MELiSSA

TECHNICAL NOTE

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TECHNICAL SPECIFICATIONS FOR THE RE-DESIGN OF THE COMPARTMENT III PILOT REACTOR

Prepared by/Préparé par	Montràs, A., Pérez, J., Peiró, E. and Gòdia, F.
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List of acronyms

CI : compartment I

CII : compartment II

CIII : Compartment III

CIVa : Compartment IVa

CIVb : compartment IVb

CV : Compartment V

HPC: Higher Plant Chamber

MELiSSA: Micro-Ecological Life Support System Alternative

UAB: Universitat Autònoma de Barcelona

UPS: Uninterrupted Power Supply

1. Context: the MELiSSA Project and the MELiSSA concept

1.1. The MELiSSA Project

Over the last 15 years several Space Agencies (i.e. NASA, JAXA, RSA, CSA, ESA) have been studying the regenerative life support systems needed to sustain long-term manned space missions.

Space exploration constraints dictate that the primary objective of the studies is to reduce the launched mass of metabolic consumables (i.e. water, oxygen, food) by increasing their recycling rates up to, ideally, closure of the gas, liquid and solid loops.

Within Europe, the main part of the work has been performed within the MELiSSA (Micro-Ecological Life Support System Alternative) project by a highly comprehensive European and Canadian scientific and technical network, coordinated by the European Space Agency (specifically the European Space Research and Technology Centre ESTEC).

Within MELiSSA, it is proposed to follow a global approach of Life Support requirements by addressing jointly the main Life Support functions, i.e.:

- Air revitalization,
- Water production,
- Waste management,
- Food production and preparation
- Quality Control and Safety issues
- Ergonomics and Habitability

With regards to the challenge of sustaining Human Life during long-term manned space missions, a stepwise engineering approach is followed in MELiSSA, starting from basic research and development studies, including preliminary flight experiments, up to a comprehensive ground demonstration of the technologies developed.

1.2. The MELiSSA concept

The MELiSSA concept is based on the duplication of the functions of the earth without benefiting from earth's large resources (i.e. oceans, atmosphere..) and from terrestrial comfort.

The goals of the MELiSSA loop are the recovery of food, water and oxygen from wastes, i.e. CO_2 and organic wastes, using light as a source of energy.

From the observation of a lake ecosystem (i.e. the identification of the elementary consumption, degradation and production functions composing this ecosystem), the

MELiSSA loop is conceived as a closed regenerative system, based on five compartments duplicating the lake ecosystem's elementary functions (see below Figure 1, further information is available at http://www.estec.esa.int/ecls).

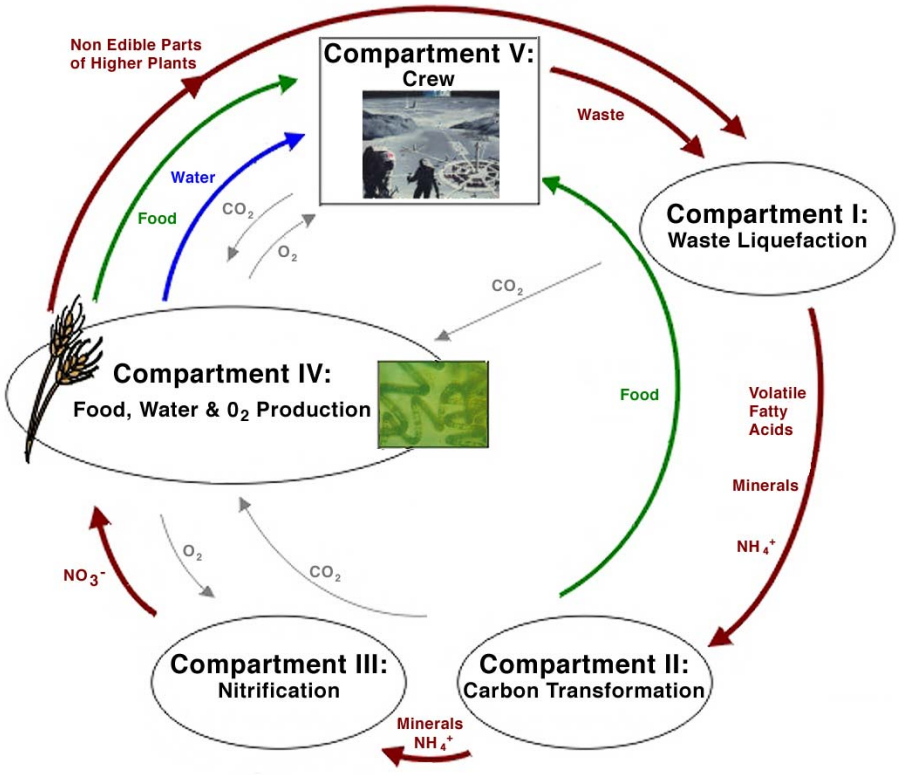

Figure 1: MELiSSA Advanced Loop Concept

Each compartment has a given objective within the complete biotransformation and connections with other compartments.

The basics are the followings:

- In Compartment I, the different waste sources are degraded in an anaerobic thermophilic bioreactor. The wastes include non edible material from plants, excess bacterial material from other compartments, fecal material, etc. The degradation yields a range of volatile fatty acids (VFA) that are transferred in Compartment II.
- Compartment II is photobioreactor where the VFA produced by Compartment I are further converted, basically to CO_2, by the photoheterotrophic growth of the bacteria *Rhodospirillum Rubrum*.
- Compartment III is responsible for the bioconversion of the nitrogen source, i.e. from ammonium NH_4^+, as produced in CI, into nitrate NO_3^-. Compartment III is a

fixed-bed bioreactor, with a co-culture of *Nitrosomonas* and *Nitrobacter* bacteria immobilized onto a solid support (beads).

- The production compartments are Compartment IVa and IVb:
    - Compartment IVa is devoted to the culture of the photoautotrophic cyano-bacteria *Arthrospira platensis* (*a.k.a. Spirulina platensis*), and is used mainly for the production of oxygen from CO_2,
    - Compartment IVb is devoted to the culture of a number of selected higher plants (i.e. wheat, lettuce and beet), for the production of food and oxygen.
    - These compartments are the closing steps for the loop, since they provide with the functions of atmospheric regeneration (converting the CO_2 generated by the crew and other bacterial compartments into O_2) and edible material generation. In addition, higher plants can also provide a way to biologically regenerate potable water through transpiration.
- Compartment V corresponds to the crew (i.e. consumer) compartment. For the first demonstration of the MELiSSA loop, it has been decided to work with laboratory animals.

The development of each individual compartment follows the same engineering logic:

- Technologies characterization in batch and continuous modes,
- Stoichiometry studies,
- Hydrodynamic characterization,
- Static Modeling,
- Dynamic Modeling,
- Control Model (for predictive control),
- Safety issues (chemical and microbiological),
- Maintenance and Dependability.

At the upper level of the complete loop (i.e. closed loop of interconnected compartments), a system approach is mandatory to achieve mass balance closure, a relevant safety of the complete system and its reliability for long term operation. This system approach is supported by a knowledge-based control leading to the development of a predictive control based management of the overall MELiSSA loop.

2. The MELiSSA Pilot Plant

2.1. Overall presentation

As expressed previously, the challenge of sustaining human life in frame of long-term missions is such that an extensive demonstration of MELiSSA on ground is a mandatory step in the process of its adaptation to space.

Owing to the state of the art at laboratory scale, the five MELiSSA compartments are progressively developed up to a pilot scale, according to a sizing scenario defined by the MELiSSA Consortium as representative of a full scale manned mission **(i.e. production of 1 eq-man oxygen, production of 20% of 1 eq-man daily diet)**.

The European Space Agency (ESA) has entrusted the implementation of the MELiSSA Pilot Plant to the Universitat Autonoma de Barcelona (UAB), with **the challenge to make it the primary European Facility for Life Support Ground-Demonstration.**

The MELiSSA pilot compartments will be integrated (i.e. connection of the gas, solid and liquid phases) within the MELiSSA Pilot Plant, with **the ultimate objective of a long-term demonstration (i.e. around 3 years of continuous operation) of the MELiSSA loop (i.e. 5 compartments interconnected).**

A new MELiSSA Pilot Plant facility has been built by the Universitat Autònoma de Barcelona., in the Departament d'Enginyeria Química, Escola Tècnica Superior d'Enginyeria (ETSE). This new facility of 214 m^2 will be devoted to the location of:

- compartments I, II, III and IVa, three Higher Plants Chambers composing CIVb, the animal compartment (i.e. CV),
- a human waste collection unit,
- a control room,
- Auxiliary equipments.

2.2. MELiSSA Pilot Plant: integration strategy

The main goal of the MELiSSA Pilot Plant described in the previous section will be achieved once all the different compartments will be operated at is final scale, in continuous mode, fully connected, under the control system, for a long operation mode. To achieve it, an step-wise integration strategy will be defined.

The closure of the MELiSSA loop is envisaged using animals as a mock-up of the crew compartment. Indeed, this is a more realistic scenario to demonstrate and study the first closure of the loop, including the effect of perturbations. The number an type of animals

to use will be defined in the corresponding study. Using animals instead of humans for this demonstration step also reduces in a great extent the feasibility of the experiments in terms of economical cost and associated safety measures.

In such scenario, the closure will be completed mainly at the level of the gas phase and water. The animal faeces and urine will not be used, that is, they will not be introduced as feed in any of the Compartments of the loop. In turn, and in order to obtain more realistic data for the MELiSSA loop operation, human faeces and urine will be collected from a group of donnors, and will be used as part of the feed material to the MELiSSA loop. In this way, the closure scenario proposed will be highly realistic, and the data obtained will enable to design future closure scenarios wuth humans.

The integration strategy within the MELiSSA Pilot Plant will follow a **step-wise approach**:

- The first steps will focus on the continuous operation of the pilot scale compartments individually. These steps will be the opportunity of additional characterization and validation activities that cannot be performed at laboratory scale, due to the level of instrumentation or the size of the hardware. The knowledge gained will potentially engender future optimization both in terms of hardware, of mathematical models and of control.
- In parallel, studies will be performed to develop the interfaces that will be necessary between the compartments. (e.g. a waste collector to collect urine and faeces, a waste preparation unit, biomass harvesting systems…)
- Then, a progressive connection of the compartments will be performed up to the ultimate closure. This progressive connection concerns all three, i.e. solid, liquid, and gas phases. Delicate issues will have to be addressed, such as, among others:
    - Prevention of any contamination of the compartments working under axenic conditions (i.e. pure mono- or multi- bacterial culture),
    - Low range of flows to be carried from one compartment to another,
    - flexibility of the design, to follow the evolution of the integration requirements and specifications
    - operator safety and high quality control.

2.3. Detailed description

The MELiSSA Pilot Plant is divided into different rooms, as described hereafter on Figure 2 and table 1. Basically, it consists of one area (9A, 9B, 9C and 9E) devoted to the bioreactors (i.e. compartments I, II, III and IVa), the waste collection unit and the animal compartment, one area (9 D) for the Higher Plants Chambers, and a central area for offices/meeting room and the control room.

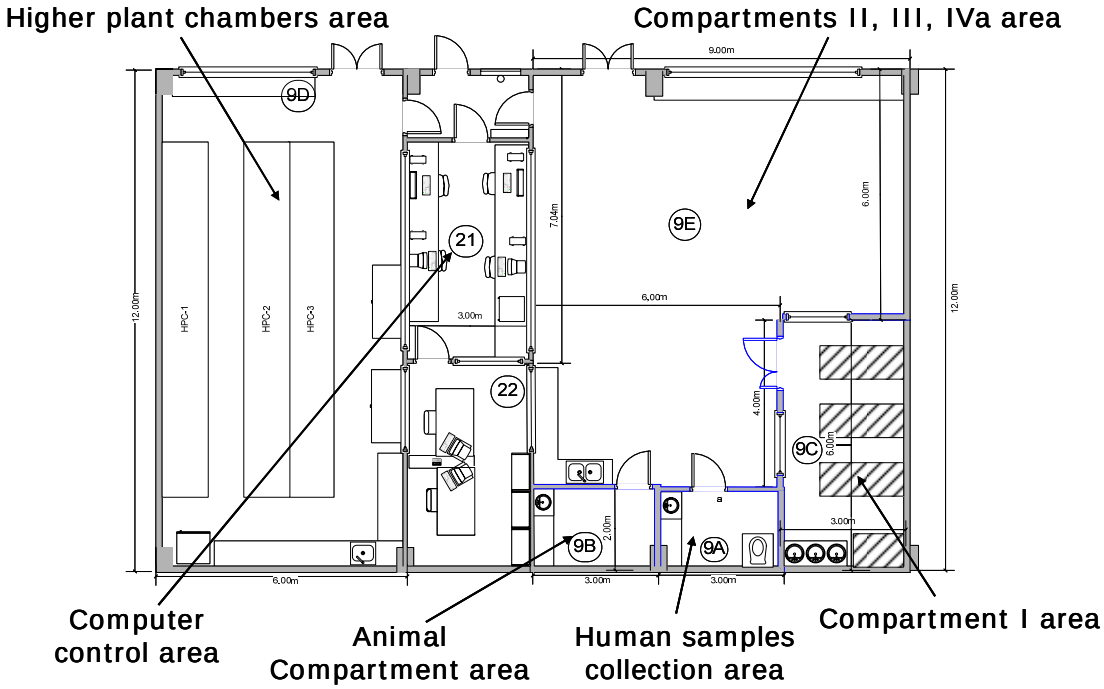

Figure 2. Basic layout of the MELiSSA Pilot Plant laboratory.

Room	Description
9E	Bioreactors area (includes compartments II, III and IVa)
9A	Human waste collection room
9B	Animal Compartment
9C	Compartment I area
9D	Higher Plant Chambers (Compartment IVb)
21	Control Room
22	Office

Table 1. Basic description regarding the distribution of the MELiSSA Pilot Plant

The document *MELiSSA Pilot Plant General Resources, Interfaces and Environment* (TEC-MCT/2006/3493/lnBLA), describes in detail all aspects of the MELiSSA Pilot Plant :

- access and design: covering sizes, maximum loads, surfaces characteristics…

- general utilities and facilities such as air filtration and ventilation, storage capacities, freezers…
- services provided by central systems, distributed over the MELiSSA Pilot Plant: steam, gas, power, cooling water..
- interfaces: with these provided services (connection types and their exact location), with additional networks (drains, gas exhausts..)..
- monitoring, alarms and safety issues.

As examples, Figure 3 provides the specific sizes of the MELiSSA Pilot Plant, and Figure 4 indicates the distribution of the different lines for power supply.

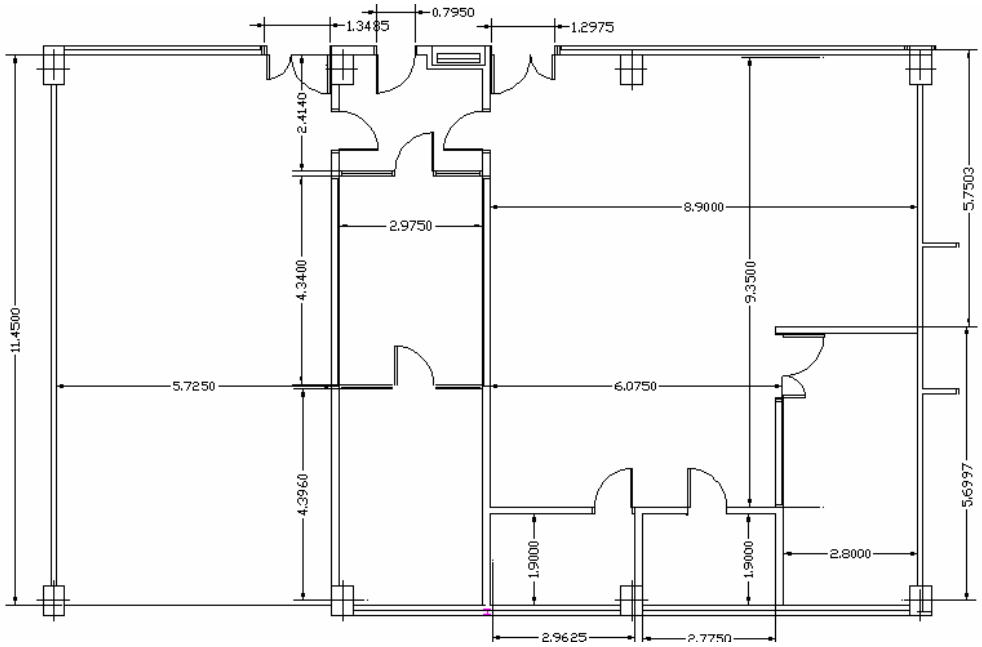

Figure 3: Sizes of the different areas in the MELiSSA Pilot Plant

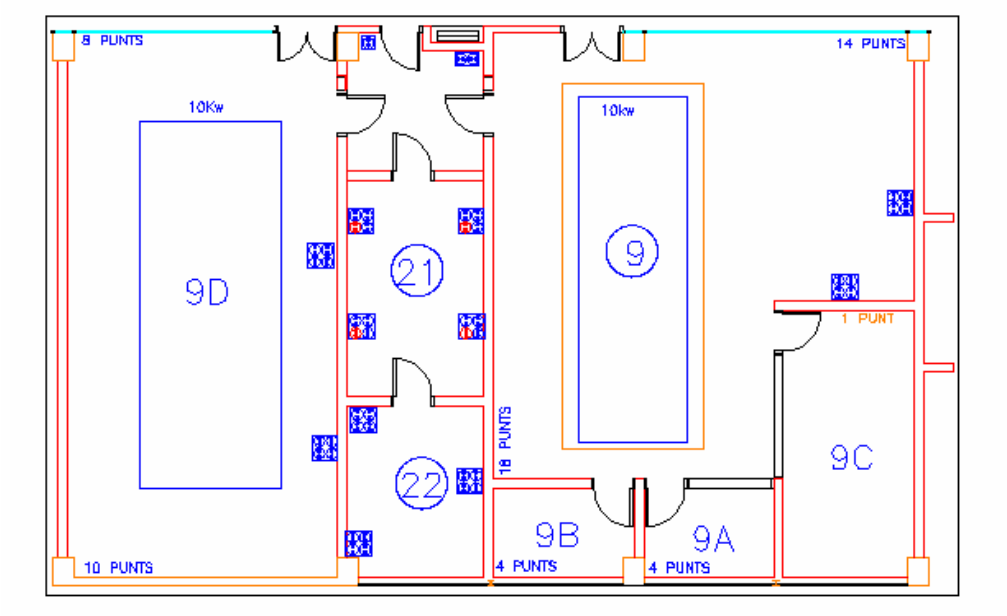

Figure 4: Distribution of the power lines in the MELiSSA Pilot Plant.

2.4. Additional technical information over the MELiSSA compartments

A brief description of each compartment in the MELiSSA loop is presented in the next paragraphs.

2.4.1.Compartment I

Compartment I, as illustrated on Figure 5, is composed of a membrane bioreactor connected to an influent feed tank and an effluent (i.e.filtrate) collection tank. The bioreactor has an approximate volume of 100 L

For the preparation of the influent, a waste preparation unit will be installed. During the integration phase, the waste preparation unit will probably be connected to the liquid phase of CIVb

Besides C-I equipment, room 9C is equipped with:

- Inert gas line to establish anaerobiosis (Helium).
- Air cooling/venting system.
- Steam line.

- Cool liquid line for temperature control and gas condensation system.
- Demineralized water.
- Tap water
- Compressed air (use of pneumatic devices).

2.4.2.Compartment II

Compartment II bioreactor will be located in room 9E. Bioreactor volume is about 50 L. A description of the reactor is given on Figure 6.

The output of C-II bioreactor, collected in an effluent collection tank, contains biomass to be further separated from the liquid output by a biomass harvesting system (today under study). The connection from the influent tank to the biomass harvesting system shall be foreseen.

Compartment C-II in room 9 will require the following services:

- Demineralized water,
- Tap water,
- Inert gas line to establish anaerobiosis (Helium),
- He and H_2 lines for gas chromatography,
- Air cooling/venting system,
- Liquid cooling supply system,
- Steam line,
- Compressed air (use of pneumatic devices).

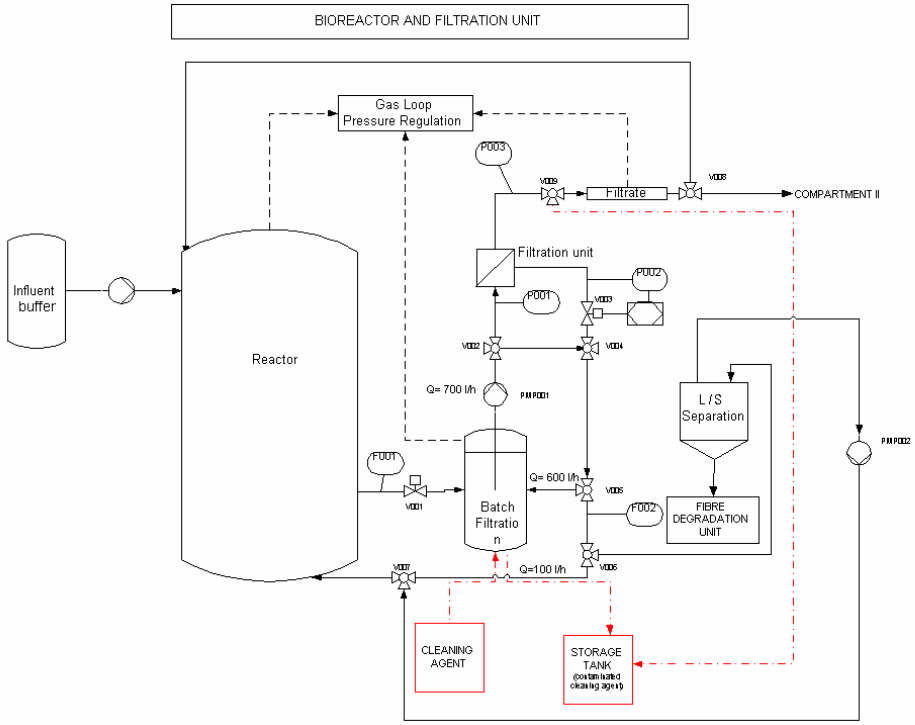

Figure 5: Schematic design of compartment I and its filtration unit.

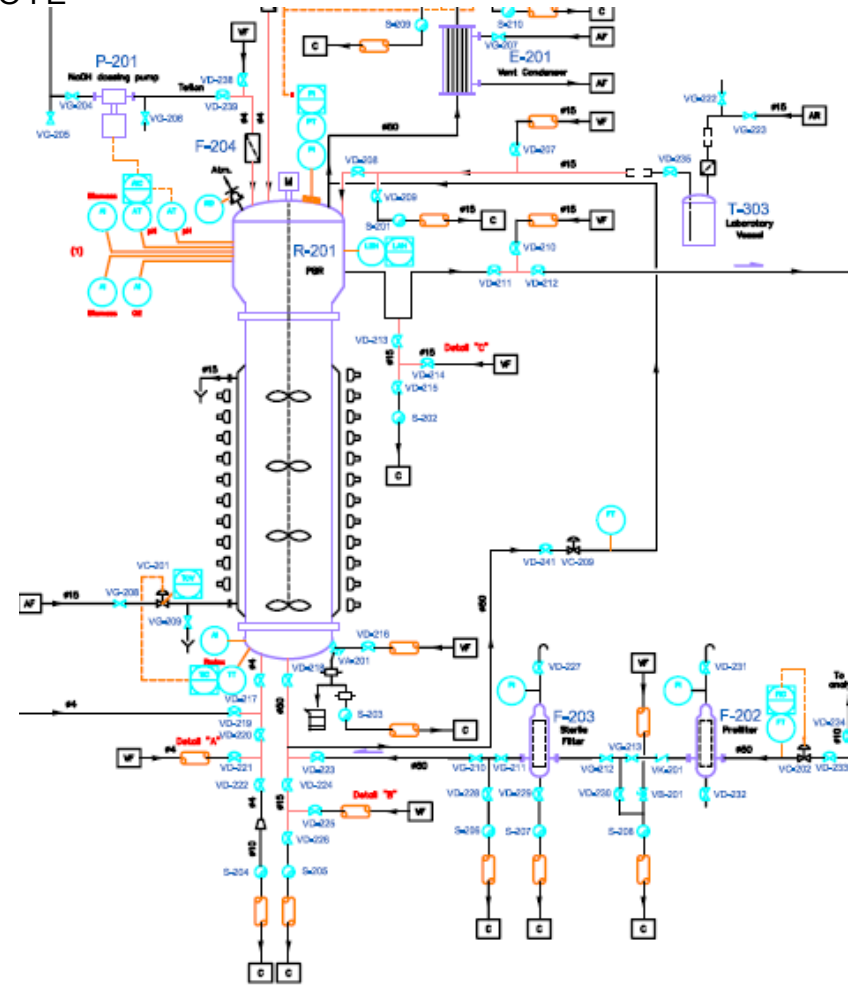

Figure 6: Configuration scheme of compartment C-II.

2.4.3.Compartment III

Compartment III bioreactor will be located in room 9E. The volume of the bioreactor is 8 L.

The present bioreactor (see Figure 7 for a schematic overview and associated picture), will be now up-graded, and the work presented here is indeed related to this up-grade.

Compartment III will require the following services:

- Demineralized water.
- Tap water
- Gas lines for independent operation O_2, CO_2, N_2.
- Compressed air as base for mixing with other gasses for independent operation and also in case of using pneumatic devices
- Liquid cooling line for output gas lines condensation.
- Steam line

2.4.4.Compartment IVa

Compartment IVa bioreactor will be located in room 9E. The volume of the bioreactor is 77 L. A schematic overview of this compartment and the equipment involved is provided on Figure 8 and associated picture.

Compartment IVa will require the following services:

- Demineralized water.
- Tap water
- Gas lines for independent operation O_2, CO_2, N_2.
- Compressed air as base for mixing with other gasses for independent operation and also in case of using pneumatic devices
- Liquid cooling line for temperature control and output gas lines condensation.
- Air cooling for lamp heat elimination.
- Steam line

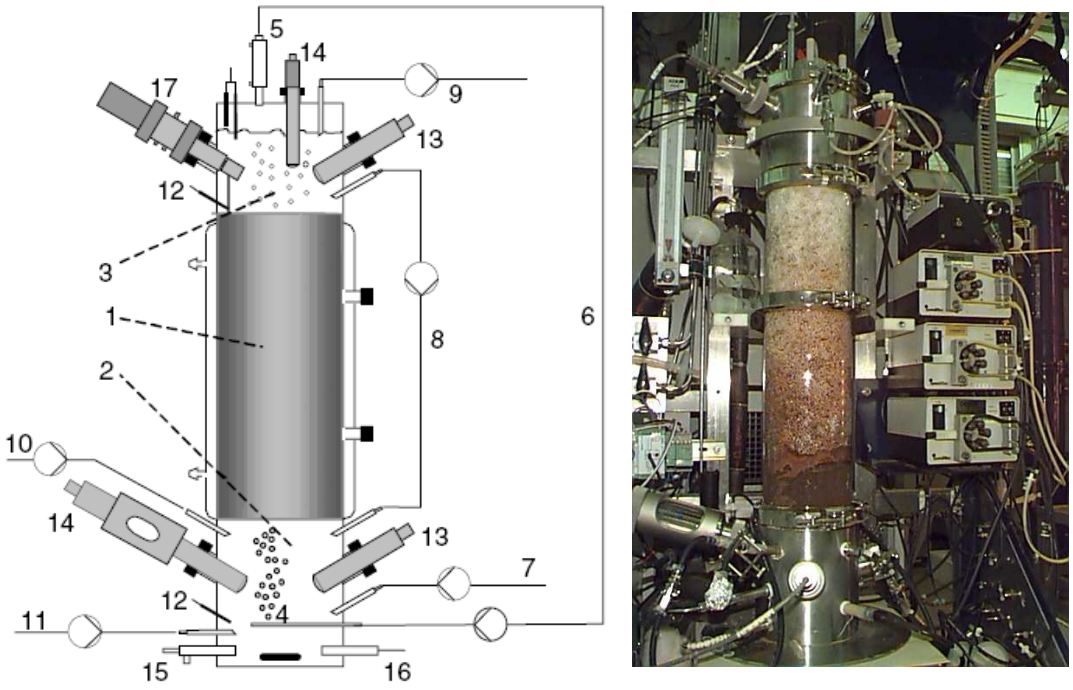

Figure 7: : Schematic overview of compartment III.

General schematic (left) and picture (right) of the nitrifying pilot bioreactor. (1) Packed-bed section with immobilized culture, (2) bottom section for aeration, liquid distribution and instrumentation, (3) top section for gas disengagement, (4) gas sparger, (5) gas exit condenser, (6) gas loop, connected to oxygen/nitrogen regulated supply to control dissolved oxygen, (7) liquid feed, (8) liquid recirculation, (9) liquid outlet, (10) acid addition, (11) base addition, (12) temperature probes, (13) dissolved oxygen probes, (14) pH probes, (15) cooling system, (16) heating system, (17) sampling device.

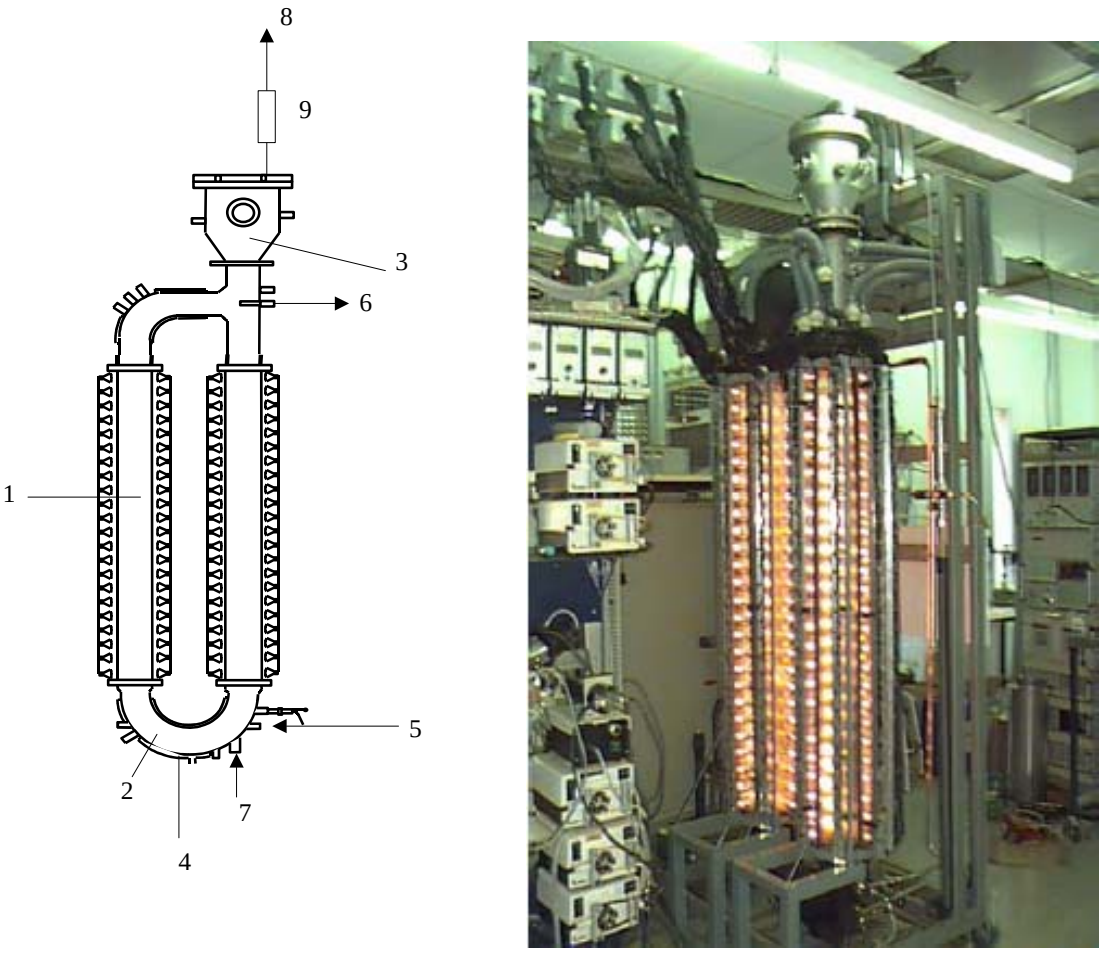

Figure 8: Schematic view of compartment IVa.

General scheme of the 77 litres photobioreactor designed for the culture of Spirulina cells. 1, transparent cylindrical parts (illuminated section) : riser (right column and downcomer (left column), 2, stainless steel connection parts , 3, gas-liquid separator, 4, external cooling jackets, 5, liquid medium inlet, 6, liquid outlet, 7, gas inlet through sparger, 8, gas outlet, 9, condenser, 10, halogen lamps.

2.4.5.Compartment IVb

The higher plant compartment C-IVb will be installed in room 9D. It will be composed of 3 Higher Plants Chambers. A schematic overview of the compartment is shown in Figure 9.

CIVb will require the following services:

- Demineralized water.
- Tap water
- Gas lines for independent operation O_2, CO_2, N_2.
- Compressed air as base for mixing with other gasses for independent operation and also in case of using pneumatic devices.
- Air cooling for lamps heat elimination and temperature control.
- Liquid cooling line for temperature control and maybe for evapo-transpiration condensation depending on chamber design (green solid line in figure 15).

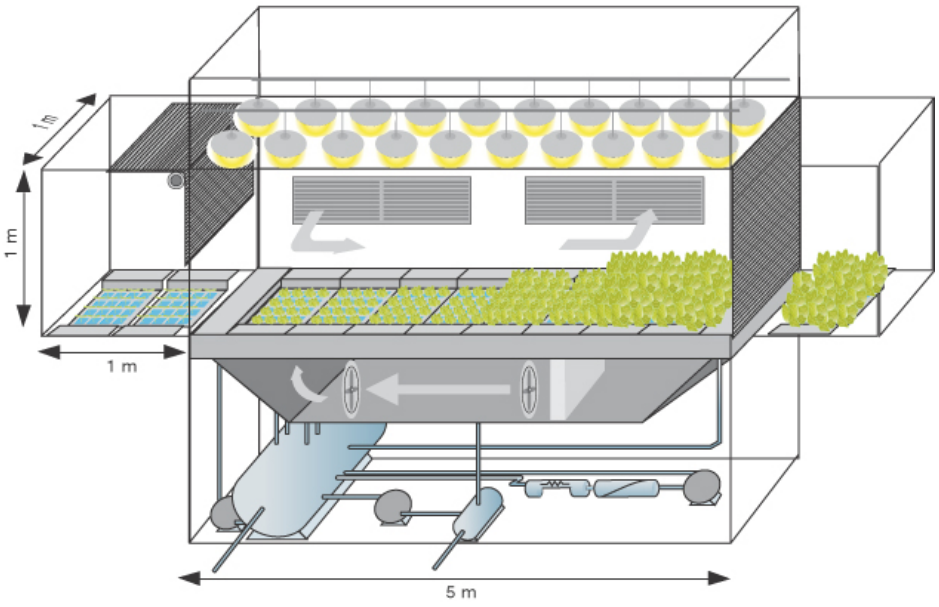

Figure 9: schematic view of the design concept for the Higher Plant chamber.

2.4.6.Compartment V

The animal compartment will be installed in room 9B. This compartment is currently under design. In principle, it will consist in an air tight cage where animals are going to live.

The animal compartment (CV) will require the following services:

- Demineralized water.
- Tap water
- Gas lines for independent operation O_2, CO_2, N_2.
- Compressed air as base for mixing with other gasses for independent operation and also in case of using pneumatic devices.
- Liquid cooling for humidity of breath air condensation.

3. Scope of the study: re-design of Compartment III

As mentioned previously, one of the compartments of the MELiSSA loop, Compartment III, is devoted to the nitrification step within the loop. Nitrifying bacteria are required in a life support system to carry out the oxidation of ammonium to nitrate. In the MELISSA loop, nitrification is carried out in an upflow cocurrent packed bed reactor where the two selected strains, *Nitrosomonas europaea* (ATCC 19718) and *Nitrobacter winogradskyi* (ATCC 25391), are immobilized on polymeric (expanded polystyrene) substratum (Biostyr®) which has an average diameter of 4.1mm. In order to avoid inhibition by light, the fixed bed was protected with thin foil. The scheme of Compartment III and a picture during its operation can be observed in Figure 7.

The operating conditions in the reactor were as follows: pH 8.1, magnetic stirring at the bottom at 400 rpm and temperature controlled at 28.0 ± 0.1 ºC. Air was supplied to the reactor by means of a sparger while oxygen enriched air was added by the control system to maintain a dissolved oxygen set point of 80%. Dissolved oxygen in the culture medium, pH and temperature were measured by means of two on-line probes located at the top and at the bottom of the reactor, whose measurements were weighed by the control system. Dissolved oxygen concentration was controlled by adding pure oxygen or nitrogen to the input gas, a solution of Na_2CO_3 was used to increase pH when necessary, and CO_2 was added when pH needed to be decreased. Total pressure in the bioreactor was kept lower than 80 mbar.

In regular continuous mode operation the reactor is fed with a given amount of NH_4^+ and is operated at a constant gas flow rate. These are the main process parameters on the reactor performance:

- Compartment III is capable of processing ammonium loads up to 1.4 kg $N\text{-}NH_4^+ \cdot m^{-3} \cdot d^{-1}$ with the minimum volume.

- The flow rate and concentration of the liquid stream entering this reactor are defined by the requirements of the MELISSA loop. As a reference for design purposes, the range of concentrations and flow rates used during tests with the current reactor design have been used. They were as follows:

Flow rate	Concentration
0.15-0.60 L/h	300-600 mg $N\text{-}NH_4^+$/L.

- Because nitrification is an aerobic process, oxygen is provided to the reactor: an approximate value of oxygen requirement can be obtained from the stoichiometry (1.5 mols O_2 /mol NH_4^+). However this value must be corrected taking into account gas – liquid mass transfer and biofilm thickness, which both have an effect on the oxygen requirements. Previous studies performed with bench scale packed-bed reactors (Pérez 2001) gave as a result an oxygen ratio of 11 mol O_2 /mol NH_4^+ as the minimum oxygen supply.

- The carbon source for cellular methabolism is supplied to the reactor from the liquid medium, as $NaHCO_3$

- Base for pH control purposes is provided: Na_2CO_3 at a concentration of 100g/L.

- For pH control purposes, although the nitrification process usually requires addition of base during stable operation, the addition of CO_2 to lower pH is required from time to time, especially in the start-up of the reactor. Also the addition of acid should be foreseen for fine-tunning of pH control under certain circumstances.

The total flow of the gas-phase in the bioreactor is kept at a constant value of 3000 mL/min, important to keep the overall hydrodynamics of the bioreactor.

The reactor has already been operated in the MELiSSA Pilot Plant over extensive periods of time, at different experimental conditions (Pérez, 2001; Pérez *et al.*, 2004). Now, taking advantage of the removal of this compartment into the new site of the MELISSA Pilot Plant, the re-design of its hardware is envisaged, in order to improve its performance, specially taking into account that some parts of the equipment need to be changed due to its intensive use, and the performance requirements for the final MELiSSA loop closure.

The purpose of the present document is to define the elements to be addressed in such a re-design work. In general, major changes from the previous hardware are not considered, since the operation of the reactor in the previous experiments was satisfactory in general. Also, no changes in sizing are required, since the capacity of the compartment already fits to the needs required for the MELiSSA loop closure. Therefore, the final goal

is to optimize the existing design, improving the necessary elements while maintaining those that have already shown good performance over the previous periods of operation.

The main driving force of the re-design of this compartment is to guarantee the continuous operation over long periods, under well controlled conditions, and in fully axenic conditions. Indeed, it has to be considered that full steam sterilization should be undertaken to guarantee the axenicity of the reactor. Additionally, it has also to be considered that the support particles currently used (Biostyr®) will require specific chemical sterilization.

Other important aspects regarding long term operation and axenicity of the reactor are the pumping devices, retractable probes, online measurements and the prevention/control of potential clogging of the packed bed bioreactor. The new design will also incorporate the necessary ports to install the on-line biomass detection system that is being developed in a parallel study, and that will allow to monitor the evolution of biomass within the packed-bed during its long term operation.

All these main considerations, as well as some more specific ones, have guided the following proposal of requirements for the re-design of Compartment III.

4. Technical specifications for the re-design of Compartment III.

The technical specifications for the redesign of the compartment III pilot reactor have been split in three main categories:

4.1 Basic requirements and definition of the hardware

4.2 Instrumentation requirements

4.3 Operation requirements

4.1. Basic requirements and definition of the hardware

The pilot reactor of compartment III is an upflow cocurrent packed-bed reactor that has a total volume of 8.1L. The reactor has three main sections. The central section is the one corresponding to the biological packed-bed. It has a total height of 520 mm and an internal diameter of 120 mm. The bottom section, with a height of 150 mm and a diameter of 112 mm, is used for gas and liquid supply, mixing and instrumentation. Biofilm and liquid mixing in the reactor was improved by liquid recirculation in addition to magnetic stirring in the bottom section.

The hardware that is to be constructed should show the same dimensions and basic characteristics as the existing one, but some concrete aspects will be modified in order to improve its operation:

1. The whole reactor should be built in stainless steel 316L, in order to allow its sterilization using pressurized steam, and avoiding the entrance of the outer light, due to the inhibition effect of light on nitrifying bacteria. For the sterilization requirements, tightness in all the sealings will be effectively guaranteed. Also, the reactor should provide all the necessary ports to host the associated instrumentation described in Section 4.2.

2. The reactor will incorporate an axial spy hole in its frontal face, as long as possible, with the following objectives: on one side, to allow the observation of the biofilm, and on the other side, to allow the observation of the lower part (mixing region) and upper part (level of liquid, gas separation) of the reactor. Lighting on the top lid will be also provided to examine the top section of the reactor.

3. The reactor will be equipped with a magnetically coupled stirrer system for its bottom section that will ensure long term contamination free operation, avoiding mechanical seals.

4. The reactor will incorporate stable stainless steel 316L or Delrin grids, preferably removable (for cleaning purposes) to allow the Biostyr beads to be retained within the central part of the same (packed-bed).

5. In order to guarantee the operation of the reactor in continuous mode during extended periods and guarantee the adequate sterilization and handing of the whole equipment, stainless steel 316L vessels will be provided for the feed and outlet liquid, total volume 40 L (working volume 30 L), both able to be steam sterilised, and being connected when possible by means of pipelines built in the same material.

6. Alternatives to the use of peristaltic pumps for both culture medium and acid or base feeding, broth removal and broth recirculation, will be proposed, guaranteeing the operating flow ranges during extended periods of time. Proposals for the design and purchase of the necessary devices are open to any technology or supplier that will fulfil the requirements.

7. Stainless steel 316L vessel and pipelines will be also provided for the acid and base storage, feeding medium and outlet, as an alternative to the use of PP or glass bottles, depending on the estimate flow and concentration of the acid and base to be used, and also depending on the availability of a solution for requirement 5.

8. Stainless steel 316L pipelines will be provided for the liquid recirculation as an alternative to the silicon tubes, depending on the availability of requirement 5.

9. The reactor should have an overpressure safety valve mounted on the top lid.

4.2. Instrumentation requirements

4.2.1. Description of the current on-line instrumentation and control of Compartment III (see Figure 7):

The current instrumentation of the reactor is summarized here. The main characteristics of the instrumentation is summarized in Table 2.

- pH sensors: both in upper and lower parts of the reactor.
- Dissolved Oxygen (D.O.) sensors: both in upper and lower parts of the reactor.
- Temperature sensors: both in upper and lower parts of the reactor.
- Pressure sensors
- Level sensor
- Gas mass flow meters:
    - O_2
    - CO_2
    - N_2
- Total gas flow meter
- Three on-line analyzers are used to measure nitrogen concentration in the liquid phase of the bioreactor:
    - NH_4^+ online analyzer
    - NO_2^- online analyzer (still under study)
    - NO_3^- online analyzer

Table 2. Description of the main instrumentation associated to Compartment III bioreactor

Monitored parameter	Measuring range	Precision	Number of units
pH	0-14	±0.1	2
Oxygen	0-100%	±0.1%	2
Temperature	4-250ºC	±0.1ºC	2
Pressure	0-1000 mbar	±1 mbar	1
Level	-	-	1

O_2 mass flowmeter	0-500 ml/min	-	1
CO_2 mass flowmeter	0-50 ml/min	-	1
N_2 mass flowmeter	0-20 l/min	-	1
Gas flowmeter	0-10 l/min	-	1
NH_4^+	0.01-255 mg N-NH_4^+ /L	±5%	1
NO_2^- (in study, not implemented)	0.02-0.5 mg N-NO_2^-/L 0.5-20 mg N-NO_2^-/L	-	1
NO_3^-	50-600 mg N-NO_3^-/L	±5%	1

The different control loops in Compartment III bioreactor are embedded in the general control architecture of the MELISSA Pilot Plant. To this effect, the electrical connections of all sensors and actuators need to be compatible with those of the quantum Schneider PLC.

The following parameters are controlled. In table 3 a brief description of the main control loops with the values of the set points and the related precision is listed.

- pH in the bioreactor liquid phase
- Liquid flow rate
- Gas flow rate
- Temperature
- Pressure
- Dissolved oxygen

Table 3: Description of the action of the main control loops associated to Compartment III bioreactor

Control loop	Set point	Precision
pH	8.0	± 0.1
Liquid flow rate	2.5-10 ml/min	To be defined
Gas flow rate	3000 ml/min	To be defined

Temperature	28.0 ºC	± 0.1ºC
Pressure	<80 mbar	1 mbar
Dissolved Oxygen	80 ± 5 %	± 5%

4.2.2.New instrumentation requirements (see Figure 11)

10. The reactor shall incorporate the standard ports where the biomass sensor currently under development by NTE will be hosted (Figure 10). The final system selected will require the use of several ports, possibly at three levels: bottom, center and top, so all of them will be provided and part of them eventually used for the final system.

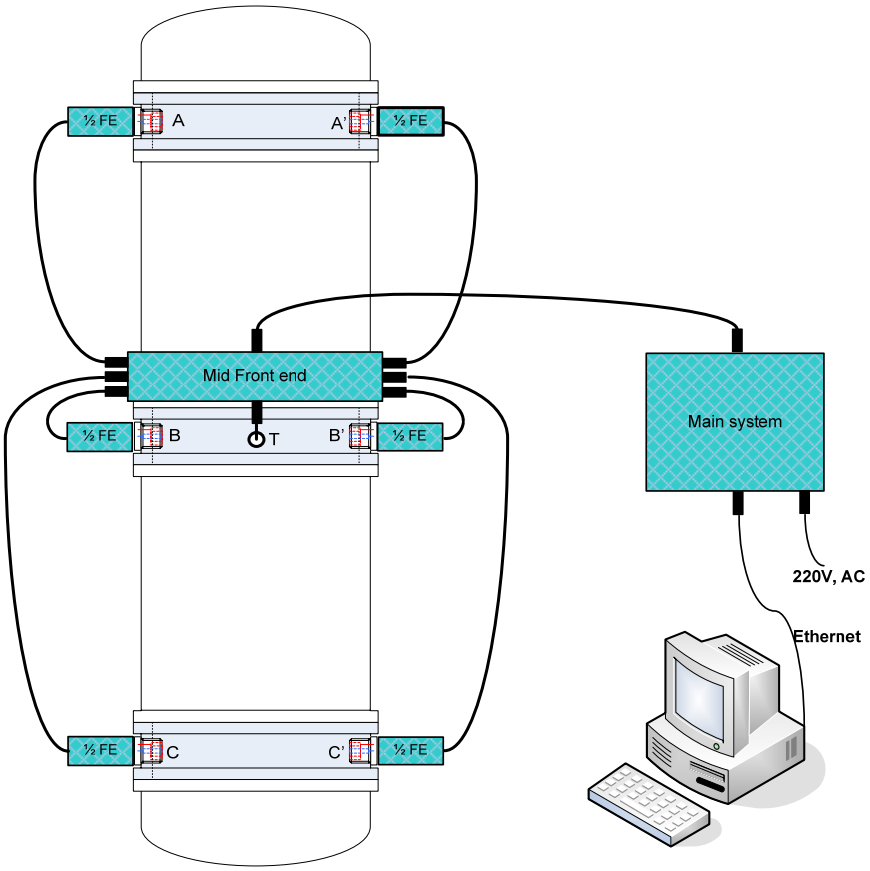

Figure 10: schematic view of the NTE biomass sensor system concept

11. The probes both for pH and D.O. should be installed in retractable housings, sterilizable without process interruption and showing hygienic connections.

12. The rest of sensors should be installed in retractable housings when available.

13. A differential pressure measurement should be implemented in the reactor in order to prevent clogging of the packed-bed due to excess of biofilm generation. This measurement will be used to foresee the needs of preventive backwashing procedures, in order to remove excess of biofilm, before severe clogging occurs.

14. The reactor shall incorporate a cellular retention device in the output line (see Figure 11), in order to retain any free biomass detached from the biofilm (low concentration expected in normal operation conditions). The retentate from this unit (typically a tangential filtration one) would be recirculated to the reactor entry, while the permeate will be pumped to other MELiSSA compartments. In this line, free of cells, an additional line will be used to feed the on-line analysers.

This system is required to guarantee both the correct performance of the online analyzers and the axenycity of the bioreactor. Typically online sampling will be required for NH_4^+, NO_3^- and NO_2^-. Proposals for the design and purchase of this sampling device are open to any technology or supplier that will fulfill the requirements.

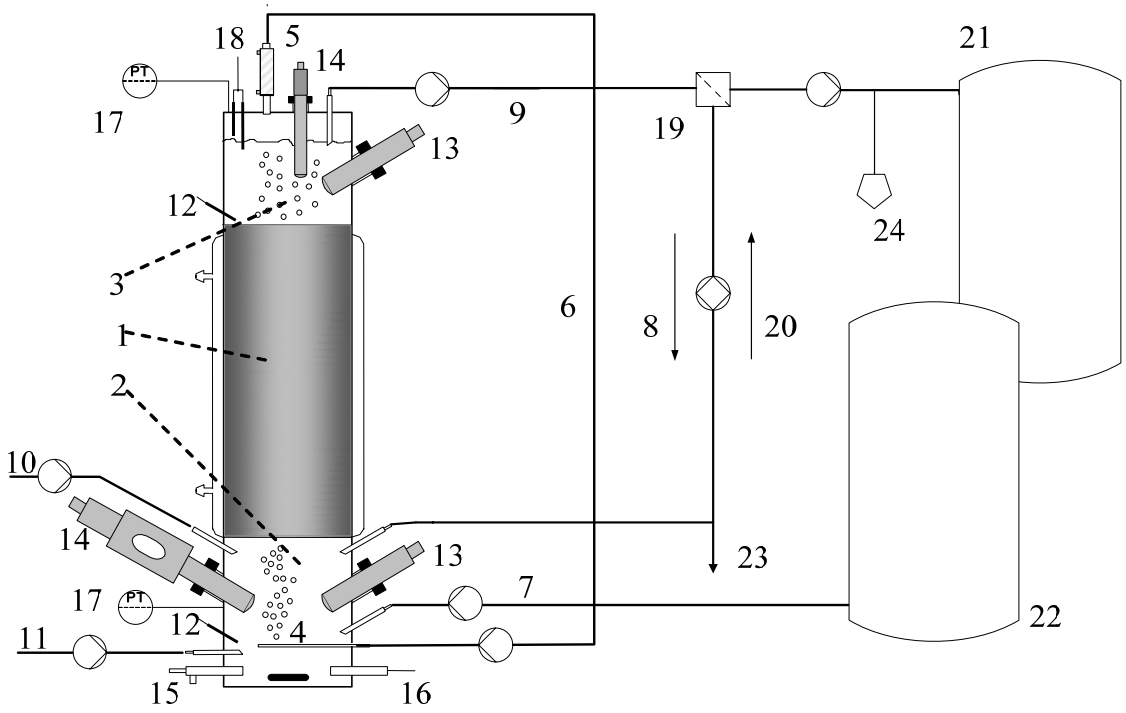

Figure 11: : Schematic overview of updated compartment III.

General schematic of the nitrifying pilot bioreactor: (1) Packed-bed section with immobilized culture, (2) bottom section for aeration, liquid distribution and instrumentation, (3) top section for gas disengagement, (4) gas sparger, (5) gas exit condenser, (6) gas loop, connected to oxygen/nitrogen regulated supply to control dissolved oxygen, (7) liquid feed, (8) liquid recirculation, (9) liquid outlet, (10) acid/CO2 addition, (11) base addition, (12) temperature probes, (13) dissolved oxygen probes, (14) pH probes, (15) cooling system, (16) heating system, (17) differential pressure sensors, (18) level sensor, (19) cellular retention device, (20) backwashing line, (21) outlet tank, (22) feeding tank, (23) detached biomass bleeding in backwash operation, (24) analysers

15. The level control should be improved, and work in a precise manner. The sensor for high level will be redundant to avoid any potential flooding, and a low level sensor should be incorporated to ensure a minimal liquid level in the reactor.

16. Flow meters should be incorporated in the most critical liquid lines (feeding, outlet, recirculation) to provide visual information on the corresponding flows.

Control issues regarding the operation of this Compartment should have to be updated in agreement with the requirements herein described; nevertheless, this update is out of the scope of the re-design work that is the aim of this technical note.

4.3. Operation requirements

4.3.1.Sterilization and loading of Biostyr® beads

17. A system to adequately sterilize the Biostyr® beads and load them into the sterile reactor should be considered, having into account that the reactor should be sterilized by pressurized steam and Biostyr® does not stand steam sterilization. Proposals for the design and purchase of the necessary devices are open to any technology or supplier that will fulfil the requirements. Alternatively, on-site chemical sterilization will be performed.

4.3.2.Inoculation

18. The inoculation of the reactor shall be performed in such a way that axenicity will not be compromised. Axenic inoculation is necessary to ensure the proper start-up of the bioreactor operation. So the reactor will incorporate the adequate inoculation port allowing steam sterilization of the same, and the corresponding system to be incorporated to bottles that will guarantee the appropriate connection with it.

4.3.3.Back-washing

Tests performed with the current reactor hardware involving extended periods of continuous operation (Gòdia et al., 2002) led to a progressive clogging of the packed-bed due to increasing biomass growth. In order to avoid this effect and remove excess biofilm, a back-washing operation should be performed periodically, i.e. liquid recirculation is reversed to facilitate the detachement of excess biomass in the packed-bed. This biomass should be removed from the bioreactor through a bleeding line, and

finally normal operation would start again. Such a process would be repeated in a cyclic pattern, in a frequency of some motnhs, taking into account that the continuous operation of the biioreactor will last for 2-3 years. This process includes the following tasks:

- Stop the operation mode.
- Disable the filtration unit.
- Start backwashing operation, characterised by:
    1. Recycling in opposite direction to flow (short period)
    2. Stop the pump
    3. Bleeding in backwashing line (system and operation to be defined)
- Recover normal operation mode

4.3.4.Long-term Operation

In all the developments above described it should be taken into account that the reactor will operate during long periods of time, and axenicity is critical to be maintained. Long-term operation will also take into account the needs for adequate CIP and SIP of the bioreactor, and the design considerations regarding ergonomics.

5. References

Gòdia, F.; Albiol, J.; Montesinos, J.L.; Pérez, J.; Cabello, F.; Mengual, X.; Montras, A. and Lasseur, Ch. (2002). MELISSA: a loop of interconnected bioreactors to develop life support in Space. Journal of Biotechnology 99: 319-330.

Pérez, J. (2001) Utilización de Nitrosomonas y Nitrobacter en forma de biopelícula para la nitrificación biológica en reactores de lecho fijo. PhD Thesis. Universitat Autònoma de Barcelona. Barcelona, Spain.

6. Comments

Comments to version 2

General comments

It seems that redesign/building activities are covering not only the bioreactor (i.e. vessel itself) and associated instrumentation but auxiliary equipment as well, i.e. feed tank, effluent tank, sampling system.... An assessment of the hardware and instrumentation already present in the MELiSSA Pilot Plant should be done to determine if part of them could be reused

Agree. We could eventually reuse some of the current equipment like peristaltic pumps, flowmeters and other small equipment, depending on the final definition of the new equipment. The reactor itself, auxiliary steel tanks and new instrumentation will be for sure built new.

In this frame of work, precise contents and limits of delivery, in term of hardware, are not fully clear. Maybe a functional scheme, or a flow scheme or general PID could bring some clarification (especially with regards to future connection to other Compartments which seems not to be taken into account).

A general scheme is included (Fig. 11, page 26).

CIII control is not mentioned as it is out of scope of the delivery; however, as the control strategy is impacting hardware design and selection, a description of the control loops should be included. It is now included (pages 24 and 25).

When possible, precise material selection should be given (e.g. quality of stainless steel...), as it will impact drastically the quotation OK, it´s specified in the text.

Under the operation requirements section, a paragraph devoted to backwash and one devoted to CIP/SIP should be added; maybe one about maintenance and ergonomy. OK, additional description is included in paragraphs 4.3.3 and 4.3.4 (pages 27 y 28).

Detailed comments

Page/paragraph	Comment
19/3	It would be easier to provide directly in the TN the additional design details given in the publication than asking people to look themselves for the publication mentioned Reference to the publication is deleted, and the details of the final design are in fact in the scheme described in Fig.7
20/use of CO2 for pH control	To ease the control of CIII, it would be probably better to deal separately with pH control on one side, biomass production on the other side. Therefore, we would recommend to use something else than CO2 for pH control. Paragraph regarding CO2 use as carbon source has been modified. Regarding pH control, we have discussed in detail, and our conclusion is now different: considering that the pH drop is due to

	the stripping of CO2 by the aeration, then it is rational to use CO2 to reset the pH value, better than another acid, also because otherwise a lack of carbon source could occur. In a way, the CO2 in the gas phase is monitoring the buffer capacity of NaHCO3.
21/2nd paragraph	Axenic would be more appropriate than sterile. The shortcut to biosafety issues is too short O.K., modified in the text.
21/3rd paragraph	First sentence to be rephrased O.K., rephrased.
21/4.1	There is no possibility to control the stirring with a magnetic stirrer in the bottom section of the reactor. An alternative should be foreseen. The requirement for a magnetic coupled mixing system has been included (page 22, paragraph number 3).
22/ 2	We should think about having some lightening system to be used when we want to see the inside of the reactor The requirement for lighting on the top lid is included in the text (page 22, paragraph number 2).
22/3	Grids should be removable, material? Modified in the text (page 22, paragraph number 4); material: stainless steel 316L (unless antenna effects are expected in NTE sensor).
22/4	Volumes for the vessels have to be provided O.K., already included (page 22, paragraph number 5).
22/5	Do you expect some design activities on this pumping issue? Is just selection of appropriate equipment, not properly design.
24/4.2.2	As the biomass sensor is based on an impedence measurement we have to be extremely careful with potential antenna effects (as the reactor is in stainless steel, impact on grids material and location to be taken into account) Assesment on this matter should be necessary (NTE)
25/13	The strategy to obtain sample free of biomass should be discussed between ESA and UAB. Filtration of the effluent to be further processed in CIVa will be mandatory. As the cells concentration in the effluent should be low, and as we have a recirculation loop on the reactor (i.e. meaning that the content of the reactor is the same than inside this recirculation loop), maybe we could foresee the appropriate filtration step, and sample this filtrate. The requirement for such a filtration step has been included (page 26, paragraph number 14) and the general in Figure 11 shows the new proposed design. Do we have any value of the total flow needed for on-line analysis? The values are 0,2 mL for NO3-, 3 mL for NH4 and 0,2-2 mL estimated for NO2-, each injection.

25/14	Ok to consider appropriate sensors for level; however, level control is out of scope Already indicated in the text (page 27).
25/16	We agree on the principle; however, we have to take into account that this device will not be used very often. Price can become an issue. We agree, we need to look for a simple system (so minimising the cost), but assurance of sterility is essential for long-term operation, and the current procedure is not perfect.

Comments to version 1

General comments

This draft document will be very difficult to use by potential manufacturers. Some mandatory information is missing or not precise enough and some information is difficult to relate to design options.

Some discussions about process options are not relevant in a document to be delivered to external companies.

These paragraphs have been removed from the document. It is also clear that this document is still not in its final form to be presented to potential manufacturers, since it still needs to be discussed with the board of consultants, when a number of points will be finalised.

Some options are fixed already. This bioreactor should work properly with biostyr beads, which does not eliminate the option to use alternative support. The document has to be updated appropriately.

This has been taken into account in the new version of the technical note.

Information shall be added about requirements with regards to:

- interfaces with the MPP and MPP control system
- MELISSA harmonized hardware
- Future integration within the MELISSA loop
- Drawings to be delivered (VISIO, autocad)
- Hardware/software/documentation to be delivered.

All these points are indeed relevant for the final ITT technical package that should be prepared. However, we did not intend to cover them at the level of definition of the re-

design of C-III. These aspects are covered by other activities, which will provide the necessary documentation to be added to the User Requirements for Compartment III. Indeed, some of them are already in preparation as part of the engineering design of the Pilot Plant document, and the integration strategy definition.

ESA considers this document version as an initial draft to be deeply upgraded before spreading.

Detailed comments

- introduction:

It is mandatory to include a paragraph providing a detailed scientific description of MELISSA and a detailed description of CIII within the loop (function, connections, control strategy…).

A paragraph has been added in the text explaining the main goals of the MELISSA project and references to publications have been added (page 1). The function of compartment III within the MELISSA loop has been defined in the text (page 1) and a brief description of the current design of the pilot reactor has been included in the section "basic requirements and definition of the hardware" (page 3).

Although the provided assessment reflects potential options for future investigations, the major part of this assessment has nothing to do in a public document and has to be removed.

We have changed this part of the text in depth, concentrating on the description of the main guidelines for the re-design of compartment III (page 1-2).

- user requirements:

Operation requirements are missing. Start-up is mentioned in UR-P-006 but should be clarified. Other operation modes shall be addressed as well, covering e.g. de-clogging of the bed, removal of the collected biomass…..Please add an appropriate requirements category.

To cover the whole range of operation of the comparment III bioreactor, the "Process requirements" category has been split in 3 different parts in which the different operation modes are addressed separately and the requirements of each one of them are described in detail (page 4-7)

UR-S should better be named sterility and axenicity management requirements.

This has been corrected in the document (see page 10)

The requirements described under UR-M should better be spread over the other requirements categories.

This has been corrected, the "Miscellaneous" category has been removed and the requirements described there have been included in the corresponding categories.

- 2.1 process requirements

UR-P-001: rationale of the load value?

Specifying the load lets us fix the capacity that the reactor is required to attain. The maximum capacity of the current design was evaluated and its value was 1.4 kg $N\text{-}NH_4^+ \cdot m^{-3} \cdot d^{-1}$. This value has to be taken into account as a reference and the new design should at least be able to fulfil this value.

Please specify 'reactor' volume and 'liquid' flow rate

An interval with an expected range of flow rate and concentration is provided in requirement UR-P-001 and was based on the values treated with the current reactor. However, for these parameters to be completely fixed, it would be necessary to have data on the ammonium concentration and flow rate within the MELISSA loop. This will depend largely on the final use of urea in the MELISSA loop and the final integration strategy adopted. However, it is considered that the provided range (page 5) should be able to fit most of the expected possibilities.

UR-P-002: shouldn't we consider in addition the possibility to use air instead of pure oxygen? In that case, as necessary gas volume will increase, we should correlate the design to the maximum gas supply and not only the minimum

This requirement refers to the necessity to supply oxygen to the reactor. The oxygen could indeed be supplied either as pure oxygen or as air and this should be defined in the specific design.

UR-P-003: what do you mean? Does it mean that we will have two gas inlets? What is the relevance of the information you provide?

The total gas flow rate entering the reactor remains constant and the ratio between the different gases can be modified to fulfil the requirements of operation.

UR-P-004: we are not sure to understand what you mean? That Na2CO3 should be avoided? What do you suggest then?
UR-P-005: which acid?

We propose that the selection of the most adequate acid and base for pH control is attained upon discussion with the consultants. This issue has been clarified in the TN document (see page 6).

2.2 requirements regarding monitoring and control
Precise which sensors should be retractable.

Those sensors that are more likely to require recalibration during operation of the reactor should be retractable (pH and dissolved oxygen sensors). This information has been included in page 7.

Should we consider TIC/TOC?

TIC/TOC could be considered as an additional analysis of the liquid phase from compartment III. However, we do not foresee an online analysis of this parameter. For this off-line analysis the liquid sampling ports would be required (requirement 26, page 9).

Sampling should be addressed more extensively: what do you want to sample? Which analyzers are you going to use?

A requirement has been included regarding the need for an adequate port where the sampling system used to obtain the sample that will be supplied to the on-line analyzers can be installed. (page 8, requirement 24)

UR-I-008 related to the biomass sensor has to be completely reconsidered and rephrased.

The user requirement has been rephrased in page 8 (user requirement 25) where it has been clarified that the reactor hardware should have a port that is able to host the biomass concentration sensor, and that further details on this sensor will be available in the final ITT document., since this is still under development within CCN7.

Should we consider a level sensor?

The use of a level sensor is necessary and this has now been included in the list as a requirement.

Why don’t you precise a number of units?

One of the backgrounds of experience within the group of consultants involved in this work is on instrumentation and control (i.e. Sherpa Engineering). For this reason, the number of units, criticability aspects, etc. have not been fixed now. Once the corresponding contribution will be done by the consultants in the final preparation of the ITT (i.e., during the meeting on the finalisation of these requirements), they will be added.

Wordings such as ‘when possible’ ‘interesting improvement’ should preferably be avoided: we have to state what we need without any flexibility; if flexibility is possible, then, it has to be clearly stated as well.

In the new version we have avoided the use of these expressions.

To avoid the use of these terms a new section has been added, namely “Adaptability of the new design” (see page 11). In this new section it is stated that some improvements should be addressed. A list of some of the improvements that we regard as interesting is provided in this section and could be open to the consultants for further suggestions.

Other requirements have been rephrased or explained more clearly to avoid the use of these words.

With regards to control issues:

- we have to provide more information on how it works otherwise doors remain open for everything,

In page 9 (user requirement 27) it has been clarified that the control loops described are to be embedded in the general control architecture of the MELISSA Pilot Plant. Indeed, we understand that the control aspects other than level 0, are not part of the ITT.

- please specify some set points for liquid and gas flow rates,

This information has been added (see table 2, page 10)

- ranges may be added

This information has been included in table 2, page 10

- references

It’s not relevant to provide the reference of an oral communication in an ITT document.

This reference has been removed.

A reference covering the overall MELISSA project should be added (e.g. web-site).

Public references to the MELISSA project have been included in the text (see pages 1, 2 and 12)

Additional references from other MELISSA teams working on CIII should be provided (e.g. L. Poughon’s work).

The following reference is included in the document:

Pérez, J.; Poughon, L.; Dussap, C.-G.; Montesinos, J.L. and Gòdia, F. (2005) Dynamics and steady state operation of a nitrifying fixed bed biofilm reactor: mathematical model based description. Process Biochemistry 40: 2359-2369.