests also suggest an upper advisable limit of *Spirulina* of about 40% of the daily food intake. According to this data about 30g of *Spirulina* per day will be consumed by the three rats. The rest of the rats diet and the oxygen demand can be supplied by the higher plant compartment. However as mentioned before, the sizing of the higher plant compartment has been done following other restrictions than to feed the rats. In consequence it will be assumed that

the higher plant compartment can supply all the rest of the food required by the rats, when necessary.

Productivity estimation of *Spirulina* to feed those rats and the concomitant oxygen production and carbon dioxide consumption can be taken from the previous *Spirulina* continuous cultures. For example, a biomass productivity of $6.9 \cdot 10^{-2}$ g biomass/l·h and an oxygen productivity of 0.1 $gO_2/L \cdot h$ were reached using a 7 L fermentor with an incident light intensity of 300 W/m^2, an illuminated surface of 0.154 m^2 and an illuminated volume of 3.64 L (Vernerey, 2000). Using this data and the Photosim simulator (Cornet, 1993), an estimation of the size of the IV compartment was done (Vernerey, 2000). Other useful data for the calculations can be obtained from the data of Martí (Marti, 1997), from which a stoichiometry in normal conditions and in carbon limitation conditions was established.

These data allow determining the requirements and productivities of this compartment, in terms of carbon and nitrogen sources consumption and oxygen production. Thus the stoichiometric data of Martí (Martí 1997) allow to calculate that for each gram of biomass produced under non carbon or nitrogen limiting conditions, 0.08 $gN\text{-}NO_3^-$ and 1.74 g CO_2 (0.47 g $C\text{-}CO_2$) will be consumed and about 1.6 g O_2 will be produced, similar to the oxygen production obtained by Vernerey 1.54 gO_2 (Vernerey 2000) .

If a complete *Spirulina* diet for rats is considered, a production of 75 g of *Spirulina* per day is necessary, and assuming an average composition similar to the one obtained in previous experiments, (such as $CH_{1.65}O_{0.53}N_{0.17}S_{0.007}P_{0.06}$ or $CH_{1.59}O_{0.59}N_{0.13}$) and nitrate as the nitrogen source, a consumption of about 6-7 $gN\text{-}NO_3^-$/Day and concomitant oxygen production of 120 g/day with a carbon dioxide fixation of 130 g/day can be expected. These gas productivities are sufficient to maintain the rats alive, if one does not take into account any prolonged increased activity period for the rats, which could increase their gas exchange rate by a factor of 2.5. Although the calculated values can be accepted as daily averages, a buffer tank will allow to cope with a temporarily increase in the rats activity. Nevertheless gas balance will have to be considered taking also into account the higher plants activity, which will provide a surplus capacity. It is still not decided if this buffer capacity will be provided by the higher plants containment facility itself or if it will be a separate tank.

The 40% *Spirulina* food in diet, suggested as the higher limit of *Spirulina* consumption by rats, has been taken as the chosen scenario. In this case oxygen production and carbon dioxide fixation will decrease proportionally ((oxygen 48 g/day, carbon dioxide fixation of 52 g/day, as well as nitrogen source consumption (2.39-2.85 $gN\text{-}NO_3^-$/day). The productivity of *Spirulina* already obtained using the 77 litres bioreactor, currently in operation at the Pilot plant, is of about 36 g/day. Therefore it is considered that this bioreactor can be used for the implementation of the Pilot plant demonstration loop.

3.5 Compartment IVb

The 20% of the supply of the HPC in a theoretical man diet has to be provided by this compartment.

In a first approach eight different plants have been considered for the MELISSA HPC: rice, tomato, potato, soybean, onion, wheat, lettuce and spinach. The edible percentage as well as the composition of these eight plants are presented in table 6 (Soucy *et al.*,1990).

	HPC (%composition)	**Edible (gdryW/W/m^2·day)**	**Waste (gdry W/m^2day)**
Tomato	**0.8**	**0.054**	**0.476**
Rice	**16**	**1.092**	**1.306**
Lettuce	**0.5**	**0.034**	**0.025**
Potato	**29.5**	**2.001**	**0.961**
Soybean	**1.6**	**0.109**	**0.060**
Spinach	**1.6**	**0.109**	**0.135**
Onion	**1.6**	**0.109**	**0.163**
Wheat	**48.2**	**3.270**	**4.973**
TOTAL	**100**	**6.78**	**8.10**

Table 6.- Percentage of each plant in the HPC and daily quantity per m^2.

In this scenario, it was supposed that the 70% of the daily diet was supplied by the HPC. The vegetables in this diet were chosen taking into account an adequate astronaut diet. As a result a percentage of the consumption of the different plants could be calculated (Poughon, 1997). This plant consumption percentage can be used to distribute the cultivation surface available among each plant assigning a surface proportional to its consumption. The percentage of each plant in the HPC is shown in table 6.

The area required for each plant on this HPC could be calculated from the data in table 7 (Drysdale *et al.*, 1994, Toki *et al.*, 1994, Poughon, 1997).

	(g edible/m2.day)	Required area (m^2)
Tomato	**18**	**2.99**
Rice	**4**	**26.73**
Lettuce	**6**	**8.35**
Potato	**33**	**20.39**
Soybean	**15**	**0.51**
Spinach	**21**	**6.03**
Onion	**22.5**	**2.90**
Wheat	**33**	**7.24**
TOTAL		**75.15**

Table 7.- Cultivating area required of each plant and total area for the proposed HPC

In order to consider the requirements of this compartment, the growth of each plant was summarized using the following equations (Poughon, 1997):

$$CO_2+NH_3+HNO_3+H_2SO_4+H_2O \rightarrow O_2+(CHONS)_{digestible}\ (CHONS)_{fibre}$$

$$CO_2+NH_3+HNO_3+H_2SO_4+H_2O \rightarrow O_2+(CHONS)_{waste}$$

Assuming the value of the ratio NO_3^-/NH_3 as 5 (Poughon, 1997), the stoichiometric coefficients of the equations above exposed for each plant were calculated (Poughon, 1997, Soucy *et al.*,1990). Thus, the global nutrient requirements could be evaluated by means of these equations and is presented in table 8.

	DM (edible)	DM (no edible)	$N\text{-}NH_4^+$	$N\text{-}NO_3$	O_2	CO_2
TOTAL (g/h)	**8.5**	**10**	**-0.114**	**-0.154**	**25**	**-32**

Table 8.- Global requirements of the HPC (negative values mean inlets and positive values outlets).

Taking into account these data and that in the MELISSA and considering an implementation in the Pilot Plant for only a 20% HP of the daily human consumption, the Pilot Plant HPC requirements are shown in table 9.

	Edible biomass (gDW/person.day)	Inedible biomass (gDW/person.day)	Area (m^2/man)
tomato	3.45	30.28	3.0
rice	69.06	82.56	26.7
lettuce	2.07	1.52	8.4
potato	126.79	60.91	20.4
soybean	6.91	3.83	0.5
spinach	6.91	8.59	6.0
onion	6.91	10.38	2.1
wheat	207.17	315.05	7.2
totals	429.27	513.12	74.33
20%	85.85	102.62	14.87

Table 9 HPC area and production

In a second approach, 27 different plants were chosen in order to supply a complete human diet (TN46.2). Out from these 27 plants the MELISSA pilot HPC would contain 8, which can almost supply complete human diet requirements (a man ingests around 650-700 gDW/day). Those 8 plants are just the same as the above explained except the change of tomato for beet. This HPC is presented in table 10.

	Edible biomass (gDW/person.day)	Inedible biomass (gDW/person.day)	Area (m^2/man)
beet	3.13	0.064	0.27
rice	60.00	67.62	6.00
lettuce	0.37	0.024	0.005
potato	20.25	5.39	0.81
soybean	116.52	142.41	5.74
spinach	2.11	0.74	0.14
onion	6.90	3.55	0.50
wheat	463.60	789.26	19.000
totals	672.89	1009.06	32.46
20%	134.58	201.81	6.49

Table 10.- HPC area and production

As a preliminary estimation it can be assumed that up to a total of 54 m^2 could be available in the laboratory for this compartment, which satisfies the room required in each of the two above mentioned HPCs. Inside it, 2 or 3 different cultivation chambers could be accommodated, such as to maintain separated incompatible plants and allow for operation of at least 2 chambers at the same time wile the third one is in service (seeding, watering, harvesting,…).

To allow for the proper collection of gas production/consumption data, the plant chambers should operate in a closed air loop. In addition, to be able to service the chambers and therefore to allow an operator to walk in, an airlock chamber system is foreseen. Thus, from this preliminary design, it can be assumed that at least one third of the surface will be lost.

4 SUMMARY OF THE CONCLUSIONS REACHED AT THE MELISSA YEARLY MEETING 2001 (CANADA).

The proposals described in the previous paragraphs, taking also into account the on going activities in respect to compartment I and the HPC, were discussed during the MELISSA yearly meeting 2001 (13-16 May 2001University of Guelph). After considering the different possibilities, an agreement was reached for setting up the final version of the **'MELISSA integration loop'** at the pilot Plant in Barcelona and therefore provide the basis for its sizing. In the following a summary of the agreements is described.

As a main practical objective it was agreed to size the integration loop such that at least it will be able to recycle the CO_2 generated by 1 man/day or equivalently the production of 3 rats/day. This allows to size the higher plants compartment (IVb). Consequently, and according to U. of Guelph calculations, this compartment will consist of 1 HPC with continuous illumination or 2 HPC with different photoperiods. Each cultivation chamber being of 15 m^2 with staggered plantation. Nitrate will be supplied by the III compartment. Ammonia will be obtained either from the II compartment or from urea, (the best point to incorporate urea in the MELISSA loop will be studied in the future). Two plant species will be cultivated, to be chosen among 3 candidates namely: kale, wheat and beet.

The biomass recycled in the first compartment will be the faecal material produced by one man/day, all the *Spirulina* and *R.rubrum* as well as all the non edible higher plant biomass, produced inside the loop. Food, urea, water and manure from the rat compartment will not be considered as part of the MELISSA loop

The first compartment bioreactor will be designed by EPAS considering a variable volume bioreactor with a working volume between 100 and 300 litres. This will allow to adjust the final volume to the kinetics of degradation finally attained, also in part function of the final design, microbial population enhancement and higher plant selection. The biomass will be feed at 2 g DW /(day·litre bioreactor) diluted about 1/10 times. However it is possible to expect improvements in the degradation efficiency and therefore those values, which have been tested previously, are only given as guidelines.

Taking into account the previous calculations and assuming that at most the first compartment bioreactor will be feed at 2 g DW /(day·litre bioreactor) for its maximum volume, that is 300 litres, the second compartment volume has to be sized at 60 L maximum as can be seen in the above calculations.

According to the higher plant average biomass composition described previously and the *Spirulina* biomass composition, it can be estimated that the amount of nitrate required for compartment IVa and IVb will be about 11. $gN\text{-}NO_3$/day. The nitrification column actually used in the Pilot Plant can reach this productivity and therefore it will be maintained in a first approximation in the integration loop.

The size of compartment IVa (77 litres *Spirulina* bioreactor) is considered adequate for its task in the Pilot Plant integration loop and therefore it will be maintained.

In a first approximation, the integration loop goal will be to test a closed gas loop between the higher plant compartment and the rats compartment.

Water loop and gas loop will be studied specifically in the coming years. It is not possible to define them without a serious study. However, there was a general agreement for the use of a pure water buffer tank that will be filled with water obtained from plants evapotraspiration activity.

5 COMPARTMENT INTERFACING

Operation of a loop of bioreactors as the one described above, requires an appropriate design of the interconnections among all of them. Their main objective is to assure proper transfer of gas liquid and solid phases among compartments, dealing with any temporary flow oscillation, providing solid, liquid or gas separation, while assuring a safe operation avoiding contamination by any undesired biological agents in any of their units. In the following a general description of the interfaces required for each compartment can be found.

5.1 Compartment I

This compartment will receive organic components to be decomposed mainly from three main different sources, human wastes (faeces and urine), microbial biomass and higher plant components. Plant biomass is assumed to be already grinded and ready to be combined with the other sources. As a first step a mixer will allow to combine the different organic components in the desired proportions, and to obtain a desired composition.

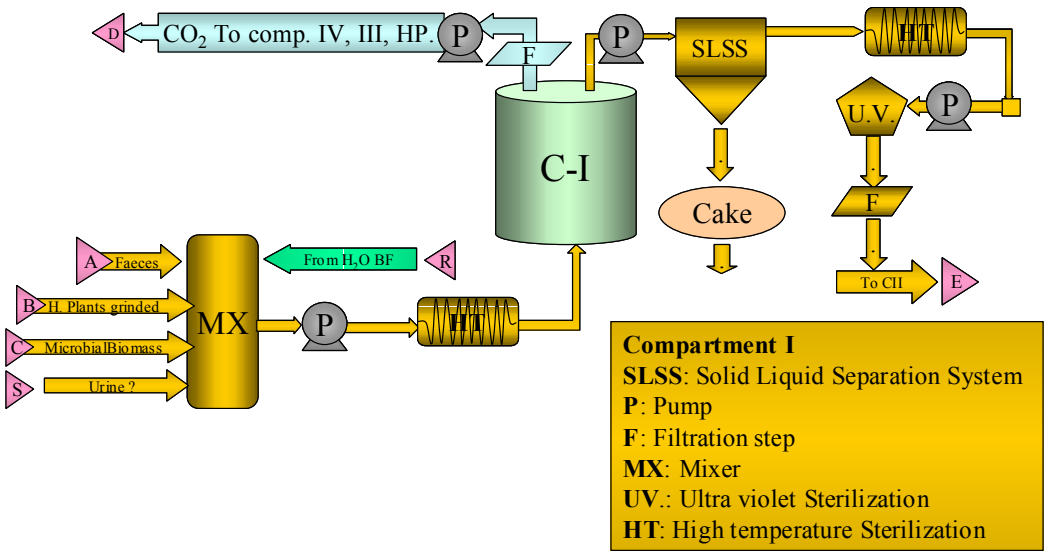

Figure 1: General overview of compartment I interconnections

The liquor will be introduced in the first bioreactor allowing the autochthonous bacteria to biodegrade it. After this first step, the produced gas will be sterilized (e.g. filtering), the low biodegradation compounds (cake) contained in the liquid phase will be removed, and the liquid effluent will be sterilized (e.g.filtering, heat) and stored in a buffer tank.

Indeed to provide an extra degree of safety against any kind of undesired contamination, another sterilization phase could be implemented consisting in a combination of methods namely thermal sterilization, UV treatment and filtration. This steps should provide a high level of protection for the rest of the compartments as well as for the human operators. A graphical representation of the interconnections can be found in figure 1.

5.2 Compartment II

Between compartments it will be necessary to provide storage capacity to allow for proper synchronization of flows between compartments and to allow to store liquid in the event of malfunction of the input pumps of compartment II and to provide a certain liquid volume stored so as to allow to maintain the flow into the second compartment during a certain time even if the output of compartment I is stopped. To this purpose a buffer tank will be installed between compartments I and II.

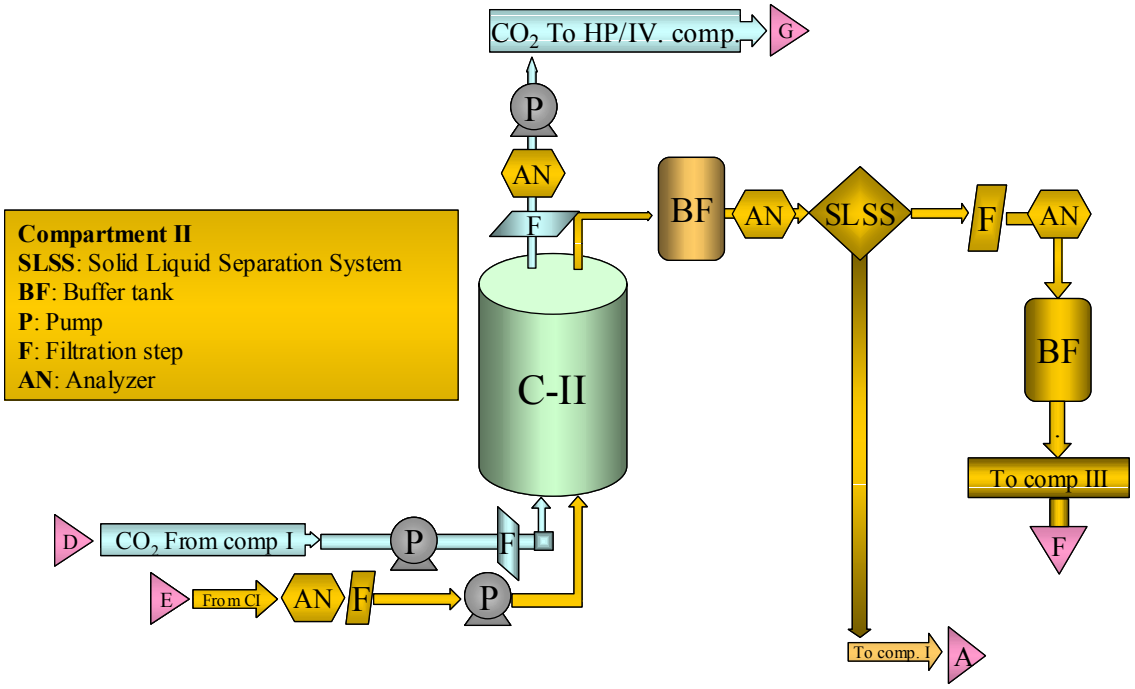

Figure 2: General overview of compartment II interconnections

After the buffer tank, an analyser is necessary and to prevent any contamination of compartment II and allow for maintenance operations in the buffer tank and analyser, a filtration step will be installed. After this step the liquid will be introduced in compartment II to consume the volatile fatty acids.

As a result of the VFA consumption biomass will be generated as well a certain amount of carbon dioxide. The produced gas will be filter sterilized and transferred to compartment IV for consumption. The liquid phase will be temporarily stored in a buffer tank, from which it will be transferred to a continuous centrifuge. As storage conditions will be of key importance to avoid media degradation due to biomass degradation, environmental conditions in the tank such as refrigeration and light intensity will have to be studied to improve conservation. Besides the reasons explained to use a buffer tank in the input of this compartment, in this case it will also allow to store the output of compartment II while the centrifuge is serviced or used for compartment IV. In this case only one centrifuge will be necessary to service compartments II and IV.

The separated biomass will be recycled through compartment I.

The liquid phase extracted from the centrifuge will be filter sterilized and stored in a buffer tank. From this one it will be transferred to compartment III.

5.3 Compartment III

The liquid flow coming from the storage tank of compartment II will be introduced into compartment II after a filtration step. This compartment will convert the nitrogen source ammonium, into nitrate, a more convenient nitrogen source for

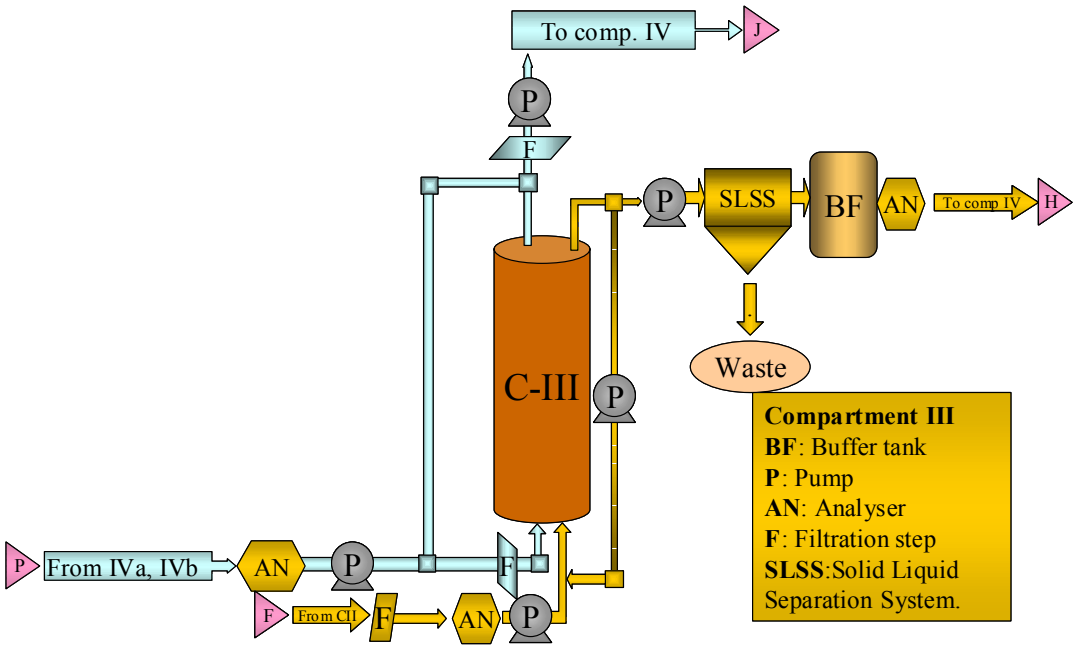

Figure 3: General overview of compartment III interconnections

compartment IV. Once the conversion is performed, detached biomass the liquid output of this compartment will be separated and sent to compartment I. The liquid will be filter sterilized and stored into the storage tank of compartment IV.

For the conversion to be possible, the *Nitrosomonas-Nitrobacter* pair will also require oxygen. To provide them a gas line rich in oxygen, but also containing some carbon dioxide to allow the bacteria to fix carbon, compartment IV gas will be filter sterilized and introduced into the compartment. The excess of gas will be redirected to the plant compartment after a filtration step. Analysers in both liquid and gas phases should be installed to measure NH_4^+ in the liquid input, NO_3^+ in the liquid output and O_2 in the gas phase.

5.4 Compartment IVa

The liquid from compartment III, after being filter sterilized and stored in a buffer tank will be introduced in compartment IVa to provide the nitrogen source and mineral nutrients. To make the biomass growth possible, carbon dioxide has also to be

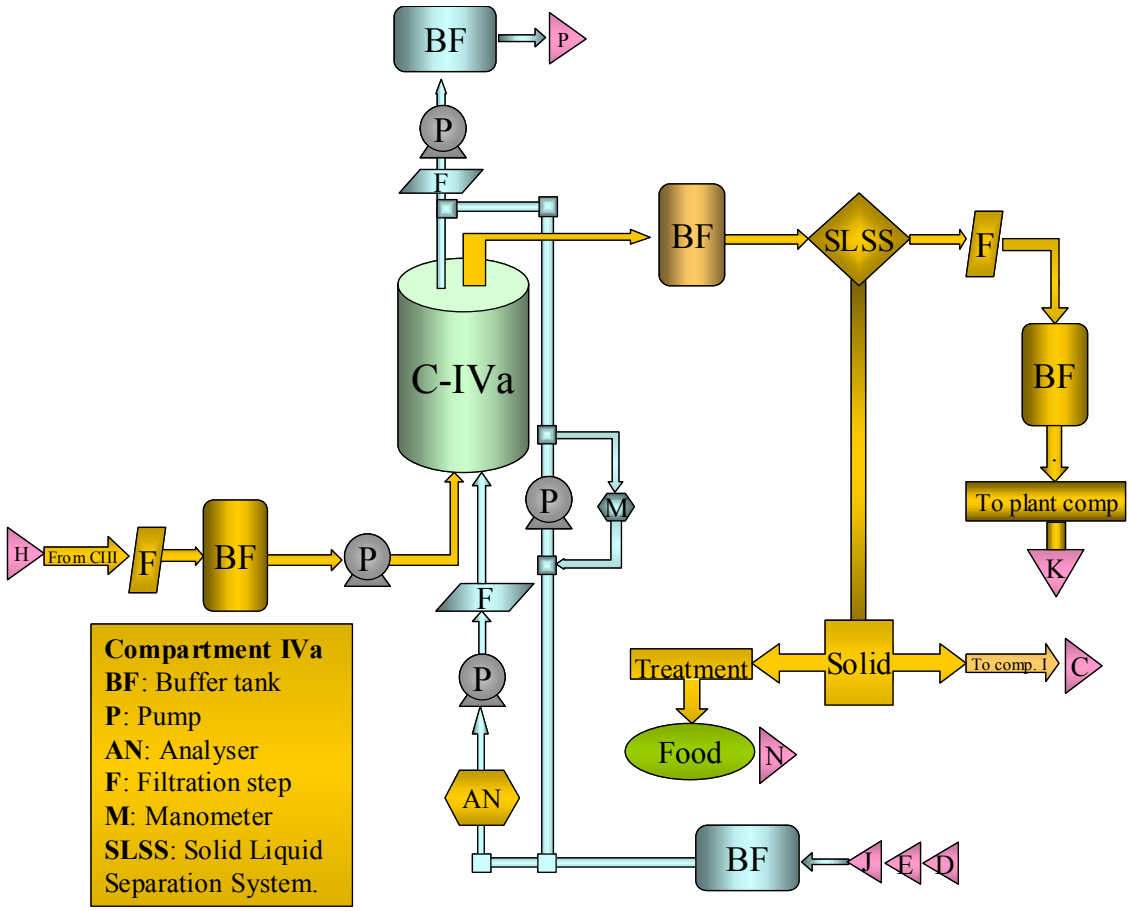

Figure 4: General overview of compartment IV interconnections

supplied. This one can be collected from the one generated in compartments I and II.

Whatever the source, it will be filter sterilized and introduced in compartment IV. The excess of gas not consumed, together with the generated air oxygen rich, will be filter sterilized and transferred to the animal compartment or to an appropriated storage tank.

The liquid output contains the edible *Spirulina*. It will be temporarily stored in a tank and subsequently a centrifugation step will allow to separate the edible biomass from the liquid phase. The biomass conservation while in the storage tank is of key importance for the biomass nutrient quality. Therefore the influence of storage environmental conditions, such as refrigeration and illumination level, will have to be evaluated to improve its conservation. This liquid will be filter sterilized and stored in a buffer tank. From the buffer tank it will be used to water the plant compartment or recycled to the first compartment in order to dilute the inlet.

The edible biomass separated in the centrifugation step will be divided in two fractions. One part will be properly treated and used as a food source for the rodents. The rest of the biomass will be either recycled through compartment I or discarded.

5.5 Compartment IVb: Higher plant compartment

The higher plant compartment will contain eight different plant species. All of them presenting different characteristics such as growth dynamics, photoperiods or nutrient requirements.

It is convenient to maintain the plants inside closed chambers so as to allow proper control of environmental conditions and perform scientific measurements such as nutrient consumption. On the other hand it can be expected that at different periods they will have to be serviced, such as at seeding or harvesting time. To facilitate all those tasks, it appears convenient to maintain the plants confined in two or tree separate chambers. This way while one is in service the others can operate normally. Those chambers will be interconnected through their gas phases and its volume can be used as a buffer tank.

The gas lines at the output of this compartment will be connected to a heat exchanger allowing to recover the plants transpiration water. This water is of high

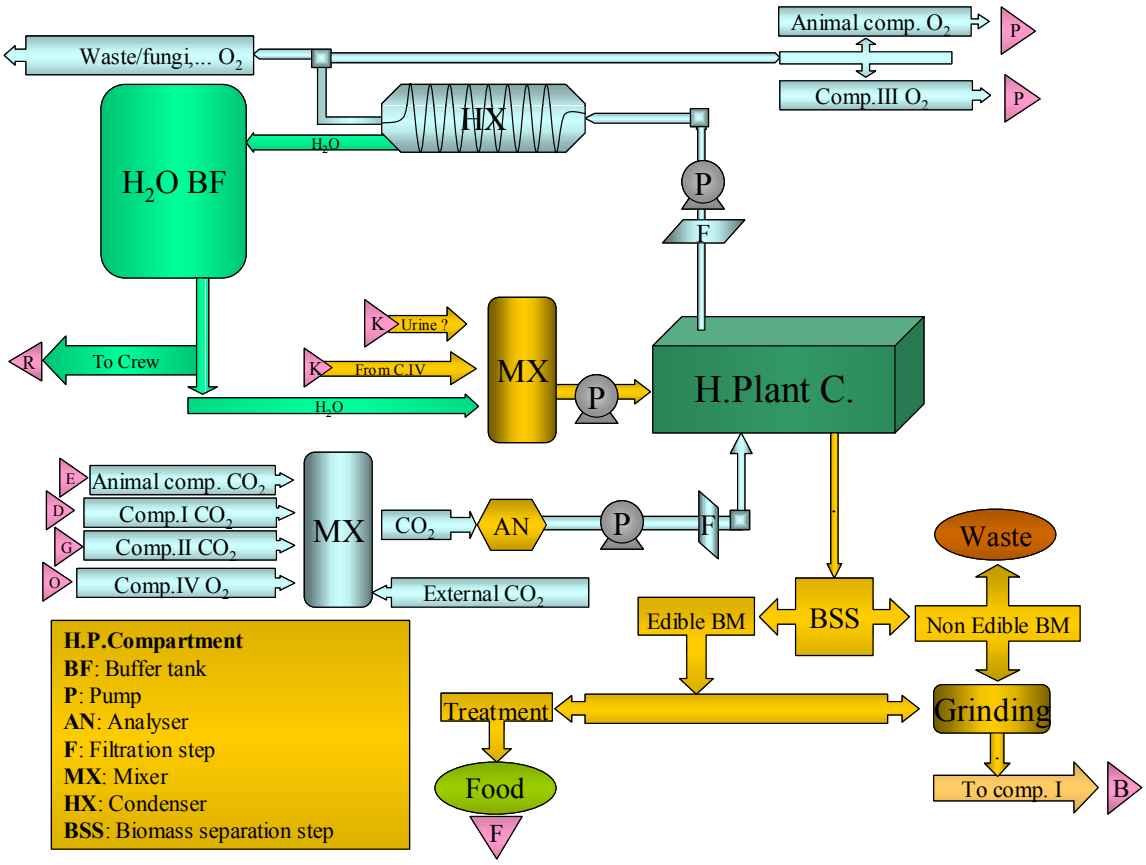

Figure 5:General overview of higher plant compartment interconnections

purity and the amount transpired per day is expected to be high. Therefore this water will be stored in a buffer tank and used to service the different compartments and, after proper conditioning (for ex. nutrient addition if necessary), also used for watering the plants.

It is clearly understood that the gas loop between the different compartments has to be further studied for safety reasons but the oxygen rich output gas flow of this compartment should be used for compartment III and/or for the rats respiration.

The plant biomass periodically collected in this compartment will be separated in edible and non edible parts. The edible fraction will be properly treated and stored to be used as food source for the rats or future volunteers. Any biomass excess will be collected with the no edible part and divided in two fractions. One fraction will be grinded and recycled in the first compartment. The rest will be discarded.

5.6 Animal compartment

Rodents will be maintained in a specific chamber designed for this purpose. The chamber will be sealed and, after a filtration step, their gas content will be recycled through the higher plant compartment, and its composition monitored.

Food supply will be prepared using plant biomass, *Spirulina* or standardised animal food source.

Solid wastes and urine from the rodents will be discarded.

The compartment set up and experimental procedures have to adhere to the current legislation for animal experimentation. In particular the following described regulations will have to be observed.

5.6.1 European Legislation

In 1986 all the state members of the European Council signed an european agreement about the protection of vertebrate animals used for experimental and or scientific goals.

From this agreement the following items can be out-lined:

- The purposes where experimenting with animals is allowed are established. Nevertheless, all kind of experiments using animals have to be notified to and approved by the corresponding administration. In this way, the experimental procedures used are controlled.
- Assuring satisfactory conditions, where all the physiologic and etiologic needs of the animals are satisfied is compulsory, as much as the experimental procedure followed allows for it.
- A special and specific training is required for all people working with experimental animals. This training will depend on the responsibilities and functions of each person.

In 1993 all the members who signed the 1986 agreement, established 4 different categories and the specific and special training of people who worked with experimental animals.

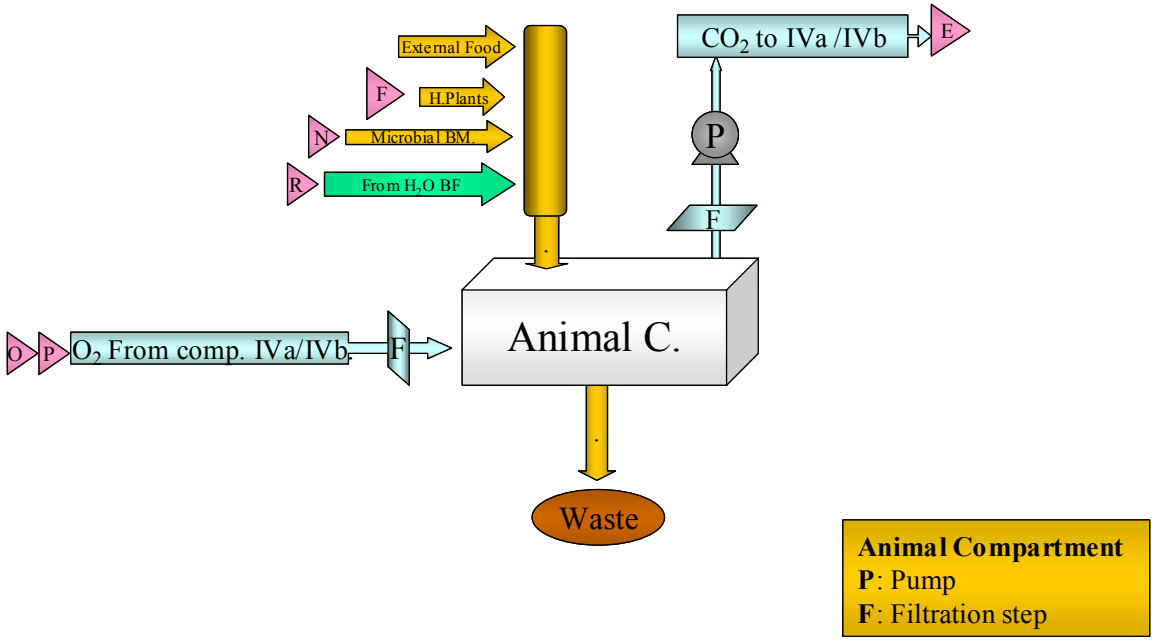

Figure 6: General overview of Animal compartment interconnections

- CATEGORY A: People who take care of the animals (keepers)
- CATEGORY B: Technicians
- CATEGORY C: Researchers
- CATEGORY D: Welfare animal advisors

In 1986 the EU established by means of the 86/609/CEE disposition the guidelines to be used in all the European states members in relation with animal experimentation.

5.6.2 Spanish Legislation

The 86/609/CEE disposition was regulated in Spain by means of the 'Real decreto 223/1998' by the Spanish government. The obligation that all the centres related with live animals experimentation (from their birth to their death) have to be registered in the correspondent administration and to quarterly report the number of animals required and the experimental procedures used in the last quarter together with the experimental procedures foreseen for the following quarter is established in those regulations.

5.6.3 Catalan Legislation

In 1988 the Catalan government approved the Animal Protection law, 'Llei 3/1988'. This law establishes that it is forbidden to do any kind of experiments with animals if the desired results or data can be obtained through other procedures that do not require animals.

In 1995 a new law, 'Llei 5/1995', that included the European guidelines, was approved. The majority of precepts of this law are developed by means of the 214/1997 decree. The use of animals for a experimental or scientific goal is regulated in those laws.

5.7 Human waste collection unit

As explained in the first section, the first compartment of the MELISSA Integration Loop has to be also fed using human wastes. Therefore a program for collecting human waste samples and a waste collection unit will be required.

The human waste collection unit will consist in an appropriated cubicle and a WC. The collection unit will have to contain a device to allow a separated collection of urine from faeces.

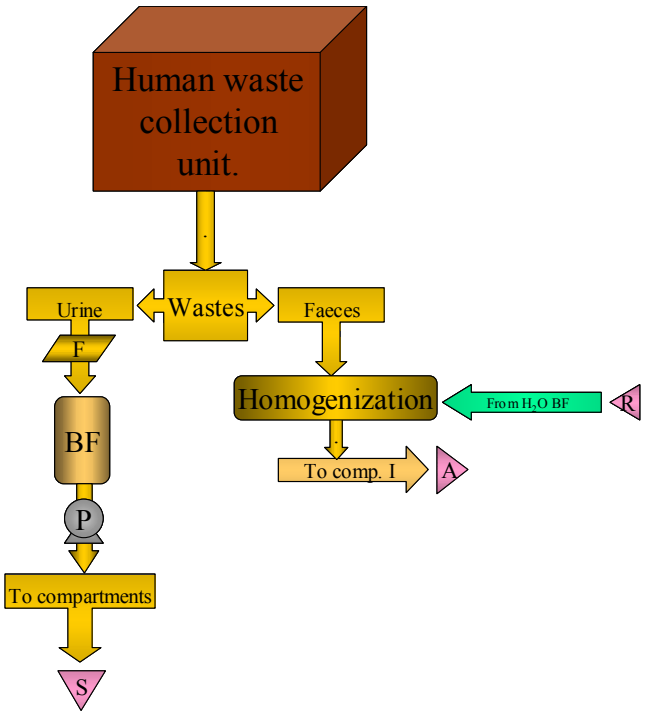

Figure 7: General overview of human waste collection unit

The urine fraction will be filter sterilized and stored in a buffer tank. Faeces will be collected, homogenised with the no edible biomass coming from the HPC and the microbial biomass coming through the whole loop and all this paste will be diluted if necessary in order to enter in compartment I. The resulting paste will be stored in a buffer tank.

The strict legislation existing in case it is considered to nourish volunteers, using a specific MELISSA diet, will also have to be considered. All this legislation is based in the Declaration of Helsinki (Recommendations guiding physicians in biomedical research involving human subjects). It was adopted by the 18th World Medical Assembly in Helsinki, Finland and was finally amended in South Africa in 1996.

6 DESCRIPTION OF KEY VARIABLES FOR EACH COMPARTMENT

For the proper operation of the interconnected loop of compartments, certain variables in each compartment are considered as of key importance and therefore will be described in the following. It is not the purpose of this section to describe the necessary control system but only to describe variables that the control system will have to take care. A more complete description of the control system will be the subject of future technical notes, either from this laboratory or in combination with ADERSA or other space companies.

As a general description all the compartments will have certain common variables controlled such as temperature, and for the bioreactors, pH and oxygen (either its presence or the lack of it), gas and liquid flows. The rest of variables measured/controlled are more specific of each one and will be described separately.

6.1 Compartment I

This compartment is in charge of the liquefaction of the wastes. Therefore Besides its high temperature and lack of oxygen the level of volatile fatty acids in its output will be of key importance, as well as CO_2 and methane. The level of volatile fatty acids will drive the growth of biomass in the second compartment. Although difficult to achieve, the measure of the biomass level in the bioreactors, is also desirable. As the volatile fatty acids are highly volatile, its content in the output gas

lines should be monitored and its existence will provide a way to test companion technologies such as biodegradation by means of a Biological Air Filter (BAF).

6.2 Compartment II

This compartment has to totally convert the volatile fatty acids into biomass. Therefore the level of those compounds in its output or better the lack of it, will have to be monitored and controlled. To succeed in this task the biomass requires light energy. Therefore the light intensity and biomass concentration are key measures to be taken into account by the control system. For the same reasons as mentioned in the first compartment the VFA content in the output gas lines should also be monitored as in compartment I.

6.3 Compartment III

The main purpose of this compartment is to convert NH_4^+ into nitrate, with nitrite as an intermediate step and using oxygen. Therefore NH_4^+, has to be monitored in the input liquid lines and NO_3^- and NO_2^- monitored in the output liquid lines. Biomass is immobilized and therefore difficult to measure. However the use of new technologies such as impedance measurements can provide a useful measurement in calculating the performance and evaluating biomass release service periods. Oxygen consumed is also desired in order to verify metabolic activity.

6.4 Compartment IV

In this compartment the main objective is to produce *Spirulina* edible biomass and oxygen using light energy, while consuming CO_2. Therefore the measurement of O_2 and CO_2 in the gas lines and light intensity and biomass concentration in the bioreactor are of key importance.

6.5 Higher plant compartment.

In this compartment plant biomass is grown as a food source while O_2 is generated and CO_2 is consumed. The plants need light energy and this one should follow certain photoperiods, and have a specific spectrum including visible and infrared. therefore the measurement of O_2 and CO_2 in the gas phase and regulation of the photoperiods and temperature is necessary. Nutrient delivery is another key issue.

Watering is required if in solid media. If in hydroponics culture, mineral nutrient concentration will have to be monitored and regulated. Among the nutrients delivered the nitrogen source (NH_4^+ , NO_3 and urine) should be measured.

6.6 Animal compartment

In this case besides maintaining the proper temperature, only monitoring of the CO_2 and O_2 is foreseen. Their concentrations should be maintained under strict limits to avoid any animal danger and also to monitor their metabolic activity.

6.7 Preliminary list of the measured and controlled variables for each compartment.

In the following a list of the variables to measure or control in each compartment has been attempted. Range indicates the range of the measurement system. Operation indicates the foreseen operational set point of the variable.

COMPARTMENT I			
VARIABLE	**RANGE**	**OPERATION**	**TYPE**
Temperature (^oC)	**0-150**	**55**	**controlled**
Pressure (atm)	**0-2**	**1**	**controlled**
pH	**1-14**	**6.6**	**controlled**
Oxygen (%)	**0-100**	**0**	**controlled**
Volatile fatty acids	**TBD**	**TBD**	**measured**
VFA in gas phase	**TBD**	**TBD**	**measured**
NH_4^+ (g N-NH_4^+ /L)	**0-5**	**TBD**	**measured**
CO_2	**TBD**	**TBD**	**measured**
H_2	**TBD**	**TBD**	**measured**
CH_4	**TBD**	**TBD**	**measured**

COMPARTMENT II			
VARIABLE	**RANGE**	**OPERATION**	**TYPE**
Temperature (^oC)	0-150	30	controlled
Pressure (atm)	0-2	1	controlled
Light intensity (W/m^2)	0-500	Variable	controlled
pH	1-14	6.8	controlled
Oxygen	0-100	0	controlled
Volatile fatty acids	TBD	TBD	measured
VFA in gas phase	TBD	TBD	measured
CO_2	TBD	TBD	measured
NH_4^+ (g N-NH_4^+ /L)	0-5	TBD	measured
Biomass	0-3	TBD	measured

COMPARTMENT III			
VARIABLE	**RANGE**	**OPERATION**	**TYPE**
Temperature (^oC)	0-150	30	controlled
pH	1-14	8.4	controlled
Bed pressure drop	0-2	TBD	controlled
Oxygen	0-100	20	controlled
CO_2	TBD	TBD	controlled
NH_4^+ (g N-NH_4^+ /L)	0-5	TBD	controlled
NO_2^- (g N-NO_2^- /L)	0-5	TBD	controlled
NO_3^- (g N-NO_3^- /L)	0-5	TBD	controlled
Biomass	TBD	TBD	measured

COMPARTMENT IVa			
VARIABLE	**RANGE**	**OPERATION**	**TYPE**
Temperature (^oC)	0-150	36	controlled
Pressure (atm)	0-2	1	comtrolled
pH	1-14	9.5	controlled
Oxygen disolv. (%)	0-100	TBD	measured
CO_2 gas	TBD	TBD	controlled
Oxygen gas	TBD	TBD	controlled
NO_2^- (g N-NO_2^- /L)	0-5	TBD	measured
NO_3^- (g N-NO_3^- /L)	0-5	TBD	controlled
Light intensity (W/m^2)	0-500	Variable	controlled
Biomass	TBD	TBD	measured

COMPARTMENT IVb			
VARIABLE	**RANGE**	**OPERATION**	**TYPE**
Temperature (^oC)	0-150	20	controlled
pH	1-14	TBD	controlled
Minerals (several)	TBD	TBD	controlled
CO_2 gas	TBD	TBD	controlled
Oxygen gas	TBD	TBD	controlled
NH_4^+ (g N-NH_4^+ /L)	TBD	TBD	measured
NO_3^- (g N-NO_3^- /L)	TBD	TBD	controlled
Light intensity (W/m^2)	0-500	Variable	controlled

ANIMAL COMPARTMENT			
VARIABLE	**RANGE**	**OPERATION**	**TYPE**
Temperature (^oC)	0-150	20	controlled
CO_2 gas	TBD	TBD	controlled
Oxygen gas	TBD	TBD	controlled
H_2O	TBD	TBD	controlled
Food	TBD	TBD	controlled
Light intensity (W/m^2)	TBD	TBD	controlled

6.8 Laboratory layout and general facilities

Incorporation of the compartments not yet in operation in the Pilot Plant will require expanding its premises. Taking advantage of a new building construction in UAB, the expansion will take place by setting up a completely new laboratory during 2002. The initial planned total surface for this new laboratory will be about 150 m^2, although final size is still under discussion.

A preliminary distribution of the room that will be available can be seen in figure 8. As a general description it can be said that about one third of the available surface will be dedicated to incorporate the tree chambers of the higher plant compartment. Another one third will be dedicated to compartments II, III and IV, in a similar way as it is implemented in the actual laboratory. The first compartment will be located in a dedicated room.

Another room will be dedicated to the animal compartment and special care will be taken to follow the governmental regulations for animal experimentation.

Human waste collection will require to set up a waste collection unit and to this purpose, a dedicated room and a specific device and storage containers, will be set up.

It is clear that a centre for data collection and control is of prime importance in the project, and consequently a special room will also be dedicated to this purpose.

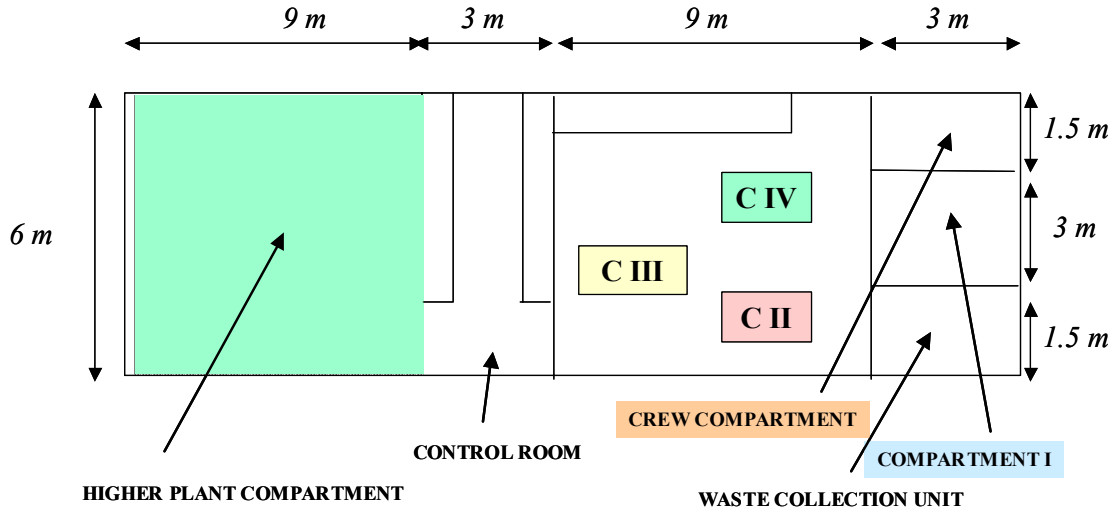

Figure 8: General layout of the expanded Pilot Plant laboratory.

7 ESTIMATED TIME SCHEDULE AND INVESTMENT PLAN

The MELISSA Pilot Plant has been successfully operating in continuous mode, three compartments. However, to finally complete the loop and analyse the degree of closure, it is still necessary to set up in the pilot plant premises, the first compartment, the higher plant compartment and the 'crew' compartment. This will result in the closure the complete MELISSA loop. To fulfil this key milestone, the envisaged work plan in the years to come is shown in figure 9.

Operation of compartments two, three and four interconnected at pilot scale will be performed during 2001. Following this step the first compartment will be implemented and connected to the previous ones during 2002. Previous experiments have demonstrated that the consortium of bacteria that best degrades human wastes is different than the one that degrades rat's wastes. Therefore in this case, it has been preferred to optimise this compartment to degrade human wastes. In consequence the human faces collection unit described above, will set up and the recollected samples, used to feed the first compartment.

Set up of the crew compartment will be done at the end of 2003 using rats as experimental animals. Closure of the basic loop including these compartments is foreseen for the end of 2003 or beginning of 2004. On the other hand, taking into account the advancement of other ESA studies (animal Holding facility, BAF,..) it is proposed in parallel, a preliminary set up of the crew compartment during 2002 using

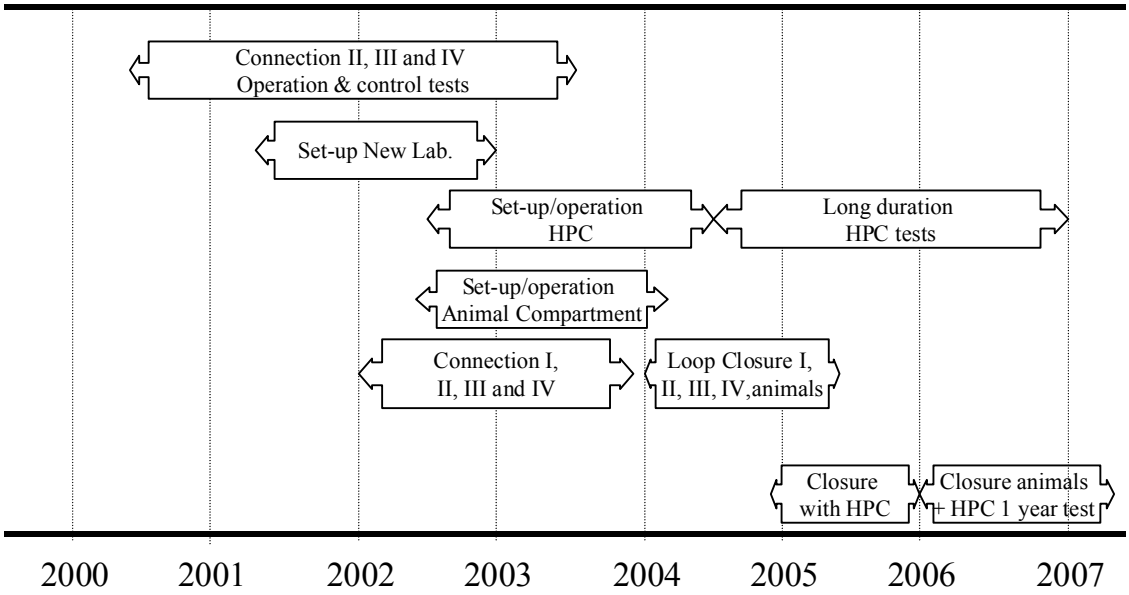

Figure 9: Pilot Plant development plan.

rats as experimental animals interconnected to a scaled dawn version of the biological air filter (BAF). This should allow to investigate associated technology (BAF).

Once the new compartments will be set up, construction of a higher plant compartment will take place during 2003. During all those periods tests will continue in the various compartments, either disconnected or in interconnected operation. After a testing period with the HPC compartment, and development and implementation of the corresponding control systems for the new compartments, closure of the loop will be assayed around 2005. During 2006 it is foreseen that it will be possible to perform a long term tests of one year demonstrating its ability to maintain alive the laboratory animals.

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