

# MELiSSA



TECHNICAL NOTE



Departament d'Enginyeria Química  
Escola Tècnica Superior d'Enginyeries  
Universitat Autònoma de Barcelona

## *TECHNICAL NOTE 75.5*

### **MELiSSA Pilot Plant: Animal Compartment design Preliminary Requirements for the animal selection**

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# MELiSSA



## TECHNICAL NOTE

### 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

UPS: Uninterruptable Power Supply

PPFD: Photosynthetic Photon Flux Density

CEEAH: Comissió d'Ètica en Experimentació Animal i Humana de la UAB

DARP: Departament d'Agricultura Ramaderia i Pesca de la Generalitat de Catalunya

## **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.

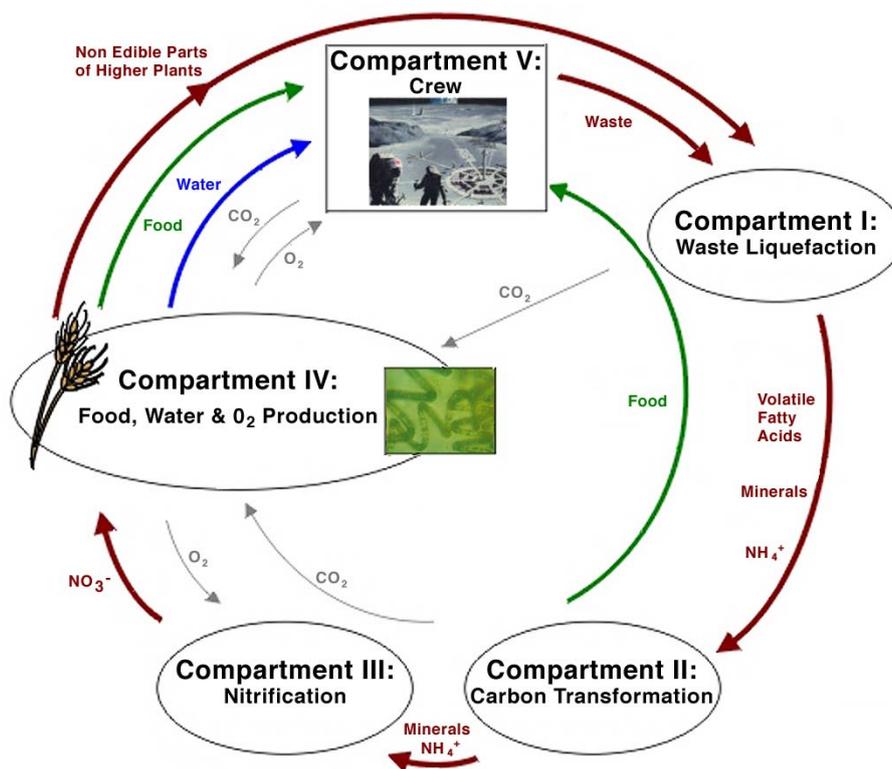
### **1.2. The MELiSSA concept**

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<sub>2</sub> 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  $\text{CO}_2$ , by the photoheterotrophic growth of the bacteria *Rhodospirillum Rubrum*.
- Compartment III is responsible for the bioconversion of the nitrogen source, i.e. from ammonium  $\text{NH}_4^+$ , as produced in CI, into nitrate  $\text{NO}_3^-$ . 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:
  - o Compartment IVa is devoted to the culture of the photoautotrophic cyanobacteria *Arthrospira platensis* (a.k.a. *Spirulina platensis*), and is used mainly for the production of oxygen from CO<sub>2</sub>,
  - o 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.
  - o These compartments are the closing steps for the loop, since they provide with the functions of atmospheric regeneration (converting the CO<sub>2</sub> generated by the crew and other bacterial compartments into O<sub>2</sub>) 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 Autònoma 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<sup>2</sup> 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 integration strategy** within the MELiSSA Pilot Plant will follow a **step-wise approach**:

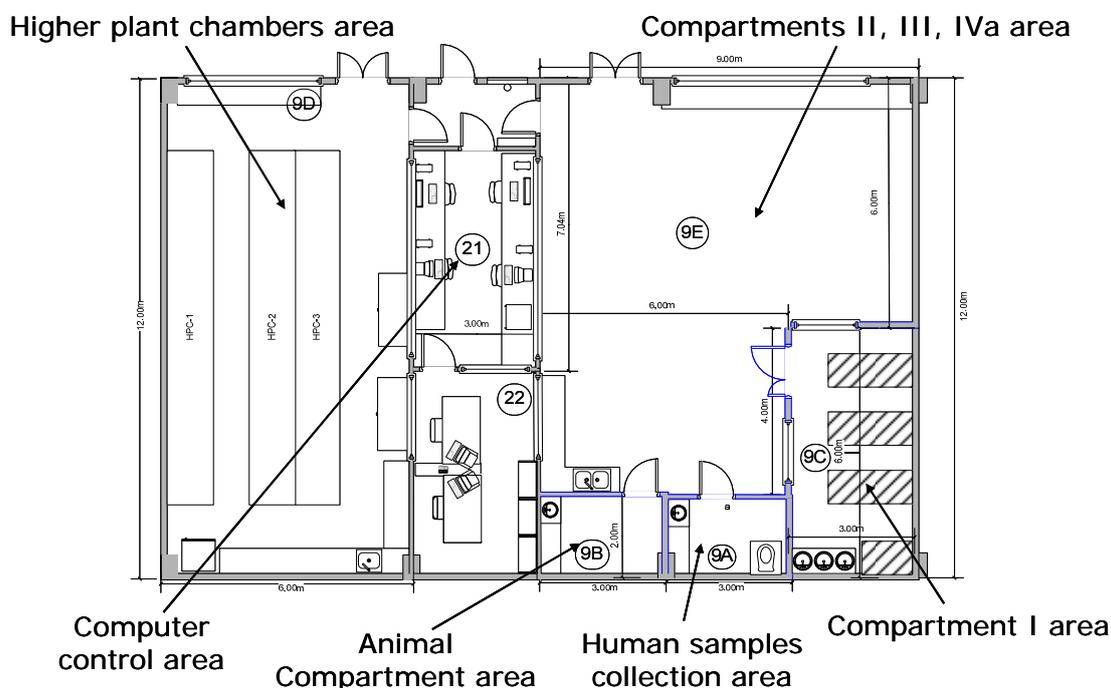
- The first steps will focus on the continuous operation of the pilot scale compartments individually. These steps will be the opportunity of additional characterization and validation activities that cannot be performed at laboratory scale, due to the level of instrumentation or the size of the hardware. 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:
  - o Prevention of any contamination of the compartments working under axenic conditions (i.e. pure mono- or multi- bacterial culture),
  - o Low range of flows to be carried from one compartment to another,
  - o flexibility of the design, to follow the evolution of the integration requirements and specifications
  - o operator safety and high quality control.

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 eases 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. Also, the animals will not be fed with the biomass produced within the MPP, but with the corresponding specific feed. 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.

### **2.3. Detailed description**

The MELiSSA Pilot Plant is divided into different rooms, as described hereafter on 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.



**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

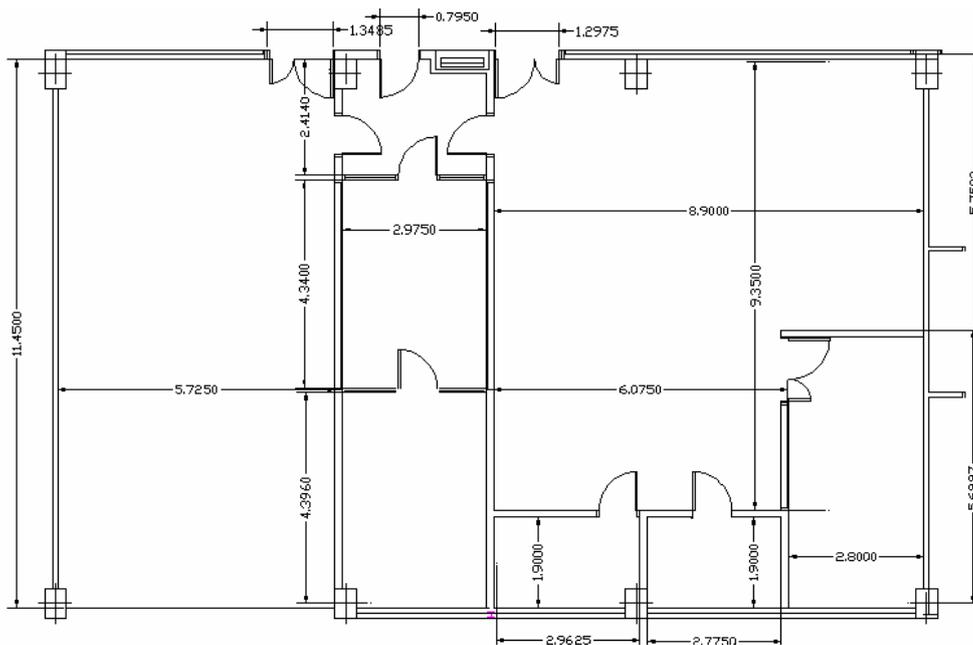
**Table 1. Basic description regarding the distribution of the MELiSSA Pilot Plant**

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**

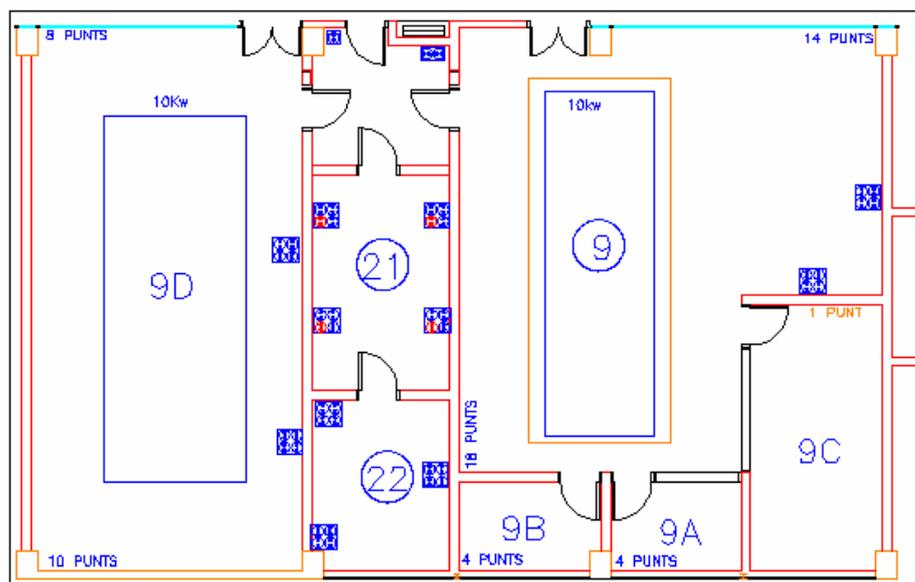


Figure 4: Distribution of the power lines in the MELiSSA Pilot Plant.

## 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<sub>2</sub> lines for gas chromatography,
- Air cooling/venting system,
- Liquid cooling supply system,
- Steam line,
- Compressed air (use of pneumatic devices).

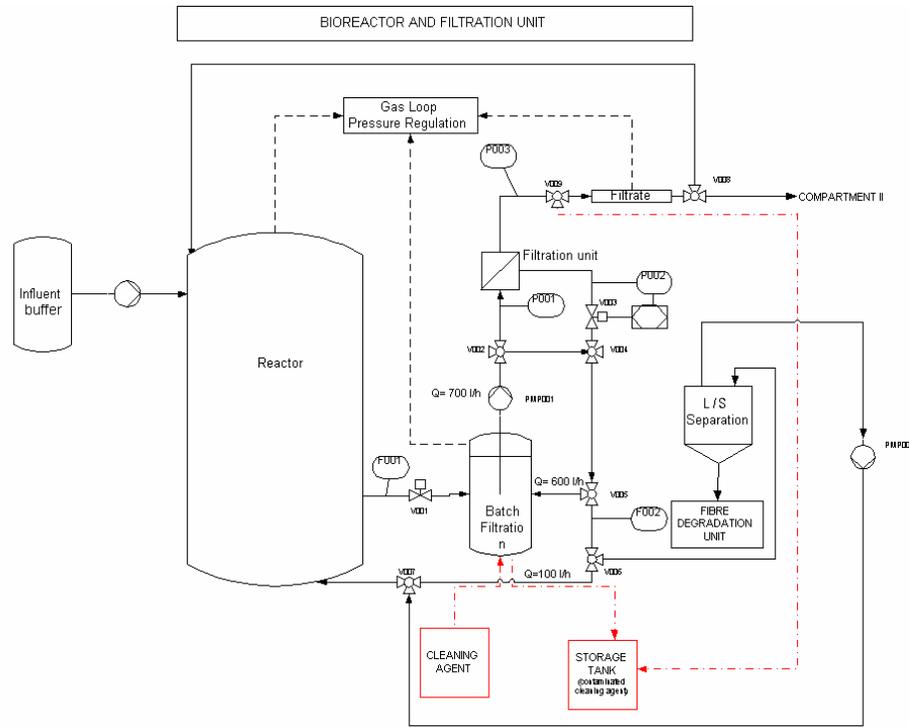


Figure 5: Schematic design of compartment I and its filtration unit.

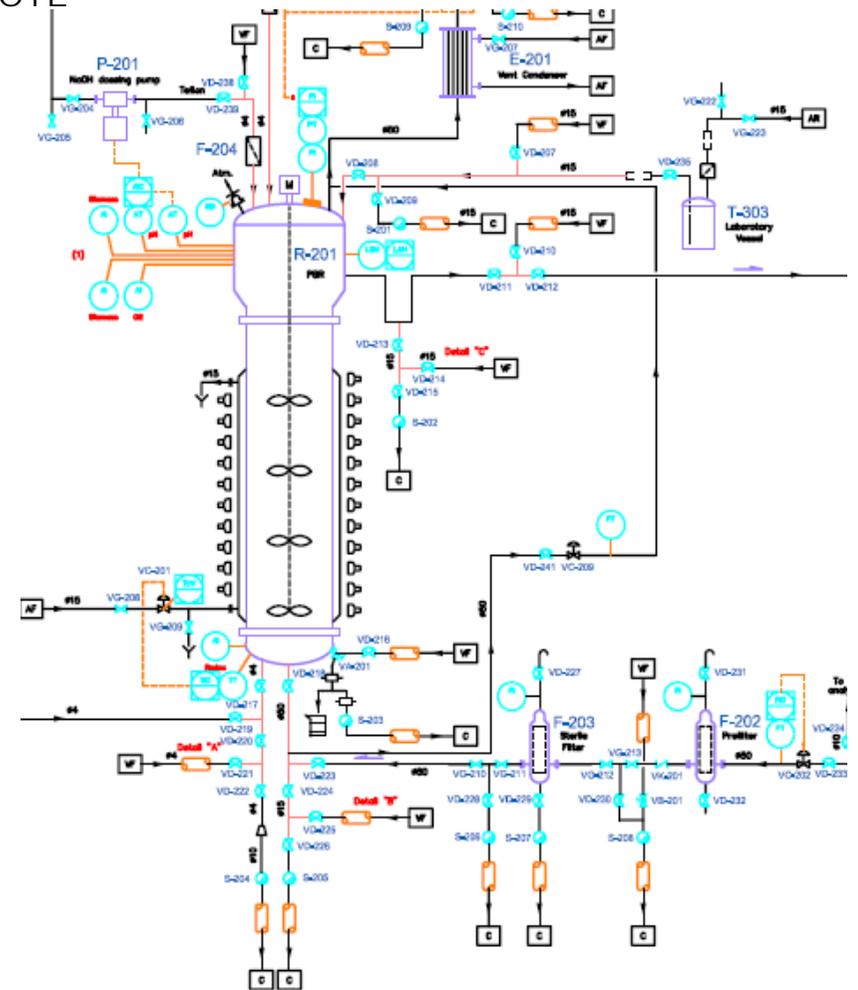


Figure 6: Configuration scheme of compartment C-II.

### 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:

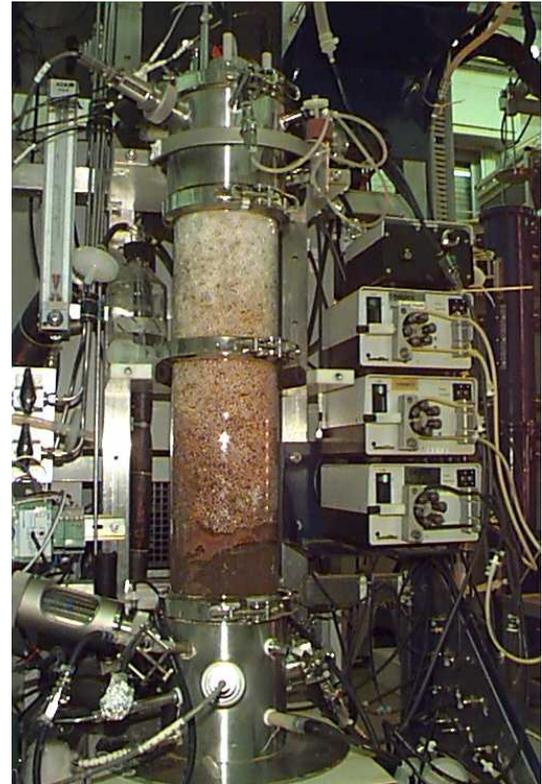
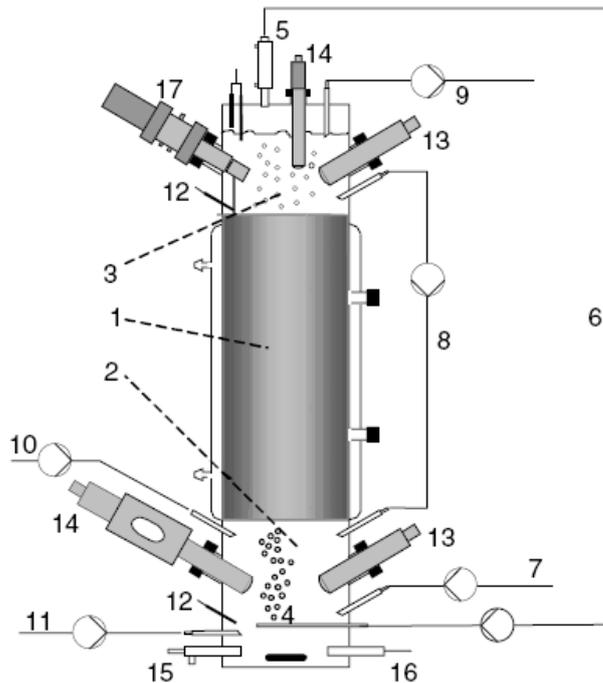
- Demineralized water.
- Tap water
- Gas lines for independent operation O<sub>2</sub>, CO<sub>2</sub>, N<sub>2</sub>.
- Compressed air as base for mixing with other gasses for independent operation and also in case of using pneumatic devices
- Liquid cooling line for output gas lines condensation.
- 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.

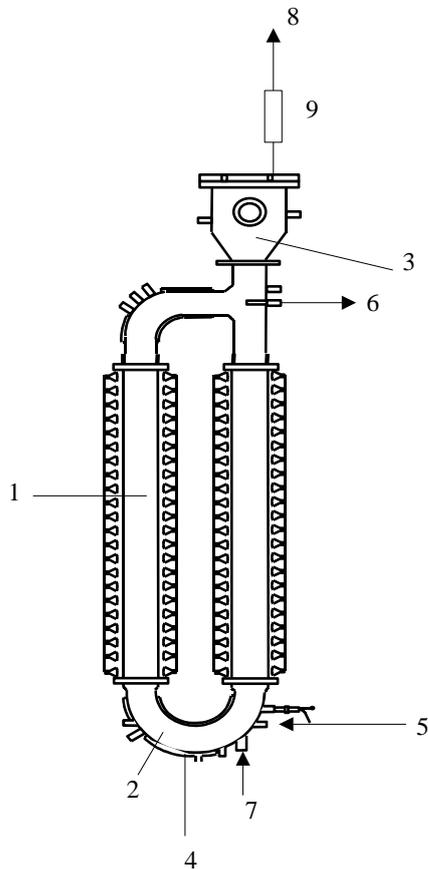
Compartment IVa will require the following services:

- Demineralized water.
- Tap water
- Gas lines for independent operation O<sub>2</sub>, CO<sub>2</sub>, N<sub>2</sub>.
- 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 pilot scale bioreactor (left) and picture (right). (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.**

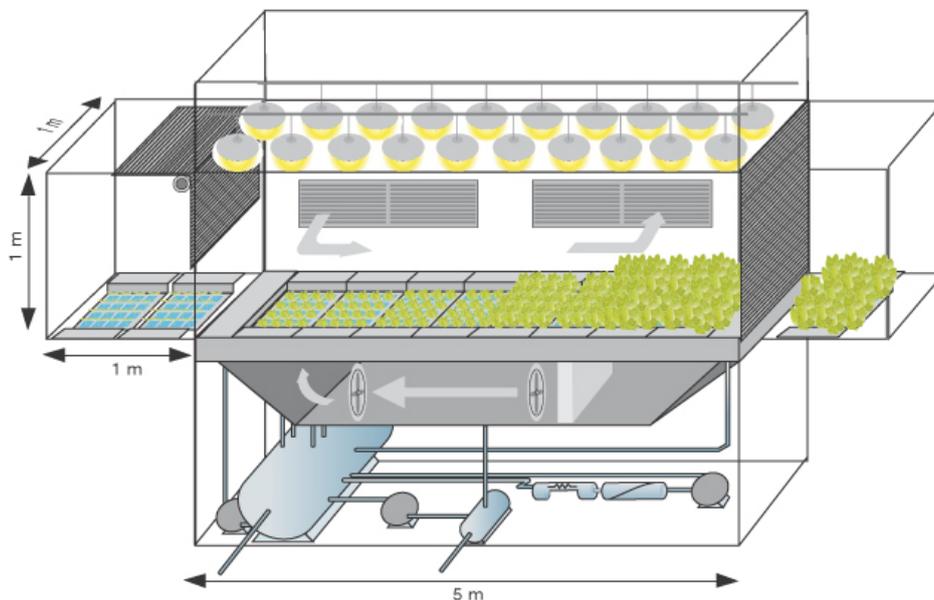
**General scheme of the 77 litres photobioreactor designed for the culture of Spirulina cells. 1, transparent cylindrical parts (illuminated section) : riser (right column and downcomer (left column), 2, stainless steel connection parts, 3, gas-liquid separator, 4, external cooling jackets, 5, liquid 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<sub>2</sub>, CO<sub>2</sub>, N<sub>2</sub>.
- 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<sub>2</sub>, CO<sub>2</sub>, N<sub>2</sub>.
- 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.
- Feed supply to animals
- Faeces and urine removal from animals

## 3. Scope of the study: preliminary requirements for the animal selection in Compartment V of the MELiSSA Pilot Plant.

The design, construction and implementation of Compartment V hardware in the MELiSSA Pilot Plant will require 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 scope of the present study is to develop the user requirements document for Compartment V hardware of the MELiSSA Pilot Plant. This target will be approached in several steps, and will involve on one side UAB and ESA, as responsible for the MELiSSA Pilot Plant, and on the other side a number of experts who will contribute with their specific knowledge on animal experimentation.

As a first step in this process, the current document provides information on the MELiSSA Pilot Plant requirements, and proposes a number of aspects that will need to be addressed specifically by the experts associated to this work as consultants. This document, entitled “Preliminary requirements for the animal selection” will then be used as a basis for the work of the experts, in order to end up with the final selection of the animals to be used and the associated hardware and operation requirements. To do this work, it is envisaged that the experts will meet a number of times with the MPP and ESA responsables. A number of questions remain open through this document, since these are the main aspects where the consultancy will be focused, leading finally to the selection of the most appropriate type of animals to perform the tests.

#### **4. Available resources.**

The availability of resources (mainly Oxygen and Water) that will be generated in the photosynthetic MELiSSA Pilot Plant compartments (Compartment IVb and Higher Plant Compartment) as well as their capacity for CO<sub>2</sub> reduction, have to be taken into account when designing compartment V. Depending on the needs of the animal compartment, some additional supplies of water or oxygen can be necessary to supplement the available water and oxygen generated by the other compartments.

Table 1 presents data from preliminary simulation studies to make a first evaluation of the resources that will be available within the gas, liquid, and solid phase. The CO<sub>2</sub> produced by the animals should also be considered in order to balance the recycling capabilities of the other compartments of the loop. The relationship among the respiratory quotient of the animals and the assimilatory quotient of the plants / cyano-bacteria has to be considered as well.

In addition, it should also be considered that several connection scenarios are envisaged. Indeed not necessarily all photosynthetic compartments will be connected to the animal compartment. In some cases it may be only the *Arthrospira bioreactor* or only the higher plant compartments (one, two, or three). This will need to be addressed, either defining a modular animal compartment with several cages allowing to adjust the number of animals to the given closure test, or by providing additional oxygen supply to that generated in the photosynthetic compartments used in a given test to reach the metabolic needs of the colony of animals used in compartment V.

<b><i>Arthrospira</i> Bioreactor (77 l, 300 W/m<sup>2</sup>)</b>	
	O <sub>2</sub> generation: 74 g/d
	CO <sub>2</sub> reduction: 78 g/d
	Biomass production: 43 g/d
<b>Higher Plants Compartment (5 m<sup>2</sup>)</b>	
Lettuce (400 PPFD)	O <sub>2</sub> generation: 69.4 g/d
	CO <sub>2</sub> reduction: 88.4 g/d
	Biomass production: 50.2 g/d
	Water recycled: 8.9 kg/d
Beet (400 PPFD)	O <sub>2</sub> generation: 71.1 g/d
	CO <sub>2</sub> reduction: 95.8 g/d
	Biomass production: 63.8 g/d
	Water recycled: 8.9 kg/d
Wheat (1200 PPFD)	O <sub>2</sub> generation: 300 g/d
	CO <sub>2</sub> reduction: 386 g / d
	Biomass production: 250 g / d
	Water recycled: 60 kg / d

**Table 1: Calculated production capabilities of different MELISSA compartments. PPFD: Photosynthetic photon flux density.**

## 5. Statistical significance

Statistical significance of the results obtained in Compartment V has to be well considered in the selection of the type of animals. This is related of course to the type of measurements to be performed on the animals as a follow-up of their evolution. A given minimal number of tested animals will be required to ensure statistical significance of the obtained results, allowing to reach founded conclusions for a given test. It will also be needed to consider aspects like gender of the animals.

The statistical requirements, together with the foreseen availability for Oxygen-CO<sub>2</sub> exchange will indeed influence on the number of animals to be used and concomitantly in the type of animals selected.

## 6. Animal monitoring and welfare

Compartment V operation of the MELISSA Pilot Plant will follow-up the evolution of the experimentation animals, both as part of the closure experiments as well as to ensure animal welfare. In addition, the existing legislation on animal experimentation will be followed strictly.

The most appropriated variables reflecting the evolution of the animals during the experiment shall be monitored. Those variables should allow certifying its health conditions and serving as early warning of any deviation. A specialized veterinary follow-up will be considered to assess those terms. The type and frequency of sampling for health check-out will be minimized in order not to disturb to a great extent the closure experiments. Also, the availability of resources from the Veterinary School at UAB will be considered at the time of defining the monitoring protocols to establish. Some possible aspects to monitor could be:

- General health aspects (blood analysis, etc.)
- Stress (for example due to noise, prolonged confinement, animal social interaction, ...)
- Variables reflecting the progress of the experiment such as increase in animal weight or consumption of resources (water, food)

The legal regulations in terms of animal experimentation shall 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<sup>1</sup>, national<sup>2</sup> and European<sup>3</sup> laws on animal experimentation. The experiment protocol has to be first presented to and approved by the local human and animal experimentation committee (CEEAH<sup>4</sup>). 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 Ministry (DARP<sup>5</sup>) for proper authorization of the proposed tests.

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<sup>1</sup>

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

<sup>2</sup>

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

<sup>3</sup>

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

<sup>4</sup>

Comissió d'Ètica en Experimentació Animal i Humana de la UAB

<sup>5</sup>

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

## 7. Experimental constraints

The selection of the animals to use in Compartment V of the MELiSSA Pilot Plant will also have to take into account a number of constraints, those already foreseen are listed below:

### 7.1. Available space

The compartment dimensions have to be adapted to the space allocated in the MPP. The surface available is of 6m<sup>2</sup> (2m x 3m) including access space for maintenance and operation. The maximum available height is 4 m. The space to host the animal compartment does not have any natural light.

### 7.2. Feeding needs

As has been mentioned, CV animals will be fed directly in their cages, according to their specific needs. They will be connected to the rest of compartments regarding water and oxygen supply, but not for solid food supply, that will need to be prepared in the MPP. Accordingly, the needs for food storage and preparation will need to be considered in the design of CV.

### 7.3. Hygiene, waste collection

All the hygiene requirements for the animals in CV will be considered. It is important here to take into consideration in the selection of the animals and in the design of their cages that such activities have to be undertaken with minimal disturbances on the closure experiments. As mentioned previously, both animal faeces and urine will not be used further in the loop, so they should be discarded directly.

### 7.4. Duration of the tests

The selection of the type of animals for CV has also to take into consideration that long closure experiments are planned, for the final demonstration of the MELiSSA loop, of 1-2 years duration.

## 8. Metabolism

In the selection of the animals for CV, it would be interesting to consider an animal with a metabolic profile as close as possible to the human one. Indeed, although this level of closure is planned with animals and not humans, the selection of animals with closer

metabolic behaviour to humans will enable to foresee more aspects in relationship to future closure experiments with humans. Particularly, similarity with human metabolism is important for two reasons: a) conversion ratio of O<sub>2</sub> to CO<sub>2</sub> and b) composition of the gas phase produced.

## 9. Operational modes for Compartment V

### 9.1. Isolated mode.

For example to collect reference experimental data of the species selected such as gas consumption/production, growth rate, stress levels... This mode of operation does not require interconnection with other MELISSA compartments but requires a supply of external resources (artificial air, artificial food, external water supply).

### 9.2. Interconnected via gas phase.

This operational mode requires a partial interconnection (gas lines, monitoring and control data...) with the other MELISSA compartments. It also requires an external supply of food and water (the quality of this supply also to be defined) and the removal of wastes.

### 9.3. Gas and liquid interconnection with the MELISSA compartments.

The requirements of this mode of operation being the same as in the previous mode plus all required to monitor and control the supply of water from other MELISSA compartments.

### 9.4. 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.

## 10. Monitoring and control of Compartment V

The environmental conditions in the compartment will have to be monitored and controlled. The following preliminary aspects should be considered:

- Concentration of main metabolic gases (O<sub>2</sub>, CO<sub>2</sub>).
- Concentration of trace gas contaminants
- Humidity levels
- Gas flow
- Cage ambient temperature

- Illumination photoperiod (awake and sleeping periods)
- Supply of food and water
- Removal of wastes (urine, faeces)
- Animal overall surveillance (video camera...)

The final definition of the animals to use and the whole Compartment V will indeed to considere how to adress these points and the possibilities to provide the corresponding equipment/instrumentation in the MPP.

## 11. Objectives of the Consultancy study

As mentioned in Section 3, scope of the study, a group of consultants will be engaged by UAB and ESA in the work on Compartment V definition, the main aspect of it being the selection of the type of animals, to be followed by the basis for the technical design of the compartment. For this, the different aspects reflected in this document should be taken into account, and considering the background provided, and the knowledge on different type of animals, it is expected that the consultancy study will yield:

- Confirmation of some of the aspects envisaged in this document
- Advices on any other aspect not considered previously in the document
- Final recommendations on animal selection: type of animals, number, conditions, etc.

## 12. Priority Matrix

As a complimentary information the various selection criteria introduced to this point, the following table provides a priority ranking among them:

Criteria	Priority*
Representativity of human metabolism: respiratory coefficient	1
Robustness of the animals (stress resistance, etc.)	1
Life time of the animal (in regards to the long term experiments planned in the MPP, from 1 to 3 year duration)	1
Potential inconveniences and drawbacks (ie, odors)	2
Representativity of human metabolism: exhausted gas phase composition	2
Easy operation and maintenance (animal feeding, veterinary follow-up, etc.)	2
Modularity vs availability of resources (ie, possibility to adapt the number of animals when oxygen resources are varying)	2
Statistical approach	2
Cost of the animal and compartment operation	3

(\*) priority levels: 1, high; 2, medium; 3, low.



### 13. REFERENCES

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### 14. ANNEXES

Annex 1: MELiSSA Pilot Plant: General Resources, Interfaces and Environment. TEC-MCT/2006/3493/In/BLA

## 15. Comments

Page/paragraph	Comment																				
9/2.2	Second and third paragraphs could be better placed after the last paragraph In the second paragraph, 'eases' instead of 'reduces'? To complete the overview of the connection of CV, it would be interesting to mention already that the animals will not be fed with the biomass produced within the MPP, and that animals urine and faeces will not be used.																				
20/2.4.6	Food supply and urine/faeces removal to be mentioned																				
23/6	Do we have any idea about the type of monitoring that can be easily performed in the MPP, probably in cooperation with the veterinary school? If yes, then, we should provide the information.																				
24/7.1	We have to mention that natural light is not available																				
24/8	Similarity with human metabolism is important for two reasons: <ul style="list-style-type: none"> <li>- the conversion ratio of O<sub>2</sub> into CO<sub>2</sub></li> <li>- the composition of the gas phase produced</li> </ul>																				
25/10	This paragraph is detailing what we should consider. To guide the animal selection, we should probably describe what we can perform.																				
26	We would suggest adding a table summarizing all selection criteria with a priority ranking. See hereafter.																				
	<table border="1"> <thead> <tr> <th>Criteria</th> <th>Priority (1-high, 2-medium,3-low)</th> </tr> </thead> <tbody> <tr> <td>Representativity of human metabolism, respiratory coefficient</td> <td>1</td> </tr> <tr> <td>Robustness of the animal (stress resistance...)</td> <td>1</td> </tr> <tr> <td>Lifetime of the animal (as we plan to have up to 1-3 year continuous operation)</td> <td>1</td> </tr> <tr> <td>Potential inconvenience (e.g. odors)</td> <td>2</td> </tr> <tr> <td>Representativity of human metabolism, Exhausted gas phase composition</td> <td>2</td> </tr> <tr> <td>Monitoring easy to perform (veterinary follow-up, feeding...)</td> <td>2</td> </tr> <tr> <td>Modularity versus availability of resources (i.e. possibility to adapt the number of animals when oxygen resources are varying)</td> <td>2</td> </tr> <tr> <td>Possibility to reach statistical approach</td> <td>2</td> </tr> <tr> <td>Cost of the animal</td> <td>3</td> </tr> </tbody> </table>	Criteria	Priority (1-high, 2-medium,3-low)	Representativity of human metabolism, respiratory coefficient	1	Robustness of the animal (stress resistance...)	1	Lifetime of the animal (as we plan to have up to 1-3 year continuous operation)	1	Potential inconvenience (e.g. odors)	2	Representativity of human metabolism, Exhausted gas phase composition	2	Monitoring easy to perform (veterinary follow-up, feeding...)	2	Modularity versus availability of resources (i.e. possibility to adapt the number of animals when oxygen resources are varying)	2	Possibility to reach statistical approach	2	Cost of the animal	3
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