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MELiSSA: THE EUROPEAN PROJECT OF CLOSED LIFE SUPPORT SYSTEM

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ABSTRACT

The MELiSSA (Micro-ecological life-support system) project is intended to be a tool to gain understanding of closed life-support systems, and consequently a knowledge base for European development of regenerative life-support systems for long-term manned missions (e.g. lunar base, Mars mission). The driving elements of MELiSSA are the production of food, water, and oxygen from the organic wastes of the mission (e.g., urine, CO_2,). Inspired by a terrestrial "aquatic" ecosystem, the MELiSSA process consists of five main sub-processes called compartments, from the anoxygenic thermophilic up to the photo-autrophic (e.g., higher plants). The choice of this compartmentalized structure is required by the very high level of space requirements in terms of robustness and safety. During the 20 years of the project, a very progressive and structured approach has been developed to characterize, model, and control the MELiSSA loop. This approach starts from the selection of the involved sub-processes, up to its predictive control. The project is structured on a Memorandum of Understanding (MOU) and is managed by ESA. It involves roughly 30 organizations encompassing Europe and Canada; eleven of these organizations, called partners, have signed the MOU: University of Ghent, University of Mons-Hainault, Studie Centrum voor Kernergie, Vlamish Institute Technology Onderzoek (B), University of Clermont-Ferrand, Sherpa Engineering, Technomembranes (F), University Autonoma de Barcelona (E), University of Guelph (CDN), IP Star (NL) and the European Space Agency. The project is co-funded by ESA, the MELiSSA partners, and local and national authorities.

The project is organized in five phases: basic R&D, preliminary flight experiments, ground and space demonstration, technology transfer, and communication and education. More than a complete status of the project, this paper presents an overview of recent achievements.

Keywords:
Closed Life Support, MELiSSA, Artificial ecosystem, ECLSS

INTRODUCTION

Long-term space missions (e.g., to Mars), including the establishment of a long-term, manned base, implies the development of a reliable life-support system including food supply and waste management. Due to the mission duration, supplying all food, oxygen, and water from Earth will result in a tremendous cost; therefore, the life-support system has to be increasingly regenerative. Presently, on board ISS, technologies are operational to regenerate clean water by appropriate treatments (e.g. SRVK, WRS). However, these techniques generally consume a lot of energy and cannot produce food, which must still be re-supplied from Earth. Food production can only be achieved by biological means, and the introduction of biological techniques opens a new set of solutions for other life-support requirements such as atmosphere, water, and waste management (Myers 1954, MacElroy 1985). A space-based life-support system must meet the rigid requirements of efficiency, mass, crew time, reliability, and safety. For the design of regenerative systems, for the study, experimental testing, and development of future manned missions, a purely engineering approach has to be followed. For the last 30 years, numerous developments of recycling technologies have been initiated by the major space agencies. However, these typically consist of recycling one product in a new consumable (i.e. water recycling, O_2 production) without a global overview of the complete life-support system. For these reasons, namely overall mass balance and, engineering approach, ESA has initiated the MELISSA project.

THE MELISSA CONCEPT

Inspired by an aquatic ecosystem, the MELISSA (Micro-Ecological Life-Support System Alternative) project has been set up to be a model for the study of regenerative life-support systems for long-term space missions (Mergeay et al. 1988). The compartmentalized structure of the loop and the choice of the several microbial processes has been done to simplify the behavior of this artificial ecosystem and allow a deterministic engineering approach. MELISSA has five major compartments (Figure 1) colonized, respectively, by thermophilic anoxygenic bacteria, photo-heterotrophic bacteria, nitrifying bacteria, photosynthetic bacteria, higher plants, and the crew.
The project is organized in five phases: 1) Basic R&D, 2) Preliminary Flight Experiment, 3) Ground & Space Demonstration, 4) Terrestrial Transfer, and 5) Education & Communication. As it was not possible to present all activities of these phases, we simply reviewed the major decisions and important results of Phases 1 to 3.

PHASE 1 – BASIC RESEARCH & DEVELOPMENT

For any closed life-support loop , the efficiency of the complete loop is totally dependent on the weakest process. The early days of the project mainly focused on the identification of the proper microbial strains. Early expectations that pure and axenic cultures could be selected for all functions were demonstrated to be impossible. This impossibility was mainly due to two reasons: waste degradation with pure cultures is limited to

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a few percent of the input stream, and the high percentage of microbial food sources is limited by the nucleic acid content and the associated risk of uric acid accumulation. For these reasons, specific attention has been devoted to the waste- degradation sub-subsystem as well as the food-production sub-system. Parallel activities related to modeling and control, as well as genetic stability, have been pursued.

The waste compartment (CI)

The liquefying compartment is the first step in the MELiSSA loop and determines the fraction of organic waste that can be recycled in the loop. In order to validate the whole MELiSSA loop, the construction of this compartment at pilot scale was necessary. The "Engineering of the Waste Compartment" started in 2001 (Hermans et al., 2000, Michel et al., 2005). The main objectives were validation of the anaerobic process, the design and construction of the first compartment at pilot scale, validation and calibration of the model, and the implementation of different control levels.

Before building a pilot bioreactor, a concept was defined and the detailed design of all parts of the pilot compartment was investigated. As a first step, a small prototype of the first compartment was built. In 2004, functional tests were performed on the hardware, providing knowledge for construction of the pilot-scale compartment. Study of the process at prototype scale was performed with human fecal matter over a representative period (4 months). Based on these prototype tests, the pilot design was updated. Hardware was selected, ordered, and assembled. Results obtained from this pilot demonstrated the possibility of reaching around 70 % efficiency (Figure 2).

Although this result could be considered successful in any other context, this value is not high enough for closed-loop systems, so major efforts have been pursued to increase conversion of fibrous material present in the waste. To achieve this goal, three technologies complementary to the first compartment were investigated: 1) fiber liquefaction by means of Fibrobacter succinogenes (Gwendoline et al. 2005), 2) liquefaction and complete sanitation of the most recalcitrant organic matter by a high pressure and temperature unit (Verstraete et al. 2005), 3) liquefaction and sanitation of the raw waste by hyper-thermophilic organisms. Results demonstrated that much higher values of degradation can be obtained (i.e. >90 %).

Food Production and Preparation

The Higher Plant Compartment or Chamber (HPC) is an important component of the MELiSSA loop (Waters et al., 2005; Favreau et al., 2005). The function of the HPC is the provision of life-support elements including CO_2 fixation, O_2 generation, potable water production, and most significantly, food production. When compared to other MELiSSA processes, the food-production sub-system presents specific challenges: 1) part of the nutritional requirements are not known, 2) it is a multi-cellular eukaryote- based technology, 3) the selection of crops and cultivars cannot be performed without a complete food-chain overview (e.g. cultivar selection, culture conditions, raw biomass processing, recipe elaboration, conservation).

Since 2007, a specific activity, "food characterization for MELiSSA", has been initiated to investigate this complete chain. Study, development, and customization of a Higher Plant Compartment (HPC) is one of the first steps toward this goal.

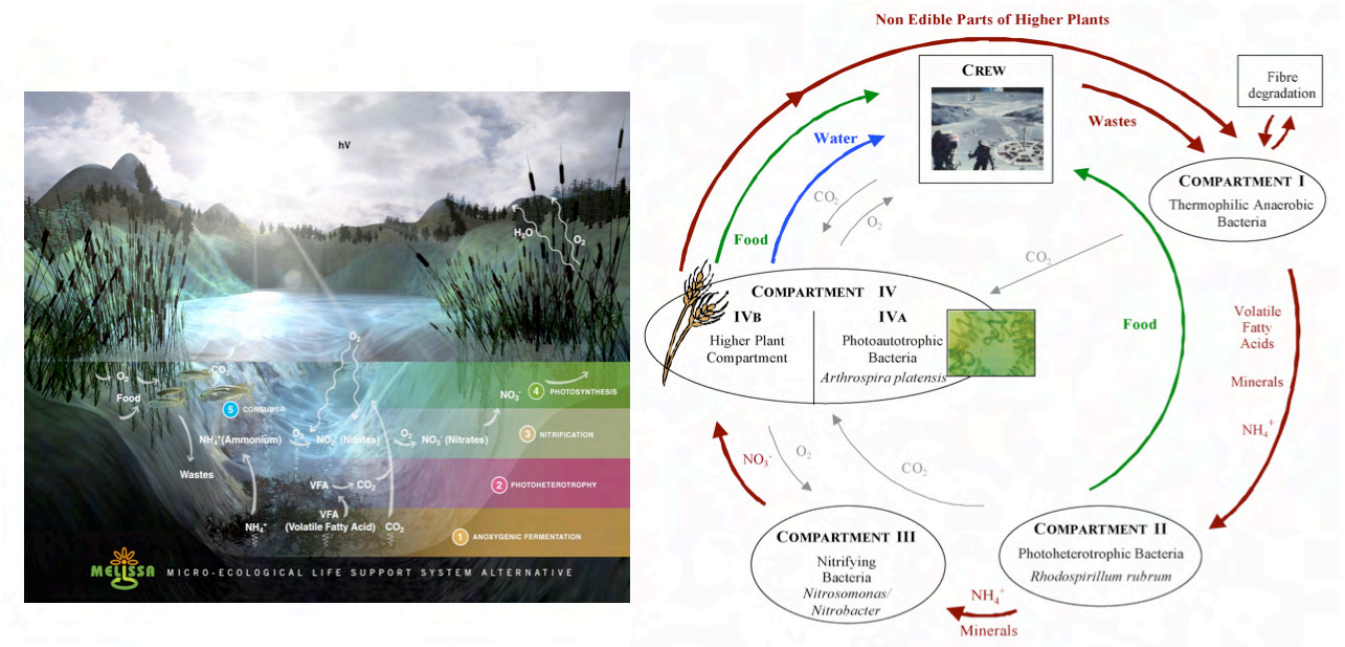


***Figure 1**: Concept of the MELISSA loop. Left-hand side: an aquatic ecosystem. Right- hand side: the compartmentalized structure.*

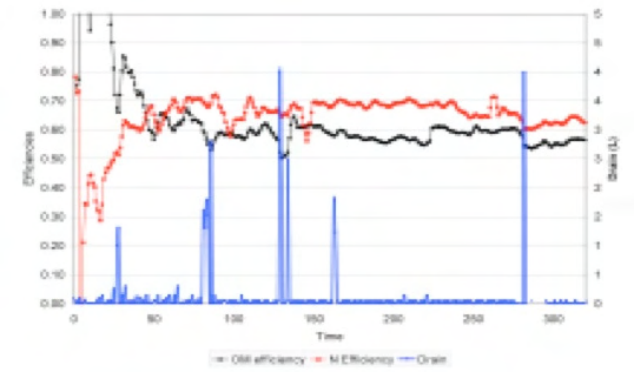


Figure 2: *Efficiency of the MELiSSA compartment 1, without the fiber degradation unit (OM : Organic matter, N: total nitrogen transformation). The drain is used regularly to reduce the percentage of non-degradable product (e.g. fibers).*

Mathematical modelling

Expansion of the MELiSSA project required a tool for carrying out steady-state and dynamic modeling of the loop, which is used by the MELiSSA partners to understand the behavior of the loop under various operating conditions, and to estimate the efficiencies and proper sizing of the loop. This mathematical model is further used for control tasks. The complete MELiSSA loop was modeled using MatLab/Simulink, and a mass-balance simulator (version 0.0.01) for the loop was released and updated (Poughon et al., 2000).

In recent years a specific effort has been made to model the higher plant compartment (Favreau et al., 2005. The main difficulty is that plants are complex organisms. Consequently, it is difficult to develop suitable structured models for them. Plant- growth modeling started in the MELiSSA project has led to several experimental and theoretical investigations (Poughon 1997, Waters et al., 2002, 2003). Main conclusions regarding the plant-growth models can be summarized as follows:

- Dynamic growth models must include expressions giving the carbon fixation rate of plants ;

- The models have a form $\frac{dx}{dt} = f(t)$ rather than a form $\frac{dx}{dt} = f(t, x)$ compared to classical microbial models. This means that the models are implicitly based on the repeatability of growth over time. This difference is important because it implies that growth rate is not proportional to the mass of the plant. Models are dependent only on environmental parameters (if the coefficients of the models take them into account) and the time constants of growth.

- Principles for the dynamic model of plant growth are similar to those used for the growth of micro-organisms : (i) the biological activity (including growth) can be described by one or more mass-balanced equations (stoichiometric approach) ; (ii) to each stoichiometric equation is associated reaction kinetics. This rate can be calculated using a specific dynamic model, which can calculate the consumption/production rates of all other compounds involved.

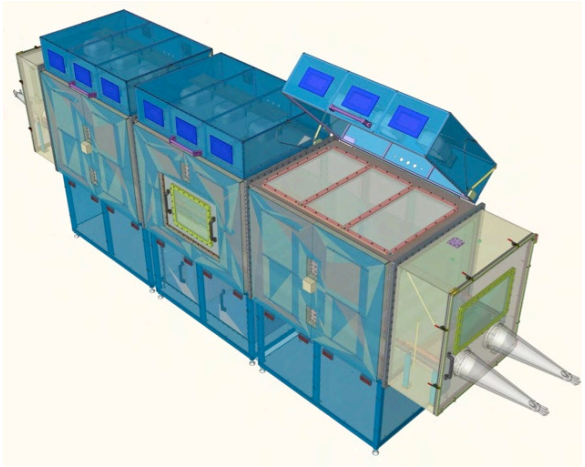
An attempt to develop an exhaustive list of the growth parameters and variables involved in the growth of plants has been made. The main conclusion is that most environmental variables (temperature, pressure, humidity, etc.) would have to be controlled to obtain optimal growth. This can be achieved only if plants are cultivated in isolated chambers. The growth itself can be controlled by light, CO_2 partial pressure, and tracked by the evolution of nutrient concentrations and CO_2 uptake. Among those parameters, lighting (PAR) is straightforward to manage, as electric lamps are used.

There are already reliable models for plant growth that have been implemented for the study of candidate crops for the MELiSSA loop, although their utilization for growth prediction and consequently for control is presently questionable.

Table I (Poughon et al., 2009) summarises MELiSSA's state of the art in terms of process characterization modelling and control.

	C1 (%)	C2 (%)	C3 (%)	C4A (%)	C4B (%)	C5 (%)
Comparison/selection of the process	80	70	95	95	20	N.A.
Process stability (without control)	0	40	30	40	10	N.A.
Steady state and mass balance	50	70	90	80	20	75
Dynamic and mass balance	20	50	60	80	5	40
First knowledge model	20	50	60	80	5	40
Calibrated knowledge model	20	40	50	80	0	N.A.
Control model	0	0	5	80	0	N.A.

Table I: *Status of current MELiSSA compartment models (Poughon et al. 2009). The % represent the % of completion of the task, N.A.: Not applicable)*



CAD drawing of the HPC Prototype.

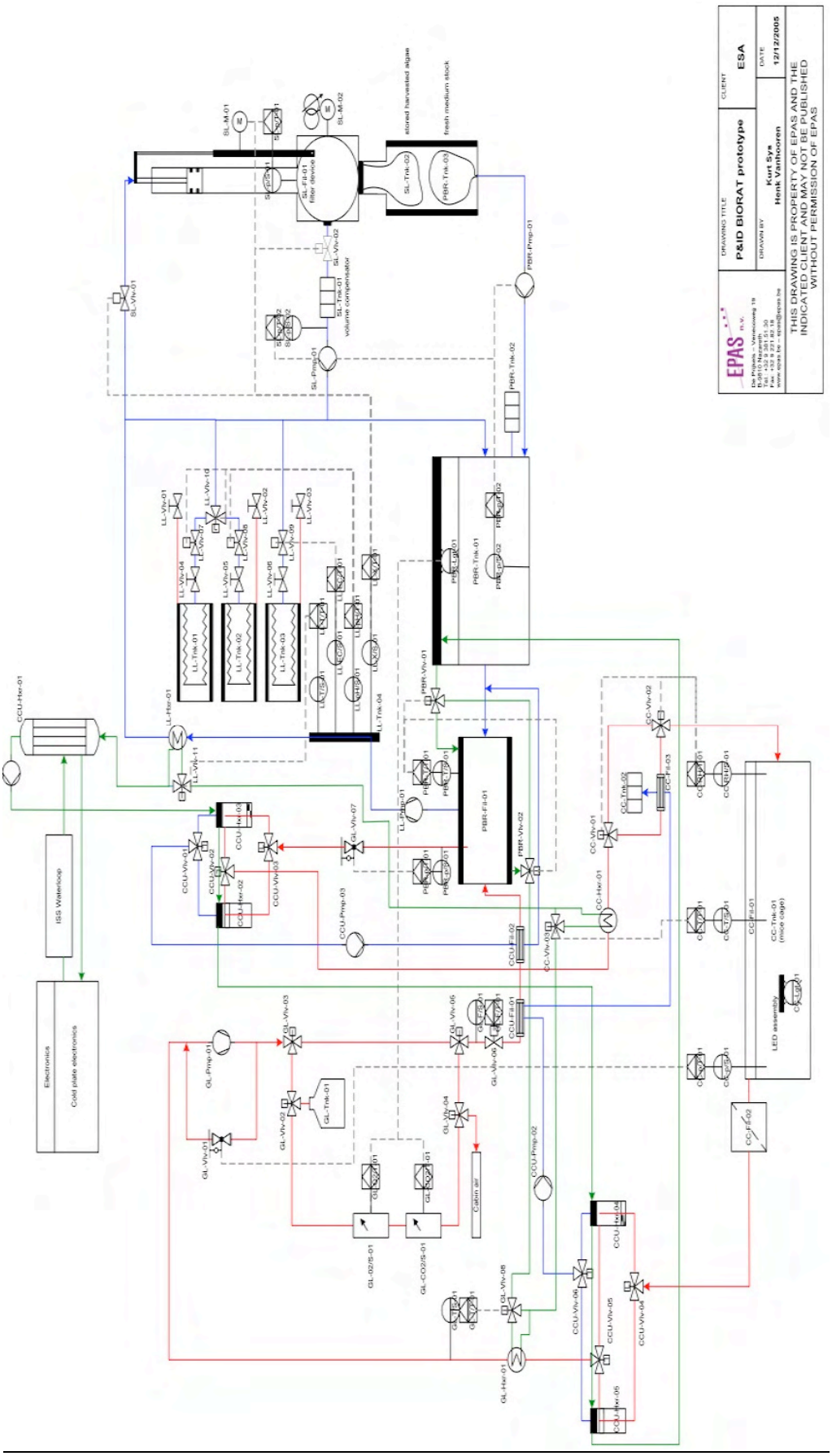


Figure 3: *Overall design of the Biorat experiment.*

Genetic stability and stress detection

For long-term operation during stress created by the space environment or cultural conditions, it is necessary to detect and potentially counter genetic evolution of organisms in the MELiSSA compartments. The MELGEN (i.e. MELiSSA Genetics) activity is focused on understanding the physiological mechanisms induced by different stresses (temperature, oxidative stress, pH, radiation, pressure, etc.) as an indicator of major genetic and proteomic evolution of MELiSSA's microbial strains (Hendrickx et al., 2005; Leys et al., 2004). At the beginning of the study the genome of the main MELISSA strains was not known; therefore, the physiology of the environmental bacterium Cupriavidus metallidurans CH34 (formerly known as Ralstonia metallidurans) has been selected in order to understand its behavior and resistance under extreme conditions. In order to estimate fine physiological changes associated with temperature or oxidative stress, flow cytometry was employed as it provides a powerful means to measure a wide range of cell characteristics.

PHASE 2: PRELIMINARY FLIGHT EXPERIMENTS

As already noted, both physico-chemical and biological processes will be influenced by the space environment. The validity of the engineering approach currently selected in the MELISSA project requires understanding and quantification of these effects. Therefore, activities of Phase 2 have been initiated in parallel with Phase 1 of the project. Currently, Phase 2 is focused on gathering knowledge about the bacteria used in the MELiSSA loop or in closed life-support systems at the global level. This includes study of genomic, transcriptomic, and proteomic data to know more about their response to spaceflight conditions with special attention to cosmic radiation, and microgravity. Phase 2 efforts also naturally integrate into the growing field of space microbiology with a special concern for bio-safety, which is of paramount relevance for life-support systems (Mergeay, 2006; Novikova et al., 2006; Wilson et al., 2008; Van Houdt et al., 2009). The use of bacteria in closed life-support systems such as MELiSSA and other alternatives was thoroughly reviewed and is regularly reassessed (Mergeay, Verstraete et al., 1988; Hendrickx, De Wever et al., 2006; Hendrickx and Mergeay, 2007; Pycke et al., 2010). The basic knowledge of MELiSSA microorganisms has taken advantage of the availability of fully sequenced genomes for Rhodospirillum rubrum (C2 of the MELiSSA loop) (Reslewic et al., 2005), the nitrifying bacteria of C3: Nitrosomonas europaea (Chain et al., 2003) and Nitrobacter winodgradsky (Starkenburg, Chain et al., 2006)i), and Arthrospira PCC 8005, the edible cyanobacteria of the C4a compartment (N.Morin et al., in preparation). After development of an optimized protocol to extract DNA from Arthrospira cells (Morin et al., 2010), the genome was sequenced at the French national sequencing center (Genoscope; Evry, France), and its annotation is almost completed. Molecular approaches were also developed for the bacteria of the nitrifying compartment in order to better understand their distribution in this bioreactor (Montras, et al., 2008). It was mainly with R. rubrum that progress was made via the construction of a DNA chip covering the whole genome of this bacteria and by developing access to its proteome (Mastroleo, Leroy et al., 2009). These developments were of importance to allow the exploitation of spaceflight experiments reported below. With regard to spaceflight experiments, efforts were carried out to evaluate new technologies such as flow cytometry (Leys, Hendrickx et al., 2004; Baatout, De Boever et al., 2006; Baatout, Leys et al., 2007), and the dosimetry of cosmic radiation (Goossens et al., 2006; Vanhavere et al., 2008). Simulated microgravity methods were also addressed in order to check their relevance to spaceflight observations and the potential for reducing dependence on spaceflight testing (Crabbe, De Boever et al., 2008; De Gelder, Vandenabeele et al., 2008; Beuls, Van Houdt et al., 2009; Mastroleo, 2009; Crabbe, Pycke et al., 2010; Leroy, Rosier et al., 2010). Two devices were used for these simulation experiments: the Rotating Wall Vessel to measure low-shear-simulated low gravity and the Random Positioning Machine. In parallel, simulations of the space-radiation environment were investigated at SCK-CEN, based on dosimetry experiments performed onboard ISS. The energetic spectrum was simulated by combining a high Linear Energy Transfer (e.g. LET) (neutron) source and a low LET (gamma) source. Different setups have been designed to assess the response of both R. rubrum and Arthrospira sp. to radiation doses equivalent to different stays onboard the ISS. Finally, in the perspective of MELiSSA compartments irrigated by effluents containing a variety of wastes including small amounts of biocides, hormones or drugs, preliminary tests were conducted on the fate of micro-pollutants (De Gusseme et al., 2009), with emphasis on the differential expression of genes in transcriptomic experiments using the R. rubrum DNA chip (Pycke et al., 2010; Pycke, Vanermen et al., 2010). All of these efforts converged and allowed the scientific exploitation of spaceflights experiments carried out in 2002 (MESSAGE1), 2003 (MESSAGE 2), 2004 (MOBILISATSIA), 2006 (BASE-A) and 2008 (BASE-B&C, BASE-D). These microbial experiments were brought to ISS by Soyuz flights for exposure to the ISS environment for about 10 days before return to Earth (De Boever et al., 2007; Leys et al., 2009; Mastroleo et al., 2009). The bacteria used for MESSAGE and BASE experiments included R. rubrum S1H as MELiSSA bacterium (Mastroleo, Van Houdt et al., 2009) and Cupriavidus metalidurans CH34 (Leys, Baatout et al., 2009) as an example of a bacterium adapted to a variety of harsh environments, including the clean rooms where satellites are built (Mergeay et al., 2009). The first experiments were conducted under drastic constraints (of volume, weight, temperature, material choice, design, security requirements) imposed by the spaceflight conditions (Leys, Baatout et al., 2009; Mastroleo, Van Houdt et al., 2009). Despite this growth, spontaneous mutants and viable counts were similar to ground experiments. Proteomic data show limited effects of spaceflight conditions, especially for C. metallidurans, although some uncommon proteins involved in acetone metabolism were found to be over-expressed in space

(Leys, Baatout et al.,2009). Transcriptomic data were mainly obtained for R. rubrum and provided information about the importance of experimental design and the effect of low doses of cosmic radiation. This effect was mainly revealed in the BASE-A spaceflight experiment where various over-expressed genes matched those found during ground tests of ISS radiation. Thus, for the first time studies showed a low dose of ionizing radiation (2 mGy) can induce a significant response at the transcriptomic level, although no change in cell viability was observed (Mastroleo, 2009). This experiment will surely stimulate further studies of effects of low-dose ionizing radiation in bacteria. These will be paramount for implementation of bioreactors in spaceflight and on planetary stations.

Transcriptomic data in the MESSAGE 2 experiment with R. rubrum revealed also some highly over-expressed, unknown-function proteins that were also observed in the Random Positioning Machine (i.e. RPM) for microgravity simulation (Mastroleo, 2009). The further deciphering of such unknown functions may shed light on some aspects of bacterial physiology in spaceflight conditions. Some of these genes (called muf) were also over-expressed in cultures of R. rubrum exposed to triclosan, a biocide used as a model for micropollutants in the MELiSSA loop (Pycke et al., 2010; Pycke et al., 2010). This suggests that some R. rubrum genes may respond to both chemical and mechanical conditions. Gene transfer via plasmid-mediated conjugation was also assayed in spaceflight with the experiment MOBILISATSIA. These preliminary experiments showed that plasmid-mediated conjugation and plasmid-mediated mobilization of small replicons could occur in both Gram-negative (E. coli and C. metallidurans) partners and Gram-positive partners (appropriate strains of Bacillus thuringiensis) at frequencies at least equal to those observed in ground-control experiments (De Boever et al., 2007). Plasmid transfer and plasmid-mediated mobilization between B. thuringiensis strains were also observed in simulated microgravity experiments (Beuls et al., 2009). At the same time, MELiSSA also developed an engineering -ocused project called BIORAT. The BIORAT project consists of a very simplified ecosystem reduced to gas exchange between a photo-bioreactor and a consumer compartment. The aim of the reactor is production of oxygen and consumption of carbon dioxide based on photosynthesis with respect to the requirements of consumers (2 mice). This means that an efficient supply of carbon dioxide produced by the mice and a good lighting system providing sufficient photosynthetically active radiation (PAR) are key parameters in designing the photo-bioreactor (Demey et al. 2000). This implies controlling photosynthetic activity but also growth and metabolism at the micro-organism level (Cogne et al. 2005). Over the last few years, specific attention has been given to the study and design of the sub-systems: - Gas Loop, - Liquid Loop, - Solid Loop and interfaces study of the Consumer Compartment. Figure 3 presents the detailed design of the complete BIORAT-prototype.

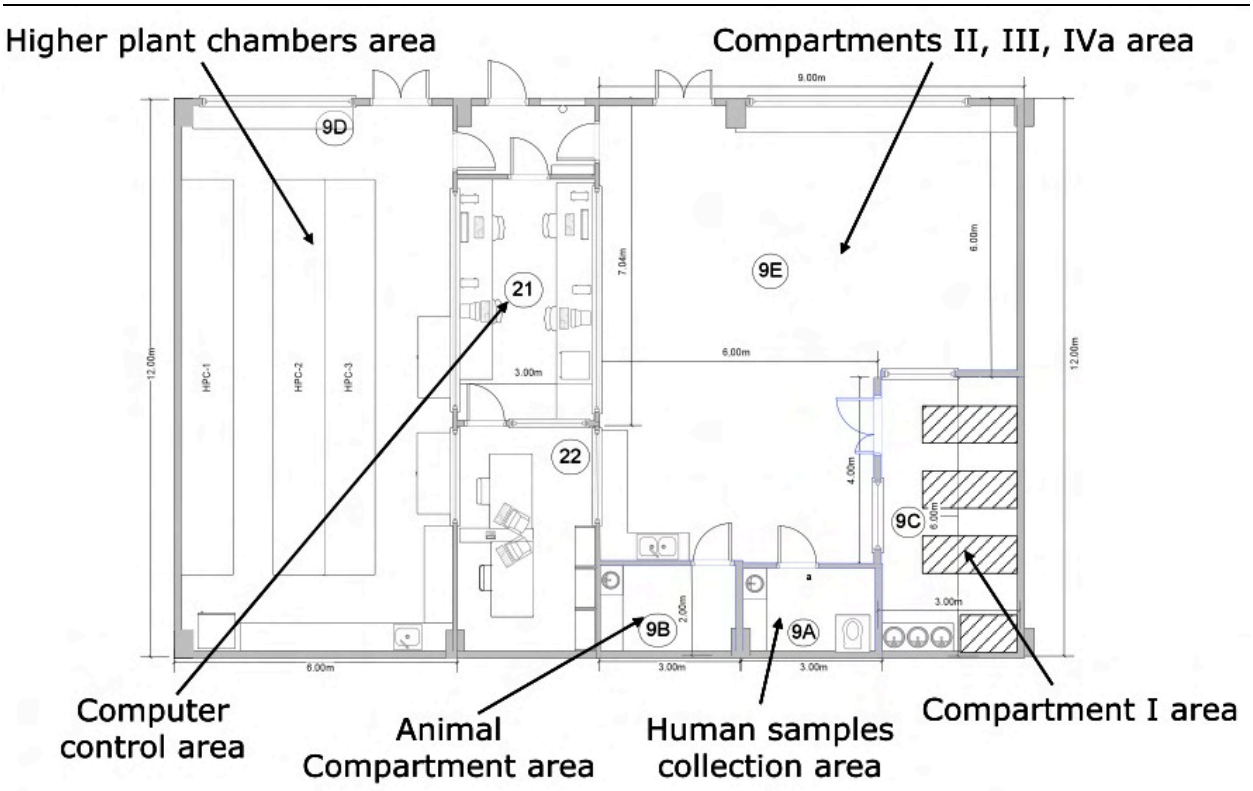


Figure 4: *General layout for the new MPP at UAB.*

PHASE 3: GROUND DEMONSTRATION

MELISSA Pilot Plant: In June 2009, the new MELISSA Pilot Plant (MPP) was inaugurated at the Autonomous University of Barcelona (UAB). The current objectives of the MPP are as follows:

- Integrate MELiSSA technologies. This can cover the installation and characterization of hardware corresponding to the different MELiSSA processes (Godia et al. 200, Creus et al. 2001). Operation and validation of all compartments in an independent manner, development and implementation of a control system, and interface equipment to ensure connection of the compartments, and operation and test of the complete loop over long periods (Poughon et al. 2009).
- Provide comprehensive test facilities where any MELiSSA-related technologies can be demonstrated on the ground. The objective of increasing the overall MELiSSA recycling rate will lead to the development of complementary technologies addressing specific aspects, e.g., fiber degradation. Such complementary technologies also will be integrated into the MPP.
- Provide access to MELiSSA-related experiments. Full understanding and control of the MELiSSA loop will require the development of specific tests as well as conduct of specific studies addressing genetic stability, toxicology, traceability, bio-safety, etc. These tests and studies will be performed in the MPP.

The current status of the NEW MPP laboratory is depicted in Figures 4 and 5.

FUTURE WORK

Based on the existing status of the project, several priorities have been identified for the coming years. These priorities are as follows:

- Development of the current modeling approach to track elements (e.g Na, P, K, Mg)
- The mechanistic modeling of higher plant crops at the same level of robustness as in microbial compartments
- The development and integration of interfaces between MELiSSA processes
- Preliminary flight experiment to understand process behavior in reduced gravity. Specific attention will be paid to bi-phasic and tri-phasic processes.
- Continuation of ground demonstrations with animals and preparation for a human-rated facility,
- Knowledge management of the accumulated know-how.

Figure 5: *Internal views of the MPP laboratory at UAB, Compartment IVa for cyanobacteria cultures.*

CONCLUSION

Over the last 20 years of the MELiSSA project, significant effort has been devoted to the experimental study of each independent compartment of the MELiSSA system, both at bench and pilot scales. The validation of detailed models for each compartment is in progress with different degrees of success depending on the compartments. It has been proved for the auto-trophic compartments that a model-based control strategy is operative, showing that deterministic control of microbial processes is possible and efficient (Cornet, 2001). The six most abundant elements in the loop, C, H, N, O, P and S are correctly followed in a theoretical simulation at steady state of the entire loop. However, further modelling investigations remain to be done at the level of each compartment, especially for the understanding and modelling of trace elements, and to validate predictive dynamic models. Deterministic control is a prerequisite of loop- sustainable existence. This robustness of a compartmentalized artificial ecosystem is materialized by the deterministic modeling and control of the interacting sub-systems. This is related to the implicit assumption that if the model is deterministic and correct, its validity range is enlarged when compared to an empirical model. This is applied to the development of the

technology of MELiSSA compartments, including microbial compartments but also the higher plants chambers, the crew compartment, food preparation, etc. The more than 20 years of MELiSSA experience has shown that such an approach is: - 1) operative, even when applied to subsystems containing living organisms, such as micro-organisms, microbial communities, or higher plants; -2) generic and capable of being tackled with different loop configurations, including all kinds of unit operations (both physico-chemical and biological).

ACKNOWLEDGMENTS
This paper is proposed as a tribute to Claude Chipaux (1935-2010), father of the MELiSSA Project.

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Acronyms

ESA: European Space Agency
ISS: International Space Station
HPC: Higher Plant Compartment
LEO: Low Earth Orbit
LET: Linear Transfer Energy
MELISSA: Micro-Ecological life Support System Alternative
MPP: MELiSSA Pilot plant
PBR: photo-bioreactor
RPM: Random Positioning Machine
WRS: Water Recovery System