# MELISSA

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***Review of the Complete MELISSA Loop and Identification of the Critical Developments***

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# 1 SCOPE

According to the Statement of Work issued by the Agency [A 1] for this project, the objective of this Technical Note is the identification and list of critical technologies for the future spacialisation of the MELISSA loop.

As per the indications in [A 1] and NTE's proposal defined in [A 2], and following a background introduction in Chapter 3, the study begins in Chapter 4 with the comprehensive review of the complete MELISSA loop and its compartments. A detailed description of the Pilot Plant as currently implemented at UAB´s premises followed by a phase analysis are the basis for the identification of the critical technologies (identified as **CT#** throughout the text).

The assumption of a MARS outpost as potential scenario for the MELISSA use in Space as a Life Support System is described in Chapter 5.

The specialisation of MELISSA through an industrial intermediate stage is discussed in Chapter 6. It also includes a functional breakdown of the Pilot Plant, which maps the envisaged hardware to the identified critical technologies and rates their degree of criticality in terms of development needs.

Chapter 7 list all these CTs which are grouped in Chapter 8 in three categories, namely scientific, industrial or space related criticalities.

Finally, Chapter 9 introduces few ideas for the utilisation of MELISSA as a Bio-regenerative Life Support System (BLSS) and Chapter 10 provides the conclusions.

This study has been carried out through an exhaustive literature review (referenced in chapter 11). Some MELISSA partners, namely UAB (E), Université Blaise Pascal (F), EPAS (B) and ADERSA (F) (this latter for what specifically concerns the functional breakdown and naming conventions) have also contributed to the identification and understanding of some of the critical technologies.

# 2 APPLICABLE AND REFERENCE DOCUMENTS

**[A 1]** **MELISSA. Adaptation for Space, Phase 1.** Statement of Work. Doc. No. TOS-MCT/2000/2977ln/CL. Issue 5. April 2001.

**[A 2]** **MELISSA Adaptation for Space-Phase 1.** Proposal issued by NTE. Doc. No. MEL-0000-OF-001-NTE. Issue 2. October 2001.

**[R 1]** **Dependability Analysis of MELISSA. Technical Note 62.7.** V1.0, ADERSA June 2002.

# 3 INTRODUCTION

The development of Life Support systems for enclosed habitats was initiated in the 50's for high altitude flight and for submarines designed for long periods of submersion. This development accelerated with the start of the manned Space flight activities by Russia and the US in the 60's.

Physico-chemical systems have been used since then for air revitalisation first. Thus, Mercury, Gemini and Apollo´s Command and Lunar Module used LiOH cartridges for $CO_2$ removal. This air revitalisation was further improved in Shuttle and MIR station leading to the present configuration foreseen in the International Space Station, with a regenerable 4-Bed Molecular Sieve and a $CO_2$ reduction using a Sabatier reactor. Water production started with the Apollo program using fuel cells, which are still used in the Space Shuttle nowadays. In this process, wastewater is vented overboard and not recycled. For longer stay periods, wastewater venting is not efficient. This led to some recycling strategies in MIR Station: hygiene water was used for re-use as hygiene water, humidity condensation was reclaimed for use as drinking water and food preparation water, and urine distillate was used as feedstock for the production of oxygen by electrolysis. The International Space Station will feature a single water stream for recycling that will follow several treatments before being re-used (Vapour Compression Distillation, Multifiltration, Aqueous Phase Catalytic Oxidation and Disinfecting). All this indicates that gas and water recycling by physico-chemical processes could be envisioned for any future space mission (Mars or lunar base). However, food production from waste recycling will not be possible by using only physico-chemical processes. These latter could be used if food supply is feasible in a regular basis, as for example space stations or even in a lunar base. However, for other long-term space missions as a Mars base, the bio-regeneration will be mandatory. In these conditions, food production is intimately linked to the capability of waste recycling.

The development of bio-regenerative life support systems was identified as a needed activity by the different countries with space development programs. Russians pioneered not only the field of bio-regenerative systems, but also Closed Ecological Life Support Systems (CEcLSS). This latter was conceived by the great visionary Konstantin Tsiolkovsky and followed by V.I Vernadsky and his more detailed analysis of biospheres. First experiments with closed ecosystems were carried out in Russia during the 50's and 60's and led to the creation of the BIOS-3 facility, located at the Institute of Biophysics in Krasnoyarsk, in Siberia. The recycling water and other gases system efficiency was 80-85% (Gitelson et al, 1991). The strategy of the Soviet program was to produce a System as closed as possible, minimising the re-supply and maximising the closure time. The US started in 1978 the Controlled Environmental Life Support Systems (CEnLSS ) program to develop biological life support capabilities. The strategy taken by NASA was to study a mixed physico-chemical and bioregenerative system that would include a plant growth chamber for food production (Mc Elroy et al, 1987). This process culminated in recent documentation describing the reference missions for future US Space activities (Drysdale et al, 1999; Drysdale et al, 2000; Hanford et al, 2001; Lange et al, 1998; Maxwell et al, 2001), where the food production of a future Mars base depends on a Plant Growth Chamber. By 1985, the Japanese National Aerospace Laboratory (NAL) started an activity with a new study group to obtain a CEnLSS that was considered partly physico-chemical. Biological Life Support Systems have been envisioned by a Japanese Space company in plans to explore Mars by the Japanese Space Agency (Ishikawa et al, 1990). Not only the Space programs were performing activities in Closed Life Support Systems, but also the submarine technology programs (Saalfeld, 1976;

Wyatt 2000) even recently considering biological processes (Diamond, 2001), were the use of algae is foreseen for air revitalisation.

In 1989, the European Space Agency Industrial Policy Committee (Environmental Control, Life Support and Habitability. Technical Dossier, Doc. No. ESA/IPC (89)) decided to start development activities in Environmental Control and Life Support Systems. Up to then, the Manned European Program was relying on US technology for Life Support (Spacelab). The European Space Agency had then initiated the development of a Environmental Control and Life Support System (ECLSS) for the European programs at that period (Hermes, Columbus free-flying and attached laboratory). This development was mainly based on physico-chemical processes. However, the European Space Agency decided as well in 1989 to start development activities of a Closed Ecological Life Support System (CEcLSS). The Industrial Policy Document above referred states that "Food production on board will reduce re-supply costs, provide more variety and, since food production would be expected to utilise the output of a (biological) waste processing system, will also reduce the need of removal of waste".

In 1988, a group of European scientists proposed in an European Symposium the use of a micro-organism based Model to develop a CEcLSS to be used in future space activities (Mergeay et al, 1988). This model was named MELISSA, standing for Micro-ecological Life Support System Alternative. In 1989, this same group of scientists began the design of this model under an ESA contract, whose results provided the main guidelines for the development of the MELISSA Model (Lasseur, 1992). Some work on CEcLSS had also been performed at that time by former Dornier and Matra, together with the Cadarache Center, in France. Some interaction was also occurring at that time between ESTEC and the Russian scientists in the field (Terskov et al, 1990) that influenced the development of MELISSA.

The MELISSA group was formed and the project started formally in 1989. The main objective of the MELISSA project was to establish a laboratory demonstration of a simple biologically closed system of plants and micro-organisms. The driving element of the MELISSA model is the reprocessing of edible biomass from waste, $CO_2$ and minerals, with the direct use of sunlight as a source for energy for biological photosynthesis. MELISSA consists of a five-compartment ecosystem (described in depth in the next sections) that allows the complete recycling of wastes generated by a crew. This MELISSA loop is based on a series of strains that were selected at the beginning of the project. Certainly, other loops could be proposed but the main purpose of the development of the MELISSA project is to have a ground demonstration set-up that will allow space engineers to develop technology to be included in future Bio-regenerative Life Support Systems (BLSS).

Several proposals were made for the installation of a BLSS in Europe, based on MELISSA results: International Biomodule (Telefax to ESTEC from Professor Gitelson, 1991; Gitelson et al, 1992) or a European Closed Ecological System CES/Hablab (Tamponnet et al, 1992; Redor et al, 1992; Tomàs et al, 1991). Finally, none of these proposals was continued and only a MELISSA Pilot Plant survived. This MELISSA Pilot Plant would be the demonstrator of the European Closed Ecological Life Support System concept. This Pilot Plant was installed in the Autonomous University of Barcelona (UAB) in 1995. Since then, it is continuously following the development activities currently supported by the MELISSA partners (some of them the originators of the model).

The MELISSA project has reached a point where all the compartments are being studied separately and some of them already well characterised. The connection between the different

compartments has already started and the complete loop will be connected in the near future. At this point, the loop will be available for experimentation. The next step for MELISSA, after comprehensive experimentation on Earth, will be its use in a Space mission.

Currently, know-how and experience on biological cultures either in microgravity (Low Earth Orbit) or transit to the Moon is very limited. It is restricted to basic research on Cell biology to know the response of the cells to the absence of gravity (Cogoli et al, 1989) and to radiation as well as to some culturing techniques with small volumes (in the order of ml) (Cogoli et al, 1999). The experiments started with the Apollo and Soyuz missions in the late 60's and early 70's carrying passive experiments -dormant seeds or spores- or more active systems such as germinating seeds or developing amphibian embryos. No biological experiments were carried out on the Moon in the Lunar Module. After Soyuz and Apollo missions, biological experiments in Space were carried out in Salyut and Mir missions as well as during Space Shuttle flights. A specific cell culturing hardware will be installed in the International Space Station (Cell Culture Unit). This limited range of experimentation did not include testing a biological system in Space conditions that could be used in a Life Support System.

This technical note reviews the envisioned MELISSA complete loop and based on this review, it identifies and lists the critical technologies that will be needed for MELISSA´s spacialisation, i.e. the installation and operation of the MELISSA loop in Space conditions. Indeed, this MELISSA´s spacialisation will depend on the scenario to be considered for the installation. Thus, reduced gravity conditions will impose requirements different from those of a Mars surface scenario. For simplification reasons this technical note discusses only a Mars based scenario, which can be considered a feasible one based on the present Space activities projections.

# 4 REVIEW OF THE COMPLETE MELISSA LOOP

## 4.1 MELISSA concept

MELISSA (Micro-Ecological Life Support Alternative) has been conceived as a micro-organisms based ecosystem, intended as a tool for understanding the behaviour of artificial ecosystems, and developing the technology for a future biological life support system for long term manned space missions.

The functional use of the MELISSA system is the recovery of oxygen and edible biomass from waste generated by the crew ($CO_2$, faeces and urea). This functionality is pursued by means of a closed loop consisting of five different compartments. Each compartment carries out part of the entire process needed to accomplish the aforementioned recovery of oxygen and edible biomass (see Figure 1).

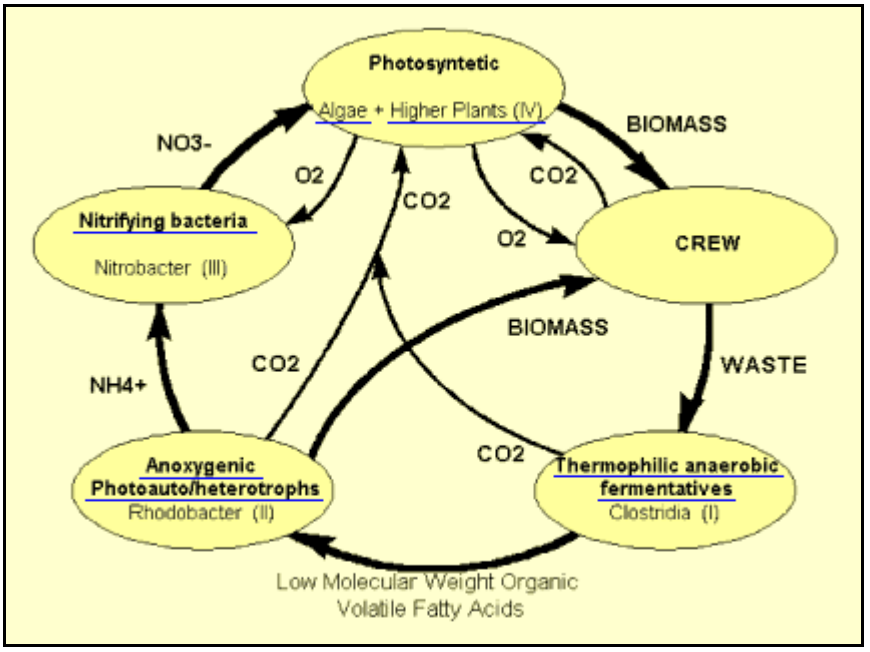


**Figure 1.-** Concept scheme of the MELISSA loop, where it is indicated the process carried out at each compartment and the main interactions between them.

In its initial design, the MELISSA loop was based on 4 axenic compartments colonised by micro-organisms: liquefying compartment, the phototroph anoxygenic compartment, the nitrifying compartment and the photosynthetic compartment, being the fifth compartment that of the crew. With this division in compartments, a micro-organism based loop is theoretically able to recover all the oxygen and edible biomass available to a crew. This loop is based in the recovery process that can be found in an "aquatic" ecosystem. However, in order to have MELISSA as a potential BLSS, it was needed to add a Higher Plant Chamber working in parallel with the alga photosynthetic compartment and also an oxidative process for the treatment of the lignified and cellulitic wastes produced by the higher plants. Including higher

plants in the loop allows simultaneously the improvement in the quality and the variety of the diet for a crew.

The complete process is therefore consisting of the following main compartments:

- The liquefying compartment, where all the wastes coming from the crew are degraded into volatile fatty acids (VFA)
- The phototrophic anoxygenic compartment, where all the volatile fatty acids are converted into mainly ammonia
- The nitrifying compartment, where all the ammonia is then transformed into nitrates
- The photosynthetic compartment consisting of the alga and the higher plant sub-compartments, where by using nitrates and $CO_2$ are producing $O_2$ and edible biomass

This edible biomass and $O_2$ is then used in the fifth compartment that generates the wastes of the loop: the crew. This process is explained only in general terms in this chapter. Other reactions which also take place in the loop and the interaction between compartments imply that the loop is not unidirectional, only. Eventually, the loop is to be a complex system with different interrelated connections between compartments aside from the main stream of the process.

## 4.2 MELISSA Pilot Plant Review

The MELISSA Pilot Plant, established at the UAB premises in Barcelona (E), is an integration plant. Project's Researchers and engineers will integrate the different compartments and elements of MELISSA in order to provide a test set-up to further proceeding with the development of the envisioned MELISSA loop.

### 4.2.1 Compartments

#### *4.2.1.1 Compartment I (Liquifying reactor)*

- *Input*. This compartment receives the wastes originated by the crew and the greenhouse as shown in **Diagram 1**. These three waste types are the following:

  1. Water from the toilet and urea
  2. Greenhouse wastes (mainly non-edible biomass)
  3. Other material coming from the daily activity of the crew: kitchen, experiments, paper, etc.

  All these wastes are grinded and mixed before being transferred to Compartment I.

- *Strains:* Common soul species are used in an anaerobic heterotrophic process. These strains allow the degradation of lipids, carbohydrates and proteins. Lignine is partially degraded with the present configuration and five technologies are being compared in order to select one that should improve this degradation rate. Among these latter, there is a Fungi compartment that is used to perform this degradation.
- *Output*. The output of the process is in the form of VFA and Ammonia in liquid phase, $CO_2$, $H_2$, $NH_3$, and VFA in the gas phase and dry weight excess in solid phase.

- *Variable measurement required at reactor.* The following variables are required to be measured to have a good characterisation of the compartment reactor:
  1. Electrical conductivity to know the salts content
  2. pH
  3. Oxidation-Reduction potential
  4. Temperature
  5. Viable Biomass in activated sludge
  6. Level of the liquid
  7. Phosphorus content
  8. Suspended solids
- *Variable measurement at input:* Composition of the material at the input (carbohydrate, lipids and protein content)
- *Variable measurement at liquid output:*
  1. Volatile Fatty Acids
  2. Ammonia
  3. Minerals
- *Variables at gas output:*
  Measurement:
  1. Volatile Fatty Acids
  2. Ammonium
  3. $CH_4$

  Main product:
  4. $CO_2$

  Small amounts of:
  5. $H_2S$

  Detection of:
  6. $H_2$
- Control loops:
  1. pH
  2. Biomass
  3. Flow
  4. Pressure
  5. Level control

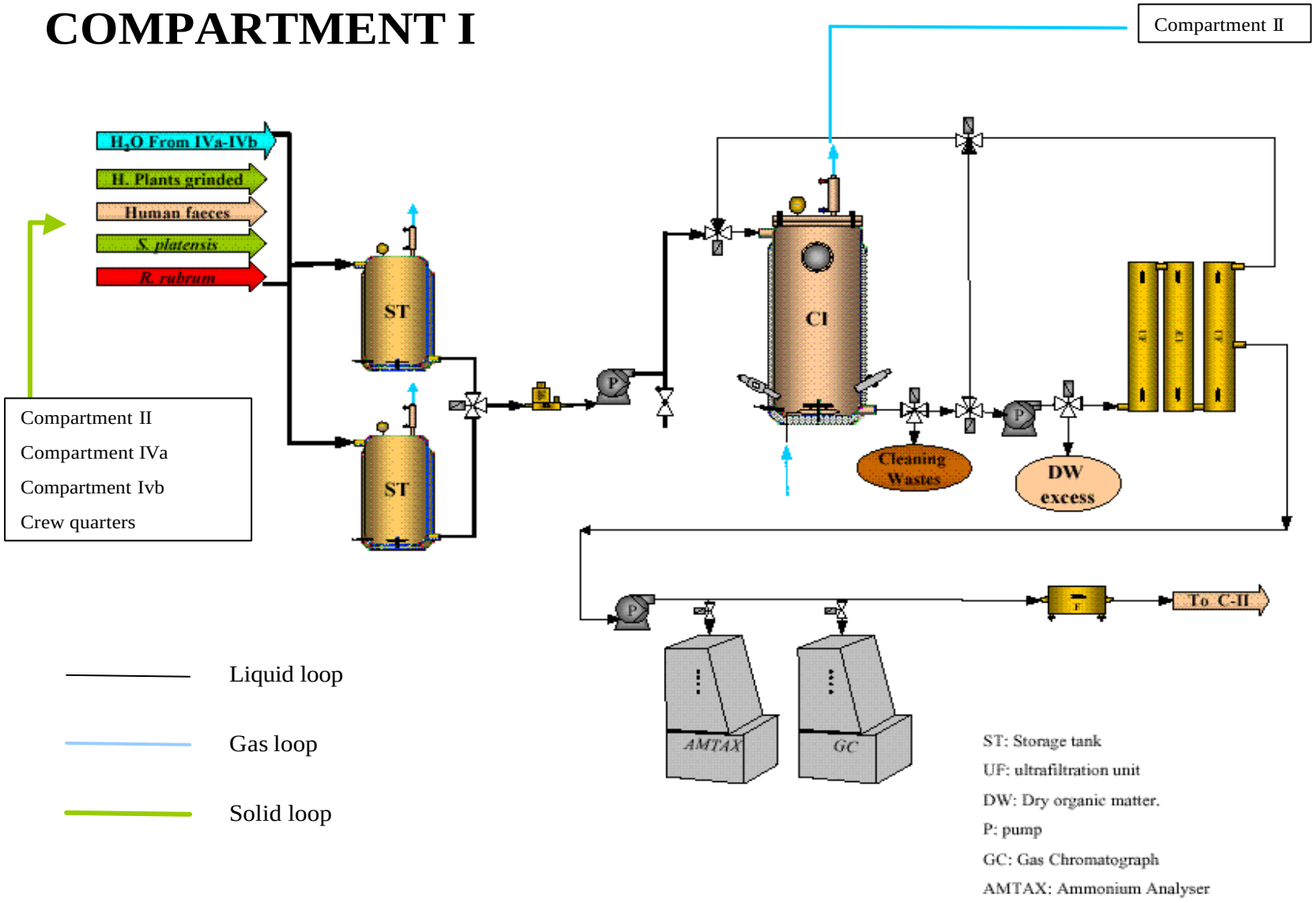


**Diagram 1.-** Schematic diagram of compartment I (Source: UAB)

#### *4.2.1.2 Compartment II (Photoheterotrophic reactor)*

- *Input*. This compartment receives the output of compartment I both in the form of gas and liquid phase as shown in **Diagram 2**.

- *Strains:* The micro-organism *Rhodospirilum rubrum* is cultured under a photoheterotrophic process. This strain allows transforming all VFAs into Ammonia.
- *Output*. The output of the process is in the form of Ammonia in liquid phase. $CO_2$ is generated in the gas phase. Dry weight excess in solid phase that can be used for food.
- *Variable measurement required at reactor*. The following variables are required to be measured to have a good characterisation of the compartment reactor:
  1. Electrical conductivity to know the salts content
  2. pH
  3. Oxidation-Reduction potential
  4. Light Intensity
  5. Temperature
  6. Level of the liquid
- *Variable measurement at input:* Composition of the flow at the input (Volatile Fatty Acids and Ammonia)
- *Variable measurement at liquid output:*
  1. Volatile Fatty Acids
  2. Ammonia
- *Variable measurement at gas output:*
  3. Volatile Fatty Acids
  4. Ammonia
  5. $CO_2$
- Control loops:
  6. Ammonium measurement loop
  7. pH regulation loop: control of pH value, variable, pH and action with a pH controller
  8. Light regulation loop: control of light intensity level appropriate for biomass, variable, light intensity and action on power supply for illumination
  9. Temperature regulation loop: control of temperature, variable, temperature and action on a temperature regulator (thermal jacket)
  10. Biomass regulation loop: Control of biomass concentration, variable, Biomass concentration and action on the input medium
  11. Liquid level regulation loop: control of liquid level to be below a certain point of the reactor, variable, height of liquid-gas interface
  12. Flow
  13. Pressure

- Alarms:
  1. Temperature exceeding range
  2. Pressure exceeding range

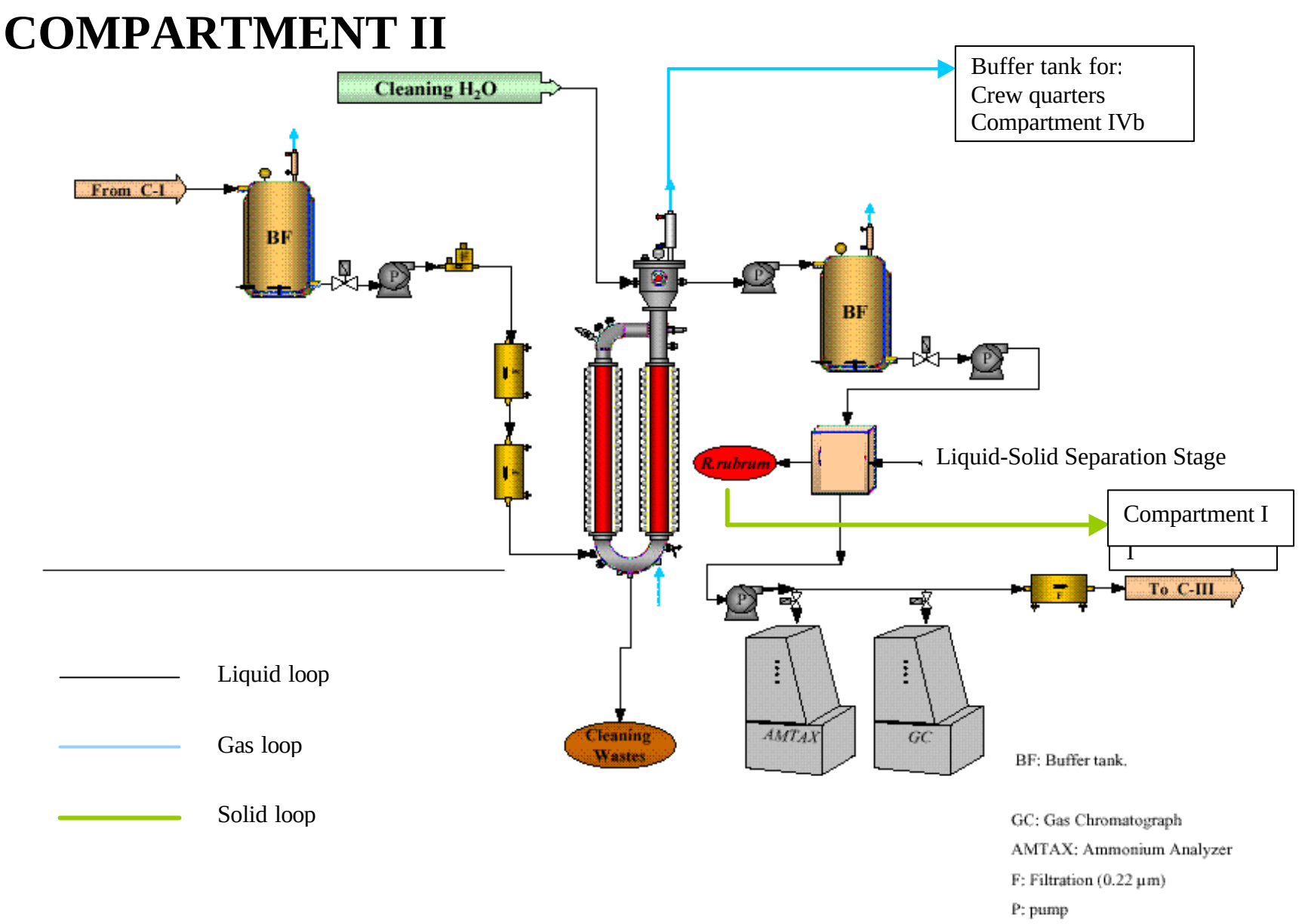


**Diagram 2.-** Schematic diagram of compartment II (Source: UAB)

#### *4.2.1.3 Compartment III (Nitrifying reactor)*

- *Input*. This compartment receives the output of compartment II in liquid phase and from a buffer tank in gas phase as it can be seen in **Diagram 3**.
- *Strains:* The process in this compartment is carried out with a co-culture of *Nitrosomonas europae* and *Nitrobacter winogradskyi*. This compartment transforms mainly the Ammonia into Nitrates. This strain needs around 6 months to reach nominal operating conditions. It is an extremely low growing culture.
- *Output*. The output of the process is in the form of Nitrate in liquid phase. $CO_2$ is consumed in the gas phase.
- *Variable measurement required at reactor.* The following variables need to be measured to have a good characterisation of the compartment reactor:
  1. Electrical conductivity to know the salts content
  2. pH
  3. $pO_2$ measurement
  4. Temperature
  5. Level of the liquid
  6. Viable biomass measurement
- *Variable measurement at input:* Composition of the flow at the input (Ammonia)
- *Variable measurement at liquid output:*
  1. Ammonia
  2. $NO_3$
  3. $NO_2$
  4. Minerals
- *Variables at input*:
  Measurement:
  1. $CO_2$
  2. Ammonia
  3. Volatile fatty acids

  Main product:
  1. $O_2$
- *Variable measurement at gas output:*
  1. Ammonia
  2. $CO_2$
- Control loops:
  1. Temperature
  2. Pressure
  3. Biomass content: objective is stable biomass content, variable measured: viable biomass and action biomass release
  4. Flow
  5. pH
  6. Level control

- Alarms:
  1. Temperature exceeding range
  2. Pressure exceeding range
  3. Biomass exceeding range

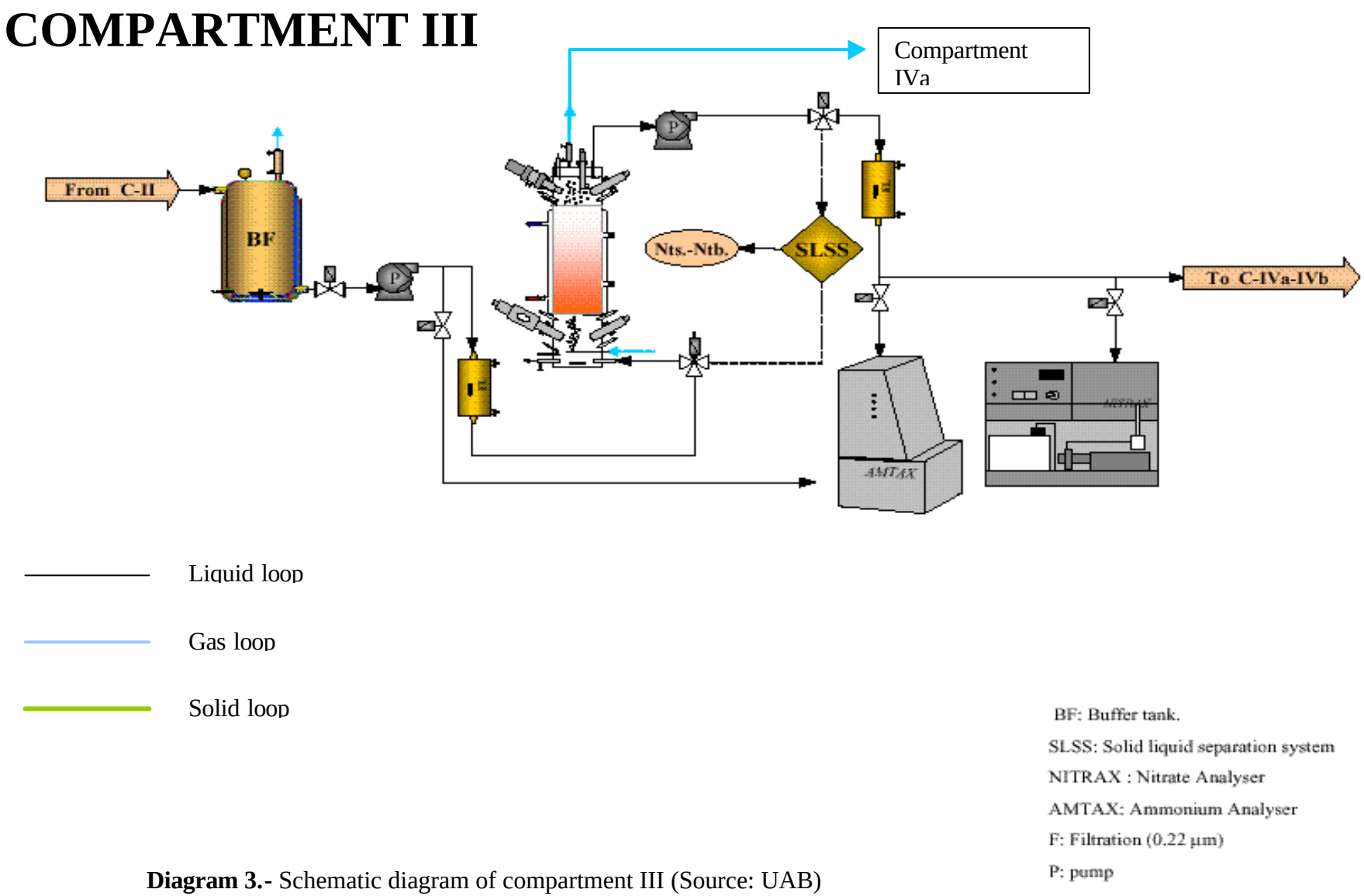


**Diagram 3.-** Schematic diagram of compartment III (Source: UAB)

#### *4.2.1.4 Compartment IVa (Photosynthetic Compartment)*

- *Input*. This compartment receives the output of compartment III in liquid phase and from a buffer tank in gas phase as it can be seen in **Diagram 4**.
- *Strains:* The process in this compartment is carried out with *Arthrospira platensis*, photautothroph microscopic algae. This compartment transforms mainly Nitrates and $O_2$ into edible biomass and $O_2$.
- *Output*. The output of the process is in the form of $O_2$ in gas phase and edible biomass in solid phase.
- *Variable measurement required at reactor*. The following variables need to be measured to have a good characterisation of the compartment reactor:
  1. Nitrate concentration
  2. Light intensity
  3. Electrical conductivity to have an idea on salts content
  4. pH
  5. Oxidation-Reduction potential
  6. Temperature
  7. Level of the liquid
  8. Viable biomass measurement
- *Variable measurement at input:* Composition of the flow at the input (Nitrate and low levels of Nitrite)
- *Variable measurement at gas output:*
  1. $O_2$
  2. $CO_2$
- Control loops:
  1. Temperature
  2. Pressure
  3. Biomass content: objective is stable biomass content, variable measured: viable biomass and action biomass release
  4. Flow
  5. Level control
- Alarms:
  1. Temperature
  2. Light regulation
  3. Biomass regulation
  4. Gas flow and pressure regulation
  5. Flow

#### *4.2.1.5 Compartment IVb (Higher Plants Chamber)*

- *Input.* This compartment receives the output of compartment III and the urea of the crew compartment in liquid phase, $CO_2$ from a buffer tank and from the crew compartment in gas phase as it can be seen in **Diagram 4**.
- *Plants:* The actual composition of the crop is still to be decided. Possible candidates are: Broccoli, Beet, Bean, Cauliflower, Carrot, Cucumber, Herbs, Kale, Lettuce, Onion, Green Onion, Peppers, Peanut, Potato, Rice, Sweet Potato, Swiss Chard, Soybean, Spinach, Tomato, Wheat, Alfalfa, Cabbage, Chilli Peppers, Mushrooms, Snow Peas and Squash. These plants will be used to convert $CO_2$ into biomass and to produce $O_2$.
  From the above list, 8 plants will be selected primarily to obtain at then a selection of 3. These 3 will be used at the Pilot Plant and could most likely be: Lettuce, Beet and Wheat with a percentage of 30% each in the final input for compartment I. The remaining 10% will be paper. These three plants are selected for different reasons: Lettuce represents a plant with high leaf surface, Beet represents a plant with a lot of root volume and Wheat is a plant with a lot of non-edible material.
- *Output.* The output of the process is transpired water in liquid phase, $O_2$ in gas phase and edible biomass in solid phase.
- *Variable measurement required at reactor.* The following variables need to be measured to have a good characterisation of the compartment reactor:
  1. $CO_2$ level
  2. $O_2$ level
  3. Light intensity
  4. Nutrient composition
  5. Air humidity
  6. Temperature
  7. Level of the liquid at the nutrient tank
  8. Pressure
- *Variable measurement at input:* Composition of the flow at the input (Nutrients composition) and at gas input:
  1. $O_2$
  2. $CO_2$
- *Variable measurement at output: Composition of nutrients in the liquid phase and at gas output:*
  1. $O_2$
  2. $CO_2$
- Control loops, which consider those loops for controlling the chamber environment and the MELISSA loop main state variables:
  1. Temperature
  2. Air Humidity
  3. Pressure
  4. Nutrient delivery
  5. Light intensity
  6. pH nutrients
  7. Flow
  8. Level control
- Alarms:
  1. Contaminant detection

2. Temperature
3. Light regulation
4. Nutrient delivery
5. Gas flow and pressure regulation

# COMPARTMENTS IVa-IVb

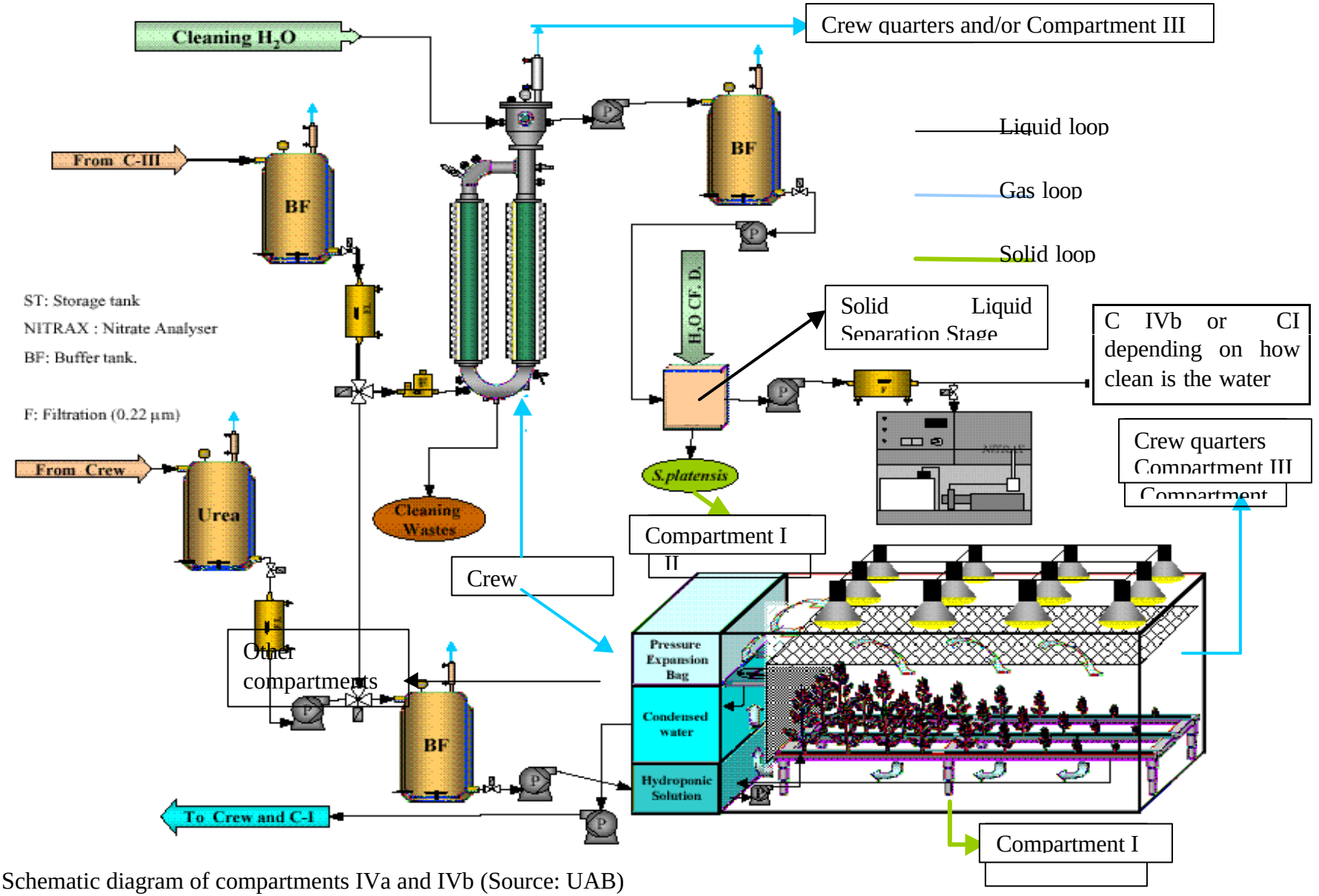


**Diagram 4.-** Schematic diagram of compartments IVa and IVb (Source: UAB)

#### 4.2.1.6 First medium-term objective of the Pilot Plant

In a first stage, the medium-term objective of the Pilot Plant is to connect an approximation of the MELISSA loop able to recycle:

- all the microbial biomass that will be generated within the loop,
- 20% of the non-edible plant biomass,
- all the faeces and urine of one person (this will be simulated by 3 rodents that eat food from outside the loop), and
- all the $CO_2$ generated by these three rodents (which will model a human breath air from the loop).

#### 4.2.1.7 Main characteristics of the Compartments

The data that is indicated in this table describes the main characteristics of the cultures in the MELISSA envisioned Pilot Plant. The data is approximate.

| *Compartment* | *Colonisation time*[1] | *Minimum Retention time*[2] | *Bioreactor or chamber volume* | |
|---|---|---|---|---|
| ***Compartment I*** | 1 month | 22 days | 300 l | |
| ***Compartment II*** | 2 days | 7 hours | 70 l | |
| ***Compartment III*** | 6 months | 17 hours | 4 l | |
| ***Compartment IVa*** | 2 days | 7 hours | 77 l | |
| ***Compartment IVb*** [3] | TBD | TBD | *Chamber Volume* TBD | *Liquid Volume* TBD |

### 4.2.2 MELISSA Pilot Plant analysis

The analysis of the envisioned Pilot Plant at UAB has been carried out by reviewing documentation (Cabello et al, 1998; Cabello et al, 2000; Demey et al, 2000; Eckhard et al, 2000; Pérez et al, 1996; Pérez et al, 1997; Poughon et al, 2000; Vernerey et al, 1998), and discussions with several MELISSA partners involved in the design of the various compartments.

The detailed analysis of the liquid, gas and solid loops have lead to the identification of critical issues that are referred to as CT#.

#### 4.2.2.1 Liquid Loop

This section describes the liquid loop of the Pilot Plant, following **Diagram 5**.

**Compartment I:**

The input for this compartment consists of:

[1] Time needed to have an stabilised culture
[2] Minimum retention time required to carry out the recycling function by the microorganism with the present configuration of the Pilot Plant
[3] This information is not available

- Water from compartment IVa and IVb. This water will be mainly that from transpiration of plants in Compartment IVb (Higher Plant Compartment) in a 95% and from liquid-solid separation stages.
- Non consumed plants grind
- Human faeces
- Arthrospira,
- Paper and
- Rhodospirilum

Some questions arise at this point:

**CT 1: Input to Compartment I definition. How are the inputs to be introduced in the bioreactor of Compartment I, in continuous or batch mode? This will imply a storage strategy. Up to date, the strategy selected is to grind the waste and freeze it in packages to be stored and introduced in Compartment I as it is needed.**

The type of process taking place at the bioreactor (thermophilic anaerobic bacteria) implies a high temperature and low oxygen concentration. The type of oxygen sensor used for the low levels demanded is a redox. The bioreactor is presently a stirred tank, but its design is under discussion.

**CT 2: Compartment I volume reduction. The process is slow, thus a big bioreactor to allow big resident times is needed. Is the design going to be improved to have a smaller bioreactor?**

Part of the output of the bioreactor will be in form of gas phase. At this output, filtering and analysis of the exiting gas will be required (gas chromatography). Such analysis should also refer to trace contaminants, as the analysis of these contaminants will also be necessary.

The exiting liquid will be filtered and measured with chromatography methods. It will contain mainly VFA and ammonia.

**CT 3: Clogging monitoring. Systems to monitor clogging and maintenance strategies will be required for the filtration of the liquid exiting Compartment I.**

**CT 4: Alternatives to chromatography. Chromatography methods will be used. This technology is not presently developed to be portable and lightweight to be used in a space mission. It also requires a lot of consumables and maintenance. This could impose a limit for the automatic operation of MELISSA in space. It is possible that for each substance a specific sensor could be developed.**

**CT 5: Gas Liquid Separation System. Separation of gas-liquid phases in reduced gravity in a stirred tank, if needed.**

There is part of output that is not recycled at this moment:

- Cleaning wastes, but these wastes are only produced between end of operation and restart

- Components that are not digested by compartment I (these are the lignine, etc.). There is presently an effort to find an alternative to allow the complete degradation of these components. Five technologies are being compared, among which degradation using fungi is being studied.

**Compartment II:**

The input to Compartment II is the liquid coming from the output of the Compartment I, mainly VFA and ammonia. If Compartment I is to be used in fed-batch, this will imply a buffer at the entrance of this compartment.

The incoming liquid needs to be sterilised. Filtering is currently the method to be used since it is deemed that no pathogens will be provided by compartment I in the Pilot Plant based on the fact that:

- The high temperature of Compartment I allows minimising the transmission of many types of viruses (they are eliminated under such extreme conditions)
- Pathogens are not likely surviving compartment I, due to enzymes that would destroy them (protease)

However, these considerations can be challenged because:

- There are viruses that will survive after dismantling, as they are able to assemble again
- The minimum amount of proteases to eliminate all possible prions is not ensured.

**CT 6: Sterilisation Methods. Sterilisation methods that could be flown will have to be developed. Autoclave technology does not seem to have the characteristics needed for this purpose.**

**CT 7: Contamination detection methods should be envisioned. Some of them might need some development work (pathogen detection). Thin films of bioactive material might be required at the different connections of the compartments (biosensors).**

**CT 8: Surface Microbe Detection. Sensors to monitor and control surface microbes are needed in MELISSA.**

The process followed in Compartment II is anaerobic, so the oxygen sensor has to be working in low oxygen levels.

**CT 9: Consumable reduction. In many instrumentation of the envisioned MELISSA loop Pilot Plant, a considerable amount of consumables are foreseen. They should be minimised as well as the maintenance workload.**

The bioreactor has to have a specific shape to allow a good illumination/volume ratio. On the opposite, the stirring of the reactor has to be ensured.

**CT 10: Design of bioreactor. Optimisation of the illumination/volume ratio versus stirring of the bioreactor.**

The direct application of light bulbs or leds to the main chamber of the bioreactor has strong impact in the thermal conditioning of the bioreactor. This is taken into account in the thermal regulation of the culture. The thermal jacket used to regulate temperature will most likely be located between the light source and the culture. Therefore, this water jacket shall be as transparent as possible to the light at a given wavelength range.

**CT 11: Photobioreactor design. The thermal jacket for this reactor will be between the light source and the culture. For long working periods, the water used for thermal regulation will have to be transparent enough for the selected wavelength range. Means to maintain the cleanliness of the water shall be ensured.**

There is a harvesting system at the output of the liquid from this compartment. This is accompanied by previous buffering before the separation of the *Rhodospirillum rubrum* from the liquid output. This compartment is a biomass-producing unit and therefore the separation method development has to be compliant with a high production rate process.

**CT 12: Solid-Liquid separation methods should be developed to be compliant with a high separation rate and possibly a continuous operation.**

The liquid output of compartment II shall only include ammonia and limited amount of VFA and trace contaminants.

**Compartment III:**

The input for this compartment will be the liquid phase being obtained in compartment II that should only contain ammonia. A buffering tank is required depending on the type of process chosen for the previous compartments (batch or continuous). The input in the gas phase is oxygen.

An important issue in this compartment is the long colonisation time for the culture. This is ranging from 3 to 6 months to get the culture working (*Nitrosomonas europeae, Nitrobacter winogradsky*).

**CT 13: Colonisation time reduction in Compartment III. Is this colonisation time to be reduced or will it be more or less kept of the same magnitude due to the type of strain used? It will largely impact on the mode of operation of the loop.**

Sterility conditions must as well be ensured at the input of the compartment to avoid contamination spreading. In the MELISSA Pilot Plant this is achieved with filtration.

In compartment's III bioreactor, the biomass is immobilised on to polystyrene beads. The liquid output of the bioreactor must contain mainly nitrate, but it also contains some biomass released from the beads in several manners:

- As a result of biomass control, by releasing the in-excess biomass for the process.
- As a result of the release of cells from the beads when dying . They typically die in the layers closer to the beads, thus releasing as well living cells.

- As a result of the cell division on outer layers.

This biomass has to be filtered by a solid-liquid separation system to avoid any presence of these bacteria downstream.

**CT 14: <u>Compartment III biomass recycling.</u> How is this filtered biomass to be treated and recycled? It is most likely going to be recycled in Compartment I.**

The following technologies might become critical and need some development effort:

**CT 15: <u>Compartment III releasing system.</u> The present method to release cells from the beads is a counterflow. This method releases the most external layers and concentrates the beads on one side of the bioreactor. This could be positive for a low gravity scenario, but it gives problems of excessive accumulation of biomass on one part of the bioreactor. It would be interesting to develop a releasing method that could be distributed in several parts of the bioreactor and acting depending on the amount of living cells measured locally. Methods like ultrasound release without disruption could be interesting.**

**CT 16: <u>On-line viable biomass monitoring.</u> Measurement of the viable immobilised biomass is a must to have a good control of the performance of the reactor. Systems to measure locally viable biomass are already available, but effort should be made to make this equipment lightweight and with lower biomass detection threshold.**

At this compartment's liquid output a very sensitive ammonia and nitrite measurement, with high resolution, is required. Both substances must not be present. Therefore, the system should be able to quickly react to that if a problem in the process is detected.

**CT 17: <u>Ammonia and Nitrate measurement.</u> A high resolution, sensitivity system will be required for the output of compartment III in order to measure low levels of ammonia and nitrite. For nitrite, this would be 0-0.6 PPM.**

Due to the pH control system taking place in this bioreactor, there is a carbon shift to carbonates. This could interfere with the measurement of ammonia.

**<u>Compartment IVa-IVb:</u>**

Both compartments are fed with nitrate from compartment III. In the case of the plant chamber (IVb), there will also be a urea feeding (urea coming from crew) that is transformed into $NH_4^+$ and $CO_2$. But this is an option, because urea could be treated by the first compartment in future designs.

**CT 18: <u>Urea treatment.</u> Is urea to feed directly after filtration the growth chamber, or will it be processed by Compartment I before? Presently, it is going to be processed in compartment I or in an added compartment.**

At the input of both compartments, there will be a buffer tank to allow a correct local control of each of the compartments. This is also applying to the buffer tanks of the other

compartments. The buffer tanks are also envisioned when one of the compartments has to be working in batch.

If there is some leak of ammonia, this could be consumed in low level by Arthrospira (Compartment IVa). The bioreactor is a fluidised bed that needs light as in Compartment II. Thus, the same requirements for designing the bioreactor appear in this compartment. This means that the temperature control will be an important issue. .

The output of this compartment will go to compartment I after harvesting of Arthrospira in a specific harvester, which will require a specific development effort due to the type of biomass. Arthrospìra contains a lot of water with a high viscous value. This might imply the implementation of different harvesting strategies from those used in Compartment II.

**CT 19: Harvesting system for Arthrospira. To be compliant specifically for Arthrospira conditions at the output.**

The Higher Plants compartment will also require some light, but also air circulation strategies. The $CO_2$ and $O_2$ partial pressure control will be very important in this case, as well as the water transpired collection. The water obtained from transpiration of plants will be used for potable water supply for the crew. The water obtained from the harvesting of Arthrospira will be devoted to water supply for plants or to dilute the input in Compartment I.

It will be important to decide how to connect this compartment and the Crew Compartment. Two options are possible:

- The connection implies two separate compartments, thus the atmosphere in the plant chamber will be different from that in the crew quarters. The plant atmosphere could then be $CO_2$ enriched (the crew is outside MELISSA).
- Atmosphere sharing between crew quarters and plant chamber (the crew is inside MELISSA).

**CT 20: Connection Crew-Higher Plant Compartment. Discussion of the connection crew-MELISSA approach is needed. This largely impacts the type of technology: one would be more insisting in atmosphere regulation to avoid hazardous levels for plants and for crew and the other would be more concentrated in filtering strategies. It seems more likely to choose sharing the atmosphere for manned operation to ease the plants harvesting operation, as well as for psychological issues. But this will depend directly on the Greenhouse strategy taken at the end.**

There will be a need for a precise pressure measurement in order to allow a good pressure control inside the loop, but more specifically inside the bioreactors. Levels of pressure below the environmental pressure should be avoided, because they normally lead to problems of contamination. Overpressure levels could also produce problems of undesired leakage to the environment.

The liquid pressure will be the same (plus hydrostatic pressure) than pressure of gasses inside the reactor. In fact, the gas pressure will be used to control the liquid pressure. For this reason, the gas pressure control is also addressed in this chapter.

Partial pressure differences in any gas will equalise by a gas flow. For instance, two free water surfaces at different temperatures will create different water vapour partial pressures, The hottest surface will be a gas source and the coldest will act as a gas drain. The water vapour diffusion originated by this partial pressure gradient will tend to equalise the pressure difference. The way to avoid these natural gradients is by applying forced gas circulation at high rate. However, this forced circulation accelerates also the process of gas transfer and modifies the whole loop kinetics.

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In the ideal loop, reactors should be open to the whole LSS atmosphere as much as possible, exchanging $CO_2$ and $O_2$ with the closed atmosphere where crew and plants are breathing.

**CT 21: Pressure management. Study of the pressure regulation of the entire loop, for each gas and for each bioreactor. The pressure management in each bioreactor will depend mainly on its design and on the design of the gas loop connections between reactors. Thus, pressure regulation in the Arthrospira bioreactor, where the walls are flexible, will differ from pressure regulation in Compartment I.**

The flows in the loop have rheologic characteristics that are different not only at different points of the loop, but at also at these points in different times of the process. This causes problems when using pumps and accessories with a working range that does not cover all the rheologic characteristics that could be found. The pH level can also change submitting the accessories and pumps to strong working conditions.

Ideally, the overall system dynamics adjustment could allow having the different reactors open to the LSS general atmosphere (possibly, some atmospheric air bubbling system to accelerate processes plus some filters to prevent contamination). The gas loop concept presented in the form of gas lines interconnecting compartments would then be substituted by an overall atmosphere reservoir where all the compartments vent and breath at an adjustable ratio.

**CT 22: Pump working range. There is a need for pumps with working conditions that are acceptable for the widest range of rheologic characteristics and pH of the flows in MELISSA. Combined sensors of flow and viscosity will be needed.**

**CT 23: Fibre degradation. The fibber degradation in compartment I is not complete and study on alternatives or complementary processes is needed.**

The pH regulation is also an important issue in the loop. The implemented regulation strategy will determine mainly the criticalities of the loop. There are two main envisaged strategies for this regulation:

- Using buffers with acid and basic products (for example, Chlorhydric acid and Sodium hydroxide) to regulate the pH. This strategy would lead to a salt accumulation in the loop. Certainly, an additional loop to fill the buffers by recycling the salts could be included. However, this approach may result in a loop's if these buffers could not be regenerated fast enough, preventing eventually a proper e pH regulation function.
- Use of substances of the own MELISSA loop to regulate the pH. For example, $CO_2$ could be used for that purpose.

**CT 24: pH regulation. Study on the pH regulation strategies: MELISSA products or external buffering. In case that the MELISSA products strategy is taken, important development work is envisioned.**

**CT 25: <u>Buffering strategy.</u> A buffering strategy will be vital to obtain a MELISSA loop compliant with the requirements of dependability. Buffer for emergency problems, to damp oscillations in flow or pressure and to store different substances.**

Filtration of substances will be a very important activity. It will be important to monitor clogging and to foresee activities to restore the normal conditions in the filter. However, some filters will be based in adsorption means and therefore, will require desorption procedures to place the substances back on the loop. This is to be considered either as a consumable or as an automated process.

Taking into account that the sensors will be working for long periods and the conditions inside the different bioreactors, cleaning means to maintain the sensor probes in correct conditions will be needed. These cleaning measures will have to be compliant with the sterility requirements not to affect the culture.

**CT 26: <u>Cleaning measures design.</u> Cleaning measures are required to maintain the probes of the sensors inside the bioreactor under correct conditions.**

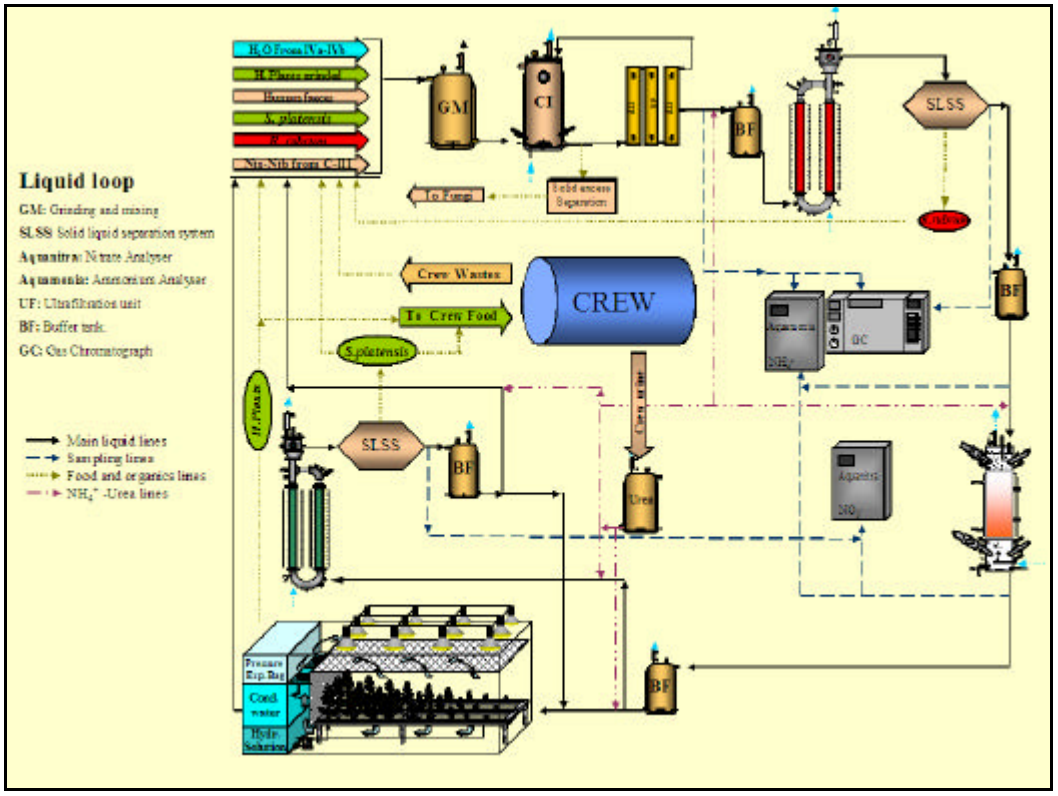



**Diagram 5.-** Liquid loop of the MELISSA Pilot Plant (Source: UAB)

### *4.2.2.2 Gas Loop*

This section describes the gas loop of the Pilot Plant following the Diagram 6.

**Compartment I:**

In the gaseous output of this compartment, there will be a mixture of gases including VFA and different amounts of $CO_2$ depending on the operational conditions.

There will be a need for a certain amount of gas at the input for the start-up of the bioreactor, but the bioreactor will need no oxygen supply.

**Compartment II:**

VFA is included in the gas that is mainly $CO_2$ at the input and low levels of VFA should be detected in the gas at the output. The detection of low levels of VFA implies the use of gas chromatography that will need some development to become portable.

**Compartment III:**

Two inputs are to be reaching this compartment: A carbon source coming from the a buffer that collects the $CO_2$ coming from compartment I and air (with variable $O_2$ partial pressure) coming from the Higher Plant Compartment (IV b). The output of this compartment is filtered and $CO_2$ is going to compartment IVa.

**Compartment IV:**

The $CO_2$ originated in the crew compartment is going partly to the Arthrospira bioreactor and partly to the Higher Plants Compartment. The HPC is also using part of the $CO_2$ coming from the buffer tank. The output of both compartments will be Oxygen that will be consumed in the Crew compartment and Compartment III.

**CT 27: Trace Gas and contaminants measurement. Trace gaseous and vapour contaminants are important measurements that should be incorporated. In addition, high resolution measurement systems are required.**

**CT 28: Airborne microbe monitoring. The detection of airborne microbes is needed in MELISSA. A system with enough sensitivity and fast measurement should be developed.**

**General discussions:**

Pumps are used in the gas loop which imply upstream pressure fluctuations. This leads to pressure changes in the bioreactors that might produce contamination problems. Along the loop, different pressure levels will have to be maintained.

**CT 29: Global pressure regulation. There should be a study related to the different levels of pressure that will exist in practice along the gas loop and means to maintain stable situation shall be envisioned.**

As already discussed when analysing the pressure regulation in the liquid loop it would be desirable to have all the reactors connected to the LSS atmosphere (including the needed filters for safety) and bubbling atmospheric air into the reactor. A possible exception can be the connection between Compartments 1 and 2.

#### *4.2.2.3 Solid Loop*

This section describes the gas loop of the Pilot Plant following the **Diagram 7**. The waste collection system will be located in the crew compartment. Urine and faeces will be obtained separately. Means to effectively separate liquid from solid phases will be needed.

**CT 30: <u>Human waste separation.</u> Study on processes to separate liquid from solid phases in human wastes will be needed at some point. Will it be a drying process, where the faeces will be frozen or dried?**

**<u>Compartment I:</u>**

Compartment I will get the solid waste from non-edible pieces of plants that are originated when food is produced. This waste will then be treated, depending mainly as to how compartment I is working (batch or continuous).

**CT 31: <u>Non-edible material preparation.</u> Study on methods of preparation of the solid wastes from food production.**

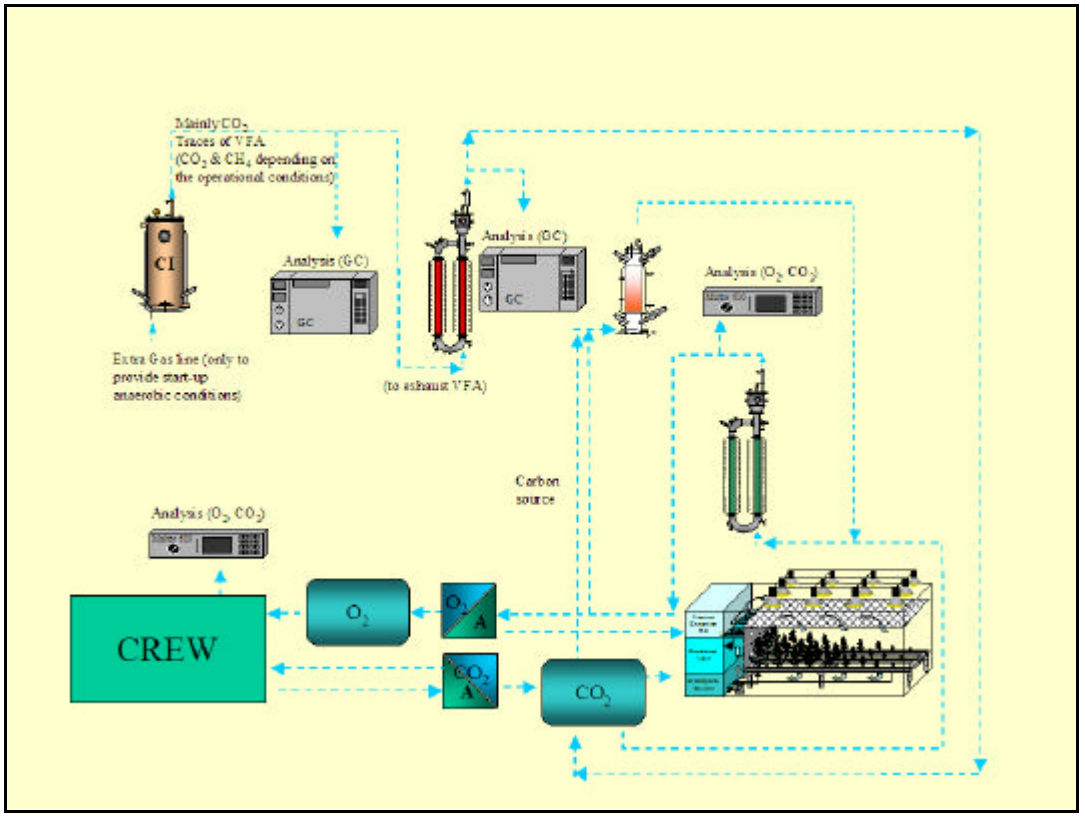



**Diagram 6.**-Gas loop of the MELISSA Pilot Plant

**<u>Compartment II:</u>**

The solids obtained in this compartment are the excess Rhodospirilum that will be used to feed again compartment I. There are two options: first, o package and put them into compartment I, and second, to feed continuously compartment I directly after harvesting.

**Compartment IVa:**

The same two options as in compartment II apply to this compartment. Arthrospira can be packaged or used in a continuous mode to feed compartment I.

**Compartment IVb:**

This compartment will produce food and the wastes from this food production will be used as indicated for compartment I.

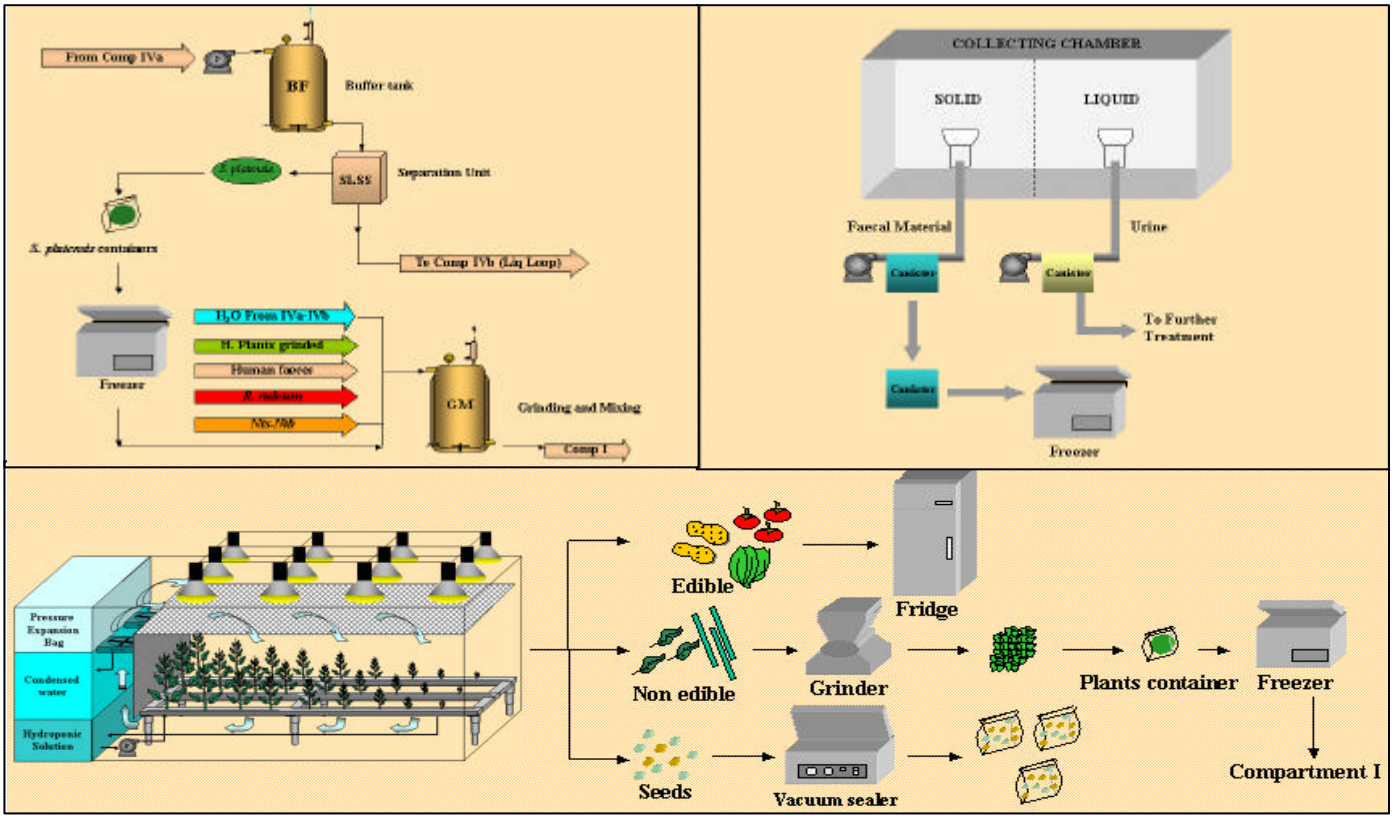



**Diagram 7.-** Solid loop of the MELISSA Pilot Plant

**CT 32: Solid Treatment. As general consideration, all the solid treatment processes need to be automated.**

# 5 SCENARIOS

The main hypothesis in order to list critical technologies of the MELISSA loop is to consider it as a Life Support System on the Mars surface. The present envisioned MELISSA Pilot Plant will likely differ to the MELISSA loop on the Mars surface. Critical technologies and other relevant issues will have to be developed and solved. Part of these critical technologies are related to the loop's intrinsic characteristics, but others will be directly related to the conditions on a hypothetical mission to Mars. It is therefore important to describe the mission scenario under which MELISSA would be working.

## 5.1 Mars outpost

### 5.1.1 Mission Scenarios

Both NASA and ESA are producing mission reference scenarios for future exploration of Mars surface. NASA has recently described three mission scenarios (Hanford et al, 2001):

- Independent exploration missions: Mars Dual Lander Architecture
- Concentrated exploration mission: Mars Split Mission Architecture
- Extended presence: Evolved Mars Base

We will only consider for analysis the two first mission scenarios, since the evolved Mars base is a mission that needs the previous fulfilment of the other scenarios. ESA has recently as well produced concept designs of the possible mission scenarios (HUMEX, Technical Note 1, Technical Note 3 and Technical Note 5). We indicate only those referring to Mars (scenario no. 1 corresponds to a Lunar base):

- Scenario 2: The 1000 day Mars mission with long term stay on Mars
- Scenario 3: The 500 day Mars mission with short term stay on Mars

For the purpose of this document, we will consider two mission concepts from NASA (Dual Lander and Split Missions) and the two mission concepts indicated by ESA (the 1000 and 500 day missions).

The exploration of Mars can provide interesting advances in displicines as geology, mineralogy, atmospheric research and exobiology. The main goal of such a mission is the understanding of the planetary formation and evolution of processes including, if possible, the evolution of life. Mars is the most similar planet to Earth and the study of its atmosphere can help understanding atmosphere on Earth. The Martian orography suggests the existence of huge amounts of water around 2 to 3 billion of years. There is speculation about the existence of certain oases or refuges, where there could be specific conditions that could permit life forms (endolithic lichens, bacteria on water layers below surface, etc.). Therefore, search for life forms, even in fossil form, is one of the main objectives of a Martian mission.

The three phases that will take place in the exploration of Mars are:

- Robotic exploration, that has already started,
- Robotic facilities for Local Resources utilisation and
- Manned Exploration Mission.

For this document only manned missions are of interest for BLSS's.

#### *5.1.1.1 NASA Missions*

The Mars Dual Lander Mission architecture employs three vehicles: a Mars Transit Vehicle, a Surface Habitat Lander (with inflatable structure) and a Descent/Ascent Lander. Both the Surface Habitat Lander and the Descent/Ascent Lander will have a common descent stage (this explains the Mission´s denomination). The Mars Transit Vehicle is used for the trip and the Surface Habitat Lander contains an inflatable structure, where the habitable volume can be expanded once on Martian surface. This Surface Habitat Lander is piloted automatically from Martian orbit to Martian surface. The second lander is the Descent/Ascent Lander that carries six crewmembers to the surface. Since this mission is conceived to be part of a multisite exploration programme, the site is not fixed.

The trip to Mars will take 180 days for the crew. The Dual Landers are sent before the crew to Mars and are positioned on Mars orbit waiting for the crew. When the crew arrives to Martian surface, the Habitat Lander will already be in working conditions and the crew will have 30 days to acclimatise. The mission duration will be 600 days. During the mission, the Transit Vehicle will be untended in Low Mars Orbit. After the mission, the crew ascents to low orbit and transfers to the Transit Vehicle and travels again during 180 days to reach the Earth.

| ***NASA Mars Dual Lander Mission*** | |
|---|---|
| **Crew size** | 6 crewmembers |
| **Transit Duration** | 180 days each way |
| **Surface Mission Duration** | 600 days |
| **Total Mission Duration** | 960 days |
| **Facilities on Martian Surface** | Surface Habitat Lander (with inflatable structure) and Descent/Ascent Lander |
| **Transit Vehicle** | Untended during mission |
| **Landing sites** | Multisite exploration |

**Table 1.-** Summary of characteristics of NASA Dual Lander Mission.

The Dual Lander Mission Power Requirements are given in Table 2.

| ***Mars Transit Vehicle*** | ***Power [kWe]*** |
|---|---|
| While the Crew is awake ("Day") | 15 |
| While the Crew is asleep ("Night") | 15 |
| ***Mars Descent/Ascent Lander*** | ***Power [kWe]*** |
| Available Power during landing | 4 |
| During Daylight | 8.5 |
| During Night | 5.5 |
| ***Surface Habitat Lander*** | ***Power [kWe]*** |
| During Daylight with Clear Weather | 18 |
| During Daylight with a Dust Storm (contingency) | 7.4 |
| During Night | 9 |

**Table 2.-** Power Generation for the Mars Dual Lander Mission.

The Concentrated Exploration Mission (Mars Split Mission Architecture) is consisting of three missions, all landing at the same location on Mars in order to build an infrastructure that will provide a safe site. For each mission, two flights preposition equipment around and on Mars before the crew transit. A cargo flight lands on Mars carrying a Mars Ascent Vehicle, an ISRU (In-Situ Resources Utilisation) plant and an Inflatable habitat. A second flight places an Earth Return Vehicle in a stable Martian orbit. At the next Mars transfer, a Surface Habitat Lander transports the crew from Earth to the surface of Mars, rendezvousing on the surface with the pre-positioned surface facilities. During the same transfer, the two flights with the pre-positioned facilities for the next crew also transit to Mars and arrive while the first crew is performing surface activities. This configuration allows having redundant facilities in case there is a problem with the first crew. The trip will take 180 days each sense, as in the previous configuration and the surface mission will last for 600 days. The crew departs from Mars with the Mars Ascent Vehicle and turns back with the Earth Return Vehicle. The second crew departs while the first is on its way back, with two more parallel flights. The cargo flights could carry rovers instead of inflatable habitat if there is no previous problem with the installed facilities.

| *NASA Concentrated Exploration Mission* | |
|---|---|
| Crew size | 6 crewmembers |
| Transit Duration | 180 days each way |
| Surface Mission Duration | 600 days |
| Total Mission Duration | 960 days |
| Facilities on Martian Surface | Mars Ascent Vehicle, ISRU plant and Inflatable habitat and Surface Habitat Lander (the three first facilities are duplicated at arrival of the crew to Mars) |
| Transit Vehicle | Untended |
| Landing sites | Single site |

**Table 3.-** Summary of characteristics of NASA Concentrated Exploration Mission.

No data on power consumption is provided for this scenario. We will consider a power consumption of a one Split mission (that is one crew) in the order of magnitude of one Dual Lander Mission. No reference as to the number of crewmembers is neither given nor the number of crewmembers staying on orbit, but we will consider a crew of 6 people and no crewmember on orbit as indicated in the Dual Lander mission.

#### *5.1.1.2 ESA Missions*

The crew size that the Agency considers for a Mars Mission is 6 for both scenarios designed (500 and 1000 days missions). ESA considers these missions as an international endeavour with the participation of USA, Russia, Japan and Europe.

The scenario 2 (1000 day mission) consists of a transit to Mars with a duration of 259 days from Earth to Mars and 301 days back, and a stay on Mars surface of 525 days. The spacecraft features four stages:

- an injection stage (4 bundled Ariane 5 central Stage provided by ESA),
- an interplanetary parent ship (for the two interplanetary flights to and from Mars, supplied by Russia),

- a lander stage (provided by NASA) and
- an ascent stage (to return to the parent ship, waiting in Mars orbit and supplied by Japan).

Four astronauts will descend to Martian surface and 2 will be kept on orbit during the 525 days in order to maintain the parent ship in workable conditions.

| *ESA Scenario 2 (1000 day Mission)* | |
|---|---|
| Crew size | 6 crewmembers |
| Transit Duration | 259 days to and 301 days from |
| Surface Mission Duration | 525 days |
| Total Mission Duration | 1000 days approx. |
| Facilities on Martian Surface | Lander stage and ascent stage |
| Transit Vehicle | 2 crewmembers stay on orbit |
| Landing sites | Multisite or single site |

**Table 4.-** Summary of characteristics of ESA 1000 day Mission.

The scenario 3 (500 day mission) consists of transit to Mars with a duration of 160 to 250 days and a surface mission of 10 to 60 days. This mission implies the use of more propellant than the scenario 2. In the scenario 3, the entire spacecraft is injected towards Mars. The spacecraft would have the same elements as the scenario 2, but with a different design for the injection stage. After Mars arrival, 4 crewmembers descend to the Martian surface and 2 members remain for 40 days in Mars orbit.

| *ESA scenario 3 (500 day Mission)* | |
|---|---|
| Crew size | 6 crewmembers |
| Transit Duration | 160 to 250 days each way |
| Surface Mission Duration | 10 to 60 days |
| Total Mission Duration | 500 days approx. |
| Facilities on Martian Surface | Lander stage and ascent stage |
| Transit Vehicle | 2 crewmembers stay on orbit |
| Landing sites | Multisite |

**Table 5.-** Summary of characteristics of ESA 500 day Mission

### 5.1.2 Landing sites

The sites that NASA is considering for robotic exploration are the following:

- HEMATITE. This is one of the three sites on Mars with detectable mineral signatures for coarse-grained hematite. This type of Hematite generally forms in water. If hematite is found, this can be a proof of previous presence of water. The scientific interest is high, but in safety terms this site is also interesting. It gives smooth, flat surface for landing in the equatorial region.
- MELAS. The Melas region is a canyon with 10-kilometer high walls (6 miles high) in the Valles Marineris region (9° South, 282° East). There is an area at the centre of this region, where it appears some kind of sedimentary rock. However, Melas is surrounded by sand dunes, being this landing site too risky. An accurate landing could provide possibly fascinating results, but a failure would place the lander in the dunes.

- GUSEV CRATER (15° South, 185° West). This crater looks like as if it were a crater lake. At some point, seemingly the water breached the crater and escaped. If this is so, the crater should be filled with sediments. The landing site is at the west part of the crater to avoid rough terrain.
- ATHABASCA VALLES (9° North, 204° West). The Athabasca Valles in the Elysium Planitia is one of the youngest outflow channels on Mars. The channel has been worn by water and has young volcanics as well, making it a prime location to look for hydrothermal deposits.
- ISIDIS PLANITIA (4° North, 88° East). It seems to be the place on Mars with oldest material exposed, near the rim of a giant impact basin. The area is expected to be rich in very old rocks and provide clues to the early environment.
- EOS CHASMA (13° South, 318° East). This region is inside the Valles Marineris outflow.

All the robotic missions planned by NASA are close to Equator due to the type of launching site used. In the case of future ESA missions, sites selected are +45° or –45° latitude and never on the Equator, due to the inclination that the ESA launchers can achieve from Guyana. This limitation would not apply most likely to a manned mission, since the injection phase of that mission would start on orbit. In the case of manned missions, the inclination of the International Space Station would influence in the sites.

From the above selection, the selection of landing sites will be driven by the following criteria:

- Scientific interest either in geology studies or in search for life exploration
- Water search for its use in future missions
- No dusty area with flat and smooth surface
- No big rocks should be on site (NASA is referring to half-meter high rocks as the threshold value for a safe landing site)

In the case of manned missions, the landing sites could be different, because the main important constraint is the need for water. Water could be used as a resource and a source for rocket fuel. However, it is interesting to know the robotic mission sites, since they will be the best-known places and therefore a highly probable landing site.

Places on Earth where tests of systems could be carried out and could help us understand Mars include:

- Death Valley, California, where Ubehebe crater and "Mars Hill" have geologic features similar to those on Mars
- Mono Lake, California, which is a 700,000-year-old evaporative lake that compares to Gusev Crater, a basin on Mars where water once was likely
- Channeled Scabland in Washington, where catastrophic floods swept through the land much like what happened long ago in the Ares Vallis flood plain where Mars Pathfinder landed
- Permafrost in Siberia, Canada, Alaska and Antarctica, where subsurface water-ice and small life forms exist
- Volcanoes in Hawaii, which are like those on Mars, though much smaller
- Rio Tinto, an old mining site in Southern Spain

### 5.1.3 Dry Antarctica valleys Environmental conditions

The main characteristics of the Mars planet are the following:

| *Mars planet main characteristics* | |
|---|---|
| **Orbit** | 227 940 000 km (1.52 AU) mean distance from Sun |
| **Diameter** | 6 794 km |
| **Martian day** | 24 hours, 37 minutes and 22 seconds |
| **Martian year** | 669 Martian days 687 Earth days |
| **Average temperature** | 218 K (-55°C) |
| **Minimum temperature** | 140 K (-133°C) (at the winter pole) |
| **Maximum temperature** | 300 K (27°C) (summer dayside) |

Mars atmosphere is so thin that it is more than a hundred times lighter than the Earth's. The Martian atmosphere has the following composition:

| *Component* | *Percentage (by volume)* | *Partial Pressure [hPa= mbar ]* |
|---|---|---|
| ***Carbon Dioxide ($CO_2$)*** | 95.32 | 5.72 |
| ***Nitrogen (N)*** | 2.7 | 0.16 |
| ***Argon (Ar)*** | 1.6 | 0.096 |
| ***Oxygen ($O_2$)*** | 0.13 | 0.0078 |
| ***Carbon Monoxide (CO)*** | 0.07 | 0.0042 |
| ***Water Vapour ($H_2O$)*** | 0.03 | 0.018 |

Mars atmosphere is also highly oxidising. Ultraviolet light, which hits the ground because Mars has no ozone layer to stop it, is almost certainly one cause, but there could be others.

Similarly to Earth, Mars has four distinct seasons. Mars orbits closest to the Sun when its southern hemisphere is tilted towards it, while the northern hemisphere is tilted towards the Sun when it is furthest away. The southern summer is therefore much hotter than the northern summer. This extra heat added to the Southern Hemisphere is a source of turbulence and strong winds. Since the atmosphere on Mars is so thin and there are no oceans on the surface to store the heat from the Sun, temperatures rise and fall abruptly.

Spring, in either hemisphere, is a time for local and regional dust storms. These storms arise as the seasonal carbon dioxide frost cap, which can extend almost half-way to the equator, sublimes in the warming spring environment. Several factors reinforce these dust storms:

- the atmospheric pressure is increasing as carbon dioxide frost (CO2) sublimes--higher pressure allows more dust to be suspended, and for a longer time;
- the temperature contrast between the frost covered surface and the immediately adjacent, recently defrosted surfaces is quite high, creating thermally-generated winds that circulate onto and off of the frost cap edge;
- similarly, temperature-driven winds arise as sublimation of frost covering sun-facing slopes and dark sandy surfaces deep within the polar region creates intense slope winds away from the higher-standing layered deposits and permanent cap.

A typical storm is about 1 million $km^2$ size and comprises microscopic particles, which move at speeds of 15-30 m/s (54 110 km/h) before dissipating after a few days. Dust devils, about 2 km width and a few kilometres high, have also been observed in the tropics by the Viking orbiters. However, the most dramatic aspect of the Martian climate is when a dust storm expands to encompass nearly one or both hemispheres. Indeed, sometimes these great dust storms can become completely global.

The observational record in the 1970s by the Viking and Mariner 9 spacecraft suggests that the occurrence of great dust storms is highly variable from year-to-year. For some years, no great dust storms occur; for other years one or even two great storms occur. For most Martian years this century there are no records of great dust storms but this does not mean they did not occur: the telescopic observations are too sparse to quantify the true frequency of occurrence.

Wind tunnel studies show that winds in the free atmosphere above the surface (a few kilometres altitude) must reach a threshold of about 45 m/s (162 km/h) to lift typical dust grains at the surface, depending on the surface roughness. All dust storms require high surface winds to start and to be sustained. Also great dust storms, in particular, always occur close to southern summer on Mars, which is the season when Mars is nearest to the sun and there is maximum solar heating.

For a dust storm to extinguish itself and the dust to fall back to the surface, the winds must drop. Computer simulations show that as the atmospheric dust load increases, the vertical mixing of the dust decreases but winds near the surface continue to increase. This makes dust storm extinction difficult to explain. But maybe dust gets transported to regions where settling out is possible or perhaps the dust is scavenged by condensation of water vapour or carbon dioxide in the Polar Regions. Or if dust storms are caused by global resonance of the atmosphere then they would naturally extinguish themselves because a dusty atmosphere would no longer be conducive to resonance.

Once autumn arrives, the polar cap of that hemisphere starts to grow again as temperature drops, sometimes reaching the middle latitudes in winter. In northern summer, clouds can form, especially around the top of volcanoes. At other times of the year, heat rising from the tropics, can make cloud bands form in this region, similarly to Earth.

### 5.1.4 Critical Technologies

From all the description given above on the expected Mars missions scenarios, we can give the following comments that will lead to the identification of Critical Technologies:

- The duration of any of the missions proposed by ESA and by NASA is over 500 days and this is clearly in the type of missions that require BLSS's.
- A Life Support System like MELISSA (a BLSS) is not needed in working conditions for the trip duration (160-300 days depending on the mission). That means that it is not strictly needed in microgravity. However, if the ESA mission concepts are taken into account, 2 crewmembers will remain for the whole trip in microgravity. This means that it should be studied how to substitute a BLSS on orbit with the same efficiency. According to the present studies, only a combined Physico-Chemical with Plant Chamber System could be used to get some edible biomass and $O_2$ recycling.

**CT 33: <u>Crewmembers on Martian orbit.</u> The need of a BLSS for the 2 crewmembers that remain in microgravity during the whole mission to Mars (ESA missions) should be analysed. If this need is confirmed then the spacialisation of MELISSA should include the microgravity scenario.**

- The environmental conditions are very aggressive and should be considered in the design of the Habitat where MELISSA will most likely be located:
  - Inflatable structures feasible for implementing the Plant Chamber Module can become unusable when considering the wind pressure that should withstand under a dust storm, with strong dust winds, of up to 110 km/h.
  - High oxidative medium. The materials shall be conceived to operate for a long period of time (several crews), withstanding this high oxidative environment, especially in NASA´s concentrated mission.
  - Low atmospheric pressure that will impose stringent requirements on the structure of an inflatable structure that would contain the Plant Chamber.

**CT 34: <u>Plant Growth Chamber Structure.</u> The structure of the Plant Growth Chamber will have to take into account the environmental conditions if its use on the Martian surface is considered. Another option would be to bury most of the structure beneath the surface. However, this option would imply the use of artificial lighting, or complex optic fibber based light transport. Dust storms are also an argument in favour of using artificial lighting.**

- A single site approach (as the concentrated mission by NASA) allows designing a MELISSA with fewer restraints. Should the multisite missions be selected, the MELISSA concept should have another design concept: a system for each mission with the same features and with flexibility to be changed depending on the site selected. For example, it will not be the same the MELISSA concept for an Equator base than for a base closer to the pole.

**CT 35: <u>Influence of site approach.</u> MELISSA space concept will depend on the type of site approach: a multisite or a single site approach. A single site approach will imply to design a more flexible system. The site chosen influences the design. It is not the same design for a deep valley or a basin (Hellas basin or Valles Marineris) with respect to a Planitia (Amazonis planitia), as it is not an area with sand dunes in the near area or a rough rocky area.**

# 6 CONSIDERATIONS ON SPACIALISATION OF MELISSA

## 6.1 Industrialisation of MELISSA

For the realisation of this document, whose main objective is to identify the critical technologies associated with the process of spacialisation of MELISSA, NTE visited different industries that use biotechnological processes to produce their products. Here are the main concerns they have indicated when suggesting the industrialisation of MELISSA, which is a previous step before its spacialisation:

- MELISSA industrial system shall ensure that sterilisation means are used between compartments in order to prevent crossed contamination between them.
- Population ageing: being MELISSA a loop and considering that it should be used during many generations of micro-organisms, the probability of system instabilities caused by mutation shall be assessed.

**CT 36: Micro-organisms population ageing influence. Assessment on the effects of the population ageing in MELISSA loop performance is needed prior to its industrialisation. Means to refresh or renew the population should be proposed, if needed. ESA is performing presently an study on this matter.**

- Scaling-up. The present Pilot Plant will be scaled for a single crewmember. Scaling-up activities are one of the most complex issues for the R&D department in companies that use biotechnological processes. That is so for a single culture, therefore it is undoubtedly a critical step for the MELISSA industrialisation, which features a complex biological system to be scaled for a 6 crewmembers habitat.
- Robustness. MELISSA will be used by professionals that are not specialists in biology or biotechnology. Therefore, it should be robust enough to endure operations by trained crewmembers with different technical profiles.
- Reliability. The system should be reliable enough to maintain a preset recycling ratio.

Even though there are other issues that should be taken into account to properly design an industrial plant (availability, maintainability, etc.), we only wanted to list those elements that were considered first priorities by the different industries when the MELISSA concept was introduced to them.

All these comments lead to critical technologies already identified in the description of the MELISSA Pilot Plant, repeated hereafter for clarity purposes:

**CT 6: Sterilisation Methods. Sterilisation methods that could be flown will have to be developed. Autoclave technology does not seem to have the characteristics needed for this purpose.** An industrial company would consider simply an autoclave. It is clear that it is not applicable for the industry.

**CT 7: Contamination detection methods should be envisioned. Some of them might need some development work (pathogen detection). Thin films of bioactive material might be required at the different connections of the compartments (biosensors).** Even if there are

methods to sterilise between compartments, there should be reliable and sensible means to detect contamination.

**CT 10: Design of bioreactor. Optimisation of the illumination/volume ratio versus stirring of the bioreactor.** This is an example of the scale-up problem that can be encountered when industrialising MELISSA before its spacialisation.

## 6.2 Spacialisation of MELISSA

Given the industrialisation of MELISSA, spacialisation refers to the conversion of the MELISSA Pilot Plant into a BLSS for a crew equivalent. The spacialisation phase will encompass the needed activities to adapt the different elements to flight qualified configuration.

### 6.2.1 PERFORMANCE OF THE FACILITY

#### *6.2.1.1 Intended use*

The main objective of the MELISSA loop as a BLSS for a mission to Mars is to recycle with the highest efficiency possible edible biomass and O2. This will allow a long stay of a crew on the Mars surface.

Water recycling is not (in principle) the intended use of MELISSA. Indeed, MELISSA will maintain a certain level of water contents along the loop, but recycling water is not its main functional objective. Other alternative systems are being proposed (Blersch et al, 2000) that could consider as well micro-organisms.

#### *6.2.1.2 Modes of Operation*

The basic modes of operation of MELISSA system will be the following:

- Transfer mode. When MELISSA is being transported to Mars.

**CT 37: <u>Transit operation.</u> During the transit to Mars, MELISSA could be either working partially or completely dismantled and assembled once on the Mars surface. It is needed to assess if having a low working level MELISSA during transit could save work in MELISSA deployment on Mars surface.**

If MELISSA is not active during transit then one have to account a setting-up period once on-site for the process to start and to stabilise (for example, process in Compartment III). Considering that in any case, the full mass of MELISSA will be transported, the best choice seems to be have an operative system during the trip to Mars.

**CT 38: <u>MELISSA setting-up strategy.</u> It is important to investigate setting-up strategies in order to reduce the time to operation.**

The MELISSA operation requires a large volume of water, which may be difficult to transport. Measures to reduce MELISSA´s water needs should be investigated during the spacialisation phase.

**CT 39: Water volume reduction. For transportation reasons, investigations on water volume reduction should be carried out.**

- Stand-by mode. This mode refers to periods when MELISSA is not attended by a crew but is waiting for its arrival (as for example periods between missions). In this mode, the lack of the crew alters the nominal operation regime of the MELISSA loop. It is important to investigate how MELISSA remains stable without the consumers and how fast the system is able to adapt to the nominal conditions, once the crew is arrived. The use of complementary physico-chemical processes could be evaluated to achieve these objectives.

**CT 40: Stand-by operations. To evaluate the stability of the MELISSA loop in long absence of the Crew.**

- Nominal mode. MELISSA loop is already being used as BLSS for a complete crew.

During this nominal mode, one can envision extra-MELISSA activities by some members of the crew. Like exploration of the Mars surface, taking place more or less regularly during the stay period. This will alter MELISSA's nominal operating conditions, as the number of crewmembers within the habitat will not remain constant, permanently. In front of this scenario few questions will have to be answered. First, how the MELISSA system (including the control system) becomes robust enough to withstand the metabolic load changes. Second, LSS for the extra-MELISSA activity needs to be investigated and it will have to be decided whether this LSS will have any relation with MELISSA (for example, re-supplying).

Similarly, one can envisage situations where the metabolic load may increase, as for example in case of crew overlapping.

**CT 41: Response to changes in the metabolic load. To investigate the MELISSA response to changes in the metabolic load do to the likely activities of the crew outside the habitat during the mission or potential crew overlapping.**

**CT 42: LSS for activities outside the habitat. To investigate specific LSS for external activities and interfaces, if any, with MELISSA.**

#### *6.2.1.3 Figures of merit*

The main figures of merit will be the following:

- Power consumption of the whole loop under different working levels
- Edible biomass recycling ratio, that can be established using the Nitrogen and Carbon recycling ratio
- Oxygen recycling ratio
- Mineral and Vitamins content loss rate
- Efficiency of the loop measured by percentage of recycling for Oxygen, Nitrogen and edible biomass.
- Mass to be transported.

- Control reaction time in front of metabolic load variations.

#### *6.2.1.4 Compartment Design and Scaling-up*

The main components of MELISSA are the different compartments. Out off the five compartments, four are bioreactors. For its proper design in the industrialisation and further spacialisation, the following issues have to be taken into account:

- Bioreactor configuration. The different processes in MELISSA will need the use of different bioreactors.
  - Stirred tank bioreactors that ensure a better diffusion of oxygen, but could live to an excessive stress on the cells. It is also valid for growing cultures, but not for stable cultures (compartment III). Previous work in microgravity has been performed (Cogoli et al, 1999).
  - Bubble columns. These are simple bioreactors, but might not present appropriate characteristics for its use on Martian surface.
  - Airlift bioreactors. This bioreactor type could be a good solution for compartments such as CIVa. Knowledge of the fluid dynamics and mass transfer in Martian gravity should be gained.
  - Fluidised bed. No bioreactor in MELISSA is using this type.
  - Packed bed columns. This is suitable for the stable population culture that we have in Compartment III.
  - etc. All these designs have already been tackled for many years in the space scientific community, but mainly for microgravity (Casas et al, 1990; Hummerick et al, 2001). This will imply an important effort on design of bioreactors for other gravity fields
- Bioreactor design features. The bioreactor will include a series of accessories and sensors whose proper working conditions need to be ensured.
- Photobioreactors. Specifically, there are two photobioreactors that will need light diffusion in the culture and that will impose stringent requirements on the shape.
- Heat transfer. The heat management of each of the MELISSA bioreactors is critical to maintain a good cooling system for the whole facility.

The gravity field influences most of these bioreactor´s characteristics. This means that a well characterised culture on ground may present a different behaviour in other gravity fields (in microgravity, Cogoli et al, 1989). Investigations in this area need to be done, as most of the work to be performed by MELISSA will be in a 1/3 g gravity field (Martian gravity field).

**CT 43: <u>Gravity influence in Bioreactor design.</u> Characterisation of bioreactors in Martian gravity fields is needed. This will be hard to accomplish on ground or on orbit and will likely need the use of missions to Mars. Being MELISSA such an important facility for a Mars mission, investigation on this field needs to start with no delay.**

Similar points will be treated as well for the Plant Chamber: type of chamber (buried or on surface), design characteristics (accessories and sensors), lighting conditions (natural sunlight or artificial light) and heat and water management in the Chamber.

**CT 44: <u>Higher Plant Compartment Illumination Strategy.</u> The Plant chamber design in MELISSA will depend on the type of illumination envisioned: a direct natural light with**

**the limitations provoked by the dust storms or an artificial illumination of the bioreactors.**

All these characteristics will follow modifications once MELISSA Pilot Plant has demonstrated the capability of the loop for a one-crewmember equivalent.

**CT 45: <u>Bioreactors and HPC scale-up.</u> Scaling-up of the bioreactors and Plant Chamber will follow after the MELISSA Pilot Plant has finally finished its demonstration phase.**

### 6.2.2 OPERATIONAL FEASIBILITY

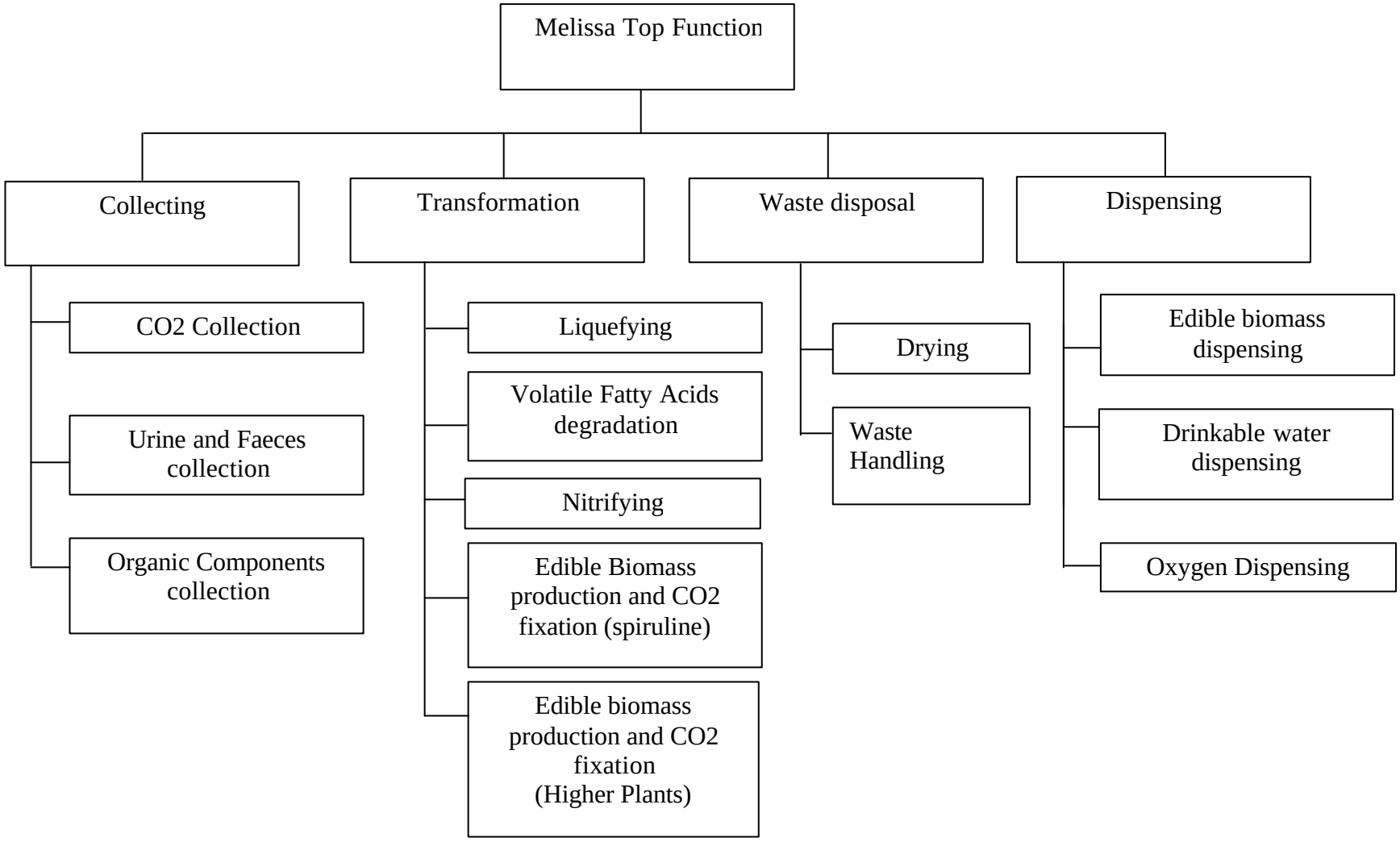
#### *6.2.2.1 Functional analysis*

The purpose of the functional analysis is to provide a tool to ensure that the identification of the critical technologies is comprehensive and adequate in depth. As such a tool, it should evolve as design choices are made.

From this point of view, the analysis is based on the functions necessary to implement the entire MELISSA loop, as shown in the MELISSA Pilot Plant description in section 4.2.

The analysis is presented in a table format, with the following fields:

- Code: function's numeric identifier function.
- Function name: function's textual identification
- Function details: descriptive text of the function's purpose.
- Related HW: the function is mapped to the actual or envisaged loop's HW. Where possible, the naming conventions follows the MELISSA Dependability Analysis in [R1].
- State of the Art: for the associated technology.
- Terrestrial interest: defines whether the associated technology can / will be developed for inherent interest in terrestrial applications.
- Space adaptation: defines whether the associated technology requires specific future development for its utilisation in Space. Four categories are defined:
  - **Solved:** hardware exists that performs equally well regardless of the gravity/pressure environment, and/or space-proven hardware is readily available
  - **Needs qualification:** Hardware exists that performs equally well regardless of the gravity/pressure environment, but no space experience is known of.
  - **Needs development:** Gravity/pressure conditions have an impact on the functioning of the item, or the solutions currently used in ground are nor suited for space environment
  - **No baseline:** No hardware is known of that provides an optimal solution for use in ground
  - **Not critical:** Gravity has no (known) effect on the item discussed.
- Process essential: either YES or NO. The lack of essential technologies prevents the functionality of the MELISSA loop.
- Mark: criticality will be rated between 1 to 5, where the highest mark corresponds to the highest criticality.


Melissa Top Function
Collecting
Transformation
Waste disposal
Dispensing
CO2 Collection
Urine and Faeces collection
Organic Components collection
Liquefying
Volatile Fatty Acids degradation
Nitrifying
Edible Biomass production and CO2 fixation (spiruline)
Edible biomass production and CO2 fixation (Higher Plants)
Drying
Waste Handling
Edible biomass dispensing
Drinkable water dispensing
Oxygen Dispensing

| Code | Function Name | Function details | Related Hardware | State-of-the-Art | Terrestrial Interest | Space Adaptation | Process essential | Mark | CT |
|---|---|---|---|---|---|---|---|---|---|
| **1. COLLECTION OF INPUT MATERIALS TO RECYCLING PLANT** | | | | | | | | | |
| 1.1 | Treatment of cabin air | A fraction of cabin air is circulated through the higher plants compartment | C0_Reactor | ISS Life SS | YES | NEEDS DEVELOPMENT | YES | 5 | CT 20 |
| 1.2 | Urine and faeces collection | | C0_Reactor | ISS Life SS | NO | NEEDS DEVELOPMENT | YES | 2 | CT 18 |
| 1.3 | Organic components collection | Grinding and storage of non-edible biomass | S2_Buffer (CIV HP) | | NO | NEEDS DEVELOPMENT | YES | 2 | CT 1 |
| **2. TRANSFORMATION (RECYCLING)** | | | | | | | | | |
| **2.1** | **LIQUEFYING AT COMPARTMENT I** | | | | | | | | |
| 2.1.1 | Liquefying | Transform the collected organic components (urine, faeces, and organic wastes) into volatile fatty acids and ammonia. | CI_Reactor | | YES | NEEDS DEVELOPMENT | YES | 5 | CT 2<br>CT 6<br>CT 5 |
| 2.1.2 | Provide mechanical containment | Ensure that the three phases are contained | CI_Reactor | | NO | NEEDS DEVELOPMENT | YES | 3 | CT 21 |
| **2.1.3** | **Provide adequate substrate** | | | | | | | | |
| 2.1.3.1 | Input mixer | Combines the different organic input components (human waste, microbial biomass and higher plants) | E1_Mixer | Under development | NO | NOT CRITICAL | NO | 2 | |
| 2.1.3.2 | Pumping | Pumping the organic mix to CI_Reactor | E1_Pump | Under development | NO | NOT CRITICAL | NO | 3 | CT 22 |
| 2.1.3.3 | Reactor stirring | Provide high oxygen diffusion | CI-Reactor | Under development | YES | NEEDS DEVELOPMENT | YES | 3 | CT 5 |
| **2.1.4** | **ENSURE PROCESS CONDITIONS** | | | | | | | | |
| 2.1.4.1 | Liquefying compartment (I) temperature control | Measures the reactors internal temperature and provides this information to the control system | CI_Reactor | Already used in space applications | NO | SOLVED | NO | 1 | |
| 2.1.4.2 | Compartment I pH control | Measures the reactor's internal pH concentration and provides this information to the control system | CI_Reactor | Already used in space applications | NO | SOLVED | NO | 1 | |
| 2.1.4.3 | Compartment I microbial activity control | Measures the biomass concentration within the reactor and provides this information to the control system | CI_Reactor | Under development. Low concentrations can be critical | YES | NEEDS QUALIFICATION | YES | 4 | CT 48 |
| 2.1.4.4 | Compartment I Pressure control | Measures the reactors internal pressure and provides this information to the control system | CI_Reactor | Already used in space applications | YES | NEEDS QUALIFICATION | NO | 2 | CT 48<br>CT 21 |
| 2.1.4.5 | Compartment I level control | Measures the compartments liquid level and provides this information to the control system | CI_Reactor | | NO | NEEDS QUALIFICATION | NO | 1 | |

| Code | Function Name | Function details | Related Hardware | State-of-the-Art | Terrestrial Interest | Space Adaptation | Process essential | Mark | CT |
|---|---|---|---|---|---|---|---|---|---|
| **2.1.5** | **COMPARTMENT I LEVEL MONITORING** | | | | | | | | |
| 2.1.5.1 | BOD | Measurement of the Biological Oxygen Demand | CI_Reactor | Off-line measurement in Waste Water Treatment Plant | YES | NEEDS DEVELOPMENT | YES | 4 | CT 48 |
| 2.1.5.2 | COD | Measurement of the Chemical Oxygen Demand | CI_Reactor | Off-line measurement in Waste Water Treatment Plant | YES | NEEDS DEVELOPMENT | YES | 4 | CT 48 |
| 2.1.5.3 | Dry Weight | Measurement of the biomass content | CI_Reactor | Electrical Impedance Spectroscopy that provides on-line viable biomass information | YES | NEEDS DEVELOPMENT | YES | 4 | CT 48 |
| 2.1.5.4 | Cellulose/faeces ratio monitoring | Measurement of the Cellulose/faeces ratio | CI_Reactor | - | YES | NEEDS DEVELOPMENT | YES | 4 | CT 48 |
| **2.1.6** | **COMPARTMENT I OUTPUT MONITORING** | | | | | | | | |
| 2.1.6.1 | Ethanol | Measurement of Ethanol | CI_Reactor | Chromatography | YES | NEEDS DEVELOPMENT | YES | 3 | CT 4<br>CT 48 |
| 2.1.6.2 | Fatty acids | Measurement of Fatty Acids | CI_Reactor | Chromatography | YES | NEEDS DEVELOPMENT | YES | 3 | CT 4<br>CT 48 |
| 2.1.6.3 | NH4 | Measurement of Ammonia | CI_Reactor | Chromatography | YES | NEEDS DEVELOPMENT | YES | 3 | CT 17 |
| 2.1.6.4 | CO2 | Measurement of carbon dioxide | CI_Reactor | | NO | NEEDS QUALIFICATION | YES | 3 | CT 48 |
| 2.1.6.5 | H2S | Measurement of Sulfhydric acid | CI_Reactor | | NO | NEEDS DEVELOPMENT | YES | 3 | CT 48 |
| 2.1.6.6 | H2 | Measurement of Hydrogen | CI_Reactor | | NO | NEEDS DEVELOPMENT | YES | 3 | CT 48 |
| 2.1.6.7 | Indigestible nutrients | Measurement | CI_Reactor | | NO | NEEDS DEVELOPMENT | YES | 3 | CT 48 |
| 2.1.6.8 | Kjeldahl-N | Measurement of N | CI_ Reactor | | NO | NEEDS DEVELOPMENT | YES | 3 | CT 48 |
| **2.1.7** | **COMPARTMENT I OUTPUT PROCESSING** | | | | | | | | |
| 2.1.7.1 | Gas Filtering | Filtering CO2 resulting from CI process | S1_Filter | HEPA filters | YES | SOLVED | YES | 1 | |
| 2.1.7.2 | Gas Pumping | Pumping CO2 to be directed to CII, CIV and CIVHP | S1_Pump | Under development | NO | NOT CRITICAL | NO | 3 | CT 22 |
| 2.1.7.3 | Liquid pumping | Pumps the liquid from CI_Reactor to S2_Solid Separator | S2_Pump | Under development | NO | NOT CRITICAL | NO | 3 | CT 22 |

| Code | Function Name | Function details | Related Hardware | State-of-the-Art | Terrestrial Interest | Space Adaptation | Process essential | Mark | CT |
|---|---|---|---|---|---|---|---|---|---|
| 2.1.7.4 | Liquid-Solid Separation | Liquid and solid waste separation from the liquid obtained in CI_Reactor | S2_Solid _Separator | Under development | NO | NEEDS DEVELOPMENT | YES | 4 | CT 12 |
| 2.1.7.5 | Liquid pumping | Pumps the liquid obtained from S2_Solid_ Separator | S2.1_Pump | Under development | NO | NOT CRITICAL | NO | 3 | CT 22 |
| 2.1.7.6 | Liquid Sterilisation | Elimination of undesired microbial organisms | S2.1_Stereiliser_UV | UV sterilisation | NO | NEEDS QUALIFICATION | YES | 3 | CT 6 |
| 2.1.7.7 | Liquid Filtering | Filtering stage | S2.1_Filter | Ultrafiltration | NO | NEEDS QUALIFICATION | YES | 3 | CT 3<br>CT 6 |
| 2.1.7.8 | Liquid buffering | Provides storage capacity for liquid to be directed to CII | S2.1_Buffer | | NO | SOLVED | NO | 1 | |
| **2.1.8** | **COMPARTMENT I WASTE EXTRACTION** | | | | | | | | |
| 2.1.8.1 | Solid waste buffering | Provides storage for the solid waste cake obtained at the S2_Solid_separator, for external disposal | S2.2_Buffer | | NO | SOLVED | NO | 1 | |
| | | | | | | | | | |
| **2.2** | **VFA DEGRADATION AT COMPARTMENT II** | | | | | | | | |
| 2.2.1 | VFA degradation | Photoheterotrophic biodegradation by rhodobacter bacteria | CII_Reactor | | YES | SOLVED | YES | 5 | |
| 2.2.2 | Provide containment | | CII_Reactor | | NO | SOLVED | YES | 3 | |
| 2.2.3 | Provide homogeneous growth substrate | Liquid steering to ensure homogeneous exposure to light | CII-Reactor | | YES | SOLVED | YES | 3 | |
| **2.2.4** | **ENSURE ADEQUATE GROWING ENVIRONMENT AT COMPARTMENT II** | | | | | | | | |
| 2.2.4.1 | Compartment II temperature control | Measures the reactors internal temperature and provides this information to the control system | CII_Reactor | Already used in space applications | NO | SOLVED | NO | 1 | |
| 2.2.4.2 | Compartment II pH control | Measures the reactor's internal pH concentration and provides this information to the control system | CII_Reactor | Already used in space applications | NO | SOLVED | NO | 1 | |
| 2.2.4.3 | Compartment II light control | Measures the light intensity in the reactor's interior and provides this information to the control system | CII_Reactor | Monitoring: Integrating sphere+ photodiode | NO | SOLVED | NO | 2 | |
| 2.2.4.4 | Compartment II Biomass control | Measures the biomass concentration within the reactor and provides this information to the control system | CII_Reactor | Under development. Low concentrations can be critical | YES | NEEDS QUALIFICATION | YES | 4 | CT 48 |
| **2.2.5** | **COMPARTMENT II YIELD CONTROL** | | | | | | | | |

| Code | Function Name | Function details | Related Hardware | State-of-the-Art | Terrestrial Interest | Space Adaptation | Process essential | Mark | CT |
|---|---|---|---|---|---|---|---|---|---|
| 2.2.5.1 | Compartment II level control | Measures the compartment's liquid level and provides this information to the control system | CII_Reactor | | NO | NEEDS QUALIFICATION | NO | 1 | |
| 2.2.5.2 | Ammonium (NH4) monitoring | Measurement of Ammonium | CII_Reactor | Chromatography | YES | NEEDS DEVELOPMENT | YES | 3 | CT 17 |
| 2.2.5.3 | CO2 | Measurement of carbon dioxide | CII_Reactor | | NO | NEEDS QUALIFICATION | YES | 3 | CT 48 |
| 2.2.5.4 | Active Biomass | On-line biomass measurement of viable biomass | CII_Reactor | Electrical Impedance Spectroscopy that provides on-line viable biomass information | YES | NEEDS DEVELOPMENT | YES | 4 | CT 48 |
| 2.2.5.5 | Kjeldahl-N | Measurement of N | CII_ Reactor | | NO | NEEDS DEVELOPMENT | YES | 3 | CT 48 |
| **2.2.6** | **COMPARTMENT II OUTPUT PROCESSING** | | | | | | | | |
| 2.2.6.1 | CO2 filtering | | S1_Filter | HEPA filters | YES | SOLVED | YES | 1 | |
| 2.2.6.2 | CO2 Analyser | Measurement of C | S1_Analyser | Infrared spectroscopy | YES | NEEDS DEVELOPMENT | YES | 2 | CT 48 |
| 2.2.6.3 | CO2 pumping to CIVHP | | S1_Pump | Gas pumps | YES | NEEDS DEVELOPMENT | YES | 2 | CT 22 |
| 2.2.6.2 | Liquid separation | Liquid solid separation | S2_Separator | | YES | NEEDS DEVELOPMENT | YES | 3 | CT 12 |
| 2.2.6.3 | Liquid filtering | Filtering stage | S2.1_Filter | Ultrafiltration | NO | NEEDS QUALIFICATION | YES | 3 | CT 6 |
| 2.2.6.4 | Liquid analysis | Measurement of ammonia concentration and other compounds | S2.1_Analyser | Chromatography | YES | NEEDS DEVELOPMENT | YES | 3 | CT 4<br>CT 17 |
| 2.2.6.5 | Liquid buffering | Provides storage capacity for liquid to be directed to CIII | S2.1_Buffer | | NO | SOLVED | NO | 1 | |
| **2.2.7** | **COMPARTMENT II WASTE EXTRACTION** | | | | | | | | |
| 2.2.7.1 | Solid waste buffering | Provides storage for the solid waste product to be directed to CI | S2.2_Buffer | | NO | SOLVED | NO | 1 | |
| | | | | | | | | | |
| **2.3** | **NITRIFICATION AT COMPARTMENT III** | | | | | | | | |
| 2.3.1 | Nitrification | Conversion of ammonium into nitrate. Nitrogen fixation done by nitrifying bacteria. | CIII_Reactor | | YES | SOLVED | YES | 5 | |
| 2.3.2 | Provide containment | | CIII_Reactor | | NO | SOLVED | YES | 3 | |
| 2.3.3 | Provide packed-bed substrate | | CIII_Reactor | Polystyrene beads | YES | SOLVED | YES | 3 | |
| **2.3.4** | **ENSURE ADEQUATE ENVIRONMENT PARAMETERS** | | | | | | | | |

| Code | Function Name | Function details | Related Hardware | State-of-the-Art | Terrestrial Interest | Space Adaptation | Process essential | Mark | CT |
|---|---|---|---|---|---|---|---|---|---|
| 2.3.4.1 | Compartment III temperature control | Measures the reactors internal temperature and provides this information to the control system | CIII_Reactor | Already used in space applications | NO | SOLVED | NO | 1 | |
| 2.3.4.2 | Compartment III pH control | Measures the reactor's internal pH concentration and provides this information to the control system | CIII_Reactor | Already used in space applications | NO | SOLVED | NO | 1 | |
| 2.3.4.3 | Compartment III DO control | Dissolved Oxygen measurement | CIII_Reactor | DO commercial sensors | NO | NEEDS QUALIFICATION | YES | 2 | CT 48 |
| 2.3.4.4 | Compartment III level control | Measures the compartments liquid level and provides this information to the control system | CIII_Reactor | | NO | NEEDS QUALIFICATION | NO | 1 | |
| 2.3.4.5 | Compartment III pressure control | Measures the reactor's internal pressure and provides this information to the control system | CIII_Reactor | Already used in space applications | YES | NEEDS QUALIFICATION | NO | 2 | CT 21<br>CT 48 |
| 2.3.4.6 | Compartment III ammonium control | | CIII_Reactor | Pumping of growing medium from Compartment II output buffer + emergency high ammonium concentration medium | YES | NEEDS DEVELOPMENT | YES | 3 | CT 17 |
| **2.3.5** | **COMPARTMENT III INPUT MONITORING / PROCESSING** | | | | | | | | |
| 2.3.5.1 | Liquid filtering | Filtering stage of liquid coming from CII | E1_Filter | Ultrafiltration | NO | NEEDS QUALIFICATION | YES | 3 | CT 6 |
| 2.3.5.2 | Liquid analysis | Measures the $NH_4^+$ concentration at the liquid input | E1_Analyser | Chromatography | YES | NEEDS DEVELOPMENT | YES | 3 | CT 4<br>CT 17 |
| 2.3.5.3 | Liquid pumping | Directs the liquid flow to CIII_Reactor | E1_Pump | Under development | NO | NOT CRITICAL | NO | 3 | CT 22 |
| 2.3.5.4 | Gas analysis | Measures the O2 concentration in the gas coming from CIVHP | E2_Analyser | Gas Chromatography | YES | NEEDS DEVELOPMENT | YES | 2 | CT 4<br>CT 48 |
| 2.3.5.6 | Gas filtering | Filtering stage | E2_Filter | HEPA filter | NO | NEEDS QUALIFICATION | YES | 3 | CT 6 |
| **2.3.6** | **COMPARTMENT III OUTPUT MONITORING / PROCESSING** | | | | | | | | |
| 2.3.6.1 | CO2 filtering | Filtering stage of the CO2 gas not consumed in CIII_Reactor | S1_Filter | HEPA filters | YES | SOLVED | YES | 1 | |
| 2.3.6.2 | CO2 pumping to CIVHP | Pumping stage to redirect not consumed CO2 gas to CIVHP | S1_Pump | Gas pumps | YES | NEEDS DEVELOPMENT | YES | 2 | CT 22 |
| 2.3.6.3 | Liquid pumping | Pumping stage at CIII_Reactor output | S2_Pump | Under development | NO | NOT CRITICAL | NO | 3 | CT 22 |
| 2.3.6.4 | Liquid - Solid Separation | Separation stage to remove dead bacterial material that includes the biomass release system | S2_Separator | Under development | NO | NEEDS DEVELOPMENT | YES | 4 | CT 12<br>CT 15 |
| 2.3.6.5 | Liquid buffering | Buffering stage | S2.1_Buffer | | NO | SOLVED | NO | 1 | |

| Code | Function Name | Function details | Related Hardware | State-of-the-Art | Terrestrial Interest | Space Adaptation | Process essential | Mark | CT |
|---|---|---|---|---|---|---|---|---|---|
| 2.3.6.6 | Liquid analysis | Measures the $NO_3^+$ concentration at the liquid output | S2.1_Analyser | Chromatography | YES | NEEDS DEVELOPMENT | YES | 3 | CT 4<br>CT 17 |
| **2.3.7** | **COMPARTMENT III WASTE EXTRACTION** | | | | | | | | |
| 2.3.7.1 | Solid buffering | Provides storage for the solid waste cake obtained at the S2_separator, for external disposal | S2.2_Buffer | | NO | NEEDS DEVELOPMENT | YES | 2 | CT 1 |
| | | | | | | | | | |
| **2.4** | **EDIBLE BIOMASS PRODUCTION AT COMPARTMENT IV** | | | | | | | | |
| 2.4.1 | Biomass production | Provides a source of edible biomass (Arthrospira Platensis) and CO2 fixation | CIV_Reactor | | YES | SOLVED | YES | 5 | CT 10 |
| 2.4.2 | Provide containment | | CIV_Reactor | | NO | SOLVED | YES | 3 | |
| 2.4.3 | Provide homogeneous substrate | | CIV_Reactor | Fluidised bed | YES | SOLVED | YES | 3 | |
| **2.4.4** | **ENSURE ENVIRONMENTAL PARAMETERS** | | | | | | | | |
| 2.4.4.1 | Compartment IV temperature control | Measures the reactors internal temperature and provides this information to the control system | CIV_Reactor | Already used in space applications | NO | SOLVED | NO | 1 | |
| 2.4.4.2 | Compartment IV Light control | Measures the light intensity in the reactor's interior and provides this information to the control system | CIV_Reactor | Monitoring: Integrating sphere+ photodiode | NO | SOLVED | NO | 2 | CT 11 |
| 2.4.4.3 | Compartment IV Biomass control | Measures the biomass concentration within the reactor and provides this information to the control system | CIV_Reactor | Under development. Low concentrations can be critical | YES | NEEDS QUALIFICATION | YES | 4 | CT 48 |
| 2.4.4.4 | Compartment IV Pressure control | Measures the reactor's internal pressure and provides this information to the control system | CIV_Reactor | Already used in space applications | YES | NEEDS QUALIFICATION | NO | 2 | CT 48<br>CT 21 |
| 2.4.4.5 | Compartment IV gas flow control | Measures the gas flow (oxygen) and provides this information to the control system | CIV_Reactor | | NO | NEEDS QUALIFICATION | NO | 2 | CT 48 |
| **2.4.5** | **COMPARTMENT IV INPUT MONITORING / CONTROL** | | | | | | | | |
| 2.4.5.1 | Liquid filtering | Filtering stage of liquid coming from CIII | E1_Filter | Ultrafiltration | NO | NEEDS QUALIFICATION | YES | 3 | CT 6 |
| 2.4.5.2 | Liquid buffering | Buffer stage for incoming liquid | E1_Buffer | | NO | SOLVED | NO | 1 | |
| 2.4.5.3 | Liquid pumping | Pumping liquid to CIV_Reactor | E1_Pump | Under development | NO | NOT CRITICAL | NO | 3 | CT 22 |
| 2.4.5.4 | Gas buffering | Buffer stage for CO2 gas incoming from CI and CIII | E2_Buffer | | NO | SOLVED | NO | 1 | |

| Code | Function Name | Function details | Related Hardware | State-of-the-Art | Terrestrial Interest | Space Adaptation | Process essential | Mark | CT |
|---|---|---|---|---|---|---|---|---|---|
| 2.4.5.5 | Gas mixing | O2 produced in CIV_Reactor is mixed with incoming CO2 | S1&E2_pump | | NO | SOLVED | NO | 1 | |
| 2.4.5.6 | Gas analysis | Measurement of the O2 and CO2 proportion of the incoming gas mixture to CIV_Reactor | E2_Analyser | Gas Chromatography | YES | NEEDS DEVELOPMENT | YES | 2 | CT 4<br>CT 48 |
| 2.4.5.7 | Gas pumping | Pumping O2 and CO2 gas mixture to CIV_Reactor | E2_Pump | Gas pumps | YES | NEEDS DEVELOPMENT | YES | 2 | CT 22 |
| 2.4.5.8 | Gas filtering | Filtering stage of the O2 and CO2 gas mixture incoming to CIV_Reactor | E2_Filter | HEPA filter | NO | NEEDS QUALIFICATION | YES | 3 | CT 6 |
| **2.4.6** | **COMPARTMENT IV OUTPUT PROCESSING / MONITORING** | | | | | | | | |
| 2.4.6.1 | Gas filtering | Filtering stage of O2 gas produced in CIV_Reactor | S1_Filter | HEPA filter | NO | NEEDS QUALIFICATION | YES | 3 | CT 6 |
| 2.4.6.2 | Gas pumping | Pumping stage of produced O2 | S1_Pump | Gas pumps | YES | NEEDS DEVELOPMENT | YES | 2 | CT 22 |
| 2.4.6.3 | Gas buffering | Storage of O2 before distribution to C0 and CIV HP | S1_Buffer | | NO | SOLVED | NO | 1 | |
| 2.4.6.4 | Liquid buffering | Buffer stage for liquid containing edible biomass | S2_Buffer | | NO | SOLVED | NO | 1 | |
| 2.4.6.5 | Liquid-solid separation | Separation of solid edible biomass (Arthrospira platensis) | S2_Separator | Separation technology under development | YES | NEEDS DEVELOPMENT | YES | 4 | CT 12<br>CT 19 |
| 2.4.6.6 | Liquid filtering | Filtering stage to obtain water for CIV HP | S2.1_Filter | Related to the Separation technology chosen for LSS | YES | NEEDS DEVELOPMENT | YES | 3 | CT 6<br>CT 19 |
| 2.4.6.7 | Liquid buffering | Liquid storage before distribution to CIV HP | S2.1_Buffer | | NO | SOLVED | NO | 1 | |
| **2.4.7** | **COMPARTMENT IV SOLID EXTRACTION** | | | | | | | | |
| 2.4.7.1 | Solid buffering | Provides storage conditions for the separated edible biomass | S2.2_Buffer | | NO | NEEDS DEVELOPMENT | YES | 2 | CT 19 |
| | | | | | | | | | |
| **2.5** | **EDIBLE BIOMASS PRODUCTION AT COMPARTMENT IV HP** | | | | | | | | |
| 2.5.1 | Higher Plants cultivation | Edible biomass production (Higher plants), CO2 Fixation | CIVHP_Reactor | | YES | UNDER DEVELOPMENT | YES | 5 | |
| 2.5.2 | Provide containment | Structural | CIV HP | Inflatable structure | NO | UNDER DEVELOPMENT | YES | 3 | CT 20<br>CT 21<br>CT 23 |
| 2.5.3 | Provide plants substrate | | CIV HP | Hydroponics culture<br>Transgenic plants | YES | UNDER DEVELOPMENT | YES | 3 | |

| Code | Function Name | Function details | Related Hardware | State-of-the-Art | Terrestrial Interest | Space Adaptation | Process essential | Mark | CT |
|---|---|---|---|---|---|---|---|---|---|
| 2.5.4 | Ensure adequate environment | Environmental settings (Temperature, humidity, pressure, illumination) | CIV HP | Greenhouse technology | YES | UNDER DEVELOPMENT | YES | 3 | CT 46<br>CT 47 |
| **2.5.5** | **CIV HP INPUT MONITORING / PROCESSING** | | | | | | | | |
| 2.5.5.1 | Gas mixing | Introduction of CO2 and O2 in the chamber´s atmosphere from the various gas sources in the loop (C0, CI, CII and CIV) and external | E1_Mixer | | NO | SOLVED | NO | 1 | |
| 2.5.5.2 | Gas analysis | Gas composition analysis stage | E1_Analyser | Gas Chromatography | YES | NEEDS DEVELOPMENT | YES | 2 | CT 4<br>CT 48 |
| 2.5.5.3 | Gas pumping | Pumping stage | E1_Pump | Gas pumps | YES | NEEDS DEVELOPMENT | YES | 2 | CT 22 |
| 2.5.5.4 | Gas filtering | Filtering stage of the gas mixture to CIV HP reactor | E1_Filter | HEPA filter | NO | NEEDS QUALIFICATION | YES | 3 | CT 6 |
| 2.5.5.5 | Liquid mixing | Mixing stage where liquid from CIV and surplus water from CIV HP condensation are combined | E2_Mixer | | NO | SOLVED | NO | 1 | |
| 2.5.5.6 | Liquid pumping | Pumping stage to CIVHP_Reactor | E2_Pump | Under development | NO | NOT CRITICAL | NO | 3 | CT 22 |
| | | | | | | | | | |
| **2.5.6** | **CIV HP OUTPUT MONITORING / PROCESSING** | | | | | | | | |
| 2.5.6.1 | Gas filtering | Filtering stage of the gas (water vapour and O2) produced inside the CIVHP_Reactor | S1_Filter | HEPA filter | NO | NEEDS QUALIFICATION | YES | 3 | CT 6 |
| 2.5.6.2 | Gas pumping | Pumping stage | S1_Pump | Gas pumps | YES | NEEDS DEVELOPMENT | YES | 2 | CT 22 |
| 2.5.6.3 | Water vapour condensation | Condensation to produce fresh water from gas vapour contents | S1_Condenser | ISS Life Support | YES | NEEDS DEVELOPMENT | YES | 3 | CT 46 |
| 2.5.6.4 | O2 distribution | O2 is distributed to C0, CIII and disposed externally | S1_Condenser | ISS Life Support | YES | NEEDS DEVELOPMENT | YES | 3 | CT 47 |
| 2.5.6.5 | Edible biomass treatment | Collection and processing into edible food source of one fraction of the cultivated plants, to be consumed by the crew | S2.2_Treatment | Food technology | YES | NEEDS DEVELOPMENT | YES | 3 | |
| 2.5.6.6 | Non-edible biomass grinding | Processing of the fraction of non-edible biomass as input for C1 | S2.1_Grinder | Industrial grinders | NO | NEEDS DEVELOPMENT | NO | 1 | |
| 3.- WASTE DISPOSAL | | | | | | | | | |
| 3.1 | Drying | Drying of the waste before entering packaging | CI_Reactor | Liofilisation | NO | NEEDS DEVELOPMENT | NO | 2 | |

| Code | Function Name | Function details | Related Hardware | State-of-the-Art | Terrestrial Interest | Space Adaptation | Process essential | Mark | CT |
|---|---|---|---|---|---|---|---|---|---|
| 3.2 | Waste handling | Packaging of the waste before entering storage to be transferred to CI when needed | CI_Reactor | | NO | NEEDS DEVELOPMENT | NO | 1 | |
| | | | | | | | | | |
| 4.- DISPENSIG | | | | | | | | | |
| 4.1 | Edible biomass supply | Supply Compartment 0 with edible biomass | C0 | Food Technology to be determined taking into account organoleptic characteristics | YES | NEEDS DEVELOPMENT | YES | 3 | CT 31 |
| 4.2 | O2 supply | Supply Compartment 0 with Oxygen | C0 | Air distribution systems<br>ISS Life Support | YES | NEEDS DEVELOPMENT | YES | 3 | CT 20<br>CT 47 |

In addition to this functional break down, and in order to close the loop, a consuming function by the Crew could also be considered. Even though this function may not be easily related with the MELISSA hardware, impacts in terms of setting-up the loop, fast reaction time to metabolic load changes, potential relation with off-habitat LSS needs, transport and landing operations etc, have been addressed in CT 38, CT 40, CT 41 and CT 42.

### 6.2.3 SPECIFIC ASPECTS

#### *6.2.3.1 Three Phase management*

MELISSA is a system where the three phases (solid, liquid and gas) are mixed and separated along the loop and in the different interactions between compartments. The main separation units needed in MELISSA (according to the description of the loop given in section 4.2) are the following:

- Liquid Gas Separation units (LGSS). This is occurring in all the different compartments with the exception of the Higher Plants, since the gas is diffused into the bioreactor medium. These separation units are simply filters with the added complexity coming from the pressure management requirements, for there is a complex distribution of pressure in MELISSA. In the case of the Higher Plants Chamber, an efficient way of collecting water vapour from air will be needed. Terrestrial green houses use forced air circulation and a cold trap for condensation and recovery of liquid water. A possible alternative, to be used if the goal is to distribute humidity instead of recovering water, is the use of salts able to adsorb and desorb water depending on the ambient humidity. The salt can be transported from points close to plants (adsorption) to drier areas (desorption)

**CT 46: <u>Transpired water recovery.</u> Water vapour recovery from air in the Higher Plants Chamber is an important technology in the frame of the MELISSA system. It needs an specific development.**

- Solid-Liquid Separation units (SLSS). This is used in Compartment II, III and IVa. In each case, the SLSS function is different:
  - Compartment II uses it to separate the biomass excess to be relocated to Compartment I,
  - Compartment III uses this unit to remove the non-viable biomass in the bioreactor,
  - Compartment IVa uses this unit to harvest biomass that will be utilised as an edible product.

  The three SLSS units will be different and require separate designs.

- Gas-Gas Separation units (GGSS). These units, even though do not separate two different phases, are included here as they are intended to separate two species, and together with the two precedent ones provide an overall overview of the separation processes between the gas, liquid and solid lines in MELISSA. In this case, the main user of the GGSS is the Higher Plant Chamber. If a combined Crew – Higher Plant Compartment is used, the GGSS will become very important. Management of Oxygen and Carbon Dioxide in an efficient way will be mandatory. This will not only include filters and sensors, but most likely will lead to modelling of atmosphere in that combined area.

**CT 47: <u>HPC Atmosphere management.</u> Atmosphere management in the Plant Growth Chamber will be a critical technology to be developed. Correct breathing atmosphere for crew vs. high efficient plants in adequate atmosphere.**

#### 6.2.3.2 Sensors

The development of sensors may be a critical issue in the spacialisation of the MELISSA loop and it is important to distinguish between state-of-the-art sensors that are currently available in the market or that will be in the near term from sensors that should be developed specifically for space.

All sensors needed in the MELISSA space system have to be identified and foreseeable systems in the near terms or available state-of-the-art systems need to be searched. Thus, wastewater treatment field (Vanrolleghem, 2001) is driving the development of portable sensors for BOD or COD. Therefore, ESA should not stress developments as these. However, there are other sensors less prone to be produced by the Industry and it is for the Space Engineering companies to develop. This strategy of listing and developing specific sensors for space has been applied in other projects (Bollan, 2000) and even in the same MELISSA development project (Elvira et al, 2001)

**CT 48: Classification of Sensors. Sensors need in MELISSA need to be listed and classified in three types: 1. Industrial available sensors suitable for flight, 2. Industrial sensors available in the near term and suitable for flight and 3. Not developed and necessary space engineering work for sensor production.**

#### 6.2.3.3 Control

All the control issues (including architecture and software) are crucial for the operation of MELISSA. These issues are being specifically studied in the frame of the same ESA project to which this Technical Note belongs.

#### 6.2.3.4 Heat management

Plenty of theoretical work has been performed on the subject of heat management for a lunar base (for example, Curwy et al, 1992). Some work has been also provided for Mars, but there are still a lot of activities to be carried out to study the heat management for MELISSA system on Mars. This MELISSA space system will be a very complex one, generating heat in a TBD manner, due to the different cultures and associated hardware. As a consequence, different temperatures at different locations will develop and these temperature differences need to be compatible with the air circulation needs for atmosphere gas composition and with control issues. This points becomes even more critical for the Higher Plant Chamber if it is to be not buried.

**CT 49: Heat Management of MELISSA space system on Mars Surface. This should be studied in detail in a separate mode for Plant Growth Chamber and the rest of the compartments.**

#### 6.2.3.5 Food Technology

MELISSA will be producing edible biomass. There could be a part of the whole system, where the edible biomass is elaborated as “food”. This means to take into account not only the dietetics, but also the organoleptic characteristics of each edible element available. This could

be envisioned as a separate activity that would lead to the production of specific food production hardware. Refer to:

**CT 31: Non-edible material preparation. Study on methods of preparation of the solid wastes from food production.**

#### *6.2.3.6 Radiation*

Radiation will affect in different ways:

- Enough protection to the cultures shall be given to avoid alteration of the strains and consequent malfunction of the system.
- UV radiation seems to be a source for the highly oxidative environment on Mars that should be taken into account in long duty elements of the system.
- High-energy particles may impair or even destroy electronic equipment. This might become catastrophic for the control system HW. Therefore, the radiation environment where MELISSA is to operate needs to be defined in order to design the appropriate hardening of sensitive equipment.

**CT 50: Radiation hazards shall be listed and countermeasures designed to avoid malfunctions of the system.**

# 7 IDENTIFICATION OF CRITICAL TECHNOLOGIES

Along the document, we have been finding different critical technologies that are in the path that will lead to the spacialisation of MELISSA. These critical technologies are listed here below:

# 8 SUMMARY

The critical technologies related to a Bioregenerative Life Support System as MELISSA, identified throughout the present study and developed in the precedent chapters can be grouped in the following categories:

1. ***Internal technical or scientific issues:*** These are issues that are intrinsically needed for the correct performance of a Bioregenerative Life Support System on ground. That means that before going to Space, MELISSA will need these issues to be solved to work in nominal conditions.

2. ***Industrial critical technologies:*** These are technologies that the industry will most likely develop in the coming years simply to satisfy the market demands.

3. ***Space critical technologies:*** These technologies are needed after the correct performance of a Bioregenerative Life Support System on ground is demonstrated and most likely will not be developed by the industry. Therefore, it would be needed that the space companies work in the issue in the coming years.

The main advantage of this classification is that the resources are better assigned, because they are separated in what is scientific issues that need to be solved by the scientific community and those technical activities that have to be solved by the engineers. These resources are better assigned as well, because developments that the industry will itself carry out will not be funded by ESA.

The risk in this classification is that the forecast of the developments to be carried out by the industry can be erroneous.

## 8.1 Internal technical or scientific issues

According to the classification given above, we list the Internal technical or scientific issues in the MELISSA spacialisation process:

CT 1: Input to Compartment I definition. How are the inputs to be introduced in the bioreactor of Compartment I, in continuous or batch mode? This will imply a storage strategy. Up to date, the strategy selected is to grind the waste and freeze it in packages to be stored and introduced in Compartment I as it is needed.

CT 2: Compartment I volume reduction. The process is slow, thus a big bioreactor to allow big resident times is needed. Is the design going to be improved to have a smaller bioreactor?

CT 5: Gas Liquid Separation System. Separation of gas-liquid phases in reduced gravity in a stirred tank, if needed.

CT 10: Design of bioreactor. Optimisation of the illumination/volume ratio versus stirring of the bioreactor.

CT 11: Photobioreactor design. The thermal jacket for this reactor will be between the light source and the culture. For long working periods, the water used for thermal regulation will have to be transparent enough for the selected wavelength range. Means to maintain the cleanliness of the water shall be ensured.

CT 12: Solid-Liquid separation methods should be developed to be compliant with a high separation rate and possibly a continuous operation.

CT 13: Colonisation time reduction in Compartment III. Is this colonisation time to be reduced or will it be more or less kept of the same magnitude due to the type of strain used? It will largely impact on the mode of operation of the loop.

CT 14: Compartment III biomass recycling. How is this filtered biomass to be treated and recycled? It is most likely going to be recycled in Compartment I.

CT 15: Compartment III releasing system. The present method to release cells from the beads is a counterflow. This method releases the most external layers and concentrates the beads on one side of the bioreactor. This could be positive for a low gravity scenario, but it gives problems of excessive accumulation of biomass on one part of the bioreactor. It would be interesting to develop a releasing method that could be distributed in several parts of the bioreactor and acting depending on the amount of living cells measured locally. Methods like ultrasound release without disruption could be interesting.

CT 18: Urea treatment. Is urea to feed directly after filtration the growth chamber, or will it be processed by Compartment I before? Presently, it is going to be processed in compartment I or in an added compartment.

CT 19: Harvesting system for Arthrospira. To be compliant specifically for Arthrospira conditions at the output.

CT 21: Pressure management. Study of the pressure regulation of the entire loop, for each gas and for each bioreactor. The pressure management in each bioreactor will depend mainly on its design and on the design of the gas loop connections between reactors. Thus, pressure regulation in the Arthrospira bioreactor, where the walls are flexible, will differ from pressure regulation in Compartment I.

CT 23: Fibre degradation. The fibber degradation in compartment I is not complete and study on alternatives or complementary processes is needed.

CT 24: pH regulation. Study on the pH regulation strategies: MELISSA products or external buffering. In case that the MELISSA products strategy is taken, important development work is envisioned.

CT 25: Buffering strategy. A buffering strategy will be vital to obtain a MELISSA loop compliant with the requirements of dependability. Buffer for emergency problems, to damp oscillations in flow or pressure and to store different substances.

CT 29: Global pressure regulation. There should be a study related to the different levels of pressure that will exist in practice along the gas loop and means to maintain stable situation shall be envisioned.
CT 30: Human waste separation. Study on processes to separate liquid from solid phases in human wastes will be needed at some point. Will it be a drying process, where the faeces will be frozen or dried?

CT 31: Non-edible material preparation. Study on methods of preparation of the solid wastes from food production

CT 36: Micro-organisms population ageing influence. Assessment on the effects of the population ageing in MELISSA loop performance is needed prior to its industrialisation. Means to refresh or renew the population should be proposed, if needed. ESA is performing presently an study on this matter.

## 8.2 Industrial critical technologies

According to the classification given above, we list the Industrial critical technologies needed in the MELISSA spacialisation process:

CT 3: Clogging monitoring. Systems to monitor clogging and maintenance strategies will be required for the filtration of the liquid exiting Compartment I.

CT 4: Alternatives to chromatography. Chromatography methods will be used. This technology is not presently developed to be portable and lightweight to be used in a space mission. It also requires a lot of consumables and maintenance. This could impose a limit for the automatic operation of MELISSA in space. It is possible that for each substance a specific sensor could be developed.

CT 7: Contamination detection methods should be envisioned. Some of them might need some development work (pathogen detection). Thin films of bioactive material might be required at the different connections of the compartments (biosensors).

CT 8: Surface Microbe Detection. Sensors to monitor and control surface microbes are needed in MELISSA.

CT 17: Ammonia and Nitrate measurement. A high resolution, sensitivity system will be required for the output of compartment III in order to measure low levels of ammonia and nitrite. For nitrite, this would be 0-0.6 PPM.

CT 22: Pump working range. There is a need for pumps with working conditions that are acceptable for the widest range of rheologic characteristics and pH of the flows in MELISSA. Combined sensors of flow and viscosity will be needed.

CT 27: Trace Gas and contaminants measurement. Trace gaseous and vapour contaminants are important measurements that should be incorporated. In addition, high resolution measurement systems are required.

CT 28: Airborne microbe monitoring. The detection of airborne microbes is needed in MELISSA. A system with enough sensitivity and fast measurement should be developed.

## 8.3 Space critical technologies

According to the classification given above, we list the Space critical technologies needed in the MELISSA spacialisation process:

CT 6: Sterilisation Methods. Sterilisation methods that could be flown will have to be developed. Autoclave technology does not seem to have the characteristics needed for this purpose.

CT 9: Consumable reduction. In many instrumentation of the envisioned MELISSA loop Pilot Plant, a considerable amount of consumables are foreseen. They should be minimised as well as the maintenance workload.

CT 16: On-line viable biomass monitoring. Measurement of the viable immobilised biomass is a must to have a good control of the performance of the reactor. Systems to measure locally viable biomass are already available, but effort should be made to make this equipment lightweight and with lower biomass detection threshold.

CT 20: Connection Crew-Higher Plant Compartment. Discussion of the connection crew-MELISSA approach is needed. This largely impacts the type of technology: one would be more insisting in atmosphere regulation to avoid hazardous levels for plants and for crew and the other would be more concentrated in filtering strategies. It seems more likely to choose sharing the atmosphere for manned operation to ease the plants harvesting operation, as well as for psychological issues. But this will depend directly on the Greenhouse strategy taken at the end.

CT 26: Cleaning measures design. Cleaning measures are required to maintain the probes of the sensors inside the bioreactor under correct conditions.

CT 33: Crewmembers on Martian orbit. The need of a BLSS for the 2 crewmembers that remain in microgravity during the whole mission to Mars (ESA missions) should be analysed. If this need is confirmed then the spacialisation of MELISSA should include the microgravity scenario.

CT 34: Plant Growth Chamber Structure. The structure of the Plant Growth Chamber will have to take into account the environmental conditions if its use on the Martian surface is considered. Another option would be to bury most of the structure beneath the surface. However, this option would imply the use of artificial lighting, or complex optic fibber based light transport.

CT 35: Influence of site approach. MELISSA space concept will depend on the type of site approach: a multisite or a single site approach. A single site approach will imply to design a more flexible system. The site chosen influences the design. It is not the same design for a deep valley or a basin (Hellas basin or Valles Marineris) with respect to a Planitia (Amazonis planitia), as it is not an area with sand dunes in the near area or a rough rocky area.

CT 37: Transit operation. During the transit to Mars, MELISSA could be either working partially or completely dismantled and assembled once on the Mars surface. It is needed to assess if having a low working level MELISSA during transit could save work in MELISSA deployment on Mars surface.

CT 39: Water volume reduction. For transportation reasons, investigations on water volume reduction should be carried out.

CT 40: Stand-by operations. To evaluate the stability of the MELISSA loop in long absence of the Crew.

CT 41: Response to changes in the metabolic load. To investigate the MELISSA response to changes in the metabolic load do to the likely activities of the crew outside the habitat during the mission or potential crew overlapping.

CT 42: LSS for activities outside the habitat. To investigate specific LSS for external activities and interfaces, if any, with MELISSA.

CT 43: Gravity influence in Bioreactor design. Characterisation of bioreactors in Martian gravity fields is needed. This will be hard to accomplish on ground or on orbit and will likely need the use of missions to Mars. Being MELISSA such an important facility for a Mars mission, investigation on this field needs to start with no delay.

CT 44: Higher Plant Compartment Illumination Strategy. The Plant chamber design in MELISSA will depend on the type of illumination envisioned: a direct natural light with the limitations provoked by the dust storms or an artificial illumination of the bioreactors.

CT 45: Bioreactors and HPC scale-up. Scaling-up of the bioreactors and Plant Chamber will follow after the MELISSA Pilot Plant has finally finished its demonstration phase.

CT 46: Transpired water recovery. Water vapour recovery from air in the Higher Plants Chamber is an important technology in the frame of the MELISSA system. It needs an specific development.

CT 47: HPC Atmosphere management. Atmosphere management in the Plant Growth Chamber will be a critical technology to be developed. Correct breathing atmosphere for crew vs. high efficient plants in adequate atmosphere.

CT 48: Classification of Sensors. Sensors need in MELISSA need to be listed and classified in three types: 1. Industrial available sensors suitable for flight, 2. Industrial sensors available in the near term and suitable for flight and 3. Not developed and necessary space engineering work for sensor production.

CT 49: Heat Management of MELISSA space system on Mars Surface. This should be studied in detail in a separate mode for Plant Growth Chamber and the rest of the compartments.

CT 50: Radiation hazards shall be listed and countermeasures designed to avoid malfunctions of the system.

# 9 MELISSA-BASED BIOLOGICAL LIFE SUPPORT SYSTEM

Based on the critical points discussed in the document, this chapter intends to present some thoughts on the possible use of MELISSA to be incorporated as the Biological Life Support System for future Space missions.

Trade-offs performed by other Agencies show that a combination of biological and physico-chemical processes could be a good option for a long duration mission (Verotsko et al, 2001). In particular, it could be interesting to combine the features of compartment I with physico-chemical processes that can reduce the biological hazards related to the malfunction of Compartment I. Alternatives can be incineration of certain wastes prior to introduction of the ashes to Compartment I.

In a real Space scenario the MELISSA steady state conditions will be often altered due to a time varying metabolic load (off-habitat activities taking some days, crew shift replacement etc.). In order to maintain these steady state conditions as permanently as possible, one possible solution would be the introduction of easy to control devices simulating the human metabolic load. Adding complementary physico-chemical reactors for air revitalisation and water recovery would be another option, permitting to react faster than the biological reactors to these temporal variations in the system behaviour. Having a faster control may also allow reducing the size of the material buffers in MELISSA.

Finally, it will be crucial to test the critical technologies, and in general the overall performance of MELISSA as BLSS, in an as much as possible realistic scenario before any Space mission. Antarctica, as already proposed by some researchers (Andersen et al, 1990), seems to be a suitable choice for this test.

# 10 CONCLUSIONS

We provide a list, which summarises the detailed list of critical technologies provided in this document and that is divided according to the types of problems indicated before. It is also important to stress that this is a list of technologies and not a list of solutions for problems. These solutions are to be addressed by the different experts involved in a Bioregenerative Life Support System development project.

## 10.1 Internal or scientific Issues

The following technical or scientific issues need to be solved for the correct performance of a Bioregenerative Life Support System on ground:

- Long colonisation time of the bioreactors. This time could reach up to 6 months. This is imposing stringent requirements on the reliability of the loop after a failure of a bioreactor.
- Fibre degradation in first compartment. Fibre remains as a waste in a first compartment and can not be degraded completely with the present designs of Bioregenerative Life Support Systems.
- Harvesting and separation technology. Separation of phases will be an important issue when all the compartments are connected.
- Pressure management of the loop. The generation of gases in the different points of a loop will produce an installation with non-localised pressure variations that will be a challenge for the designers.
- Design of bioreactors. This will be an important factor for the scaling-up of the plant. For example, illumination vs. aeration that will influence on the geometry of the bioreactor
- Packaging and storing strategy for input to first compartment
- Recovery of water from transpiration of plants
- pH regulation. The use of external buffers might imply either a continuous supply or a regeneration. Use of redistribution of substances in a loop to regulate pH is being studied.
- Dependability of the system: availability, reliability and maintainability of the system.

## 10.2 Industrial Critical Technologies

We have assumed that the following critical technologies are to be developed in the coming years to respond to the market demands:

- Improvement on technology for calibration in sterile conditions.
- Systems to monitor automatically clogging of membranes
- Substitution or improvement of Gas Chromatography and Mass Spectrometry (lighter and simpler systems)
- Fast and accurate contamination detection methods (pathogens)
- Polyvalent pumps to cope with liquids with wide range of rheologic characteristics
- Technologies for high efficiency in separation of phases
- Measurements of low levels of Ammonia and Nitrite (high resolution, high sensitivity)
- Trace gaseous and vapour contaminants measurement with enough resolution
- Airborne microbes detection

- Sensors to monitor and control surface microbes
- Improvement of ion detection systems

## 10.3 Space Critical Technologies

The following critical technologies will need a development process by the space companies in the coming years in order to fulfil the requirements set by the hypothesis used in the work (A Bioregenerative Life Support System on Mars):

- Solid-liquid separation in 1/3 gravity and in microgravity
- Gas-liquid separation in 1/3 gravity and in microgravity
- Design of bioreactors in 1/3 gravity and in microgravity
- Sterilisation methods that can be flown
- Connection between a Plant Compartment and the Crew Quarters. The crew will need a suit to enter the Plant Compartment if it is a separated environment, but the growth of the plants might be enhanced with specific atmosphere different to that of the crew. Just on the opposite, direct access to the Plant Compartment would be good for crew health issues, but the efficiency in growth will diminish.
- Use of solar radiation on Mars surface. It can either be sunlight directly, but with problems on intensity level due to dust storms or illumination with specific wavelength and considering another electrical energy supply
- Heat transport and management. This will be specific for Mars surface.
- Calibration of dissolved gas measurements. Reduced gravity or microgravity will impose conditions that will be different form those, under which these systems are designed and calibrated.
- Plant Compartment structure. The type of soil and the conditions (dust storms) will influence this design with specific rigidity requirements.
- Dependability of the system on Mars surface
- Design of the transport to Mars surface: already started cultures in bioreactors or frozen starters to be used on Mars surface
- Set-up on Mars surface
- In-situ Resources Utilisation issues
- Protection against radiation of the compartments
- Water mass reduction
- Control response in case of variable load
- Interface with EVA
- Fast setting up strategies

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