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C-IVa: status and refurbishment recommendations

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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).

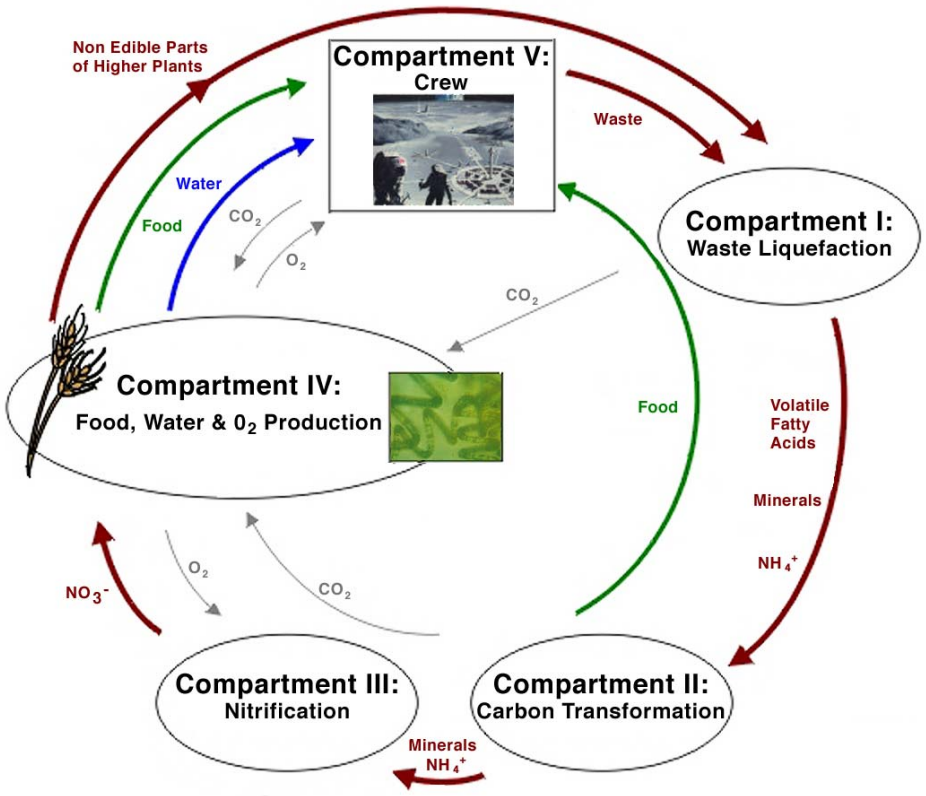

Figure 1: MELiSSA Advanced Loop Concept

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.

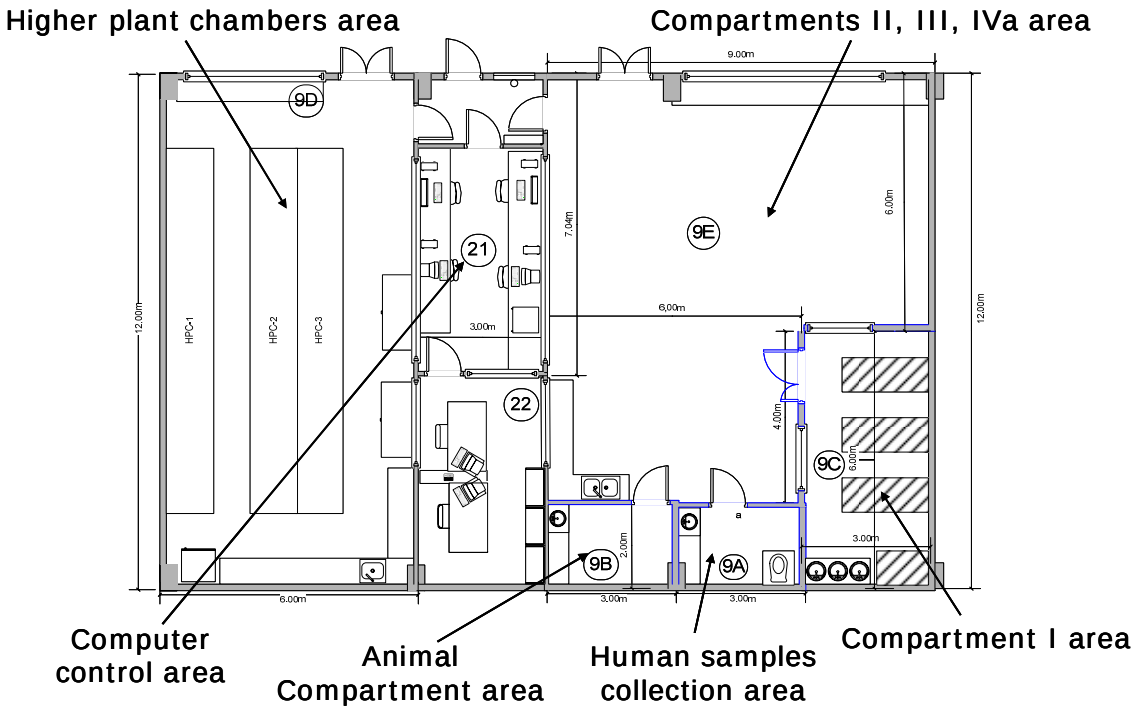

Figure 2. Basic layout of the MELiSSA Pilot Plant laboratory.

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.

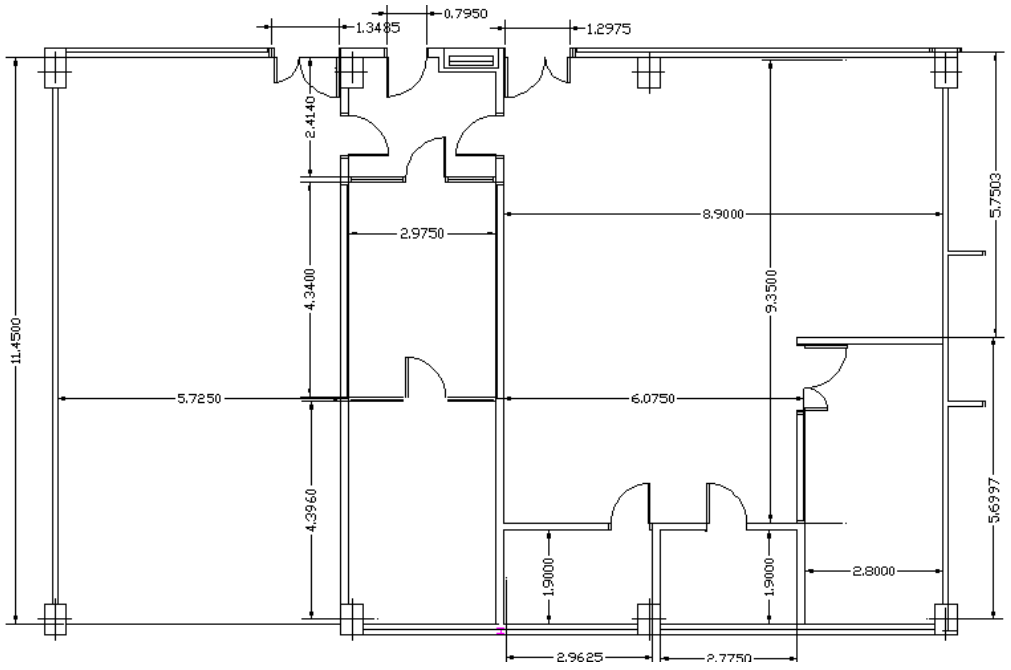

Figure 3: Sizes of the different areas in the MELiSSA Pilot Plant

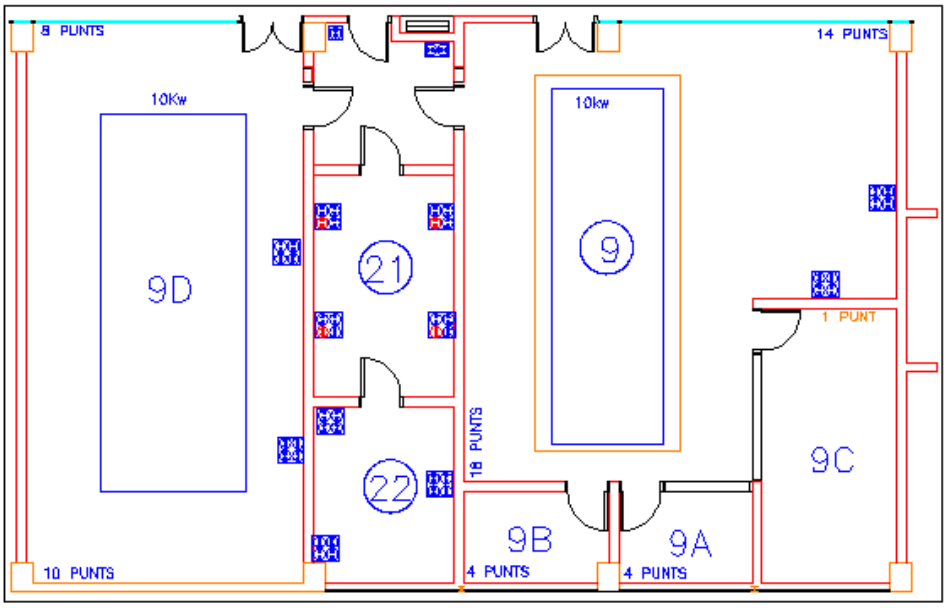

Figure 4: Distribution of the power lines in the MELiSSA Pilot Plant.

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).

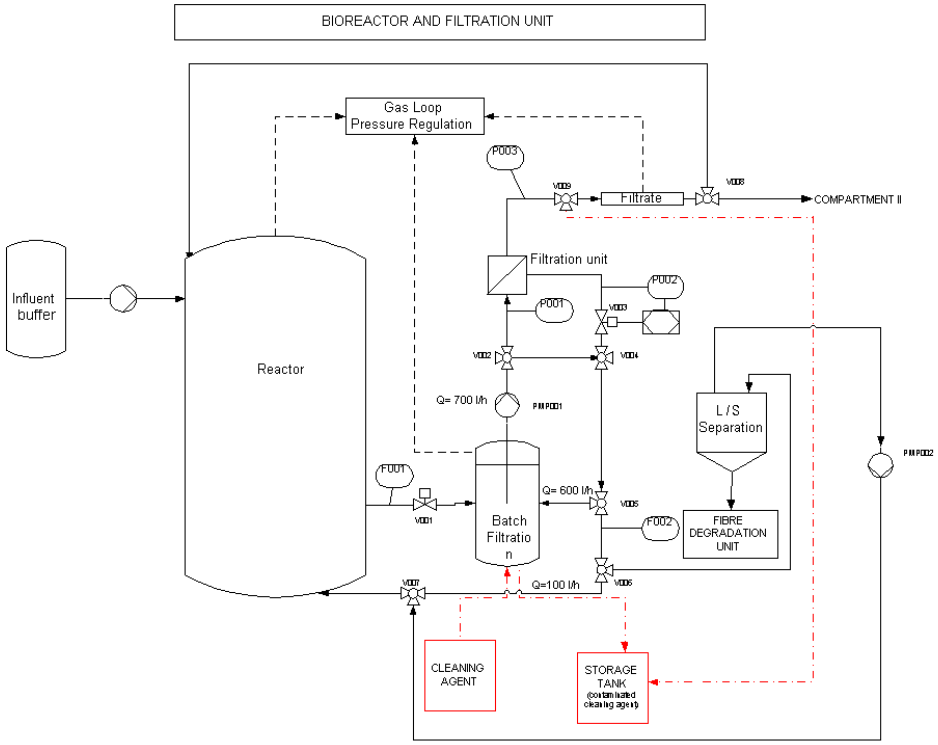

Figure 5: Schematic design of compartment I and its filtration unit.

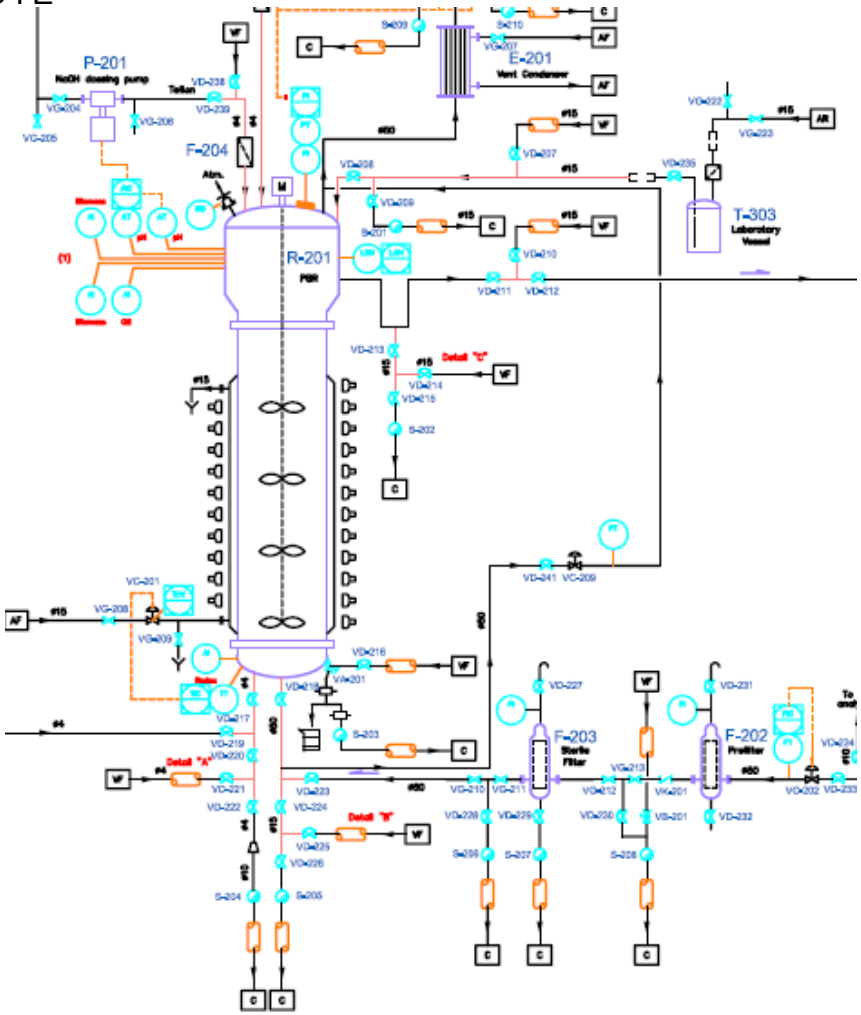

Figure 6: Configuration scheme of compartment C-II.

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

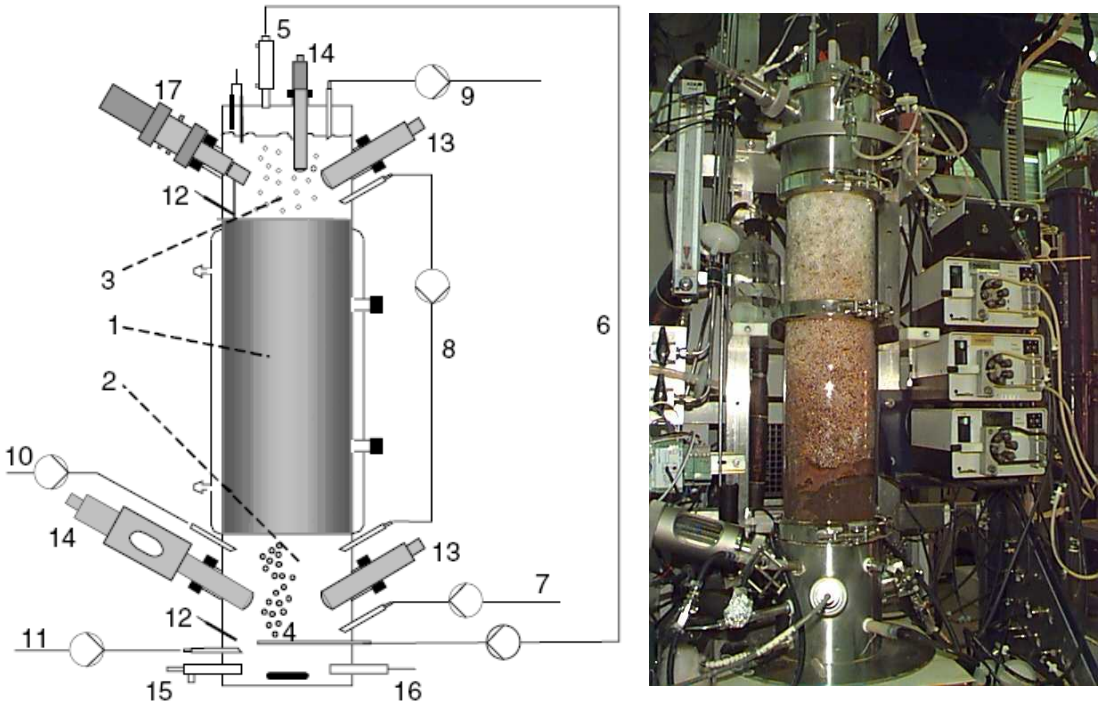

Figure 7: : Schematic overview of compartment III.

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.

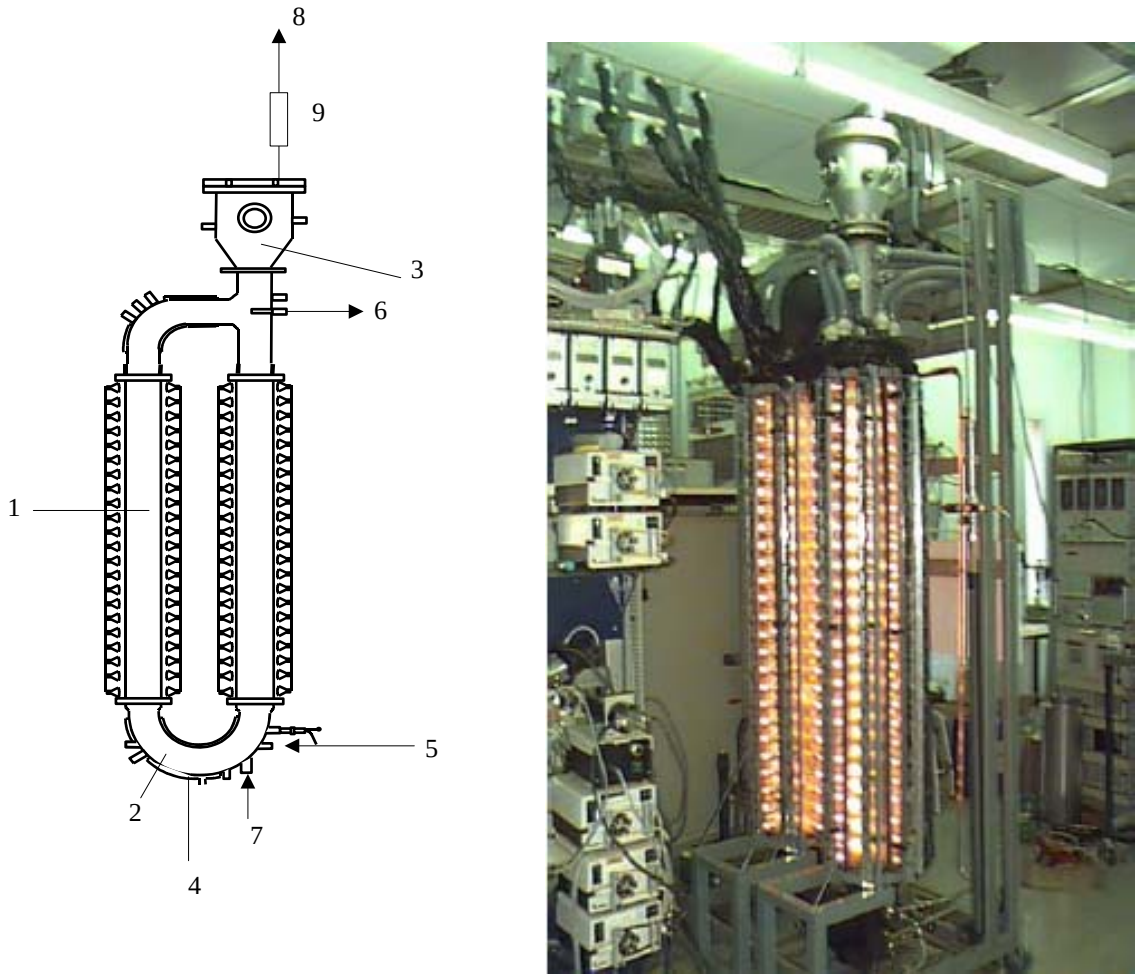

Figure 8: Schematic view of compartment IVa.

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).

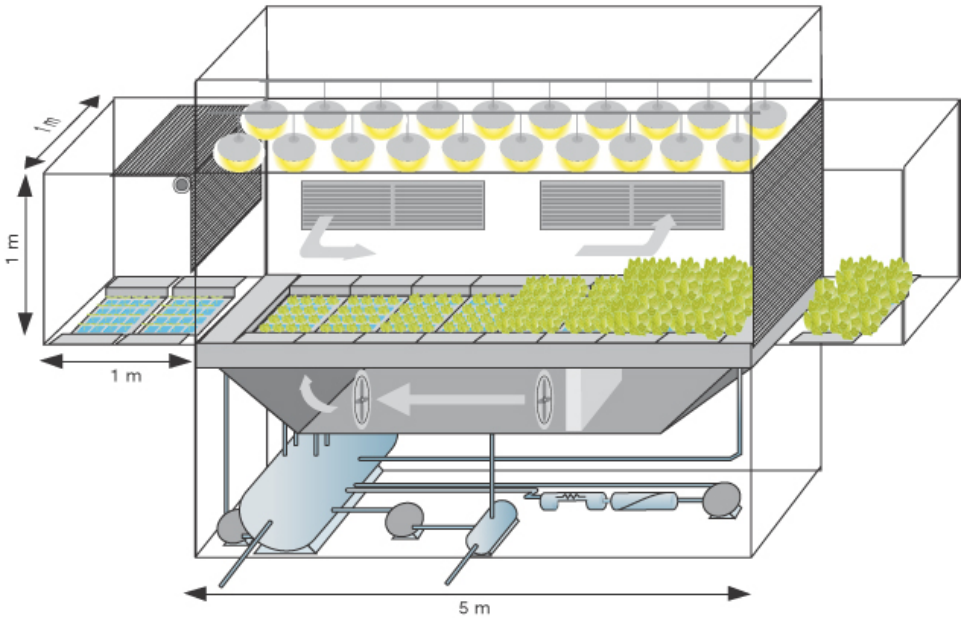

Figure 9: schematic view of the design concept for the Higher Plant chamber.

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: status and refurbishment recommendations for Compartment IVa

In the MELISSA loop compartment IV has the task to provide food and oxygen to the crew as well as to remove the carbon dioxide produced during respiration and as a result of the degradation activities in other compartments. To perform this task this compartment was divided into compartment IVa, colonized by a cyanobacteria, and compartment IVb, were higher plants are used. The combination of those two compartments will allow to provide a proper diet to the crew as well as to properly adapt the dynamics of the oxygen consumption and carbon dioxide generation to the human activity.

Compartment IVa is colonized by *Arthrospira platensis* (*a.k.a. Spirulina platensis*). This cyanobacteria was initially selected due to its well known characteristics of growth conditions and biomass quality and composition. It has been used as a protein source since ancient times and today is industrially produced and commercialized as a dietary food complement. *A. platensis* is a photoautotrophic organism which fixes carbon dioxide and produces oxygen using the energy of light. Those characteristics makes it a perfect organism to be used is a closed ecosystem such as MELISSSA.

Compartment IVa is the better known compartment in the loop, as it was the first to be studied. Initial studies by UBP led to the development of a mathematical model describing light distribution inside bioreactors and its interconnection with *A. platensis* growth . Based on this model one of the MELISSA partners developed a model predictive control routine that allows to control biomass productivity according to the

user requirements. This control law was tested in the Pilot Plant using a 7 litres airlift bioreactor.

Based on the previous experience of operation of the 7 litres bioreactor, a new bioreactor 10 times bigger in volume (77 litres) was designed, built (Figure 10, Bioengineering AG, Wald, Switzerland) and installed in the Pilot Plant (Vernerey *et al.*2001). For its design the special characteristics of those photoautotropic cultures were taken into account such as the sensitivity to the shear stress, the cooling requirements or the illumination distribution and light transmission. In order to take into account the light transmission characteristics the model developed by Cornet *et al.* (1998) was used (Vernerey *et al.* 2001). The newly developed bioreactor is shown in Figure 8.

The pilot-scale photobioreactor used for the Arthrospira sp. cultures is a 77-L external loop air-lift bioreactor. A detailed description of the scale-up, design, associated instrumentation and physical characterization of this photobioreactor has been already reported (Vernerey *et al.*, 2001).

The illuminated part of the bioreactor consist of two cylinders, being respectively the riser and down-comer, made of polyamide tripan, a transparent plastic foil material, each column with a 15 cm diameter and 1.5 m height. Both cylinders are connected at their lower and upper parts by U-shaped stainless-steel sections with an external jacket for the temperature control, where all the instrumentation is located.

Illumination system consists of 350 halogen lamps (Sylvania, 12V, 20W) distributed homogeneously around the plastic cylinders. A voltage regulator allows for providing different light intensities to the bioreactor.

Liquid media input is done by peristaltic pumps (Reglo-Analoge MS 2/6-160; MS 4/6-100, Ismatec SA, Glattbrugg, CH) from one of the two 50L buffer tanks, through a liquid filter (0.22 µm KVGL04HB3 Millipore, Billerica, MA, USA) to ensure sterility and then to the bottom part of the bioreactor. A U-tube located at the top part of the bioreactor allows the output liquid to flow by gravity, while maintaining a constant culture volume.

Gas phase is injected from the bottom of the riser column, in the right side of the reactor. The riser is also provided with a stainless-steel section for gas phase separation. The connecting u-shaped parts connecting the two columns are made of stainless-steel and provide temperature control by means of an external jacket. Gas circuit is composed by 4 mass flow meter-controllers (Bronkhorst High-Tech BV F202D-FA-44-V, Ruurlo, NL) that measure and regulate both the input CO_2-enriched air responsible of the culture agitation and the output gas flow. An IR analyzer for CO2 coupled to paramagnetic analyzer for O_2 (Maihak, Multor 610, Hamburg, DE) measures on-line the composition of the gas phase.

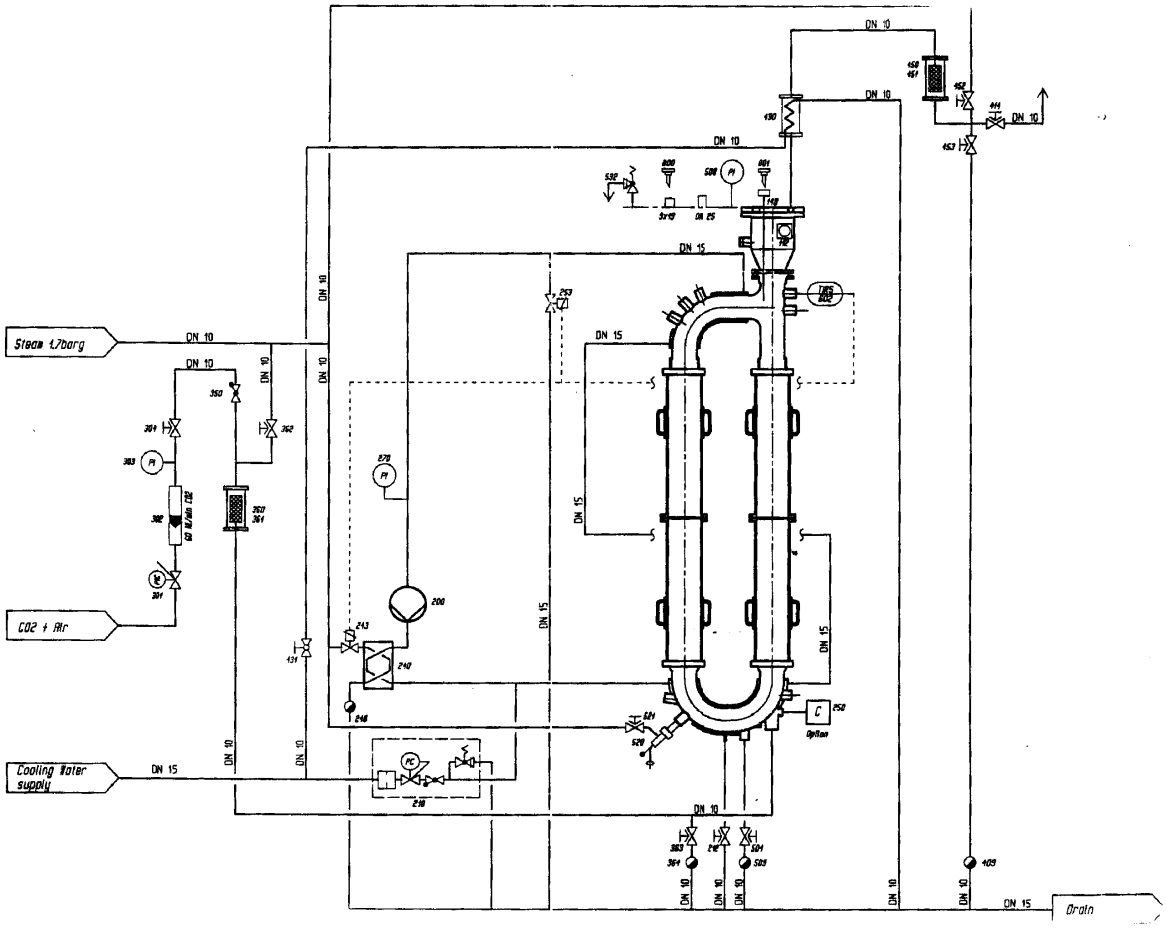

Figure 10: General overview of CIVa reactor.

After its installation and validation of the proper operation of its instrumentation and control system a physical characterization of the reactor was performed, including all the characterization of mixing hydrodynamics, residence time distribution and gas-liquid mass transfer characteristics. Continuous operation of the compartment for long operation periods (up to six months) has been achieved, under high operational stability. In addition, this Pilot reactor has been used to perform the first tests of connection between two compartments of MELISSA at the Pilot Plant level (Compartments III and IVa).

More recently, the control hardware of this compartment was further improved as a result of the work developed under contract with ESA for this specific issue, with the objective of having a high performance control system able to control all the foreseen compartments of the MELISSA loop as also described in a previous section.

The reactor has already been operated in the MELiSSA Pilot Plant over extensive periods of time, at different experimental conditions, such as flow-rate, illumination rate, or composition of the culture medium. The results obtained provide limits for the operational feeding rates in the reactor, and ammonium and nitrates concentrations required for its proper operation. Table 2 summarizes the most common ranges of bioreactor operation obtained using Zarrouk culture medium.

Table 2. Main variables associated to Compartment IVa bioreactor operation

Variable	Operational range	Stand alone setpoint	Units
Biomass productivity	up to 27	10	$mg \cdot L^{-1} \cdot h^{-1}$
Biomass concentration	variable	1	g/L
Recirculation gas flow	1.5-3.5	2.2	nL/min
Dilution rate	0-0.05	0.01	h^{-1}
Liquid flow	0-3.85	0.77	L/h
Light intensity	0-225	Under productivity control	W/m^2
pH	8.5-10.5	9.5	pH units
Temperature	20-40	36	ºC
Input CO_2 concentration	0.036-5	2	%

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 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.

Other important aspects regarding long term operation and axenicity of the reactor are the pumping devices, retractable probes, online measurements or pressure control.

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

In the next table, the detailed summary of the aspects analysed on the hardware of Compartment IVa used previously and proposed alternatives to be considered in the re-design is provided.

Scope	Reason	Alternatives
- Substitution of plastic walls	- Difficult operation (installation and sterilisation) - Inefficient control of volume, then altering dilution rate - Risk of leakage - Risk of breakage by overpressure - Risk of release of gas to environment by safety valve opening	- Glass cylinders, maintaining upper and lower steel parts /Glass cylinders in a new vessel
- Foam control	- Foaming risk - Risk of exhaust filter clogging and overpressure	- Foam detectors - Antifoam feedings (auto / manual) - Foam breakers - Redundant outlet gas filter
- Level control	- Volume and foam control	- Load cells or weighting platform - Differential pressure
- Retractable and redundant pH probes	- Long-term calibration - Redundancy	- Existing ports / new ports
- Sterile cleaning of Biomass probe	- Axenicity	- Gas filter in the specific air inlet
- Retractable / Redundant / Alternative biomass sensor	- Long-term calibration - Redundancy - Alternative technology testing	- Existing ports / New ports - Outlet biomass module (shared among compartments/ dedicated) - New sensor in vessel (bottom?)
- Retractible D.O. probe	- Long-term calibration	- Existing ports / new ports
- Sterilizable and redundant pressure sensor	- Axenicity - Redundancy	- Check compatibility of current PIC with steam - Repair or replace analogue PI - Two ranges: sterilisation / Operation

Scope	Reason	Alternatives
- Pressure control improvement	- Inadequate control based on input and output flows	- Independent outlet control
- Sampling probe	- Axenicity	- Revise configuration
- Inoculum transfer system	- Axenicity	- Steam sterilizable flexible connections (shared among compartments/ dedicated)
- Inoculum reactor	- Guarantee adequate growth	- Lab fermenter 10L (shared among compartments)
- Lighting system	- Guarantee adequate performance	- Revise the status of the lamps / Stands / Electric cabinet / Hot air extraction / Wiring (external company)
- Temperature control	- Avoid damage to steam solenoid valve	- Substitute steam heating by electric heating - Modify temperature control
- Flow control	- Guarantee precise liquid flow control	- Revise balances and use weight for flow control
- Balances refurbishing	- Adequate appearance and cleaning	- Painting or coating upper surface
- Feeding and harvesting vessels	- Substitute bottles	- Stainless steel tanks - Plastic sterile bags
- Loop gas piping	- Guarantee stable and resistant pipelines	- Stainless steel pipelines and valves accordingly - Include Gas loop recirculation (second step) - Include compressor for closed loop (second step)
- Control of pressure and flow in line to analyzers	- Stable and reliable measurement	- FIC and PIC
- Control of shifting in/out gas analysis	- Automatic analysis	- Control strategy development

The scope of the proposed work does not include any control aspects, since this work is the responsibility of one of the partners in the MELiSSA project, and the same kind of control approach is followed in all compartments. However, the instrumentation and hardware provided should allow a number of specific control actions, as described further on.

Finally, it has also to be mentioned that the MELiSSA Pilot Plant is running a parallel contract for the design of Compartment II of the MPP. Since this is also a

photobioreactor, and some of the requirements and associated instrumentation have similarities, the development of the present work will be coordinates to that corresponding of C-II. This coordination will be done by the MPP. Also, the company undertaking the work described here will need to take into account the existence of a harmonized list of basic instrumentation for the MPP, to be provided to the selected company in the design phase. With this harmonization, it is pursued that the basic instrumentation in the MPP (T, pH, PO_2 probes, etc.) is the same in the different compartments.

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

The technical specifications for the redesign of the compartment IVa 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 hardware that is to be upgraded should have the same dimensions and basic characteristics as the existing one, but some concrete aspects will be modified in order to improve its operation (the main elements under redesign are shown in Figure11):

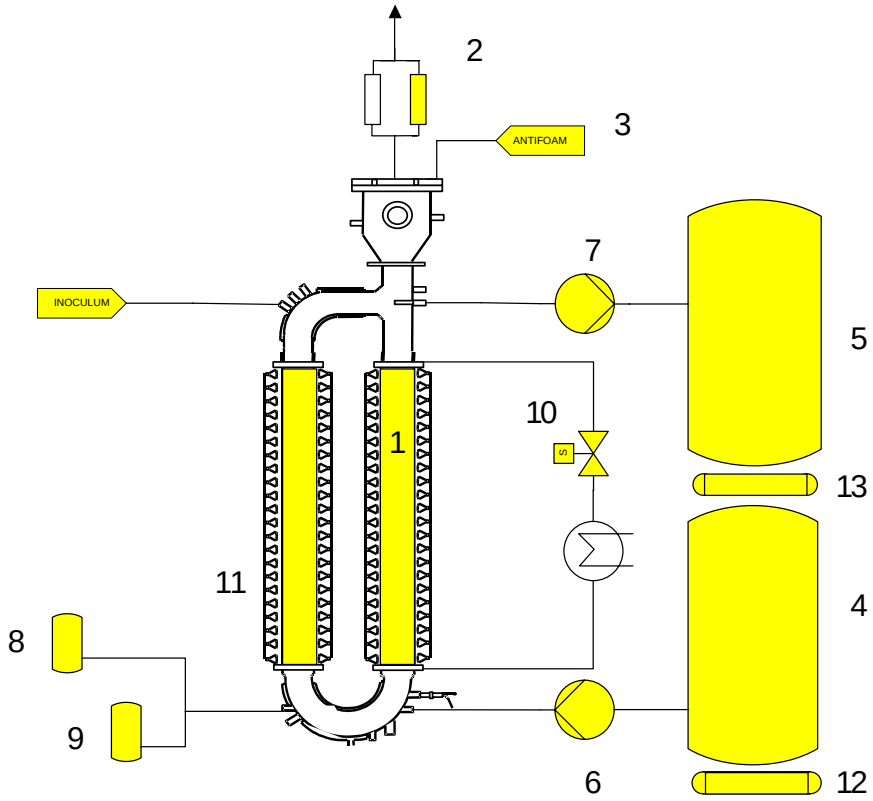

Figure 11: : Schematic overview of updated compartment IVa hardware:

(1) Gas-lift columns, (2) Outlet filters, (3) Antifoam feedings, (4) Feeding tank, (5) Harvesting tank, (6) Feeding pump, (7) Harvesting pump, (8) Acid storage tank, (9) Base storage tank, (10) Temperature control system, (11) Lighting system, (12) and (13) Weighting scales.

1. Alternatives to the existing plastic walls of the reactor should be provided, in order to reduce the difficulty of operation (installation and sterilisation), inefficient control of volume (then altering dilution rate) and risks of leackage and breackage by overpressure. Glass or another rigid material should be evaluated in order to substitute the current material of the walls, but maintaining if possible the upper and lower steeel parts of the reactor. For the sterilization requirements, tightness in all the sealings should be effectively guaranteed. Also, the reactor should provide all the necessary ports to host the associated instrumentation described in Section 4.2.
2. The redesign of the vessel will take into account the need of some new ports for the instrumentation now required, and for the design of these ports the particular features of this culture should be also considered, such as avoiding dead zones where potential formation of clusters of polysaccharides could take place.

3. Redundant outlet filters should be provided in the gas outlet, in order to cope with potentially clogging and making feasible independent sterilisation if clogging occurs. Other alternatives like foam breakers could be also evaluated as an alternative for further foam containment.
4. Antifoam feedings will also evaluated, in combination with foam detectors (see item 25) as an improvement for further foam control.
5. A gas sterilising filter will be incorporated in the air supply for cleaning the biomass sensor, or sterile air will be provided to that position after the current air inlet filter.
6. Current sampling probe will be evaluated regarding the quality of the same for guaranteeing the axenicity.
7. 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 120L (working volume 90L), enough capacity for 24 hours at the highest flow. Both should be able to be steam sterilised, and being connected when possible by means of pipelines built in the same material. As an alternative to the steel vessels, sterile plastic one-use bags would be evaluated, considering investment and operation costs, and also the suitability of these bags to fulfil the requirements for the feeding and harvesting tanks, specifically the need to be maintained at 4ºC.
8. Alternatives to the use of peristaltic pumps for both culture medium and acid or base feeding and broth removal, 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.
9. 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 6.
10. Provide containment vessels for acid or base spills contention if necessary depending on the solution for requirement 9.
11. Stainless steel 316L pipelines and valves accordingly will be provided when adequate for the gas piping, so guaranteeing stability and resistance.
12. Temperature control should be improved, avoiding the current design where steam damages the solenoid inlet valve. Alternative electrical heating or steam through a different valve will be evaluated and implemented.

13. The overpressure safety valve will be revised for adequacy to axenicity purposes, and eventually a rupture disk will be evaluated as an alternative.
14. The status of the illumination system, based on two external frames including halogen lamps, should be revised in order to upgrade its quality and safety when needed, both considering the electrical cabinet, wiring, stands and hot air extraction system.
15. Existing weighting scales for inlet liquid feed should be refurbished, considering both the status and coating of structure, legs and weighting platform.
16. Weighting scale or an alternative like differential pressure measurement should be provided for measurement of weight in the harvesting tank, considering as an alternative to reuse one of the existing for the inlet liquid feed.
17. Weighting scales for acid and base will be provided, in order to record the consumption of those feeds, and eventually implement further control actions.
18. Pressure release valve will be revised in order to check its sizing to assure enough capacity.
19. No-return valves will be implemented in the diverse gas pipelines to protect mass flowmeters from accidental steam input.
20. The current skid of the reactor should be refurbished to repair the sloping of the vertical structure.

4.2. Instrumentation requirements

4.2.1. Description of the current on-line instrumentation and control of Compartment IVa (see Figure 12):

The current instrumentation of the reactor is summarized here. The main characteristics of the instrumentation is summarized in Table 3.

- pH sensor: in upper part of the reactor.
- Dissolved Oxygen (D.O.) sensor: in upper part of the reactor.
- Temperature sensor: in upper part of the reactor.
- Head pressure sensor: in upper part of the reactor
- Biomass sensor: in upper part of the reactor
- Inlet gas mass flowmeters:

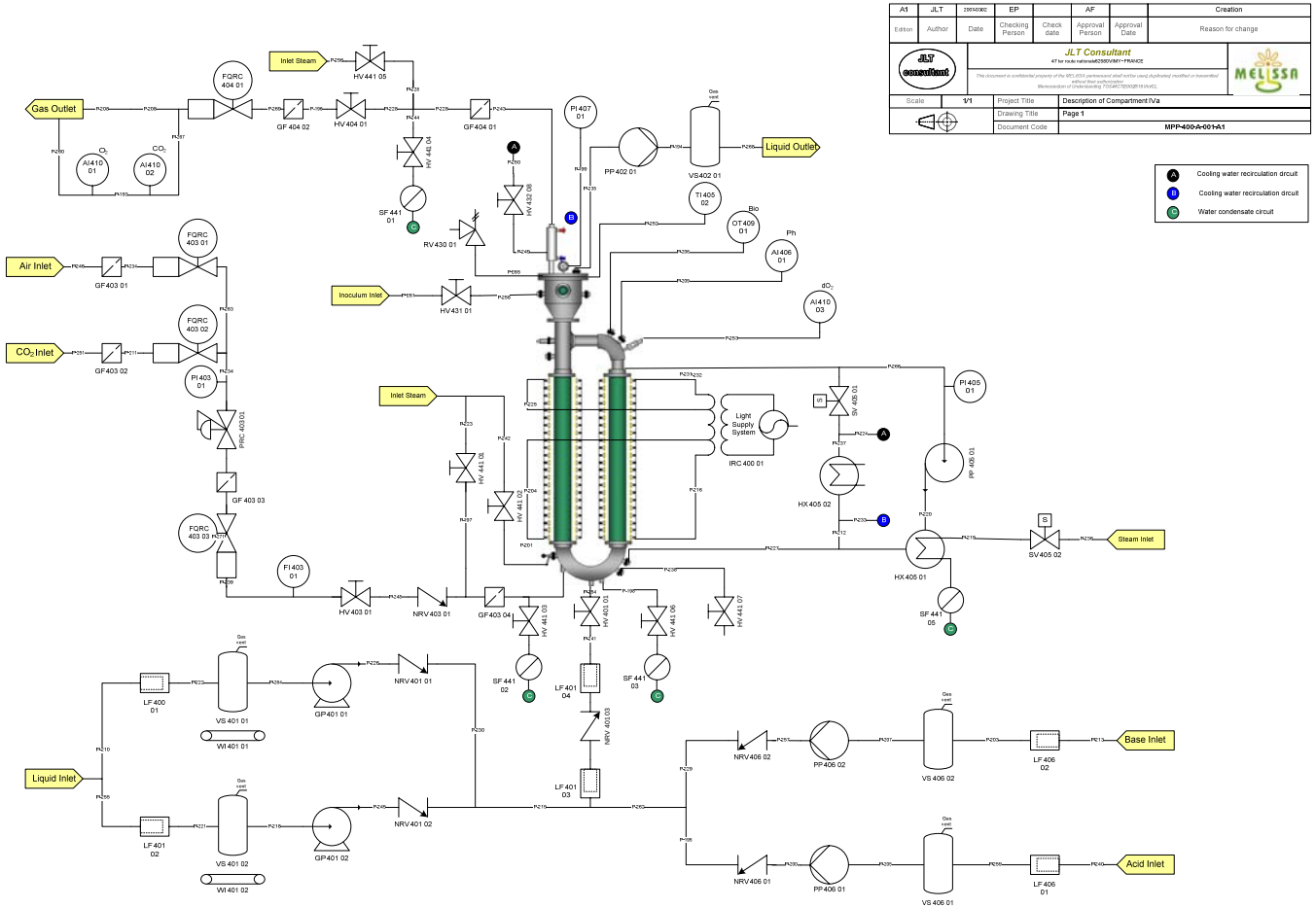

Figure 12: Schematic view of CIVa reactor and instrumentation

- Air
- CO_2
- Total gas

- Inlet total gas volumetric flowmeter

- Inlet gas pressure sensors:

- Air
- CO_2
- Total gas

- Outlet total gas mass flowmeter

- A gas on-line analyzer is used to measure O_2 and CO_2 concentration in the gas outlet phase of the bioreactor.

Table 3. Description of the main instrumentation associated to Compartment IVa bioreactor

Monitored parameter	Measuring range	Precision	Number of units
pH	0-14	±0.1	1
Oxygen	0-100%	±0.1%	1
Temperature	0-150ºC	±0.3ºC	1
Head Pressure	0-1500 mbar	±1 mbar	1
Biomass	0-2 UA	0.1 UA	1
Air inlet mass flowmeter	0-30 NL/min	0.5 NL/min	1
CO_2 mass flowmeter	0-5 L/min	0.5 NL/min	1
Total inlet gas mass flowmeter	0-30 L/min	0.5 NL/min	1
Total inlet gas volumetric flowmeter	0-30 L/min	0.5 NL/min	1
Inlet Air Pressure	0-4000 mbar	±100 mbar	1
Inlet CO2 Pressure	0-4000 mbar	±100 mbar	1
Inlet total gas Pressure	0-2500 mbar	±100 mbar	1
Total outlet Gas	0-30 L/min	0.5 NL/min	1

flowmeter			
CO2 conc. in outlet gas	0-20 %	1 %	1
O2 conc. in outlet gas	0-25 %	1 %	1

The different control loops in Compartment IV 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
- Light control
- Inlet liquid flow rate
- Outlet liquid flow rate
- Inlet gas flow rate
- Outlet gas flow rate
- Temperature
- Pressure
- Dissolved oxygen (only monitored)
- Liquid level (not controlled overflow)
- Biomass

Table 4: Description of the action of the main control loops associated to Compartment IV bioreactor

Control loop	Set point	Precision
pH	9.5	± 0.1
Light	133 W/m^2	3 W/m^2
Inlet liquid flow rate	2.5-10 ml/min	***To be defined***
Outlet liquid flow rate		
Inlet gas flow rate	2,1NL/min	0.05 NL/min
Gas outlet flow rate	2,1NL/min	0.05 NL/min
Temperature	36.0 ºC	± 0.1ºC
Pressure	<80 mbar	1 mbar
Dissolved Oxygen	not controlled	-
Liquid level	not controlled 77 L	-
Biomass	0.5-1.5 g/L	5%

4.2.2.New instrumentation requirements

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

22. pH electrode should be redundant, because pH is considered a critical variable for this compartment, that means to provide one additional pH sensor, located in the lower part of the reactor.

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

24. A new biomass sensor should be installed based on a different technology of the existing one, in order to provide a redundant but also alternative measurement, the location of this new probe to be defined.

25. Foam detectors should be implemented in the top part of the reactor, to prevent potential foam to arrive to the gas outlet causing filter clogging and overpressure.

26. Temperature control should be improved, avoiding the current design where steam damages the solenoid inlet valve. Alternative electrical heating or steam through a different valve will be evaluated and implemented.

27. Flow and pressure indication and control will be implemented in the gas pipeline to analyzers, in order to assure stable and reliable measurements.

28. The implementation of pressure indicators upstream and downstream the inlet feeding filter will be evaluated, in order to prevent clogging of the same during long-term operation.
29. Level control should be implemented, and work in a precise manner. Both load cells or differential pressure systems will be evaluated as alternatives.

30. Current pressure sensor should be revised to check its compatibility with steam sterilisation and axenic conditions. Considering the criticality of pressure control for this reactor, a second redundant probe should be implemented, the location of the same to be defined**.** Also range of measurement of both sensors should guarantee enough precision to be used both during sterilisation and operation conditions.
31. The implementation of mass flowmeters both in feeding and harvesting lines should be evaluated as an alternative of the improvement of feeding and harvesting flow control by means of weighting (see actions on control herebelow).
32. The implementation of a light intensity or power consumption sensor will be evaluated and its connection to the PLC in order to control the potential malcfunction of the lighting system and allowing the corresponding alarm strategy.

The proposed hardware will allow to perform a number of control actions, to be defined out of the scope of this work, as mentioned earlier. For information, some of these actions are:

- Alarms linked to the gas flowmeters signal processing should be checked for alarm conditions and their priorities
- Adequate control of shifting inlet/outlet gas analysis will be implemented
- Pressure control should also be improved, being independent of inlet and outlet gas flows, but implementing complete closing of inlet gas flow if triggered by a high pressure alarm.

- Liquid flow control both in feeding and harvesting will be improved. For this purpose, the use of weighting scales measurement for the control of feeding and harvesting pumps will be evaluated and implemented accordingly.
- pH control will be improved to guarantee that acid or base pumps are stopped in case of pH out of range.

4.3. Operation requirements

4.3.1.Inoculation

33. 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.

4.3.2.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 consider the needs for adequate CIP and SIP of the bioreactor, and the design considerations regarding ergonomics.

5. References

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