Universitat Autònoma de Barcelona

TECHNICAL NOTE 87.2.5

MELiSSA Pilot Plant: Compartment V User's Requirements

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Reference/Réference	MELiSSA Pilot Plant Frame Contract 19445/05/NL/CP
Issue/Edition	0
Revision/Révision	0
Date of issue/Date d'édition	30/09/08
Status/Statut	Final

APPROVAL

Title *Titre*	**MELiSSA Pilot Plant: C-V User requirements**				Issue Edition	0	Revision Révision	0

Prepared by *Auteur*	Peiro, E. and Gòdia, F.	Date *Date*	30/09/08
Checked by *Verifié par*	Fossen, A.	Date *Date*	30/09/08
Approved by *Approuvé par*	Gòdia, F.	Date *Date*	30/09/08

Approved by customer *Approuvé par le client*	Lamaze, B.	Date *Date*	02/04/09

CHANGE LOG

Issue/*Edition*	Revision/*Révision*	Status/*Statut*	Date/*Date*
0	0	Final	30/09/08

Distribution List

Name/*Nom*	Company/*Société*	Quantity/*Quantité*
Brigitte LAMAZE	ESA	2 hardcopies + electronic version

TABLE OF CONTENT

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

MPP: MELiSSA Pilot Plant

UAB: Universitat Autònoma de Barcelona

ITT: Invitation to Tender

SPF: Specific Pathogen free

DPTE: transfer system for animal isolators

RQ: Respiratory Quotient

WBC: White Blood Cell count

RBC: Red blood Cell count

FELASA: Federation of European laboratory Animal Science Associations

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).

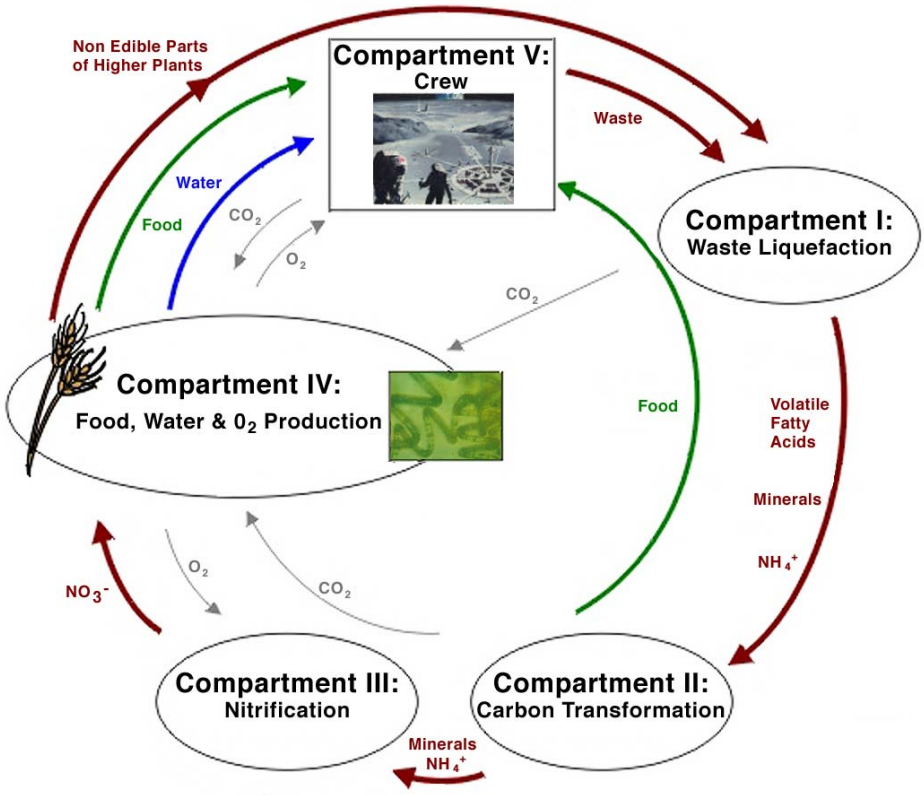

Figure 1: MELiSSA Advanced Loop Concept

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 its final scale, in continuous mode, fully connected, under the control system, for a long operation mode. To achieve it, a 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 and 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.

The aim of connection of compartment V with the rest of the loop is to demonstrate the closure of the gas loop and of the water loop. 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 donors, 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 with 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.

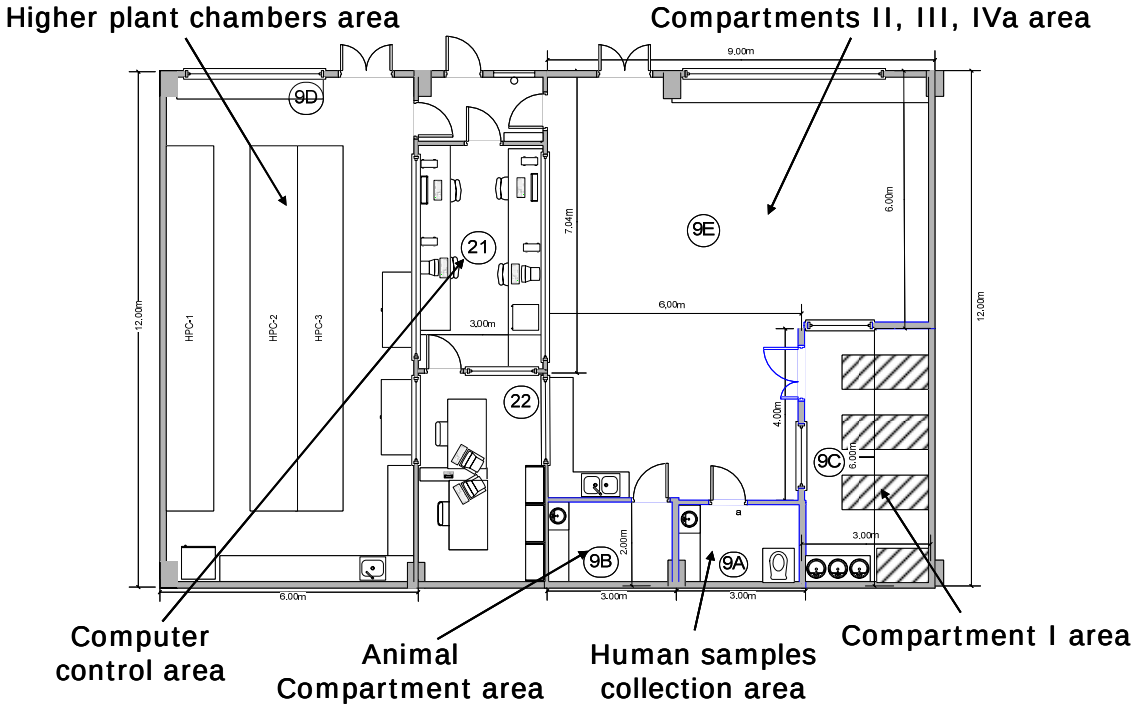

Figure 2. Basic layout of the MELiSSA Pilot Plant laboratory.

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), here attached as Annex 1, 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.

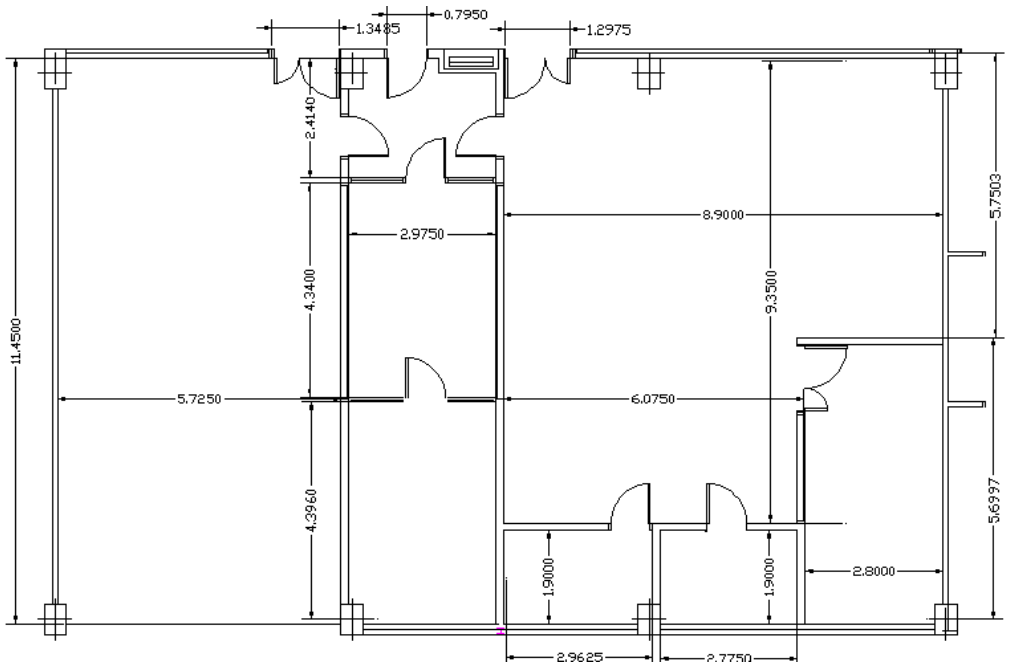

Figure 3: Sizes of the different areas in the MELiSSA Pilot Plant

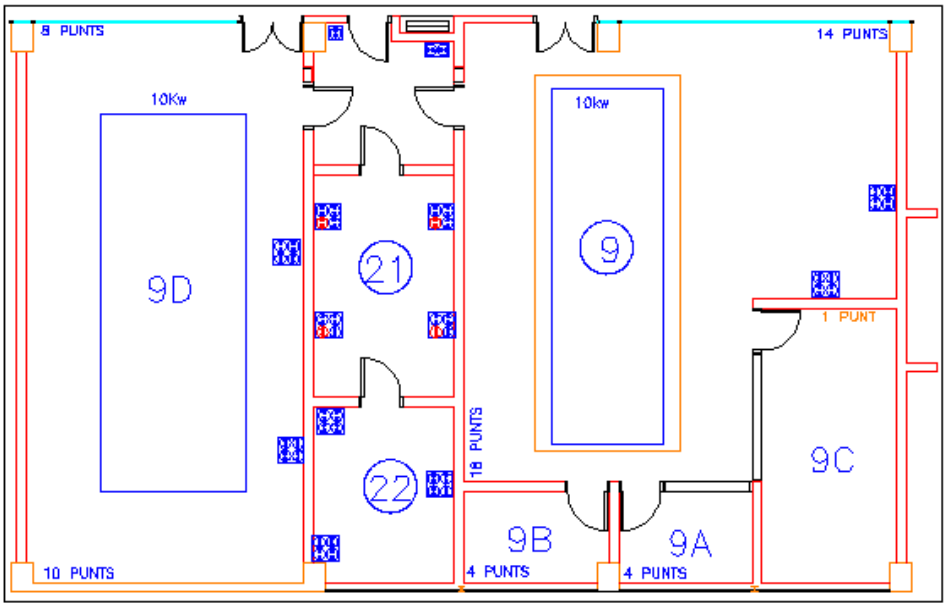

Figure 4: Distribution of the power lines in the MELiSSA Pilot Plant.

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, underlining the features impacting the engineering design of the MELiSSA Pilot Plant.

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).

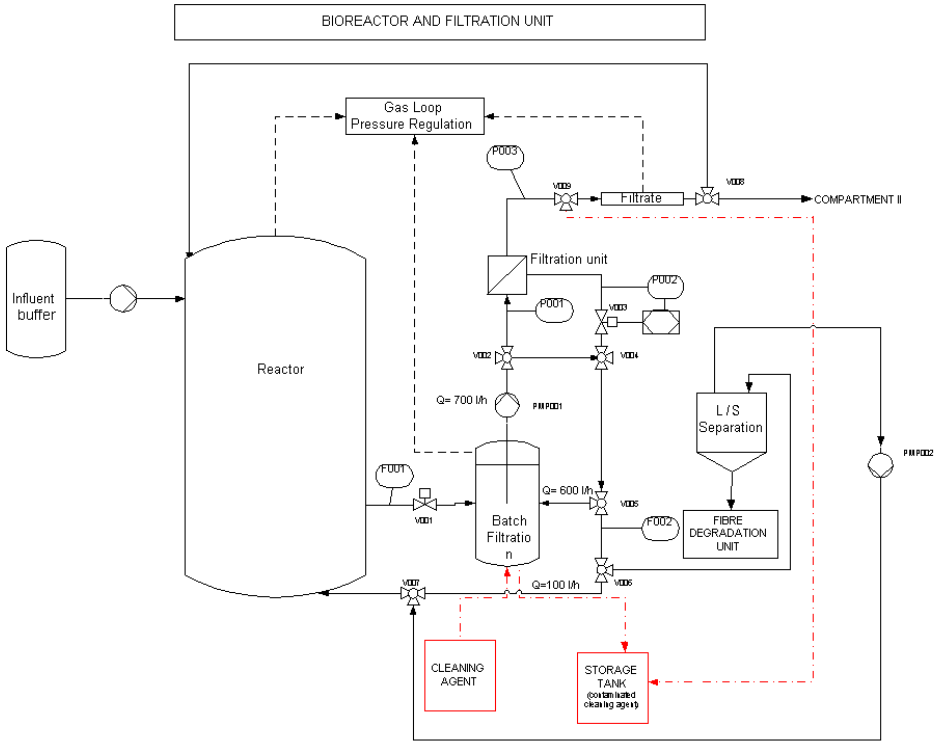

Figure 5: Schematic design of compartment I and its filtration unit.

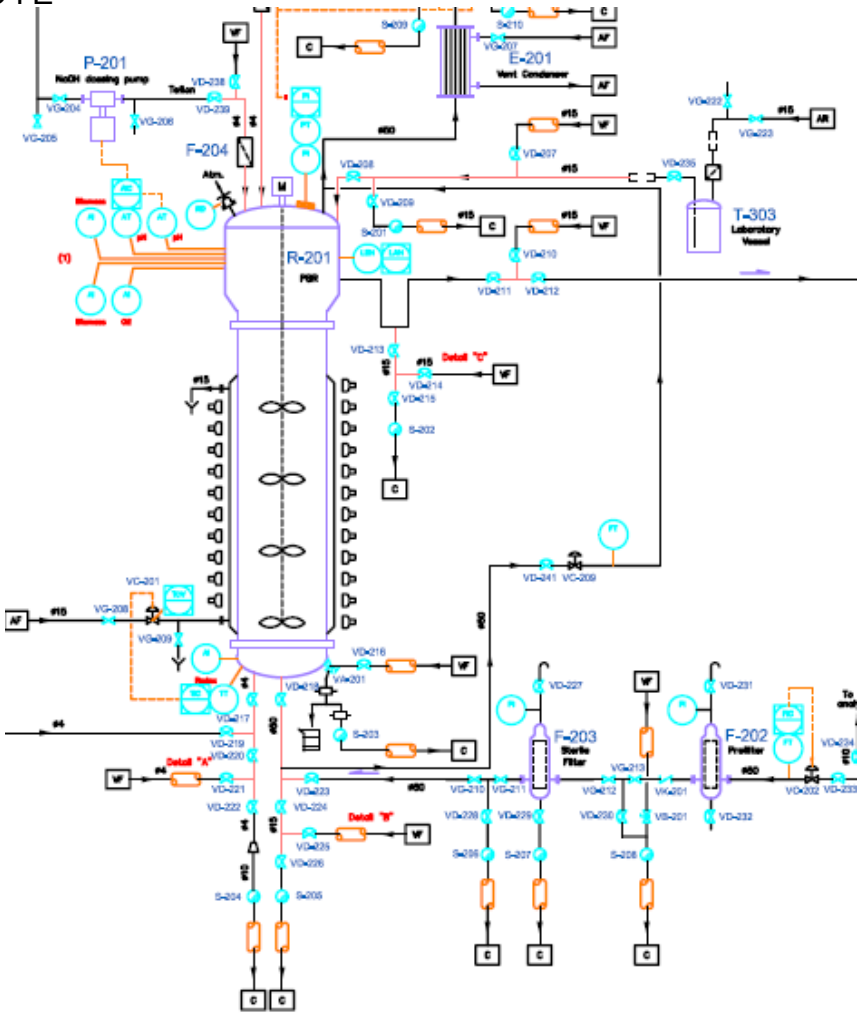

Figure 6: Configuration scheme of compartment C-II.

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 will probably be upgraded. Nevertheless the configuration of the existing equipment, (see Figure 7 for a schematic overview and associated picture), can be used as a reference to evaluate the equipment that will be part of the upgraded compartment in the Pilot Plant.

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

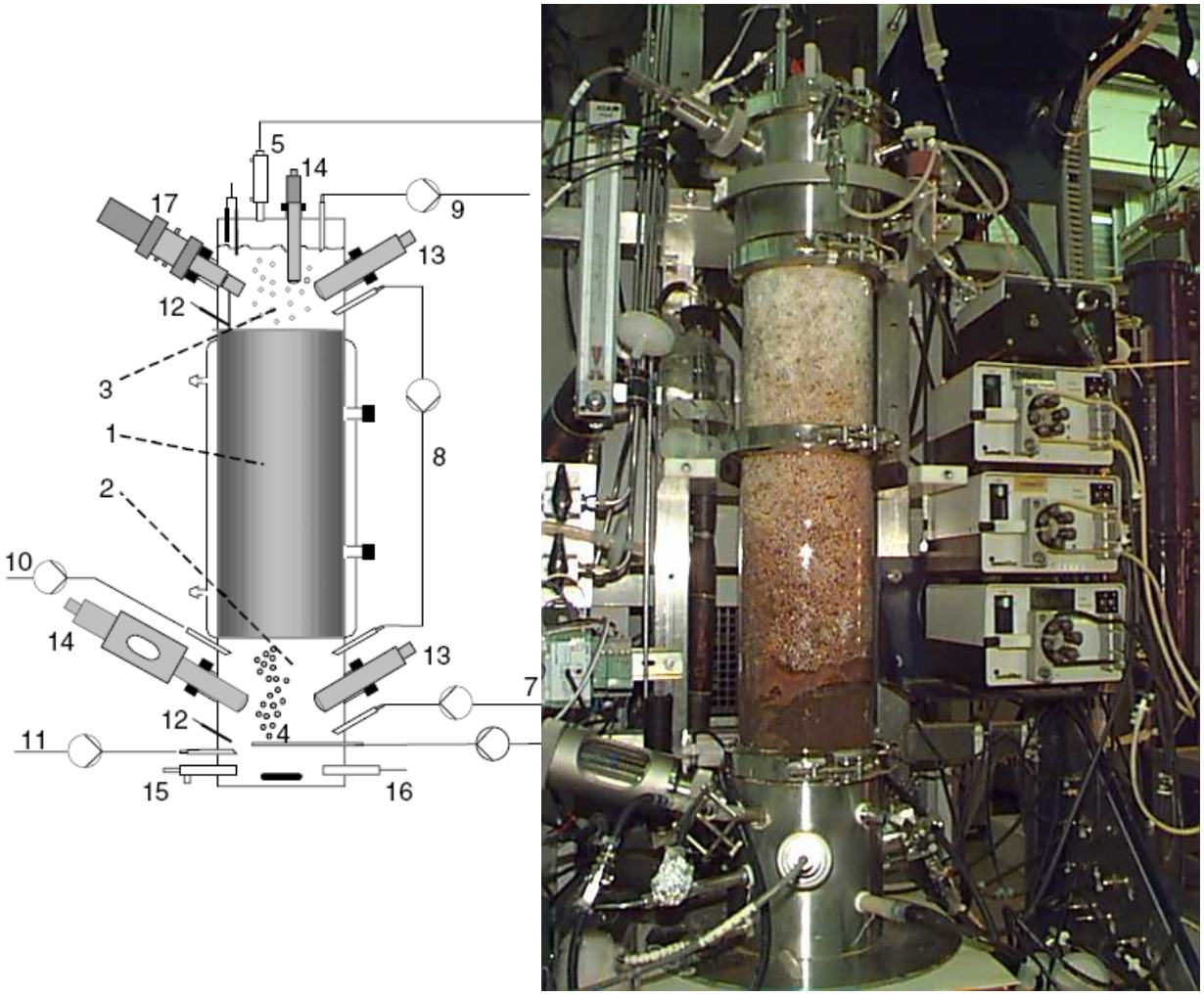

Figure 7: : Schematic overview of compartment III.

General schematic of the nitrifying bioreactors for bench (right) and pilot (left) scales. (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.

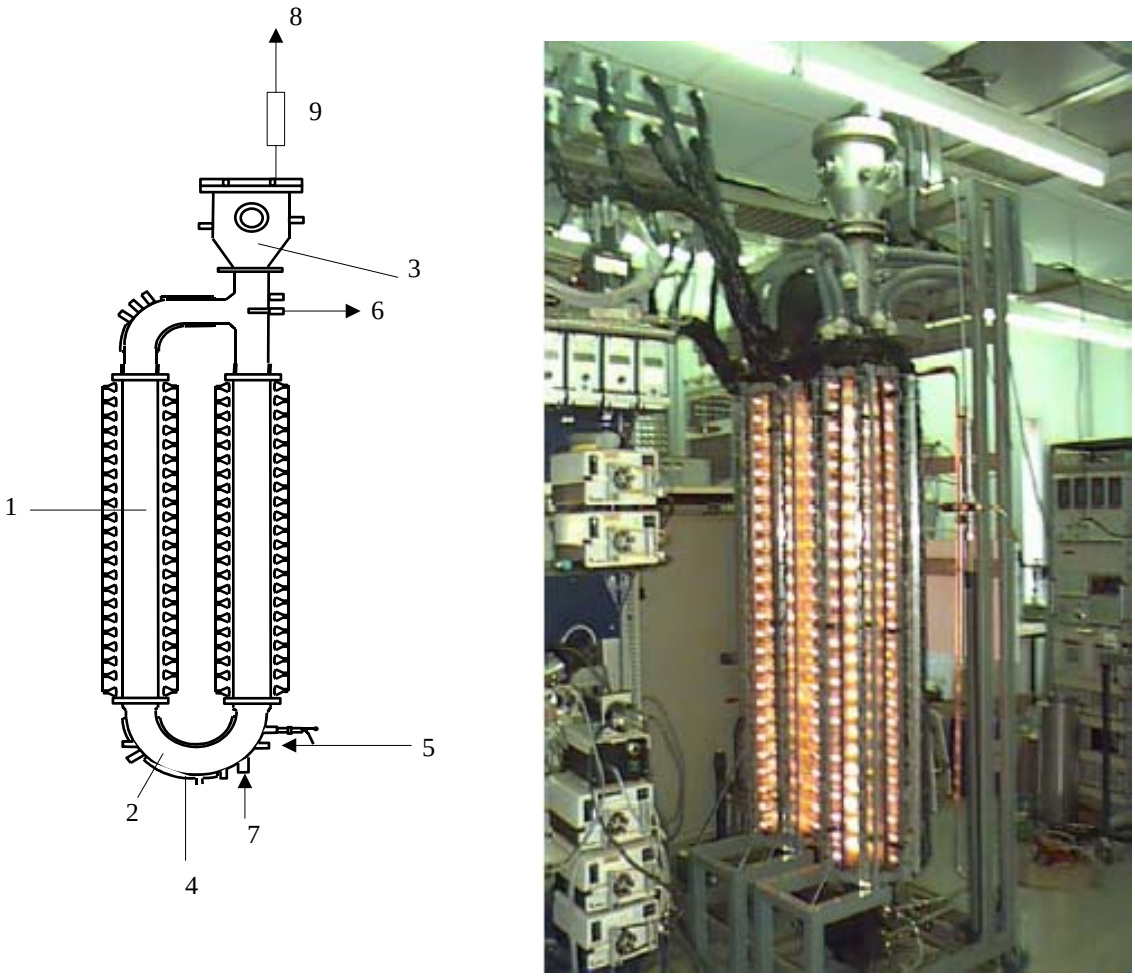

Figure 8: Schematic view of compartment IVa.

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).

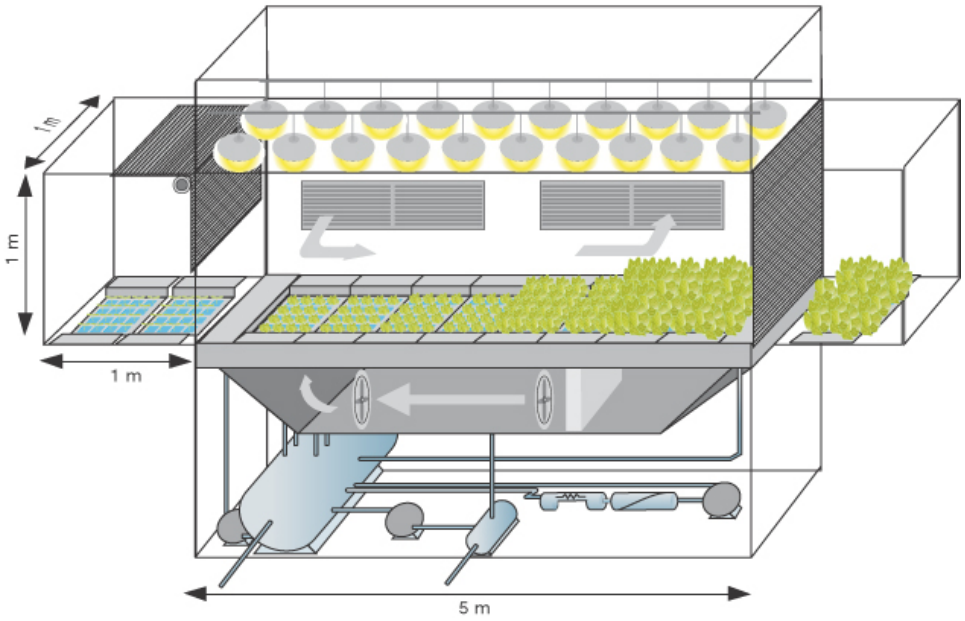

Figure 9: schematic view of the design concept for the Higher Plant chamber.

2.4.6.Compartment V

The animal compartment will be installed in room 9B. This compartment is currently under design, and in fact this TN is focused on this topic. 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 removal of breathed air.
- Feed supply to animals
- Faeces and urine removal from animals

The MELISSA Pilot Plant, is aimed at demonstrating on ground the MELISSA loop according to a specific demonstration scenario. The scope of the animal compartment in the MELiSSA Pilot Plant is to be used as a crew mock-up in that ground demonstration phase. In particular, the animal compartment will be involved in the demonstration of the following aspects of the MELISSA loop:

- closure of the gas loop;
- partial closure of the liquid loop.

Especially the following functions shall be covered:

- production of air
- recycling of carbon dioxide
- production of potable water,

with the main focus on the two first ones.

3. Preliminary aspects for the design of Compartment V in the MELiSSA Pilot Plant.

The design, construction and implementation of Compartment V hardware in the MELiSSA Pilot Plant involve a number of steps:

1. Definition of the user requirements for Compartment V.
2. Selection of a company to develop the detailed design and construction of the corresponding hardware, through the corresponding ITT.
3. Delivery and installation of the hardware in the MELiSSA Pilot Plant.
4. Functional characterization of the equipment and final acceptance.
5. Operation of Compartment V as an independent compartment, to test its operation, control, stability, etc.
6. Connection of Compartment V to the MELiSSA loop, according to the defined integration strategy

The present document defines the user requirements document for Compartment V hardware of the MELiSSA Pilot Plant, as the step 1 of the previous list , being the basis for the step 2.

3.1. Animal model selection

The documents "TN 75.5. MELiSSA Pilot Plant: Animal Compartment design Preliminary Requirements for the animal selection" and "Animal Model for MELiSSA Pilot Plant" (MPP-TN-08-5001) have covered the definition of the animal model taking into consideration first all the relevant conditions for this selection and then the study of several animal models. Were taken into consideration the constraints regarding mainly space availability and respiratory parameters, in concrete:

- the O_2 consumption for 1 individual
- the O_2 consumption per kg for each species
- the number of animals equivalent to one human being (versus the specific criteria of oxygen consumption)
- The surface of the cages or pens needed according to the recommendations of the ETS 123 Council of Europe Convention for the protection of animals used in research.

It was concluded that the laboratory rat would be the most suitable animal model to achieve the objectives of the study. Despite its small size, and that the value of O_2 intake / kg for this species is far different from humans, it is possible to house a sufficient number of animals that will mimic the intake of a human in one isolator. The number of animals will also allow statistical treatment of the data obtained. The best biological model to mimic adult spaceman is a cohort of fully grown adult rats, and in order to simulate the same consumption as a human, 40 animals weighting approximately 500 g should be housed.

3.2. Animal population management

Female rats from the most common strain of outbred laboratory rats (Wistar Rats), could fulfil the requirements. Although female rats will show hormonal variations due to their sexual cycle, these variations should not affect the experiment. However, females in this species are smaller than males and this will allow optimization of space.

.

Such cohorts should arrive into the system at 14 weeks old and stay for the test until 30 weeks old. It is necessary to consider that the life span of rats is around 1 year. Only some housing systems with a restricted diet allow rats to live for two years. Rats reach sexual maturity at 9 weeks and in consequence the animals proposed for this experiment will be the equivalent of humans between 20-50 years of age.

After their stay in the experimental unit, the animals will be humanely killed in order to conduct the pathology analysis and a new cohort will take their place immediately. In this way, there will be three sets of animals during the year maintaining a consistent consumption of O_2. Animals from the same cohort could be replaced all at once or in stages. The advantage of renewal in stages is that there will be animals with different weights and ages at the same time which will provide a more stable gas exchange than that provided by animals all in the same age group (i.e. all young or all old). However, it is necessary to consider the risk of mixing new animals with animals that have been living in the facility, in case of any potentially infective disease that could affect one animal. This risk will be very low because the animals will be SPF when supplied. If it is possible that the full production of the MPP will be set up gradually, the number of animals to start the study could be lower at the beginning and more animals could be incorporated later as the production of oxygen from the plant increases. A full health monitoring control should be performed on the animals before being introduced in the MPP animal compartment.

The presence of a long lifespan animal model is therefore replaced by the presence of a cohort of animals regularly renewed and monitored. The rational for this protocol is that the animals will be present in the unit long enough in relation to their life-span to detect any health problems caused by living under the experimental conditions. However, their stay in the unit will not be long enough for animals to show any diseases relating to old age.

In terms of O_2/C O_2 exchange it is necessary to consider that laboratory rodents are nocturnal. This means that their activity period is at night. An adaptation of the plant O_2 production (diurnal) to the highest period of animal O_2 consumption (nocturnal) needs to be considered.

3.3. Housing: Isolators

To keep the animals isolated from the exterior, the use of commercial isolators is recommended. These airtight compartments are built in hard or flexible transparent material. Supplied with two independent engines to regulate the air flow inside the chamber, they can be kept in positive of negative pressure (for the rigid systems). The access to the system, once the animals are isolated inside, is through an airtight door, called a DPTE door. Airtight transfer compartments are available. They allow the caretaker to feed and exchange animals and bedding without any effect on the composition of the ambient air of the isolator.

The chamber will be settled in the room 9B of the MELiSSA Pilot Plant (see the dimensions and access possibilities in Fig.2, page 10): the ground place available for the system is 1,20 m x 2,2 m in a 2m x 3 m room. The door to access the room is 1,000 cm wide.

Regarding capacity, the provider should preferably offer an already commercially available solution. However, some specific characteristics of the project and the limitation of the available space available will probably
require some tailor made components.

3.4. Control group

The Compartment will also include a control group of rats. This group should be housed in exactly the same conditions and breathe the standard air of the laboratory. The number of animals for this control group would in principle be the same as the MPP group to facilitate statistical analysis. Some physiological parameters should be collected to compare the two groups in order to better establish the environment is not casing any ill health. The control group should be housed in a similar isolator to the one used by the test group but not connected to the MELISSA loop. By working with rats of well established strains it will be possible to do a very homogenous distribution of animals between the two groups.

4. Scope of the study: User's Requirements for the Compartment V of the MELiSSA Pilot Plant.

4.1. Requirements for air tight chambers used for long term rat housing

4.1.1.Chambers (Isolators)

1. Environmental conditions: rats need to be housed in controlled environmental conditions. This requires that temperature and humidity are monitored and maintained in the range of those recommended by the European Convention ETS 123 (20-24 ºC and 55%±10% humidity). In addition, the photoperiod recommended is 12:12 light/dark hour period.

2. The project includes the acquisition of two isolator chambers systems, with transfer modules. The rational for the two isolators (or two units) is that one will serve for the experimental group of animals while the other one will serve as a control group of animals. The only difference between these two groups will be that the control group will receive filtered air from the room.

3. These chambers will be used to house gradually between 12 to 40 rats in each chamber, each in a different controlled atmosphere.

4. The global system should be air-tight and include hermetic transfer doors and airlocks for the introduction of food and material and the disposal of wastes.
The hermeticity of the system will guarantee the controlled inner atmosphere and minimise as well the risk for the MPP related to the presence of bacteria from the faeces as well as skin of the animals, even if this is not a risk specific to the use of animals (humans show a similar microbiota). Tests should be performed after the installation of the system to ensure the compartment is hermetic

5. The connection for the air supply with the MPP will require the installation of HEPA filters to avoid any contamination from the plant to the animals and vice versa.

6. The system should include blowers to maintain a permanent HEPA filtered air flow. As the purpose of the study is the quality of the pulsed air, the system must allow to modify it in order to be able to control the pulsed air and to take it into a buffer compartment.

7. The extracted air shall be analysed and the extracting engine should allow the connection to HEPA filters to prevent contamination of the pilot plant. Tests should be performed after the installation of the system to ensure the efficacy of the HEPA filters.

8. The system should be able to accept the introduction of monitoring systems to analyse the air composition or other environmental parameters. The following aspects should be considered:

- Concentration of main metabolic gases (O_2, CO_2).
- Concentration of trace gas contaminants (CH4, NH3, VOCs, etc.)

- Humidity levels
- Gas flow
- Cage ambient temperature
- Illumination photoperiod (awake and sleeping periods)
- Animal overall surveillance (video camera…)

9. The rats will be housed in cages complying with the guidelines on the annexe A of the European Convention ETS 123. For this, at least 20 cages should be introduced in the chamber.

10. The surface should also include a place for handling the cages and the animals for simple examinations, feeding and watering. A small scale should be included to weight the animals regularly.

11. The chambers will be settled in the room 9B of the MELiSSA Pilot Plant: the ground place available for the system is 1,20 m x 2,2 m in a 2m x 3 m room and the door to access the room is 1,000 cm wide. The system should allow the handling of a trolley to carry the transfer module.

Taking into account these constraints, a possible solution is the installation of two identical chambers (one for the control groups an another for the MPP group) positioned one on top of the other. A platform will be required to access the top one.

4.1.2.Cages

Cages to maintain rats are commercially available and there are several options in terms of the materials they are made of. These cages should include the following details per isolator:

12. Number of cages: 20 cages for rats with about 900 cm² ground surface and 18 cm high o similar. The recommendations of the European Convention ETS 123 regarding the space requirements for laboratory animal advice group-housing for rodents. They should be in groups of at least two or three compatible animals in a 900 cm² cage including vegetal bedding and shelters, as the minimal ground surface requirement for a 500 g rat is 450 cm². That gives minimal dimensions of the cages at about L: 480 cm l: 290 cm.

13. If this dimension can not totally be respected, a special authorization will have to be asked in the application for the ethical agreement to run the study with rats in a lower space. In that case, a minimal surface requirement of 300 cm² per rat could be accepted

but a enrichment environment program will have to be added to the project. If absolutely necessary, this adaptation to less space will mean to be able to keep same cages with 3 rats per cage or alternatively smaller cages with 2 rats per cage.

14. They must be made of transparent material, resistant to extreme pH disinfectant exposure and resistant to high temperature sterilisation in autoclave.

15. They should contain a stainless steel adapted cover to close the cages and hold the feed and the watering bottles.

17. They should provide watering bottles resistant to extreme pH disinfectant exposure and to high temperature sterilisation in autoclave, and with stainless steel tops adapted for rats. Alternately, a filtered water line should be provided.

18. They should contain stainless steel label supports

19. Spare cages: considering that there will be two isolators and that all the materials need to be sterilized before used, at least 20 extra cages and all their components will be provided to allow the necessary changes to clean the cages once a week.

4.2. requirements regarding Identification

20. Each rat should be individually identified. The method could be an ink tattoo on the foot or the injection of an electronic micro ship. The second system offers the chance to establish a completely computerized data management system. In addition, each cage should display the identification of the rats housed.

4.3. requirements regarding Feeding

21. The food will be a commercial pellet specific for SPF adult rats administered *ad libitum*. These pellets are designed to limit the fattening effect observed with normal foods.

22. The two best options to maintain a similar RQ to humans and also, as mentioned, to limit the risk of obesity and other pathologies due to an excess of proteins are:

Diet 1: Proteins 17. 3% - Glucides 53 % - Fat: 3.15 %
Diet 2 (expanded pellet) : Proteins 14.5 % - Glucides 61.5 % - Fat : 2.6%

The normal average food intake is 25g/rat/day. It´s considered that Diet 2 will best mimic space travel conditions and will also better preserve animal health.

4.4. requirements regarding Watering

23. Filtered clear water will be provided in commercial feeders adapted to the cages or through a general pipeline. In the former case, the caretakers will regularly fill the bottles and pay attention to the quality of the water. Each rat will drink about 70 ml/day.

4.5. requirements regarding Waste disposal

24. In cages with two or three animals, the bedding should be changed once a week to avoid the concentration of ammonium in the ambient air of the chamber. Clean cages, filled with sterile bedding will be introduced in the isolator through the compartment using the DPTE door. The dirty cages will be taken away by the same procedure.

4.6. requirements regarding biological parameters

The purpose of the study is to demonstrate that it is possible to maintain life in good health conditions. For this reason it will be necessary to evaluate several physiological parameters:

25. Weighing: the rats will be weighted on arrival. They will be randomized into two groups with a homogenous distribution by body weight. Then, during all the protocol, a weekly weighing will be organized.

26. Food intake: The food intake will be measured twice a week for each cage, not individually by weighing the feeder to calculate the amount of remaining pellets.

27. Blood samples: blood samplings are scheduled for every cohort at T0, T+8weeks and T+16weeks. For every sample, a blood count of red and white cells is performed with a special attention to WBC, RBC and Hb.

28. Activity recording: a system for the monitoring of activity can be established to compare the resting and activity periods between the two groups.

29. Health monitoring: To assess that there are not any undesired biological contamination.

4.7. requirements regarding Health monitoring and sanitary status

30. The rats should be Specific Pathogen Free animals, purchased from a good breeder and a health report should be provided on delivery. A complete health monitoring should be performed by the provider before sending the animals to guaranty the SPF health status on arrival.

31. In addition, health monitoring tests should be organized at the reception of each group of animals and at the end of their stay, and additional specific test will be carried out if the animals show any symptom of disease. A basic serology and microbiology testing will be necessary. It´s recommended to follow FELASA guidelines for rodents in experimental units. If considered necessary, it is possible to determine by specific molecular techniques (FISH or PCR) the presence of those microorganisms that could be considered a hazard to the MMP.

32. An emergency procedure should be defined to be able to prevent any lethal adverse effects of the MELISSA loop on the animals' health.

33. The system must include a security system in case of accidental stopping of the engines. Additional alarm systems will be necessary to detect failures affecting critical environmental parameters.

34. The air composition in the cage should not differ from that of humans. The correlation O_2/CO_2 will be the same. However, it is necessary to consider that there will be a significant concentration of NH_3, and a low concentration of methane, as a result of the presence of animal urine and faeces respectively.

Therefore, NH3 and CH4 must be kept as low as possible to preserve animal health achieved through the appropriate frequency of change of animal bedding. In addition, rats like other mammals secrete volatile compounds, such as pheromones, used for social communication, that have to be taken in consideration as well.

35. Safety mode: this mode should be automatically engaged any time that the animals' survival is in danger. For example power failure or malfunctioning of the equipment, exhaustion of supplies… This mode would set default conditions (for example allowing an input of external air, …) assuring animal survival until operators can take appropriated action.

4.8. Legal requirements and training

36. According to current local legislation, animals for experimental purposes can only be housed and used in registered centres. The Universitat Autonoma de Barcelona is already registered as a user centre for laboratory animals. However, it will be necessary to register the laboratory where the animals will be housed as a satellite of the main UAB facility. It will be necessary to establish the supervision schedule by an animal welfare officer and to have the services from a veterinarian to supervise the health conditions of the animals. This service might be provided by the UAB animal facility (Servei d'Estabulari).

37. The legal regulations in terms of animal experimentation should be taken into account as well. Any experiment with animals will have to be previously approved by an external commission. From the legal point of view, animal experimentation has to be in agreement with the local[1], national[2] and European[3] laws on animal experimentation. The experiment protocol has to be first presented to at approved by the local human and animal experimentation committee (CEEAH[4]). This request has to include a description of the experimental procedures, personnel involved with proper training and facilities to be used. The committee shall, in turn, issue the corresponding report to the Regional Ministery (DARP[5]) for proper authorization of the proposed tests.

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1 Generalitat de Catalunya Decret 214/1997 (DOGC 7-8-97), DOCG num. 2450 7 Aug. and Catalan Law 5/1995, (DOGC 10-7-95).

2 BOE RD. 223/1988 14 March, and O.M. Oct. 1990.

3 EU council ETS 123 and European Directive 86/609/CEE, (DOCE 18-12-86).

4 Comissió d'Etica en Experimentació Animal i Humana de la UAB

5 Departament d'Agricultura Ramaderia i Pesca de la Generalitat de Catalunya

4.9. requirements regarding personnel

38. The following tasks will required these approximate times:

- Daily supervision: 1h/day
- Refilling water bottles, food etc: 1 h /twice a week
- Cage changing, weighting of the animals, of the food etc. once a week: 8 h/week
- Other tasks (for example, sterilizing, preparing material to and from the UAB animal facility, etc): 6 h/week

Total estimated time per week: 21 hours.

39. At least one trained technician accredited to work with experimental animals will be required to maintain the animals This person should hold a FELASA category B course qualification. This course is provided in several institutions in Spain and one of them - RCC CIDA - is based near to UAB.

40. A specific training of the personnel in the daily task operating the isolators will be provided by the chamber supplier.

4.10. requirements regarding long-term operation

41. The equipment provider(s) should facilitate details about the usual delivery time for pieces and other reparations.

42. In all the developments above described it should be taken into account that the compartment will operate during long periods of time, and contamination is critical to be limited. Long-term operation will also take into account the needs for adequate cleaning and disinfection of the chamber and the transfer module, and the design considerations regarding ergonomics.

43. The use of disinfecting or cleaning agents will be discussed and limited as much as possible in order not to transfer undesirable substances into the MELiSSA loop.

5. REFERENCES

Lasseur, Ch.; Verstraete, W.; Gros, J. B.; Dubertret, G. ; Rogalla, F. MELISSA: A potential experiment for a precursor mission to the Moon Advances in Space Research, 18:11, 111-117. 1996

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Tranquille, N. ; Emeis, J. J. ; de Chambure, D. ; Binot, R. ; Tamponnet C. *Spirulina* acceptability trials in rats. A study for the "Melissa" life-support system Advances in Space Research, 14:11, 167-170, 1994.

Albiol, J.; Gòdia, F. TN 75.5. MELiSSA Pilot Plant: Animal Compartment design Preliminary Requirements for the animal selection

Vergara, P; Vidal, S. MPP-TN-08-5001. Animal Model for MELiSSA Pilot Plant

6. ANNEXES

Annex 1: MELiSSA Pilot Plant: General Resources, Interfaces and En vironment. MPP-TN-08-0001.

7. COMMENTS

MELiSSA Pilot Plant: Compartment V User's Requirements

Comments

General comments

Reference document: to be updated
Updated MELiSSA Pilot Plant Frame Contract 19445/05/NL/CP

Detailed comments

Page/paragraph	Comment
19/Section 2.4.6, second paragraph	We propose to eliminate this requirement (*Liquid cooling for humidity removal of breathed air*), it has not been taken into account in the isolators, because apparently is not needed; maybe later in the integration? It was decide to maintain the Req. as it was written, maybe not for the isolator in its current design but for the future could still be valid.
20/Section 3.1, first paragraph	Is this reference (MPP-TN-08-5001)a MPP internal one? TN might be confusing "MPP-TN" is one of the standard types of doc. already approved in our MPP-QAP-0001 SOP for documents and Records control
20/Section 3.1, first paragraph	(… *the conclusions of the study*) Of which study? Can you rephrase, please? O.K., text rephrased: "the study of several animal models"
21/Section 3.2, third paragraph	(… *the risk of mixing new animals with animals*) Please precise the type of risk Precised in the text: "in case of any potentially infective disease that could affect one animal".
23/4.1.1, bullet 4	Modified to be coherent with the final manufacturing: airlocks in stead of transport modules

23/4.1.1, bullet 7	(*The extracted air will also be studied …*) If it is a requirement, then we should write: the extracted air shall be studied (maybe analysed is a better wording) Text rephrased: "The extracted air shall be analysed …"
23/4.1.1, bullet 7	The requirements are then that, first, the compartment should be airtight, and secondly that any extracted air should be HEPA-filtered; performance of tests is requested but this is not a user requirement Agree; request of tests included after requirements 4 and 7 (tightness and HEPA filtration respectively)
24/Section 4.1.2, bullet 13	(*If this dimension can absolutely not be respected…*) Wording issue? Do you mean "not totally"? Yes, amended: "*If this dimension can not totally be respected …*"
25/Section 4.1.2, bullet 15	(*… a stainless steel adapted top*) What for? Better say "cover" and complete the use: they are used to avoid the animals leaving the cage and to hold the feed and the bottles of water: it´s the common kind of cover for cages. Text rephrased: *"…a stainless steel adapted cover to close the cages and hold the feed and the watering bottles"*
25/Section 4.1.2, bullet 19	Extra cages: Any requirement on storing conditions? Not really, as they need to be sterilised before use.
27/Section 4.7, bullet 33	(*Additional alarm systems will be necessary to control environmental parameters*) I think there is a wording issue: alarms are not a mean to control environmental parameters; please rephrase Agree. Rephrased: "Additional alarm systems will be necessary to detect failures affecting critical environmental parameters".
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.