II. CLOSED ECOLOGICAL SYSTEMS IN EXTREME ENVIRONMENTS

CURRENT AND FUTURE WAYS TO CLOSED LIFE SUPPORT SYSTEMS: VIRTUAL MELiSSA CONFERENCE, GHENT (B) (3-5 /11/2020). A REVIEW

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Abstract: The 3-days international virtual MELiSSA conference (November 3 to 5, 2020) about the closed life support systems was organized by the University of Ghent and IDDUP in collaboration with ESA/ESTEC (European Space Agency) and MELiSSA Foundation, and welcomed around 150 papers. The first day addressed the welcomed contributions of the 5 major space agencies, the plants & photosynthetic microorganisms used for life support in space, the simulation of planetary ecosystems as well as MELiSSA inspired circular economy and education. The second day focused on Ground demonstration and analogue testing, Photobioreactors and photosynthetic microorganisms characterization, Modelling and Systems design, Organic wastes process and Metabolome where a real breakthrough was observed as well as in human microbiome in closed environments. The last day addressed: 1) the complex problematics of urine as a major source of nutrients, 2) Terrestrial synergies, and 3) some Physical, Chemical and Microbial contaminants linked to space research.

Keywords: MELiSSA conference, closed life support system

INTRODUCTION

From November 3 to 5, 2020, the international MELiSSA conference was organized by the University of Ghent and IDDUP, on line. It was an opportunity to listen again to life support representatives from the major space agencies: China, ESA (Europe), JAXA (Japan), NASA (USA) and Russia, as well to worldwide life support actors from all TRL levels (Academic research to flight hardware demonstrators). Taking into account the contents of other life support related conferences, the MELiSSA conference is becoming a unique international platform of exchanges. Although open to any life support developments, the talks were mainly focused around MELiSSA project activities. The talks addressed three major families of questions:

- which renewable food sources (edible plants and microbes) are available to cover the needs of a space crew and could be grown in space conditions?
- how to reach a high degree of closure via the processing of solid, liquid and gaseous wastes and consequently to ensure a circular and viable system in space conditions?
- which are the effects of the space conditions on life support systems and especially confinement, space radiations and microgravity?
- how to maintain these very long term efforts, via and in collaboration with terrestrial applications, leaded these days by the challenges of circular economy?

In this respect, we may recall that life support developments for space exploration does need a very long development time, too long to maintain a

continuous and high funding from the space agencies only. Consequently, continuous applied and collaborative applications (as circular economy that will remain the main echo) are absolutely essential. Therefore, a substantial and essential part of the conference addressed the terrestrial applications.

The Covid linked conditions of confinement imposed to the conference also illustrate in some way the importance of these questions on the earth! Indeed, the conference had a great “virtual” success with major space stakeholders being represented and with more than 220 registrations, a peak audience of 183 attendees and 150 papers and posters!

This collaborative spirit is also reflected in the opening presentations of the first conference day. The life support programs from the 5 major space agencies (China, ESA, JAXA, NASA and Russia (IBMP)) were presented, as well as the program from several national agencies involved in the MELiSSA project (Belgium, Italy and Switzerland). It may be recalled here that all the presentations of the former MELiSSA conferences in Roma and in Lausanne are available on the website of the MELiSSA Foundation, including a large number of technical documents (i.e. Technical Notes).

Based on a French scientific flight experiment of growth of microalgae embarked in 1987 [1] on the Chinese launcher “Long March” (in itself a sign of international collaboration almost “in tempore non suspecto”!), MELiSSA started from an unsolicited MATRA (now AIRBUS) proposal to ESA. Taking

into account the collaborative spirit of the project as illustrated since the early days of MELiSSA, by the progresses made in University Blaise-Pascal of Clermont-Ferrand (now UCLermont-Auvergne) in the characterization of light transfer during the growth of photosynthetic bacteria and microalgae [2, 3, 6], a Memorandum of Understanding was elaborated with ESA in charge of the project coordination. This was later followed by exchange of information and collaboration with other space agencies as well as by coordinating space flights from Baikonour, Kourou or Cape Kennedy and by the creation of the MELiSSA Pilot Plant (Claude Chipaux Laboratory) in the University of Barcelona (UAB) [4, 5, 7]. In 2012, the MELiSSA partners decided to create the MELiSSA foundation to select and support MELiSSA PhDs and post-doc.

From 2002, ten MELiSSA flight experiments have been performed, three more are manifested. Six of them have been selected via the International Life Sciences (ILSRA), and two in direct collaborations with Russia. The MELiSSA approach started from simple experiments (e.g. tubes, Petri dishes, on board ISS and ground analysis of the return samples) to recycling process demonstrators via smaller scale process demonstrators (e.g. ARTEMIS). It is worthwhile to note ARTEMIS was the first successful microbial photo-bioreactor in space. Today, three experiments are approved for flight: ARTEMIS-C, as an evolution of ARTEMIS, URINIS as an in orbit demonstration of nitrification of Urine and WAPS (Water Across Plants) to investigate crop growth under space environment.

The three days of the very rich conference will be evoked hereunder via a selection of communications and quotations mostly very much summarized due to available space constraints. Yet the full program of the 3 days conference, all the presentations in slides and the full book of abstracts are on the website of the MELiSSA Foundation as well as a link <https://www.melissafoundation.org> allowing the access and therefore the informative dialogue between the Conference and the present report.

1. CONFERENCE FIRST DAY: Major topics

A. Plants & photosynthetic microorganisms for life support in space

B. Simulation of planetary ecosystems

C. MELiSSA inspired circular economy

D. Education

A. Plants for life support in space

Plant Characterization Unit (Enginsoft, Italy). Edible biomass production in plant compartments was thoroughly addressed by the development of

appropriate tools as the Plant Characterization Unit that aims towards the development of state to the art Plant Chamber and collection of scientific data for mathematical model elaboration. These will monitor carefully separated aeroponic and hydroponic parts of the plant compartment, the delivery of nutrients and will ensure the early detection of any leakage and their prevention.

From UNapoli, the full controlled growth of tuberous plants (potato) from tuber to tuber in dedicated space compatible compartments was reported. It is aimed to develop a nutrient delivery system of PFPU (Precursor of a (microgravity) Food Production Unit). The study is carried on by an Italian consortium led by Thales Alenia Space, Italia. In parallel, the ReBUS project, an Italian project coordinated from UNapoli, intends to launch a national research line for the creation of a BLSS in Space. The research aims at minimizing the use of exogenous resources while maximizing both the use of the *in situ* available resources (i.e. Lunar and Martian soils, water, and gas present in the atmosphere) and the recycling of the organic matter produced in the system itself (e.g. crop residues, crew physiological waste).

In parallel and harmonized with these Italian efforts, a Plant Characterization Unit (PCU), a hydroponic research facility with closed atmospheric and liquid compartments developed in Norway aims to characterize crops such as lettuce, wheat, soybean and potato with respect to O₂ and CO₂ production and consumption, nutrient consumption and biomass production. Additionally, activities to upgrade the crop-cultivation chamber of the MELiSSA Pilot Plant (MPP) were initiated, aiming at improving its performance by capitalizing on the Norwegian PCU development.

From UNapoli again: Seed orientation affects seedling development in hardware for experiments in space and therefore deserves a major attention for the preparation of space experiments involving plants and is a typical example of basic research with important consequences. The same research team involved in a study about micro-tomato observed that high temperatures affect pollen fertility more than altered gravity.

In the same spirit, a NASA talk also reports an effect of microgravity on plants: Predictions on several days of growth in partial g levels using the proposed used model show impaired growth response. Plant gas exchange has been extensively modelled in 1g conditions, but it is the first time it was modelled in reduced gravity.

As a complement to space-grown vegetal food, microgreens defined as edible seedlings of different plants (vegetables, herbs, or grains, including wild species) and have been shown to contain considerably higher levels of essential vitamins and antioxidants compared to their mature counterparts. Reported results obtained in Belgium (SCK•CEN) focused on microgreens from radishes and cabbages.

B. Simulation of planetary ecosystems

In the context of Interstellar Lab concept EBIOS (Experimental BIOregenerative Station), the first space-inspired village on Earth, a Bioregenerative Life Support System (BLSS) has been designed to sustain life in an enclosed environment in USA. In this respect, the BLSS is planned to be constructed on the territory of the Mojave desert: a hostile environment with extremely small annual rainfall and highest temperatures on the North American continent.

C. MELiSSA inspired terrestrial circular economy

The SEMILLA platform as a means to create environments to develop the Circular Economy concept

Circular Economy (CE) is a new paradigm where the traditional 'take, make, waste' production and consumption model turns into another where three principles drive the action of hubs: design out waste and pollution, keep products and materials in use, and regenerate natural systems. The first of SEMiLLA IPStar CE Hubs has been created in Malaga (Spain) with the participation of the University of Malaga.

The same concept of space-inspired resource recovery from wastewater treatment was illustrated with the Biomakery concept at the La Trappe brewery (SEMiLLA IPstar), situated on the premises of the OLV van Koningshoeve Abbey, the Netherlands. The beer is brewed according to the Trappist principles with an active community of 22 monks involved. As a consequence, 320 m³/day of brewery and cheese production process water and an estimated 18 m³/day of municipal water, from the monastery, need to be treated.

SEMiLLA IPstar studied also the Amsterdam case: A preliminary feasibility study concluded that the MELiSSA technologies could recover nutrients and water from the municipal wastewater and hence offsets the requirements for an average of 2 kg of vegetable crops, per person per day.

D. Societal and Educational aspects

The INSPIRATION project is an application of MELiSSA for food and water recycling in

Congo (RDC) proposed by SCK•CEN (Mol, Belgium) and set up with a local NGO. It is aimed to combat malnutrition by promoting and supporting local production of *Spirulina*. This project aims to experiment alternative resources utilization for the production of *Spirulina* in collaboration with local university researchers. As an example, the use of struvite, a cheap by-product of waste water treatment, is being tested as an alternative nitrogen and phosphorus source.

The MELiSSA Foundation (MF), an essential part of the MELiSSA educational programme, mainly selects PhD students ready to make a 4 years' doctorate on a MELiSSA topic and to constitute the Pool Of MELiSSA PhD (or POMP) or "POMPers". Every 2 years, MELiSSA summer universities (MSU) are organized. In such a summer university organized in 2019, around 15 involved young scientists: POMPers and other MELiSSA PhD students attending this MSU, organized a common research project animated from ETH Zürich and called IGLUNA. The 2021 MELiSSA Summer University will take place in Varna, Bulgaria.

IGLUNA 2020 is a project coordinated by the Swiss Space Center which aims to stimulate PhD student education and exchange through an international and multidisciplinary project. The purpose is to demonstrate technologies to sustain life in an extreme environment for a space habitat. The project started in September 2019.

The point of view of a philosopher (UVincennes) as a totally external and attentive observer about the ethical spirit and the internal organization of MELiSSA attracted also the attention: an approach to a philosophical ethnography of human spaceflight with the implications and visions of the future that some spaceflight technologies are conveying as well as an inquiry into the role MELiSSA might have in public education.

From ESA management, a science-fiction inspired description concluded this first day of the conference as a Life Support Systems making-of "Red Safari" (a book from the Weyrich Editions).

2. CONFERENCE SECOND DAY: Five major themes were addressed during this day also animated by contributions from Bulgaria.

- A. Ground demonstration and analogue testing
- B. Photobioreactors and photosynthetic micro-organisms characterization
- C. Modelling and Systems design
- D. Organic wastes process & refining
- E. Metabolome, microbiome

A. Ground demonstration and analogue testing

The work carried out in the MELiSSA Pilot Plant in Barcelona (UAB) and presented here focuses on the successful integration of the liquid and gas phase of a nitrifying packed-bed bioreactor, an air-lift photobioreactor and a rats' isolator as a mock crew. The liquid effluent of a nitrifying packed-bed bioreactor colonized with a co-culture of nitrifying (aerobic) bacteria *Nitrosomonas europaea* and *Nitrobacter winogradskyi* was connected to a photosynthetic air-lift photobioreactor colonized with the cyanobacteria *Limnospira indica* PCC8005. Additionally, the gas phases of the air-lift photobioreactor and the rats' isolator were connected

in a closed gas loop. The main goal of this integration step is focused on fulfilling the oxygen requirements of the animal compartment hosting three rats, at different biomass concentration in the air-lift bioreactor that in turn is fed by the effluent from the nitrifying bioreactor. A step further in the integration of the different compartment phases is achieved by coupling the gas phase of the nitrifying bioreactor to the already integrated gas loop containing the air-lift photobioreactor and the animal compartment gas phases in a closed gas loop system (Fig1). Therefore, in this configuration, the air-lift photobioreactor provided simultaneously the oxygen required for 1) nitrification and 2) respiration of the animals.

Fig 1.: The air-lift photobioreactor and the rats' isolator (C5)

A communication from Russia (Siberian branch of Academy of Sciences) described the recycling of human metabolites (urine and faeces) via combination of physico-chemical (H_2O_2) and biological processes). The food value of salicorn was also emphasized.

The IBMP (Moscow) main talk evoked also the treatment of organic wastes and especially the disposed hygienic means using microbial technologies that have much advantage for application in spaceflight. This would diminish the volume of organic wastes. The biological hazard of the wastes will be controlled and the system may be compatible with the other biological ELSS (greenhouses). The biogas created during biodegradation may be used as energy carrier and the water obtained in the biodegradation processes may be reused.

A report on LunAres Research Station (Poland) described a new hygiene module using grey-water treatment during analog missions with a dry toilet where the composting is primed with coconut husk fibers.

By contrast, the Japanese agency (JAXA) wishes to develop an in-situ (Moon) resource utilization (ISRU) technology as essential for sustainable exploration. The goal is to realize lunar farming with TansaX and so to construct a system that can more efficiently produce the necessary supplies when compared to the transport cost from the earth.

B. Photobioreactors and characterization of photosynthetic life support microorganisms

Three types of photosynthetic microorganisms are currently considered for life support studies: the cyanobacteria and especially the edible *Limnospira indica* PCC8005 (formerly *Arthrospira platensis*) the inhabitant of C4a and involved in the successful ARTEMIS-B flight (December 2017) as well as in the BIORAT ISS project, the Alphaproteobacterium *Rhodospirillum rubrum* foreseen to colonize the C2 compartment of the MELiSSA loop and also edible eukaryotic microalgae considered for space food as *Chlorella vulgaris* and *Chlamydomonas reinhardtii* (reports from Riga and Stuttgart).

In the **ARTEMIS-B flight (Belgian Nuclear Research Center (SCK.CEN)) experiment**, four space compatible photobioreactors inoculated with the cyanobacterium *Limnospira indica* PCC8005, were integrated in the ISS Biolab incubator and operated in batch mode for duration of 1 month. The experiment was performed in parallel on ISS and on ground. The Arthrospira-B flight experiment showed it was possible: 1) to build, qualify, and operate a photobioreactor in space, 2) to restart the cyanobacterial cultures in the bioreactor in ISS after a period of storage and upload, 3) to maintain an axenic photosynthetic active culture under space conditions, 4) to monitor online the O₂ and biomass production of the culture, and 5) to implement successfully ground commands to adapt bioreactor conditions and allow several crew interventions. Space conditions had no significant negative effect on the microbial photosynthetic bio-process. A second report from the SCK•CEN relying on the expertise of this institution about the response to radiations illustrated the remarkable resistance of *L.indica* PCC8005 to Gamma radiations.

Characterization of oxygen production from photobioreactor for ISS cabin technology demonstrator (RUAG Slip Rings SA). BIORAT Phase B2 project consists in the development of an on board technology demonstrator preliminary designed for the ISS cabin to be hosted in EDR2 facility. This project phase started on December 2018 and will be running for 30 months. This Bread Board Model consists in a dedicated photo-bioreactor for the continuous cultivation of *L.indica* PCC8005, in a membrane module for the exchange of oxygen & carbon dioxide between the liquid phase in the liquid loop to the gaseous phase of the ambient air. These life test results allowed to gain experiences on long duration algae cultivation (27 days) and to perform correlations of the existing mathematical model of the cultivated algae growth to the actual measured values of the liquid loop BBM.

The Alphaproteobacterium *Rhodospirillum rubrum*: Investigating volatile fatty acids conversion to CO₂ by the MELiSSA bacterium *Rhodospirillum rubrum* in various culture conditions (a report from SCK•CEN)

The second compartment (C2) of the ESA's Micro-Ecological Life Support System Alternative project was designed to mainly process the volatile fatty acids (VFAs) coming from the first compartment but also to produce CO₂ from the related catabolic process. Removing VFAs from the water stream is crucial to ensure a good rate of

nitrification in the third compartment while CO₂ supply is needed for the fourth compartment where higher plants and cyanobacteria are grown. C2 is inhabited by *Rhodospirillum rubrum*, a purple proteobacterium. In this work, we set up a simple and robust protocol for batch cultivation of *R. rubrum* in anaerobic conditions with 80 µmol/m²*s halogen illumination, and allowing CO₂ production follow-up using a standardized colorimetric method. Preliminary results put forward a net production of CO₂ in light anaerobic conditions when acetate was used a sole carbon source. Surprisingly a similar net production of CO₂ was also measured when using a mixture (9:1) of acetate and propionate (124 mM net C concentration) while photo-assimilation of propionate was previously reported to be dependent on CO₂ fixation. Ongoing work includes the introduction of butyrate as extra carbon source and follow-up of VFAs consumption using ion exchange chromatography.

A report from a consortium around UNantes described the production of *Rhodospirillum rubrum* at the pilot scale and the challenges towards its industrial large scale production

The aim of the study is to emphasize challenges towards the industrial production of an edible biomass from *Rhodospirillum rubrum* for human nutrition and cholesterol treatment. Heterotrophic growth condition for *R. rubrum* is driven under anoxic conditions. The growth is then mainly driven by bacteriochlorophylls and carotenoids for light absorption. Culture medium reveals also a complex composition, and growth rate is found to be highly related to light intensity and pH value. Therefore, setting an industrial production of *R.rubrum* requests to select the adequate technology as well as a good understanding of *R.rubrum*'s metabolite response to culture conditions. Possible scaling up of the culture has been demonstrated on HECTOR photobioreactor, but still can be optimized in terms of latency phase, mixing and management of biofilm. The use of an intensified culture technology (ISOS) has been demonstrated on this strain, and could allow a viable industrial production.

C. Modelling and Systems design

ALISSE Tool Status and Perspective for Space and Earth development

ALISSE, a managing tool from Sherpa MELiSSA partner, provides a decision-making tool to support ECLSS trade-off with three major aspects: Reference Model defines the integration of life support system models with their environment, Metrics describes any

Environmental Controlled Life Support Systems (ECLSS) and Workflow allows to assess all the criteria by chaining different simulation models & tools.

Global Control Loop of MELiSSA Life Support System

A control system that ensures the survival of the crew while minimizing the external resources was developed by Sherpa engineering. In particular, it was looked at the quantity balance between the production of matter and the consumptions. To do so, a dynamic model of the compartments was used to calculate the flow exchanges of the MELiSSA Life Support System. The flows considered are the possible critical flows for the operation of the loop: O₂, CO₂, water, food. A complete model of the MELiSSA loop is shown and is composed of the different compartments, the chemical reactions between the different materials, and the materials flowing between the compartments. In its previous work, UCLermont-Auvergne has developed a static model of the entire loop. Based on this work, the present model was developed and validated by simplifying the compartments model and adding a dynamic behavior and a global control. We demonstrate the feasibility of the loop control, and present a dynamic and modular control system that ensures the crew survival in different malfunctioning scenarios.

Application of the energy cascade model (MEC) on Lettuce crop grown in controlled environment at two different scales: A small growth chamber and a vertical farm. The aim of this study from UNapoli was to evaluate whether the Energy Cascade Model (MEC) can be equally and reliably applied on lettuce cultivation trials conducted in facilities at different scales. Therefore, the outputs of the MEC model were evaluated against data collected during experiments conducted in two different controlled environment facilities: a small growth chamber at the University of Naples Federico II and a multi-layer vertical farm (UAg Farm) at the University of Arizona Controlled Environment Agriculture Center (UA-CEAC). Both experiments were conducted on green and red-leaf 'salanova' lettuce under nominal and off-nominal growing conditions. The study mainly focused on the effect of different air VPDs obtained fixing the temperature and changing the relative humidity during the cultivation trials. Results showed that these environmental parameters had significant effect on evapotranspiration rate and stomatal conductance and impacted the predicted output the most with significant differences between

differently coloured leaves. Preliminary results confirm that MEC model can be a suitable solution for monitoring and controlling edible plant growth in Advanced Life Support (ALS) systems.

Exploring the impact of irregular metabolic efficiencies and the space environment on the survivability of a regenerative life support system through agent-based modeling. The survivability of a regenerative life support system during long-duration missions in interstellar space was simulated in this study from UDelft using an agent-based model (ABM) of MELiSSA. In this ABM, humans, plant plots and bioreactors are modeled as virtual agents that interact with each other. The ABM approach enables the introduction of heterogeneity at the level of agent attributes, and thus allows for the study of complex interactions that cannot be observed in more traditional equation-based modeling techniques. The behaviors are modeled according to the major chemical reactions and productivities of each MELiSSA compartment. In a first series of simulation experiments, the following environmental stressors were conceptually simulated: cosmic radiation reducing metabolic functionality, and particle impact causing loss of life support system elements.

D. Organic wastes process & refining Design and control of a bioanode for CO₂ recovery in regenerative life support systems

This study from UGent demonstrates the utility of a microbial electrochemical cell (MEC) to drive carbon recovery towards CO₂. Bio-anodic oxidation of fermentation effluent through an MEC has the potential to favour CO₂ production over methane, and provide in situ pH control by electro-migration of hydroxyl ions produced at the cathode across an anion exchange membrane. Results were presented about a scale up from a 0.008 to 0.08 m² bio-electrochemical cell with customized control and automation features implemented to improve process stability, and gas recovery. Overall, total COD removal efficiencies were achieved above 80 % and VFA removal efficiencies of 95 to 100 % could be achieved in both permeate and defined medium tests. Coulombic efficiencies between 75 and 90 % were achieved indicating a strong selection for the bioanodic (CO₂ producing) process. Under continuous operation with fermentation effluent this system has reached VFA removal efficiencies above 95 %, total organics removal efficiencies of 88 %, with 60 to 70 % of fed organic carbon recovered as CO₂.

Microbial analysis of the MELiSSA waste degradation compartment 1 (C1) and isolation and identification of C1 dominant bacteria: a study from ULeuven animated by a POMP2 fellow.

In the C1 compartment of MELiSSA, a thermophilic (55°C) anaerobic microbial consortium liquefies the solid waste to produce CO₂ for the C4 compartment and produce ammonium, volatile fatty acids (VFA), and minerals for the C2 compartment.

The data demonstrated a highly stable community composition in C1 reactors up to day 1454 after inoculation. Over 2,500 different operational taxonomic units (OTUs) were identified. The C1 core microbiome, OTUs shared between all 3 reactors was composed of 500 OTUs which represented 93% of the total bacterial community. Seven of these shared OTUs, related to *Caproiciproducens*, *Thermoanaerobacterium*, Unclassified *Ruminococcaceae*, *Lactobacillus*, *Fonticella*, *Leuconostoc*, and *Moorella*, accounted for 75% of the total C1 bacterial community, with *Caproiciproducens* and *Thermoanaerobacterium* being the most dominant. Nine different strains were isolated of which one of them appeared to be the dominant C1 bacterium *Thermoanaerobacterium*. The organism was most related to the obligate anaerobic *T. thermosaccharolyticum*. Other isolates are related to the facultative anaerobic *Lactobacillus* and *Bacillus*, and to the obligate anaerobic *Clostridium baratii*.

The Stephan Angeloff Institute of Microbiology at the Bulgarian Academy of Sciences illustrated the biodegradation of various wastes by four communications covering a broad recycling horizon:

- Two-stages anaerobic digestion of organic wastes – laboratory experimental studies and mathematical modeling: a very extensive study about microaerophiles isolated from various sources to degrade toilet paper and similar material;
- Two-phases system for anaerobic digestion of corn extract for biohydrogen and biomethane production;
- Cellulose wastes management by microbial degradation;
- Anaerobic biodegradation processes for organic waste utilization followed by algal biomass accumulation.

E. Metabolome, microbiome

The metabolome of MELiSSA (UMons)

The complexity of MELiSSA is high but stoichiometry-based modelling may help to look at parallel or complementary pathways. This may allow to stabilize the loop. In this respect, Omics are a major part of the molecular toolbox for dissecting the MELiSSA metabolome. Proteomic analysis is

required for the identification of metabolic pathways in the MELiSSA loop compartments, and there after genome wide mutant library for the validation of the metabolic pathways and Metabolomics through Nuclear Magnetic Resonance and LC/Gas Chromatography/Mass Spectrometry. As an example, “Omics” were used to analyze the response to nitrified urine as a nitrogen source for cyanobacteria. Perspectives of metabolomics approaches include: a better analysis of the response to space related stresses (radiations, microgravity), an insight in the effect of metabolites accumulation in the loop (but also of contaminants!), the completion of metabolic characterization of MELiSSA compartments

Impact of a closed life support system on human microbiome and health: managing symbiosis (INRAE, France)

From early life onward, environment dominates over host genetics in shaping the human microbiome, which in return interacts with the immune system, playing a fundamental role in modulating risks of chronic diseases. Within confined environments, such as a closed life support system, human are exposed to drastic changes compared to non-confined environments. Specific space-flight conditions such as radiation and microgravity will further modify airborne, equipment and human microbiomes. In such conditions, bioprocesses involved for food production, product recycling, wastes processing and refinery, surrounding inhabitants, might increase emergence of new microbial contaminants, such as viruses and other pathogens. Finally, long-term consumption of pre-processed food might deeply influence the gut microbiome. These specific changes and their impact on microbiome and immune homeostasis will have to be monitored, in flight or ground experiments, to address the role of the microbiota in maintaining immune function and health of the crew in such confined environments. Closed life support systems, with the possibility to control the environmental input, give a unique opportunity to precisely measure the impact of surrounding bioactive elements on human microbiome, immune system and health. Air spraying of particular mix of beneficial bacteria or nutritional supplements might mitigate or prevent development of chronic diseases due to the alteration immune function during confinement. With the aim of enabling discoveries on the role of gut microbiome in human health and disease, MetaGenoPolis research projects lead to the translation of recent discoveries into applications in nutrition, prevention and therapeutics towards an improved human health

through innovative strategies to restore host-microbe symbiosis. The INRAE unit already collaborates with University Hospital Tübingen on the ICELAND research project with the objectives to measure the effects of prolonged stay in an environment with limited antigen diversity (Concordia station, Antarctica: 3500 m above sea level, 1000 km off the coast, 12-13 participants for one year in Antarctica, 9 months locked on Concordia.) on human adaptive immunity and the gut microbiota during two consecutive seasons, including a nutritional supplementation to counteract or prevent potential health consequences.

3. CONFERENCE LAST DAY: main thematics

A. Terrestrial synergies

B. Urine treatment for the MELiSSA loop

C. Physical, Chemical and Microbial contaminants

The day was inaugurated by a magistral presentation of prof. Willy Verstraete (UGent) **“Resource recovery: the Power of change”** available on the website.

The past: Experiences in Resource Recovery.

The present: What drives the cyclic economy?

The future: The magics of upgrading.

As a conclusion: What will urban water and waste management look like in 2040? The top priorities will be

- 1) Reclaimed water (climate change) and
- 2) Zero disposal (shortage of goods).

“We have a plenty of tools, but still there is a long way to go, especially about information and education”.

A. Terrestrial synergies: a real and definitive trend

Design and simulation of circular water systems: the UWOT model.

This study from KWR Research Institute (NL) focuses on recent advances that include: (a) a model application for the (re-)design of a Dutch circular water neighborhood in Limburg, which combines DW, WW and runoff retention measures; (b) an application in Athens quantifying the effects of a novel sewer mining option; (c) a broader demonstration for the regional case of Westland, where rural circular water technologies are combined with urban interventions to close the regional water cycle. The two latter cases are part of the NextGen EU project.

Grey water recycling from space to earth

This report from the company FGWRS® observes that terrestrial environment issues may present major

synergies with the space objectives. With Sherpa Engineering (member of the MELiSSA consortium) a simulator was created allowing to review, size and predict the functioning of a grey water recycling process paired with energy recovery in collective use buildings (hotels). The company has a demonstrator that runs continuously on 4 hotel rooms in Monaco and all the parameters are collected and analyzed (water quality, temperature, treatment rate, ...). This ESA technology was showcased on the Monaco Pavilion for the Dubai 2020 expo and in the training locker rooms of the Roland-Garros 2020 tennis tournament.

Grey water re-use during music festivals using a mobile constructed wetland and a mobile drinking water system: (Grey) water treatment and re-use is an essential prerequisite for a sustainable music festival, often operated on a decentralized location. In such areas these temporary events are typically not connected to a sewage network. In this study at the Ghent University, a combination of a vertical flow based mobile constructed wetland (area of 15 m² and a volume of 15 m³) and a membrane based drinking water system was developed (and tested for 3 years) to treat grey water (GW) as well as grey and black water (GW+BW) at different festivals in Flanders (Belgium). The drinking water system was able to further polish the GW and produce water that met the Belgian drinking water standards.

“Microalgae-based biofaçade as a solution to support sustainable access to food, energy, and water in urban centers” (UNIV NANTES, France)

&

“BIOFACADE: a symbiosis between microalgae production and buildings” (XTU architects, France)

These two reports on biofaçades use mainly spirulines (cyanobacteria) (also cultivated in the MELiSSA loop). Photo-bioreactor performances are known to be first limited by light. From the early days of MELiSSA, UCA have progressed in the characterization of light transfer. Based on these efforts GEPEA, UNantes has been able to design and characterize multiple photo-bioreactor geometries, leading to a semi-industrial platform Algosolis, unique in Europe. More recently large scale-up of photo-bioreactors are popping-up with several impacts on environment: thermal insulation, carbon reduction, waste recycling. The most advanced technology is currently integrated in the large XTU building, in Paris for full scale demonstration and commercial utilization.

A model for the global carbon cycle (University of Lausanne). Main hypothesis, followed methodology, used sources: CCS (carbon capture & storage) and CCU (Carbon Capture & Utilization) processes are believed to be part of the global solution to climate change and it is aimed at evaluating the possible contribution of CCS and CCU to climate change mitigation processes. In order to do so, the Simulink-based PhiEMI software was used to build the model and to perform simulations based either on actual data collected from the existing literature or on hypothetical scenarios in the world future. Main results: A complete model of the global carbon cycle was presented and is composed by the natural carbon cycle and the anthropogenic carbon cycle, included the most recent technological processes which can the anthropogenic carbon cycle, i.e. CCS and CCU. While the existing literature on the cycle of carbon dioxide presents a certain number of analyses of the natural carbon cycle, the anthropogenic carbon cycle, or a combination of the two, there are no publications which include CCS and especially CCU. In light of their increasing importance, it is therefore necessary to create a new model of the carbon cycle which incorporates these options.

Consolidating the Swiss activities and rationale for ALSS and MELiSSA development (Earth Space Technical Ecosystem Enterprises SA). In 2019, over twenty Swiss stakeholders, active in the field of Advanced Life Support Systems (ALSS), including those directly involved in the R&D and transfer of related space- and Earth-based technologies, expressed their strong interest in the ongoing MELiSSA project of ESA by endorsing a Position Paper.

B. Urine treatment in the MELiSSA loop

Nitrify to support life: MELiSSA's development path of an essential process. (UAntwerp)

A nitrification-based process is a crucial nitrogen (N) conversion step in most regenerative life support systems. A crew member consumes 9-19 g protein-N per day, and excretes the same amount, mainly under the form of urea in urine (7-16 g N per day). Nitrification is pivotal to multiple purposes in long-duration human spaceflight, ranging from stabilization of waste streams to the production of useful compounds: water, N₂ as inert gas, and/or nitrate for food production.

Extensive testing occurred on the two important streams, namely urine and effluent from the upstream MELiSSA C1 unit (anaerobic fermentation), with formulated media and real waste streams. Functional nitrification has been achieved with open, undefined consortia, as well as with synthetic, gnotobiotic communities, i.e. enabling an increased level of

process predictability and safety. The process was shown to enable refinery and concentration of nutrients from urine, as well as downstream water recovery from urine, condensate and grey water.

Long-term stability was demonstrated in the MELiSSA pilot plant in Barcelona for more than a year. Several runs showed that the process effluent was suitable to produce microalgae and crops. Ongoing work focuses on (i) dealing with urinary organics, (ii) avoiding base addition, (iii) coupling to the food and oxygen production compartments, and on (iv) producing inert gas. Two new flights are currently under preparation, being **URINIS A** and **B**. The challenge addressed here is to obtain in-flight activity in the ISS, both in batch incubations as in a first continuous reactor test.

Combining (bio)-electrochemical processes and nitrification for urine recycling in Space (UGhent)

Currently, on board of the ISS, only water is recovered from urine using physical-chemical processes and hazardous chemicals, while the nutrients are concentrated in toxic brine. Therefore, a novel treatment train for water and nutrient recovery from urine was investigated, combining (bio)electrochemical processes and nitrification. Since ammonia volatilization can pose a hazard to the crew, the urine (33% dilution) was first stabilized to prevent urea hydrolysis into NH₃/NH₄⁺ (total ammoniacal nitrogen, TAN) during storage. By recirculating the fresh urine over the cathodic compartment of an electrochemical cell, the pH was increased to 12 due to water reduction at the cathode (7 kC L⁻¹ urine to reach pH 12). The high pH inhibited urea hydrolysis for over 18 months and triggered precipitation of bivalent cations (>85% Ca²⁺ and Mg²⁺ removal), reducing the downstream scaling potential. Subsequently, stabilized urine was treated in a microbial electrolysis cell (MEC) in order to remove the organics (chemical oxygen demand, COD). Up to 85% of the COD was removed, generating energy in the form of hydrogen gas at the cathode. A current production of 6-20 mA was obtained at COD loads of 200-340 mg COD d⁻¹ and HRT between 2.5-6.7 days, corresponding to coulombic efficiencies of 25-45%. The MEC community was dominated by, amongst others, members to the genera *Geobacter*, *Pseudomonas*, *Arcobacter* and *Comamonas*, known to comprise electroactive bacteria, i.e., bacteria capable of transporting electrons to an electrode. The treatment train yielded a stable nitrate-rich nutrient solution, suitable for microbial protein production based on photosynthetic microorganisms. Moreover, by integrating electrochemical cells in the treatment train,

the use of chemicals and the logistics related to their storage can be minimized.

Other contributions about the Urine treatment may be quoted:

- Control of pH and process modelling contribute to stable alkalinity-limited urine nitrification. A report from a POMP fellow from Eawag (Switzerland) to be consulted on the MELiSSA Foundation website as well as the four following ones.

- Opening the 'yellow box': Main organics in urine and their fate during nitrification and microalgae cultivation (UAntwerp).

- Urine nitrification in space: assessing the effect of space conditions on synthetic nitrifying communities (UGhent).

- How to gradually acclimate MELiSSA's nitrification compartment to urine mixed with VFA-rich anaerobic digestion effluent in the C1 compartment of the MELiSSA loop (KULeuven).

- Adaptation of activated sludge biomass for nitrification of concentrated urine (Wroclaw University of Science and Technology).

C. Physical, Chemical and Microbial contaminants

Monitoring microbiological quality and safety through diagnostics: the MIDASS experience (a report from a company in Lyon)

Life support systems are a key to support long-haul space flights. It comes at the price of mastering the microbiological risk (by addressing the "space-cificity" of antimicrobial resistance), especially with high biomass fermenters like MELiSSA. Terrestrial applications are a very good learning tool as well as offering disruptive innovations for a sustainable planet. Agility to partner with terrestrial industry is a key. Antimicrobial resistance should not be underestimated in long-haul flights.

MATISS-1-2: Microbial aerosol tethering on innovative surfaces in the International space station &

Broad-spectrum-applications metal-free and durable antimicrobial polymer coatings embedding bio-sourced antimicrobial agents for confined inhabited space stations.

These two reports originated respectively from Lyon and Luxembourg attracts the attention on the microbial contamination in space stations and on the need to find creative bio-sourced solutions.

Towards an inventory of eco-toxicological effects metals and alloys on marine environments after over fifty years of launching activities (from an UMontpellier report).

For what concerns launching activities in general,

focus was particularly made on metallic compounds, especially aluminum that enters among main propellants composition: Indeed, it first appeared from foreign literature databases mining (the case of the MIR station, for instance), that the return rate of metallic part could globally reach 7.7% exceeding largely those of organic compounds. Our analysis confirmed that according to experimental conditions, corrosion and/or bio-corrosion were responsible for liberating (bioavailable) metals in sea water. However, our study did not show direct short term effect of (bio)corrosion on filterers (i.e. mussels).

This analysis confirmed that according to experimental conditions, corrosion and/or bio-corrosion were responsible for liberating (bioavailable) metals in sea water. It is suggested that further experimental assessment is to be conducted in more appropriate conditions of pressure, temperature and oxygen content aiming at modelling deep sea ecosystems and with longer incubation periods to derive final eco-toxicological conclusions.

CONCLUSION

Started as a MELiSSA contribution to the Space Microbial Workshop 2005, organized by SCK.CEN in Mol, Belgium, the European Life Support Workshop took momentum in 2009 with the inauguration of the new MELiSSA Pilot Plant in Barcelona, Spain.

However, at that time, the talks were still mainly European, centralized around four countries (BE, SP, FR, IT), and with a limited community (around 50). No terrestrial applications were included. Later-on, the so-called MELiSSA workshop took amplitude and consolidation with two major editions, in Lausanne, Switzerland, 2016 and as joint conference with AgroSpace conference in 2018 in Rome, Italy. It is fair to mention, that during the same period, the European Space Agency, considerably consolidated the project organization, involved countries (12 in 2018), involved organizations (around 50), and Memorandum of Understanding partners (15 partners today).

As advocated in the ESA conclusion talk, MELiSSA project carries the European values of:

- good science & engineering;
 - environment and ecosystem understanding and protection;
 - education and multi-cultural integration.
- MELiSSA know-how has great potential for societal challenges, with the need to engage citizens to show economic benefit by exploring market identification.

We are entering an historical era where ESA and the space sector have the opportunity to reinforce the role of technology to preserve life and resources for future generations.

The next MELiSSA conference is currently planned in Toulouse, France, in November 2022, jointly organized with the “Société Française de Génie des Procédés”.

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REFERENCES

1. Dubertret G., M. Lefort-Tran M, C. Chipaux, The Chinese flight experiment: Ecological algal system in microgravity conditions – Preliminary results, Proc. of the 3rd European Symposium on Life Sciences Research in Space (ESA SP271), Graz, Austria, 1987.
2. Mergeay M., W. Verstraete, et al. First expression of the MELiSSA concept, a micro-organisms based model for CELSS development, Proc. of the 3rd Symp. on Space Thermal Control & LSS, Noordwijk (NL), 1988, 66-68.
3. Lasseur, C., W. Verstraete et al., MELISSA: a potential experiment for a precursor mission to the Moon, Adv. in Space Res., Vol 18, 1996, 111-117.
4. Gòdia F., J. Albiol, et al., LSS/MPP MELISSA: a loop of interconnected bioreactors to develop life support in space, J. Biotech., Vol. 99, 2002, 319-330.
5. Gòdia F., J. Albiol, et al., The MELISSA pilot plant facility as an integration test-bed for advanced life support systems, Adv. in Space Res., Vol. 34, 2004, 1483-1493.
6. Poughon L, Laroche C. et al. *Limnospira indica* PCC8005 growth in photobioreactor: model and simulation of the ISS and ground experiments, Life Sci Space Res (Amst). 2020 25:53-65
7. Ilgrande C, Mastroleo F et al, Reactivation of Microbial Strains and Synthetic Communities after a Spaceflight to the International Space Station: corroborating the Feasibility of Essential Conversions in the MELiSSA Loop, Astrobiology Astrobiology. 2019 Sep;19 (9):1167-1176.

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