

CURRENT AND FUTURE WAYS TO CLOSED LIFE SUPPORT SYSTEMS

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ABSTRACTS
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ORAL PRESENTATIONS

ABSTRACTS

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Recycling Space Organic Waste with Fungal Biodegradation: Advancing Sustainable Plant Growth on Lunar/Mars Regolith

The integration of Bioregenerative Life Support Systems (BLSS) and In Situ Resource Utilization (ISRU) offers a promising path for sustainable food production on the Moon and Mars. The ISRU strategy aims to minimize terrestrial input into a BLSS by using native Lunar and Martian regolith as a plant growth medium. However, these extraterrestrial soils differ significantly from fertile terrestrial soil and require modification before they can support plant growth. In the context of space exploration and bio-regenerative life support systems, biological waste generated by crew members

- such as human waste, food residues, hygiene towels, and inedible plant parts - can be considered a valuable resource for recycling. These organic wastes can be processed to recover not only water but also compounds that can serve as fertilizers or compost, enhancing the physical and chemical properties of regolith to support plant growth and enable in-situ food production. To achieve this, organic waste must undergo physical, mechanical, and biological degradation. Fungi are well-known for their ability to degrade a wide range of organic matter, including recalcitrant compounds such as cellulose, lignin, oils, and hydrocarbons. They play a critical role in nutrient recycling in ecosystems and are among the main decomposers in composting processes. Fungal strains, particularly non-pathogenic ones with minimal spore production, are ideal for treating organic waste in space missions. This study explores the potential of using fungal degradation to produce compost from organic waste generated during space missions. Based on NASA data (Life Support Baseline Values and Assumptions Document, NASA/TP-2015-218570), a mixture of wastes, named Space Organic Waste (SOW), was formulated to simulate those generated during missions to the International Space Station (ISS). This mixture included food scraps, cellulose paper towels, and plant cultivation residues. A fungal consortium of 10 strains of the University of Pavia Fungal Collection, already known for their capacity to grow on plant debris, lignin, and cellulose, was built based on the ability of each fungus to grow on SOW. Solid-state fermentation was performed by inoculating SOW in sterile

plastic bags with a humid fungal consortium at a 4:1 SOW: inoculum ratio (fresh weight). Control bags contained SOW without fungal inoculum. The bags were kept at 22°C for 60 days, with weekly manual mixing. The biodegradation capacity of the fungal consortium was assessed by measuring the dry weight reduction and fungal biomass increase. The elemental and chemical composition of both initial and fungal-degraded dry SOW was analysed to evaluate its potential as a fertilizer/soil improver for Lunar/Martian regolith, using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) and Nuclear Magnetic Resonance (NMR) spectroscopy. Special attention was given to lipid content and profile using ¹H-NMR spectroscopy. After 60 days, SOW dry weight decreased by approximately 30%, accompanied by a threefold increase in fungal biomass. Despite initial heterogeneity, the fungi-degraded SOW showed a significant increase in macro and microelements (N, Na, K, P, Mg, Fe, Al, Zn, Mn, Cu), mainly due to their progressive concentration in the reduced dry matter and the contribution of fungi. The ¹³C-CPMAS- NMR spectrum of the initial SOW revealed a composition predominantly composed of polar aliphatic molecules, with O-alkyl-C accounting for 62-64%, followed by alkyl-C (18%), methoxyl-C and C-N groups (10%), and minor quantities of carboxylic and aromatic compounds. Fungal degradation was associated with the selective preservation of hydrophobic lipid molecules, while bioavailable carbohydrates and proteins were primarily degraded. Although the lipid content remained similar between the initial (23.7% dry weight) and fungi-degraded SOW (23.2% dry weight), significant changes in the lipid spectrum were observed. Triglycerides underwent hydrolysis, unsaturated fatty acids were oxidized, and new NMR signals appeared, corresponding to oxidized free fatty acids. The results suggest that microfungal consortia could be effectively utilized for the bioconversion of organic waste into fertilizers, making a meaningful contribution to the development of bioregenerative systems that support astronaut survival by enabling in-situ recycling of organic matter during space missions.

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Aquatic Mosses for Bioregenerative Life Support Systems in Space: A Study on Radiation Tolerance and Biofiltering Potential

The development of Bioregenerative Life Support Systems (BLSSs) is fundamental to sustaining human presence on long-duration space missions. The ESA-funded Moss On Mars (MOM) project investigates the use of aquatic bryophytes as novel, multifunctional organisms within BLSSs frameworks, focusing on their ability to act as biological filters and environmental regenerators. These non-vascular plants, known for their simplicity and robustness, show promise due to their low maintenance, oxygen production, natural filtering capacity, and inherent tolerance to mild chemical stressors. Their unexplored potential in space biology makes them attractive candidates for closed-loop life support. The MOM study analyzed the physiological and biochemical responses of selected moss species exposed to different levels of ionizing radiation, aiming to assess their suitability for atmospheric recycling (CO₂ uptake and O₂ release) and contaminant removal. Among three initial

candidates (*Taxiphyllum barbieri*, *Leptodictyum riparium*, *Vesicularia montagnei*), *T. barbieri* was identified as the most promising based on preliminary trials. It was subsequently subjected to X-ray treatments (1, 10, and 30 Gy), to determine its tolerance relative to control conditions. X-rays (6 MV-energy photons) were chosen as the reference radiation due to their comparable biological effectiveness to spaceborne low-energy protons and considering that this represents the first irradiation experiment involving aquatic mosses. Findings are focused on the ability of *T. barbieri* in maintaining key physiological functions post-irradiation, to evaluate its role as a radiation-tolerant component in BLSS designs. These insights pave the way for integrating mosses into space habitat systems, enhancing sustainability through improved air and water quality management. Further investigations may explore additional applications such as

biomaterial production, radiation shielding, Martian soil biostimulation, and even food supplementation in extraterrestrial environments. This research was conducted

within the framework of the MOM project (CUP: E77G24000350005), supported by the Italian Space Agency under the EISI Study initiative (IDEA I-2024-03402).

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MELiSSA Pilot Plant: Contributing to MELiSSA Loop Closure

The MELiSSA Pilot Plant aims to achieve the complete integration of the MELiSSA loop through a stepwise approach, progressively connecting the different streams of its five compartments. This contribution presents recent progress including the interconnection of up to four compartments of the Pilot Plant. Specifically, it includes the complete gas closure and liquid phase interconnection of: Compartment 3 (an ureolysis and nitrifying packed-bed bioreactor), Compartment 4a (an air-lift photobioreactor for the culture of the edible cyanobacteria *Limnospira indica* with concomitant O₂ production), Compartment 4b (a 5 m² higher plant growth chamber, capturing CO₂, producing O₂, drinking water and food) and Compartment 5 (an animal isolator with rats as a mock-up crew). Results from multiple long-term continuous operation experiments under controlled conditions are presented. In line with the stepwise integration strategy, recent efforts have been focused on integrating the higher plant chamber. Firstly, the higher plant chamber was integrated with the crew compartment and secondly Compartment 4a was also integrated. For the first time in the MPP, the two oxygen producing compartments were successfully integrated with the crew compartment, achieving full gas-phase closure. The system showed high robustness and reliability over long operation periods

(several months) under a dedicated control system, a fundamental part of the MELiSSA loop operation to coordinate the different compartments. The performance of the system has been analyzed, with extensive on-line instrumentation for the main variables, under different experimental conditions. Subsequent work has focused on the liquid phase integration of the ureolysis and nitrification compartment to the oxygen producing compartments (the photobioreactor and the higher plant chamber). Diluted synthetic urine was used as influent to Compartment 3, as a preparatory step toward the use of human urine. The performance of the photosynthetic compartments with nitrified urine as an influent has been evaluated. The ongoing objective is the full integration of the four compartments: complete gas closure and liquid phase integration using synthetic urine as influent to Compartment 3. The experimental conditions planned for the upcoming integration test campaign are also presented. In conclusion, the MELiSSA Pilot Plant has made significant progress in recent years, consolidating its development program and producing a collection of data highlighting the feasibility and maturity of the MELiSSA loop as a regenerative life support system.

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Impact of super-elevated CO₂ concentration on biomass and oxygen production of kale cultivated in the Higher Plant Chamber of the MELiSSA Pilot Plant

Plants are expected to play a crucial role in future crewed space missions by providing food, recycling water, and regenerating air. The MELiSSA (Micro-Ecological Life Support System Alternative) project is focused on developing and integrating a regenerative life support system for long-duration space missions. Its ground demonstration facility, the MELiSSA Pilot Plant (MPP), operates a closed-loop system composed of interconnected compartments, each dedicated to specific functions. One of these compartments is the Higher Plant Chamber (HPC), a 5 m² cultivation module initially tested with *Lactuca sativa* (lettuce) as the reference crop. To expand the crop portfolio, new experiments were conducted using kale (*Brassica oleracea* L. convar. *acephala* var. *sabellica*). The primary objective of this study was to characterize the performance of kale in the HPC, with a particular focus on oxygen generation and biomass production. Results were compared to those previously obtained with lettuce under identical operation conditions and a baseline CO₂ concentration of 1,000 ppm in the gas phase. Two CO₂ concentrations were tested in this study:

1,000 ppm and 10,000 ppm. Understanding plant performance under elevated CO₂ conditions is essential, as such scenarios may occur when coupling the HPC gas phase to the gas phase of other compartments of the MELiSSA loop. Kale seeds were germinated for 14 days before being transferred to the HPC, where 100 plants were cultivated hydroponically for 28 days under staggered growth conditions. Plants were grouped into four age categories (7, 14, 21, and 28 days post-transplantation) to assess continuous crop production. Hoagland nutrient solution (electrical conductivity: 1.9 dS m⁻¹; pH: 5.9) was delivered using a nutrient film technique (NFT) system. Under the tested conditions, oxygen production rate reached approximately 3 g O₂ h⁻¹ under both CO₂ concentrations tested. Although shoot dry weight and leaf area presented higher values under elevated CO₂ concentration, these differences were not statistically significant. It was concluded that elevated CO₂ concentrations in the gas phase did not significantly impact the oxygen production rate. When compared with previous experiments using lettuce, kale demonstrated a 1.5- fold

increase in oxygen production and biomass growth. Furthermore, notable differences were found in nutrient consumption, both in absolute terms and when normalized to

biomass. These findings underscore the potential of kale as a promising candidate for inclusion in biological closed-loop life support systems.

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Not only roots: Plant response to gravity stimulus to retain flower orientation

It is widely accepted that long-term space missions must be as much as possible independent from terrestrial supply and that human settlements on the Moon and Mars rely on plant-based LSS. In this perspective, successful fulfilment of the seed-to-fruit and the seed-to-seed cycles in space will be essential not only to provide fresh food for the crew, but also to produce viable seeds to be used for the cultivation of plants over time. Fruits and seeds are the end points of the complex process of plant sexual reproduction. Their production relies on the successful achievement of a long series of subsequent specific steps highly affected and driven by the interaction with the environmental factors. Plant reproduction occurs in the flowers; therefore, to produce fruits and seeds it is essential that all flower parts are perfectly developed (receptacle, sepals, petals, stamens, pistil) and each reproductive process (including micro- and macro-sporogenesis, pollination, micro- and macro-gametogenesis, fertilization, embryogenesis, fruit and seed maturation) is fulfilled. On Earth, countless combinations of morphological traits (e.g. size, colors, shapes) characterize the flower corollas of the different species. Floral traits are associated with pollinator interaction and interspecies competition for pollen mobility between flowers. In addition to the vexillary action of the corolla, also flower orientation plays a fundamental role in completing the reproductive cycle successfully. The role of gravity in flower orientation phenomenon is intuitive and mostly taken for granted. Although limited, experimental

evidence highlights that this phenomenon can vary significantly among species. FLOS (FLOWer for Space) is a project funded by ASI (Italian Space Agency) to investigate possible effects of altered gravity on pollen functionality and flower development and orientation. In this study, we investigated the effect of clinorotation and different initial flower positioning on final corolla orientation. We used *Viola tricolor* as model species, whose flowers are edible either fresh in salads or candied. The corolla is clearly zygomorphic and vertically oriented when plants are grown under natural conditions. Before the experiments, plants were all grown under the same controlled environmental conditions. When plants reached the stage of flower induction, we performed several tests in which flowers of different individual plants were subjected to different combinations of gravity and light directions. Corolla orientation and peduncle bending were measured with digital image analysis and a digital inclinometer. Results showed that plants respond to gravity stimulus to retain flower orientation and that gravitropism in flowers is dominant on light tropisms. Further morpho-anatomical analyses suggested that the process of gravitropic bending primarily occurs in the flower peduncle. Overall, experimental data confirm that plant characterization activities aimed at cultivating plants in space must include research on floral morphological and functional traits. Such findings can step forward scientific knowledge on biological process so far neglected also on Earth living plants.

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Space-Fed, Space-Ready: Innovations in Astronaut Nutrition and Extraterrestrial Agriculture

Space agriculture is essential for sustaining human life during prolonged missions, yet the extraterrestrial environment poses significant challenges to crop nutrition, soil safety, and astronaut health. This study evaluates the nutritional quality and safety of crops cultivated both in Low Earth Orbit (LEO) and on lunar regolith simulants, identifying deficiencies in key minerals such as calcium and magnesium, alongside variable antioxidant profiles. We document the physiological impacts of spaceflight induced nutrient shortfalls including altered genetic expression in Ca-regulated bone pathways and assess the emerging incidence of gastrointestinal dysfunction, notably increased intestinal permeability (leaky gut), which impairs nutrient uptake and immune homeostasis. Furthermore, our analysis of

plant growth on lunar regolith simulants highlights risks of heavy-metal uptake (e.g., aluminum, chromium) and subsequent toxicity to astronauts. To mitigate these compounded risks, we propose integrated strategies: bioengineering of nutrient-dense, metal-tolerant crops; biofortification approaches to bolster mineral content; incorporation of high-antioxidant species to support oxidative stress resilience; and pharmacogenomic guided supplementation for individualized astronaut nutrition. By uniting insights from plant stress physiology, genetic regulation, and sustainable extraterrestrial agriculture, this work advances practical solutions for nourishing and protecting crews on deep-space voyages and future lunar or Martian outposts.

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Water recovery from aerosol streams with an eye to microgravity

The water is a valuable resource specifically in the case of the space shuttle, which is a closed system: mass - e.g., water - cannot be made-up during the space-trips. Water harvesting with membranes from aeriform streams is a recently developed operation, which attracted attention for recovering water by the dehydration of, e.g., cooling tower and flue gas streams. A device was designed and built for operating in steady state also focusing on its next utilization in microgravity. The appropriate design of the membrane module, in addition to the suitable choice of membrane type and packing and the water particles transport inside the membrane harvester are indeed crucial aspects to be considered for making this technology successful also in microgravity. Thus, targeted solutions were investigated to promote water harvesting by means of hydrophobic porous membranes (pore size of 0.1-0.5 microns) for keeping the liquid fraction of water

- even the small size particles - contained in aeriform streams. The gas fraction and the water vapour still permeate the membrane without any separation of the different molecules; this separation is not of interest in the present case and the size of the various gas and vapour molecules is significantly lower than the membrane pore size. The lecture will also discuss obtained results as a function of the main variables affecting the

separation process such as, e.g., feed stream humidity and oversaturation, and feed location (capillary lumen or module shell side). Specific solutions are investigated to promote water harvesting for different angles of inclination of the membrane humidity separator. The appropriate design of the membrane module together with the suitable choice of membrane type and related configuration (i.e., hollow fiber) are indeed crucial aspects that have been considered to make this technology successful. The overall efficiency in separating the liquid water exceeds 99%. All variables and parameters play an important role in the specific field of interest in microgravity; therefore, specific attention has been paid on them addressing some similarities and differences from separators on the Earth and in microgravity condition. For instance, one of the most significant challenges to be faced in microgravity is the water removal from the membrane module owing to the absence of gravity. This requires specific measures in designing of the membrane unit, which also includes geometrical and fluid-dynamics considerations. The separation performance was kept for long-time, measures lasted various months. Acknowledgment: European Space Agency and Thales Alenia Space Italia S.p.A. are gratefully acknowledged for funding this research under the project MELISSA PFP (Precursor of Food Production Unit).

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Data management strategies within the MELISSA Plant Characterization Unit

The Plant Characterization Unit (PCU) located at the University of Naples enables the MELISSA community to perform experiments on multiple plant species in a fully controlled environment. The data generated in this facility allow for precise characterization of plant physiological processes under varying parameters, which will be used to validate and calibrate mechanistic models of plant growth and development in Bioregenerative Life Support Systems (BLSSs) to support human presence on long-duration space missions. The amount of data generated by the PCU facility is such that a dedicated data management activity was devised, to standardize and harmonize procedures for data collection, storage and reusability. The objective is to streamline data sharing and

collaboration among researchers and institutions within the project, via the creation of guidelines and a platform to store and retrieve this data. The different data types will be presented, with their applications for modeling purposes, and challenges pertaining to reusability and storage. The data transformation approach will be detailed, particularly the processes used to transform raw data into usable datasets. The first steps in developing an open-source searchable, accessible, and reusable repository, including a complete set of metadata, will be described. This work was performed within the PaCMan (Plant Characterization Unit for closed life support system Maintenance, subsystem integration, review and scientific deployment) project, funded under ESA Contract 4000115852/15/NL/AT.

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Advanced, Intelligent, and Functional Environmental Control for Managing Regenerative Plant Gas Exchange Fluxes in Bioregenerative Life Support Systems.

Advanced, Intelligent, and Functional Environmental Control for Managing Regenerative Plant Gas Exchange Fluxes in Bioregenerative Life Support Systems. A novel approach to designing plant growth units could enable precise, functional

control over plant physiology, optimizing their contribution to regenerative fluxes in bioregenerative life support systems for space (Figure). Plants can be grown in fully artificial environments, even in space, provided that i) environmental

variables are maintained within physiologically acceptable ranges, ii) water and nutrients are supplied as needed, and iii) pathogens are effectively controlled. This discussion focuses on the first condition, assuming the other factors are optimally addressed. Over the past few decades, numerous facilities have been developed for plant growth under fully controlled conditions on Earth and in space, all aimed at regulating a predefined set of environmental parameters. During operation, these systems must demonstrate the ability to maintain the set points established based on plant requirements. Regenerative fluxes can be assessed retrospectively, based on factors such as gas exchange over varying time periods, plant biomass production, and the carbon accumulated in the biomass. We believe that a paradigm shift in the design and construction of growth facilities could significantly enhance system performance in terms of controlling regenerative fluxes. To this end, we are upgrading our growth chamber to: i) measure environmental variables in real time; ii) calculate the CO₂ and water fluxes exchanged by the plant canopy in real time; iii) make all data available for algorithmic processing; and iv) develop an intelligent algorithm capable of autonomously adjusting the chamber environment to regulate the CO₂ and water fluxes exchanged by the canopy. In our twin growth chambers, we can now measure and control air temperature (ranging from 15 to 35°C), CO₂ partial pressure (up to 2500 ppm), relative humidity (35-90% RH, depending on air temperature), light intensity (up to 600 μmol quanta m⁻² s⁻¹, adjustable based on spectrum via three Heliospectra Model Elixia C-plate dimmable lights), and

spectrum. Additionally, we can measure CO₂ and water exchange rates at time intervals of 3-4 minutes. Growing an arugula canopy (*Eruca vesicaria* L.), we demonstrated that: a) The chambers can effectively function as a closed system for measuring the canopy gas exchange. b) The response of the technological system to changes in CO₂ partial pressure and relative humidity within the chamber is very rapid, allowing the system to reach a new equilibrium, for example, after an external injection of CO₂, within two to three minutes. This time frame is fully compatible with the objectives of measuring and functionally controlling gas exchanges through an automated system managed by an AI algorithm. c) The plants' response to fluctuations in CO₂ partial pressure, light intensity, and relative humidity within the available technological system is rapid, allowing the system to reach a new equilibrium within minutes. d) The system is capable of accurately calculating the rate of photosynthesis over a three-minute period by performing linear regression on the CO₂ concentration data. e) The system can reliably calculate the rate of transpiration over a five-minute period by performing linear regression on the gravimetric water loss data. We will provide data demonstrating the system's performance in terms of gas exchange in an arugula canopy during light-dark transitions, as well as in response to changes in [CO₂], light intensity, temperature, and their combinations. Additionally, we will present a logical framework for how these data can be used to enable intelligent control of the bioregenerative fluxes produced by higher plants in BLSS.

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Assessment of Airflow, CO, Accumulation, and Thermal Stress in Lunar Modules Using CFD

Maintaining the health and performance of astronauts on long-term missions, inside limited environments like the Lunar Gateway, calls for ongoing air quality and thermal condition control. Natural air circulation is greatly limited in low or zero gravity environments, which lowers the efficacy of passive mixing and lets local CO₂ concentrations develop, particularly around astronaut heads during sleep. With effects on cognitive ability and breathing quality, these concentrations might surpass allowed limits and the simultaneous inadequate heat removal can cause thermal discomfort and extra stress. Inspired by the configuration of the European Space Agency (ESA) I-HAB

module, this work investigates air behavior, CO₂ distribution, and thermal distribution using computational fluid dynamics (CFD). Point sources of heat and CO₂ replicate the crew's resting metabolic emissions; the interior geometry incorporates some important elements based on past technical guidelines. The results stress the need of design considering the cabin geometry and crew position as well as the application of intelligent environmental control systems. Computational fluid dynamics shows to be a vital instrument for safe and efficient living in extraterrestrial environments, improving the quality of sleep and breathing in lowered gravity conditions.

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Bioregenerative Life Support Systems for the Moon: Italy's Pioneering Project Supported by the Italian Space Agency

One of the main challenges to be faced in the realization of lunar missions involving the presence of astronauts concerns the capacity to supply resources. Currently, the resources needed for short-term space missions on the ISS are entirely transported from Earth and waste materials are only partially recovered and

recycled (e.g. urine). However, for technical and economic reasons, this will not be possible for missions beyond low orbit, where it will be necessary to significantly increase the regeneration of resources in situ. Therefore, the planning of long-term missions should concur

with the development of self-sufficient systems capable of carrying out the following tasks: complete recovery/reuse of water, air regeneration, reduction of volatile organic compounds, food production, nutrient and resource recovery and waste recycling. In such context, the Italian Space Agency selected and financed four projects with the aim of developing Bioregenerative Life Support Systems (BLSS) and waste recycling technologies for the Moon. BIOLUNA, which is led by Thales Alenia Space Italia S.p.A., aims at developing a dynamical model for a BLSS that includes a crew, a greenhouse for the production of fresh food, and an algae photo-bioreactor for oxygen production. Mass and energy flows are regulated by a control system based on artificial intelligence (AI) algorithms, which aims to minimize buffers and energy consumption. Models and control laws are empirically validated. The main goal of the project BEATRICE (coordinated by the University of Rome La Sapienza) is the construction of a bioregenerative system to be studied, prototyped and tested in analogue missions using speleological environments. The system is based on the treatment of wastewater and the consequent generation of electrical energy through Microbial Fuel Cells (MFC), as well as on the autonomous cultivation of edible plants that will use the energy generated by the MFCs and on the use of regolith for ISRU (In-Situ Resource Utilization) applications. REGOLIFE by the

Scuola Superiore Sant'Anna has the scope to study whether the microbiota of plants and earthworms could induce modifications of the lunar regolith similar to terrestrial soil (pedogenesis). In particular, the response of different organisms (*A. thaliana*, earthworms, microbiota) will be tested to the lunar regolith; as well as their potential beneficial effects as biofertilizer. The impact of lunar gravity on the composition of the plant microbiota and on the characteristics of seeds and plants will be also investigated. The activities of the projects will be carried out in a reliable and automated technological platform, which will be designed and developed by the research team. The BioMOON project (VERITAS SpA) aims at realizing a lunar biorefinery, in which all waste produced by the crew members is used to produce bioenergy and nutrients useful for the sustenance of the astronauts themselves in a close loop. The integrated system consists of bioreactors with immobilized cells of microalgae, anaerobic photosynthetic bacteria (PNSB) and granular methanogen groups that in lunar gravity conditions convert the organic waste and CO₂ into compounds and/or molecules for human nutrition (microalgae), energy metabolites (hydrogen) and chemical precursors (methane). In conclusion, these projects will pose the bases for the technologies which will ensure the sustainability of human in future space missions to the Moon, and beyond.

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Optimisation of Plasmas Assisted Oxygen Production for Mars with Transfer Learning

Oxygen is a critical element for long-term Mars mission planning. Efficient in-situ oxygen production reduces the wet-mass needed for life support and propellant systems, hence facilitating long-term stay and lighter return missions. Low-temperature plasma presents an innovative approach to In-Situ Resource Utilization (ISRU) on Mars by enabling efficient CO₂ conversion to oxygen and other value-added products, which is favoured by the high CO₂ concentration and low-pressure conditions of the Martian atmosphere. Unlike solid oxide electrolysis systems like MOXIE which require high temperatures (~1000 K) and pressures (1 bar), plasma technologies make the most of Mars's unique atmospheric conditions to achieve improved energy efficiency and scalability since they operate at lower pressures (~600 Pa) and high CO₂ concentration. However, optimizing the plasma reactor parameters is challenging due to the complex plasma

chemistry, particularly the lack of precise knowledge about some reaction rate coefficients and the interactions between various species. In this context, Machine learning (ML) offers a promising way to enhance the efficiency of CO₂ conversion by identifying key plasma-chemical pathways and optimizing reaction rate coefficients. This work looks into the application of transfer learning to build a surrogate model for Mars plasma aided CO₂ conversion. Starting from synthetic data generated by a 0-D kinetic global chemistry model, LoKI developed by IPFN a surrogate model was developed for various operational parameters. That tunes the acquired knowledge based on experimental data. Specifically, the idea is to explore the possibility of providing a hybrid learning methodology where we can use the experimental data as high confidence data while relying on synthetic data to extend to unknown values.

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Bioconversion of Plastic Waste into Edible Protein: A Bio-Inspired Solution for Circular Life Support in Space

Long-duration space missions demand closed-loop life support systems that can efficiently manage waste, recycle resources, and ensure sustainable food supply. At Norbite, we are developing a nature-inspired biorefinery that uses insects specifically *Galleria mellonella* to transform unrecyclable plastic

waste into high-value outputs, including edible proteins, lipids, and biofertilizers. This process mimics the larvae's natural ability to degrade synthetic polymers, enabling the valorisation of polymeric waste including packaging, textile, etc., generated onboard. Our modular, container-based system offers a scalable

and adaptable platform for integration into confined environments such as space habitats. By coupling waste reduction with food and nutrient generation, the technology contributes to circular bioeconomy principles suited for extraterrestrial life support. In this presentation, we will outline recent advances in plastic degradation by insects, the safety and

nutritional potential of the resulting biomass, and how this system could be engineered for space conditions. The Norbite approach bridges synthetic waste recycling with onboard food production, offering a novel paradigm for sustainable missions beyond Earth.

TOR BLOMQUIST

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Centralized Testing Facility for space food production, handling, and bioregenerative processes

Exploration missions beyond Low Earth Orbit (LEO) pose unique challenges, especially in providing a sustainable food system for extended crewed missions. Due to resource constraints and food acceptability issues, relying solely on Earth resupply missions is impractical and not always feasible. As a result, in-situ food production is essential for sustained human space travel. While current space food research shows promise in a variety of areas, there are still important technological gaps, particularly in macronutrient-rich crop production, post-harvest management such as food storage and processing, and cohesive bio-regenerative strategies. To address this issue, we propose a Centralized Testing Facility for Food System Research, that will interlink all major food production functions into one ground test demonstrator to serve as a hub for multidisciplinary research and inter-agency and industry collaboration. This

facility will foster diverse research and innovation in food production, post-harvest management, and bio-based material utilization. Such a facility will provide the unique opportunity to systematically test and evaluate the interactions between various food research, technologies, and systems such as food processing, food storage, side stream management, and equipment innovation. This approach will promote cohesion and integration, and prevent research from occurring in isolated silo activities. The facility aims to serve as a proof of concept of a versatile closed-loop food production system, specifically designed for future lunar habitats, allowing for comprehensive testing and acceleration of any related research. Furthermore, this provides an opportunity to evaluate new food production and management technologies, assessing their merits and potential integration into future exploration architectures.

IULIAN BOBOESCU

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Enabling Microbial and Microalgal Functional Ingredients for Terrestrial and Space Applications

Introduction: Microalgae and cyanobacteria convert carbon dioxide into functional ingredients such as proteins, carbohydrates, lipids, pigments and secondary metabolites. Their conversion efficiency is higher than that of higher plants, while requiring less nutrients and fresh water. This makes these microorganisms very interesting candidates for regenerative life-support applications. However, due to the presence of protective structures such as the cell wall, the bioavailability of these ingredients is limited. Thus, certain processing strategies are required to enable their potential. Aims: Completely novel processing technologies are required however, as low-gravity environments and confined space exploration modules prevent the use of conventional approaches. Using mild treatment strategies such as external fields in combination with 3D printed microfluidic channels could provide a solution to these challenges. For instance, when combining microfluidic channels with laser, electric, inertia and acoustic fields, precise manipulation, extraction and even separation of microalgal cells

and their components can occur. Materials and Methods: The present research employs high frequency (2MHz) acoustic standing waves to harvest microalgal cells in a targeted fashion based on their biomass composition. Subsequently, lower frequency (0.5MHz) acoustic pulsing waves are used to gently disrupt the microalgal cells, providing access to their inner components. The separation potential of the released cell structures based on their acoustic properties was investigated both in silico as well as in vitro. Results: Acoustic fields were used to manipulate and mildly open *Tisochrysis lutea* microalgal cells in order to further fractionate their main cell components. Precisely designed microfluidic chips were manufactured and tested to assess and maximize the separation efficiency of these approaches. Conclusion: Learning how to harness these phenomena could enable various bioprocess applications, from novel functional food ingredients to the development of bioactive compounds for both space as well as terrestrial applications.

GIORGIO BOSCHERI

Senior system engineer - Thales Alenia Space Italia

MICROx2: A Microgreens Greenhouse for Lunar Surface Missions

MICROx2 is a pioneering project aimed at demonstrating the feasibility of cultivating microgreens on the lunar surface as part of a bioregenerative life support system (BLSS) for future human space exploration. Commissioned by the Italian Space Agency (ASI), the project focuses on efficient crop production within a

human rated, pressurized habitat to enhance astronaut nutrition and sustainability in long-duration missions. The project investigates plant growth under lunar gravity and radiation conditions while optimizing resource utilization through controlled environment agriculture (CEA) techniques. Key

technological elements include an autonomous hydroponic cultivation system, LED-based lighting strategies tailored for microgreens, and an advanced environmental control system to regulate temperature, humidity, and CO₂ levels within the greenhouse module. MICROx2 follows a structured timeline, with initial laboratory testing on Earth, leading to integration with future lunar surface missions. The outcomes will contribute

to understanding plant physiology in reduced gravity and serve as a precursor for larger-scale crop production in future lunar and Martian habitats. This presentation will provide an overview of the project's objectives, current progress, and high-level technical challenges, outlining the roadmap toward sustainable space agriculture.

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Microbial electrochemical cell integration in the MELiSSA loop: enhancing carbon conversion for improved waste treatment

Efficient carbon recovery is a key objective of the MELiSSA project, which aims to develop closed-loop life support systems for long-duration space missions. A central challenge lies in the complete conversion of complex organic waste, originating from the crew compartment and the inedible fraction of cultivated plants, into CO₂. To achieve this, two compartments of the MELiSSA loop work in tandem: Compartment 1 (C1) facilitates the primary degradation of crew-generated waste into volatile fatty acids (VFAs), while Compartment 2 (C2) ensures the complete oxidation of these VFAs into CO₂, enabling carbon recovery. A microbial electrolysis cell (MEC) has been proposed as a promising technology for the C2, offering efficient VFA oxidation while minimizing gas-liquid mass transfer limitations. Additionally, MECs provide a route for full CO₂ recovery in a compact and energy-efficient format, which is essential for space applications. One of the main challenges in this approach is determining the optimal organic loading rate (OLR) that the MEC can handle without inducing unwanted methanogenesis.

Methanogens may compete with the anode for electrons, leading to methane production instead of CO₂, thereby reducing recovery efficiency and disrupting loop integration. In a first 40-day experiment, a two-chamber MEC separated by an anion exchange membrane was inoculated and operated with raw C1 effluent in the anolyte at an OLR of 175 mL/day. A phosphate

buffer saline solution was used as catholyte. A fixed potential of -0.1 vs Ag/AgCl was maintained at the anode thanks to a potentiostat to optimize microbial VFA oxidation. Under these substrate-limited conditions, the system achieved 93% total COD removal, and the produced biogas was composed predominantly of CO₂ (77%), with only 7% methane during steady-state operation indicating effective limitation of methanogenic activity. Notably, the system maintained stable pH on the anode side without external pH control, thanks to alkaline generation at the cathode. The system was comprehensively characterized through physico-chemical analyses, electrochemical monitoring, and 16S rRNA gene-based microbial community profiling. To evaluate the system's response to increased substrate availability, a second test was conducted at an OLR of 350 mL/day, which also resulted in a reduced hydraulic retention time (HRT). This phase highlighted the competitive dynamics between electrogens and methanogens, emphasizing the importance of carefully balancing flow rates and loading to prevent methane formation. Overall, these findings demonstrate the technical feasibility of integrating an MEC into the MELiSSA loop, offering dual benefits: enhanced carbon oxidation and passive pH stabilization both crucial for the development of resilient, long-term bioregenerative life support systems in space.

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KAROL BRESLER-PRZYBYB

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LUNA facility FLEXHab - Simulation and research testbed for future human lunar missions

With the global push to revisit the Moon and make it a permanent site for human exploration and possibly habitation, Earth-based training centres and technology testbeds are becoming more relevant than ever. As part of a larger global vision for human-lunar exploration, The German Aerospace Center (DLR) and the European Space Agency (ESA) are jointly constructing a lunar analogue facility, named LUNA, at the European Astronaut Centre premises in Cologne. This facility provides capabilities for astronaut training, engineering, and scientific research to a wide range of users. It serves as a lunar surface research and training centre where complex, end-to-end lunar missions can be simulated on Earth under realistic

conditions. This facility provides capabilities for astronaut training, engineering, and scientific research to a wide range of users. As such, it is a lunar surface research and training facility, where complex end-to-end lunar missions can be simulated on ground under realistic conditions. The LUNA facility allows the full training process in order to simulate the Future Lunar Surface Operations for the upcoming crewed and robotic missions, as well as the validation of robotic systems and the cooperation between humans and robots. Moreover, new technologies and activities for In-Situ Resource Utilization (ISRU) will be explored, together with new operational concepts, training techniques and protocol development. A key

component of this vision is the analogue habitat adjacent to LUNA, known as FLEXHab, which enables the simulation of multi-day missions aligned with upcoming lunar exploration scenarios. It is designed to support comprehensive simulations of integrated Moon operations for crews of up to four people, providing a unique operational environment with human-in-the-loop capabilities. While the LUNA hall focuses on lunar surface activities, the habitat offers an optimal environment for all intravehicular operations. It enables immersive simulations while providing realistic amenities required for space mission

training, such as airlocks, suit ports, exercise equipment, crew quarters, and leisure areas, even though it is not a flight-ready solution. The work outlines the design philosophy of the FLEXHab, its relevance for the LUNA facility within the context of future lunar exploration missions, implementation and deployment of the system with initial simulation runs. Possible expansions of the habitat system next to the LUNA facility is also discussed, which would offer even greater simulation opportunities.

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GREGORY CAPORASO

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The microbiomes of human excrement composting: toward safe human waste cycling for closed-system horticulture

Composting is the human-managed, microbially driven, aerobic degradation of organic materials, including food scraps, yard and garden waste, and animal (including human) excrement, resulting in a safe, nutrient rich soil amendment. Human excrement composting (HEC) in particular will be critical for closed-system horticulture, as it provides a mechanism for cycling nutrients from human excrement for reuse in plant growth. Over the past two decades our ability to investigate complex communities of microorganisms, or microbiomes, has benefited from major technological innovations that have illuminated our microbial world and the human microbiome. Some of these technologies have also been applied to understand and optimize the safety and efficiency of composting systems and to understand their microbiology. Before HEC systems can be deployed off-Earth, critical challenges will need to be addressed ranging from automated safety monitoring through understanding of which microbes (if any) will need to be included as inoculants for completing the composting reaction when organisms can't simply be sourced from the environment. Microbiome profiling technologies, including rRNA profiling, metagenomics, metatranscriptomics, and metabolomics, along with more traditional techniques including culturing and quantitative PCR, can help address these challenges. My lab has recently carried out the most highly replicated and densely sampled microbiome profiling experiment focused on human excrement composting (<https://doi.org/10.1093/ismeco/ycaf089>). In this study, fifteen biological replicates were collected from existing composting toilet users, and the microbiomes of these were tracked through weekly sampling for one year during mesophilic composting. Species-

specific and pan-enterovirus qPCR assays, 16S rRNA profiles, and culturing experiments illustrated fifteen unique microbial community compositions followed a similar trajectory from fecal-like to soil-like microbiomes over the year, though the rate and extent of change differed dramatically across replicates. We observed near complete elimination of *E. coli*, but observed increases in abundance of the spore-former *C. perfringens*. This highlights the limitations of mesophilic composting and the potential of optimizing this reaction for safety. I will conclude this presentation by describing an ongoing follow-up experiment, where we are currently carrying out thermophilic composting of human excrement in eight biological replicates, all of which have achieved and maintained internal temperatures of over 55C for at least three weeks, exceeding US EPA guidelines for Class A pathogen reduction in compost. This will be the most detailed microbiome study of human excrement composting ever performed, as far as we are aware, and in addition to providing insight into the biology of the reaction we hypothesize that this will serve as a biodiscovery platform which we can mine for thermostable, biotechnologically relevant enzymes, microbes, and microbiomes. I am a global leader in microbiome science, most widely known for leading the development of the QIIME 2 microbiome data science platform (<https://qiime2.org>). I look forward to engaging with the MELiSSA community to learn how microbiome science techniques can aid in the development of closed life support systems for space travel and terrestrial applications. Additionally I hope to use this meeting to begin networking with researchers in the EU, in preparation for spending sabbatical time in France in the 2026-2027 academic year.

MARTIN CERFF

Project engineer biological life support systems - Blue Horizon SarL. Advances on *Limnospira*-based materials for 3D printing

Technologies for regenerative life support systems are required for the crew during deep space missions such as Mars transit or permanent settlements on the Moon or Mars.

Among others, these technologies are investigated in the Micro-Ecological Life Support System Alternative- (MELiSSA-) project, which is developed by ESA and partners. Their fundamental role is to support the crew with their physiological daily needs which are the production of oxygen, recycling of carbon dioxide, water and fresh food, mainly by means of plants and microorganisms such as cyanobacteria. Ideally, the material loops are closed and/or the waste streams are used in manifold ways to minimize

upload of materials. Therefore, valorization of generated organic waste streams can help to increase flexibility of the crew and reduce upload mass. The cyanobacterium *Limnospira* is one of the key players within the MELiSSA concept which is commonly referred to as *Spirulina*. It plays a key role in the regeneration of air and water. Spent biomass, i.e. harvested after oxygen production, is recognized as food supplement. Beyond, spent biomass also has the potential of being further

treated as solid organic waste and valorized as biomaterial: e.g. as a thermoplastic for 3D printing of small parts or acting as binder in regolith-based composite materials to contribute to permanent settlements on Moon and Mars. Regolith is an abundant in-situ resource on the surface of Moon and Mars. The current goal of the OW-ink project is to deeper characterize Limnospira-based materials, investigate potential applications and develop the first steps towards a space architecture. Here,

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KEETA: A novel development of self-sustainable insect-based protein and 3D food production system for crewed deep space missions.

KEETA is a space food production system that was developed as part of NASA's Deep Space Food Challenge (DSFC) program under the 3-year crewed Mars mission requirements. The system uniquely utilizes Asian Palm Weevil (*Rhynchophorus ferrugineus*) from its micro-ecosystem's rearing module as an ingredient in protein-based meals, which are produced via a 3D food printer and processor. The feasibility of the system was tested by real-time trials using the prototype's full operational cycle in a simulated environment alongside computational evaluations that refined the operations of the insect rearing platform and improved the parameters for food preparation.

Outputted food products were then nutritionally analyzed and sampled by 30 qualified panellists using a 9-point hedonic scale for palatability response and edibility evaluation. The result demonstrated the engineering feasibility of the prototype system, with the successful cultivation of Palm Weevils and subsequent processing into an edible protein source. During the 28-day testing period, the rearing process required approximately 13.61 litres of water and 4.67 kilograms of feed substrate to produce a sufficient number of larvae for one month's worth of ingredients for a single

crew. The 3D food printer is also capable of utilizing the harvested protein source, with the ability to dispense up to four materials simultaneously within the same printed layer, supporting highly optimized time-saving concurrent printing of multiple food items that use different ingredients or independently customized settings, sufficient convection heat for real-time cooking is then implemented internally with the printing process through a built-in oven. Official lab analysis showed that produced daily meals contained approximately 180 grams of sago worm meatballs, which provided 14 grams of insect-based protein and supplements to fit the NASA daily dietary intake requirements of 58 grams (d35% of the total daily energy intake or 0.8 g/kg) per day for total concentrated protein. The sensory test yielded a 69.33% acceptance rate, with panellists slightly preferring the meatball (6.27 ± 1.95 on the hedonic scale), making it a suitable new menu item. The ongoing study can increase the viability of the 3D food printer and streamline the system for space applications, along with the development of new menus to suit wider preferences, with options to enhance the final output to suit long-term deep space missions."

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STEFANIA COZZOLINO

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Simulated MELiSSA C3 effluent as sole nitrogen source for kale and lettuce cultivation in a closed-loop hydroponic system (C4b).

The MELiSSA (Micro-Ecological Life Support System Alternative) program, funded by the European Space Agency (ESA), aims at developing a closed-loop Bioregenerative Life Support System (BLSS) to sustain human life in long-duration space missions, such as lunar or Mars bases. Within the MELiSSA Pilot Plant (MPP) at Universitat Autònoma de Barcelona (UAB), compartment C3 (nitrifying bacteria) generates an effluent containing residual nitrogen (N) compounds, primarily urea (10-40%), along with organic substances (mainly organic acids and amino acids) contributing to chemical oxygen demand (COD). This study evaluated the feasibility of using laboratory-simulated MELiSSA C3 effluent as the sole N source for cultivating leafy vegetables (kale and lettuce) in a closed-loop hydroponic system under controlled environment conditions. The simulated effluent contained 10-40% residual urea and up to 40% of

chemical oxygen demand (COD) compounds, primarily organic acids and amino acids. A factorial experiment was conducted with three replicates, testing:

- two leafy vegetable species: kale (*Brassica oleracea* L. convar. acephala var. sabellica, cv. Nero Palmizio di Toscana) and lettuce (*Lactuca sativa* L. cv. Grand Rapids);
- four urea concentrations (0, 10, 20, and 40%);
- presence or absence of COD compounds.

All nutrient solutions were standardized to contain equal total nitrogen level, regardless of N form (nitrate, ammonium, or urea). Plants were grown under controlled conditions for 28 days, monitoring physiological parameters and nutrient solution composition throughout the cultivation period. At harvest, crop yield and edible biomass quality were assessed. Results showed that moderate urea concentration (10%) significantly increased

biomass production in both crops. Furthermore, the presence of COD compounds reduced nitrate accumulation in kale leaves, suggesting a potential improvement in crop nutritional quality.

These findings support sustainable nitrogen management strategies and circular resource recovery strategies, contributing to the advancement of BLSS-oriented space agriculture.

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Miniaturized and Monitored growth chambers for Cyanobacteria Culture in Space: MIMOCYCS

MIMOCYCS is an experiment proposed to demonstrate the growth of the cyanobacterium *Limnospira indica* in miniaturized culture chambers (cassettes) equipped with individual led-based illumination systems and monitoring devices allowing to have a continuous follow-up of cell growth and photosynthetic activity. The experiment is based on three components. First, the biological component of the experiment, *Limnospira indica* (commonly known as *Spirulina*) has been selected due to its relevance in the context of Life Support Systems in long-term missions in Space.

Particularly this cyanobacterium has been extensively used in the scope of the MELISSA project, a unique project developing a circular life support system to support sustainable human exploration in Space (Lasseur et al. 2010). In such a context, *Limnospira indica* enables three relevant functions in life support: CO₂ capture, O₂ production and generation of food (*Limnospira indica* has a very high protein content and it is already used as food component in Earth). Second, the proposal is leveraging from an existing payload already used in the EMCS of ISS (Manzano et al. 2020), called FixBox. Even the payload was developed and used for a different purpose, the use proposed here only requires the design and construction of new cassettes to be placed in its periphery, while the core of the hardware will be maintained with minimal modifications. This circumstance greatly facilitates to prepare an experiment that can be ready and validated in a feasible period of time. Third, an innovative cassette architecture enabling both individual illumination in multiple units (five) within the same experiment as well as on-

line recording of cell growth and pigment contents will enable to generate additional knowledge for the characterization of *Limnospira indica* growth in Space, contributing to the design of future life support systems. Of particular interest, is the capacity of measure on-line cell growth as well as pigment contents associated to the photosynthetic activity of the cells. Finally, the experiment is designed to operate at axenic (i.e., pure) culture conditions, a critical condition to ensure the quality of the obtained results. The specific objectives of the proposal are a) define all the biological components of the experiment: inoculum preservation conditions, culture media composition, cell growth kinetics; b) design and construction of the new miniaturized transparent plastic cassettes (growth chambers) to be incorporated into the core existing hardware of the FixBox payload. The design of these cassettes is crucial for the success of the experiment both for technical and scientific purposes. Technical because needs to be watertight but has to allow the release of internal gas and scientific because needs to provide the proper mix of the biological components. These cassettes will have embedded their own miniaturized illumination and monitoring elements (photonic detectors); c) Test the proper operation of the cassettes integrated in the FixBox and tests the overall performance of the experiment; d) Adaptation and compatibility of FixBox hardware to new experiment configuration and flight requirements, e) certification and validation of the experiment; f) performance of the experiment and ground control; g) pre and post-flight analysis.

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VERONICA DE MICCO

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Are there main trends in plants responses to ionizing radiation?

The stepwise space exploration strategy envisages three high-level destination goals: Low Earth Orbit (LEO), the Moon, and Mars. The realization of manned missions to these destinations requires the development of sustainable closed artificial ecosystems, known as Bioregenerative Life Support Systems (BLSSs). In BLSSs, higher plants are key organisms for resource regeneration (oxygen production and carbon dioxide removal through photosynthesis, water recovery via transpiration, and waste recycling) as well as for food production. Depending on mission scenarios, as duration and distance increase, onboard food production shifts from microgreens or leafy greens (which mainly provide vitamins, minerals, and antioxidants) to staple crops to ensure adequate caloric intake. However, cultivating plants in space is challenged by various environmental stressors, remarkably ionizing radiation, which can alter plant development and metabolism. This affects not only the nutraceutical value of edible plant parts but also the plants efficiency as regenerators, impacting the input/output balance of the BLSS subsystems. While plants exhibit greater radio-

resistance than animals, a standard behaviour has not been identified yet for a multiplicity of reasons.

Indeed, historically, the effects of radiation on plants have been less studied than other space-related factors like microgravity. Furthermore, experimental results are often difficult to compare, as studies have used different endpoints, plant materials, radiation types, and doses, with different protocols in radiation analogues accelerators for which space fidelity remains a challenge. Here we summarize the main findings from over 15 years of research conducted within ESA (European Space Agency) and ASI (Italian Space Agency) projects. These studies investigated the effects of low- and high-LET (Linear Energy Transfer) radiation (at doses ranging from 0.3 to over 25 Gy) on the morpho- anatomical, eco-physiological, biochemical, and nutritional traits of various plant systems (e.g., seedlings, microgreens, adult plants) across different botanical families, including Asteraceae, Brassicaceae, Fabaceae, Lamiaceae, and Solanaceae. The overall analysis indicates that dry seeds show high radio-resistance, though species- and radiation-specific

responses in growth and development were observed. Nonetheless, some general trends in the variation of parameter groups are emerging. These studies are critical for understanding the mechanisms of radio-resistance, the impact of radiation on the ability of plants as regenerators in BLSSs, and on the nutritional quality of fresh food produced onboard. The findings also help define shielding requirements during the cultivation cycle of plants in different space contexts. Finally, they offer insights into designing nutritional countermeasures to protect astronauts from radiation exposure by incorporating fresh plant-derived foods, enriched with antioxidants that are enhanced by radiation itself. Part of the results presented here are based on activities within the projects: Space It Up!, funded under grant Prot. CI-2022 DSR-042 for Attività spaziali (thematic area 15) by the Italian Ministry of University and Research (MUR), as part of the program Partenariati estesi alle università, ai centri di ricerca, alle aziende per il finanziamento di progetti di ricerca di base, Contract n. 2024-5-E.0 - CUP n. I53D24000060005; B-22-

00112 / Exploring the radioresistance and radiosensitivity mechanisms of edible plants (RadioPlant2) and Bio_08_DeMicco / Assessing radiosensitivity of higher plants and mechanisms for radioresistance at different phenological stages (RadioPlant), performed in the frame of Program Advisory Committee for Biophysics and Radio-Biology, program FAIR Facility for Antiproton and Ion Research in Europe GmbH, GSI Helmholtzzentrum für Schwerionenforschung GmbH (Darmstadt, Germany). Acknowledgements go to ASI (Italian Space Agency) and ESA (European Space Agency) who supported some of the experiments within the MELISSA (Micro-Ecological Life Support System Alternative) PhD POMP program with the project Radiation effect on plants. Acknowledgements also go to Prof. Mariagabriella Pugliese (Dept. Physics of the University of Naples Federico II), and Istituto Nazionale Tumori IRCCS Fondazione G. Pascale for support in X-ray treatments. Keywords: food countermeasures, bioregenerative life support systems, ionizing radiation, plant traits

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MARTA DEL BIANCO

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MOONRICE: Cereal Crop Production For Future Planetary Bases

The development of growth systems for plant cultivation in space aims at minimizing the resources required in terms of energy and volume. The optimization of the plant architecture and physiology in a controlled environment will be fundamental to maximize resource use efficiency and reduce waste. The selection of specific crops and cultivars combined with genetic approaches can speed up the process of obtaining space-adapted plants. In this context, for example, the generation and selection of crop dwarf varieties, which makes crops compatible with efficient vertical farming systems, is a rapidly expanding field. An ideal space crop, however, should also be highly productive, optimised for growth in a closed environment and resistant to space stressors. Most calories in the human diet come from complex carbohydrates, obtained mainly from the

cultivation of cereals. Among cereals, rice is currently the main source of energy for the world population. Rice has also interesting nutraceutical characteristics: it contains small and readily digestible starch, does not contain gluten and, depending on the variety, can provide fibre, proteins, vitamin B, iron, and manganese. Rice is also a high yielding cereal and can be grown with soilless methodologies (e.g., hydroponics), which are currently considered the most adapted technologies and potentially applicable to space exploration scenarios. Starting from the definition of the rice ideotype for future space applications, the project Moonrice aims at using gene editing (CRISPR-Cas9) to create new rice varieties with specific attributes that enhance crop performance in future planetary bases.

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Angle dependence in the plant gravitropic response

Research on gravitropism has been dominated by two main ideas: that gravity is perceived through the sedimentation of starch-rich plastids within specialised gravity-sensing cells (Starch-statolith hypothesis), and that tropic growth is driven by auxin asymmetry across the graviresponding organ (Cholodny-Went hypothesis). Our recent work on gravity-dependent, non-vertical growth in lateral organs in Arabidopsis, has highlighted the importance of a third, even older concept in gravitropism: angle dependence. However, the mechanistic basis of how statolith sedimentation, and eventually Cholodny-Went driven auxin asymmetry, translates into angle dependent gravitropic behaviour remains unexplored. Here, using a combination of cutting edge vertical confocal imaging with time lapse tracking

software, we characterize for the first time the dynamics of gravisensing in the columella of the Arabidopsis primary roots. We observed that statolith sedimentation across individual tiers of columella cells occurs according to the angle of displacement from the vertical axis. We also demonstrate how statolith sedimentation leads to angle dependent PIN3/7 polarization in specific columella domains. This detail analysis shows that different PINs/columella tiers play distinct roles in establishing the asymmetric auxin gradient at different angles. Our findings provide a fundamental framework to further explore the mechanisms that regulate angle dependent gravitropic responses in both primary and lateral organs, with major implications for crop improvement.

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Mapping the paths of human space exploration, a life science prospective

Human space exploration is one of the most effective drivers for scientific research and technological innovation. In view of the steadily growing international relevance of human space exploration beyond Low Earth Orbit, the Italian Space Agency (ASI) is continuously reinforcing the network of the national scientific community and industrial stakeholders for the success of future Moon and Mars exploration missions. This requires tackling unsolved issues related to the effects of long-duration space flights and developing the required, enabling technologies. ASI has activated four working groups with the participation of national experts from the scientific community on the macro-areas of space life sciences: integrated physiology, microbiology, biological systems for life support, and radiations. The groups have analysed the current scenario of space life science research and have identified the most relevant

objectives and key issues to enhance the national contribution and competitiveness towards enabling human deep space exploration in collaboration with international partners and agencies. In this contribution, we will present and discuss the results produced by the working groups, with emphasis on the long-termed, strategic initiatives that have spurred from the output of their analysis of the field. To fulfil these goals, ASI will implement a Space Research Centre devoted to Space Life Sciences. The ASI Centre for Space Life Sciences will perform research and act as a coordination centre for a national research network. The Centre will also act to promote aggregation, meeting, and teaching activities for investigators in this field. It will be equipped with laboratory instrumentation and innovative space simulation facilities.

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Speculative Designer - Frederik Deschuytter

The Body Gardeners

As space agencies advance plans for extraterrestrial habitation, this speculative design research investigates how regenerative life support systems (RLSS) transform perceptions of human bodily materials from waste to vital resource. In contemporary Western contexts, bodily materials like feces, sweat, hair, and skin cells are framed as waste - byproducts to be discarded - reflecting historically and culturally grown attitudes that separate humans from their biological processes and environment. Advancements in RLSS research, exemplified by ESA's MELISSA program, require a new framework that positions the human body as an integral component of engineered ecologies. These systems suggest a tighter metabolic entanglement between human physiology and synthetic ecologies, challenging traditional boundaries between body, technology, and environment. The Body Gardeners' speculates on a future where body waste is systematically harvested, cultivated, and ritualized in space habitats. The project envisions how bodily waste streams become central to survival. In

interplanetary contexts, body materials transition from waste to treasured resource, generating novel rituals and demanding new ethical frameworks. This human-environmental symbiosis, where bodily materials become essential contributions to community survival, is a paradigm shift that eventually challenges human's exploitative ecological relationships to planet earth nowadays. In this project by Pleun van Dijk & Frederik Deschuytter, these speculative scenarios are explored through a combination of objects and short films, each examining one specific bodily material - hair, sweat, or skin - and its role in an interplanetary context. The scenarios depict the emergence of 'body gardening' as a hybrid practice integrating biology, ecology, and technology, reframing the body as an integral component within complex metabolic exchanges that sustain life itself, inviting critical reflection on how our present technological and ecological choices shape possible futures both on Earth and beyond.

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The potential of insects in bioregenerative systems for space

The prospect of supporting permanent crews on extraterrestrial surfaces, as envisioned by the ongoing Artemis program and projected for manned missions to Mars, requires the ability to regenerate at least part of the primary resources necessary for survival, including food. In recent years, research and solutions for cultivating plants in space environments have multiplied, aiming to appropriately integrate astronauts' diets with fresh food. This fresh food is essential to ensuring a balanced nutritional intake capable of compensating for the qualitative limitations of prepackaged and preserved rations. Our studies on

Bioregenerative Life Support Systems (BLSSs) for space missions have led to the development of systems and procedures for growing plants in controlled environments without soil, under stringent cleanliness conditions to prevent contamination. While part of the plant production is intended for consumption, the residual plant matter (roots, stems, leaves) can be processed through bioconversion mediated by decomposer organisms. Our research introduces a novel approach within the space context, focusing on the decomposer insect *Hermetia illucens*, commonly known as the black soldier fly. Its larvae are highly

efficient at converting organic waste into plant compost. As part of the ReBUS project, funded by the Italian Space Agency (ASI), we demonstrated the feasibility of raising this insect in containment conditions replicable in space. The larvae were fed a mix of organic waste, referred to as Space Organic Waste (SOW), which includes food residues and paper wipes, mirroring the organic waste composition (based on NASA data) generated during ISS missions.

Additionally, we incorporated inedible parts of vegetables cultivated as fresh food for the crew. We evaluated the bioconversion performance based on insect fitness and efficiency in degrading the organic material. Results showed that the insect readily adapts to conditions replicable in BLSs for recycling mission waste. Chemical analyses revealed a high degradation efficiency of the organic component (87.2%), with a notable 46.1% reduction in cellulose, primarily from paper wipes. The *H. illucens* degradation process of SOW led to notable changes in the concentrations of both macro and micro elements, resulting from the loss of dry matter during degradation and the assimilation of elements into the biomass of *H. illucens* larvae. The larval-degraded SOW was then used as a soil amendment for lunar and Martian regolith simulants, demonstrating its effectiveness in promoting the growth of *Lepidium sativum* L. microgreens. Moreover, the possibility of introducing *H. illucens* into BLSs opened an intriguing perspective: using this insect to produce protein-rich food on-site. These larvae contain essential amino acids and other nutritional components that are scarce in plants. *H. illucens* larvae are already widely used in animal feed and, in some

cultures, are accepted for human consumption. We are exploring the potential to rear these larvae using waste from microgreens cultivation (roots and basal stems). Nutritional analysis of the larvae flour raised on SOW highlights not only a rich protein content but also significant levels of fatty acids, dietary fiber, vitamins (notably B vitamins crucial for nervous, immune, and skin health, as well as vitamin A), antioxidants, and essential minerals. We are also investigating the potential to enhance the nutraceutical value of *H. illucens* flour by feeding the larvae antioxidant-rich waste. In the BIOMIRATE project, funded by ASI and recently launched, we aim to bioengineer lettuce with the MYB-like *PhAN4* gene, which regulates anthocyanin accumulation a purple pigment with natural antioxidant properties. This initiative aims to produce biofortified lettuce with enriched antioxidant content in both leaves and roots. This would achieve, on one hand, an 'ideotype' (an optimized variety for space applications) of lettuce more resistant to space stress, particularly radiation, and, on the other hand, pigment-rich roots grown under controlled conditions that can be used for rearing larvae. This experimental approach aims to produce insect flour enriched with antioxidant components. Such flour could then be proposed as a high-value ingredient for creating functional foods for astronauts, such as hamburgers, meatballs, and crackers. The purpose of our research is therefore to combine the biodegradation efficiency of this insect with its potential in high-value-added food formulations. This innovation represents a significant advancement in the efficiency and sustainability of resource regeneration systems for space exploration.

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Roadmap for Advancements for Menstrual blood Management in reduced gravity (AMMITY)

Menstrual fluid in the context of spaceflight is currently considered waste and is often avoided during space missions through the use of hormonal contraception. However, with the prospect of longer space missions, there is a growing need for alternative solutions to manage menstrual blood while also exploring its potential as a valuable resource. This valorisation necessitates the development of a menstrual blood collection device, cleaning and sterilization methods, and the identification of optimal applications within the spacecraft's life support system. All of these steps need to align with the constraints of an astronaut's environment, including reduced gravity, minimisation of resource use as well as crew comfort and privacy. The AMMITY (Advancements for Menstrual blood

Management in reduced gravity) project, hosted by SpaceShipFR, aims to address the challenges of collecting and transforming menstrual blood from waste to resource. Parabolic flight tests conducted as part of the project have provided valuable insights into the design requirements for an effective menstrual collection device in microgravity, while protocol testing during analogue missions gave clarity on device comfort and effective cleaning measures. These findings contribute to AMMITY's comprehensive roadmap for integrating menstrual blood management into spacecraft life support systems. Advancements in menstrual blood collection and revaluation will also be key for terrestrial applications.

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Acoustic Levitation Photobioreactor: Enhancing Cyanobacterial Cultivation and Oxygen Production

Cyanobacteria are microorganisms capable of photosynthesis, converting carbon dioxide into oxygen while being a viable food source. This makes them a promising candidate for sustainable life support systems in long-term space missions and closed environments [1, 2, 3]. However, a major limitation in photosynthesis and oxygen production is light availability, with up to 60% of light lost within the first centimeter of culture at standard optical densities (about 1). This study seeks to optimize light exposure for cyanobacteria cultivation in bioreactors. To address this challenge, we developed a device that enables cyanobacteria to grow in sheet-like structures floating freely within the culture medium. This is achieved through acoustic

levitation, a method that uses ultrasound waves to trap small objects, such as cells or particles [4, 5]. The system integrates acoustic levitation into a photobioreactor equipped with probes for real-time monitoring of pH, redox potential, and partial oxygen pressure (pO₂). For this study, we used the cyanobacterium *Limnospira indica* PCC 8005, cultivated in Zarrouk medium [6], as it is extensively utilized in the MELISSA project [3, 7]. Our findings establish the potential of acoustic levitation to enhance light penetration in cyanobacteria cultures, offering a promising pathway to optimize oxygen production in bioreactors for closed environments and long-term space missions.

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Antimicrobial Coating : Protecting and Improving Human Space Flights

Abstract Long-duration space missions require robust closed-loop life support systems. Despite advancements in air and water recycling, microbial threats including biofilms, fungi, and pathogens continue to pose significant risks. This proposal investigates whether incorporating antimicrobial surface coating can significantly enhance the microbial stability of spacecraft environments. The central hypothesis is that passive, long-acting antimicrobial coatings embedded in structural and operational surfaces will reduce microbial colonization in critical systems. This research will focus on a theoretical assessment of integration within spacecraft systems. Through a structured review of contamination pathways and microbial hotspots identified in prior missions, the study will map viable use cases for antimicrobial coatings across habitat surfaces, air systems, water systems, and life support infrastructure. The outcome will be a prioritization framework and technical roadmap for future implementation and testing.

1. Background and Rationale Microbial contamination is a persistent risk in space habitats. Biofilms are especially problematic in microgravity, forming resilient colonies on surfaces within water systems, ventilation ducts, and medical infrastructure. The International Space Station (ISS) has reported recurring microbial growth in potable water systems and on surfaces, despite stringent sanitation protocols (Ott et al., 2004; Castro et al., 2004). Pathogens and fungal growth can compromise both structural materials and crew health, as documented in studies linking microbial accumulation to material degradation and respiratory concerns. Conventional cleaning methods are insufficient to maintain long-term sterility in closed-loop environments. Closed habitats naturally support microbial proliferation due to high humidity, continuous human activity, and recycled air and water systems. Passive antimicrobial protection embedded in surfaces provides a way to mitigate this risk without demanding crew time or consumable resources.

2. Objectives Identify critical zones within space habitats where microbial proliferation poses operational or health risks. Assess the compatibility and theoretical efficacy of coatings across different materials and subsystems. Develop a prioritization framework and integration roadmap for targeted application in future mission scenarios.

3. Methodology and Application Areas The study will adopt a qualitative and systems-based approach grounded in literature review, mission reports,

and internal simulation data. It will incorporate access to ISS analog habitat models and previous testing results conducted under ESA-relevant environmental and material compatibility standards. These resources will enhance the accuracy and applicability of theoretical assessments.

Mapping Microbial Hotspots Review of documentation from ISS and analogous environments to locate recurrent contamination zones, including air circulation paths, potable water systems, and plant growth areas.

Functional Use Case Analysis Evaluation of where and how Q-FIELD™ coatings could theoretically be applied to suppress microbial growth, including on control interfaces, medical tools, food preparation surfaces, and textiles.

Material and Integration Review Analysis of the compatibility between Q-FIELD™ formulations and materials commonly used in spacecraft construction, including polymers, composites, and habitat linings, guided by performance data from ESA-standard testing protocols.

Implementation Strategy Development Drafting a roadmap for deployment, outlining coating protocols, reapplication intervals, and areas with highest return on integration.

4. Expected Results A mapped inventory of microbial hotspots in long-term mission scenarios. A categorized list of high-priority surfaces and systems suitable for antimicrobial coating. A proposed framework for phased implementation of coatings into current and future mission designs.

5. Contribution to MELISSA Objectives The MELISSA program emphasizes sustainable human presence in space through regenerative life support.

Antimicrobial coatings address an unmet need by stabilizing the biological conditions within those systems. By providing a structured roadmap for integration, this study contributes directly to MELISSA's goals of environmental control, safety, and long-term autonomy.

6. Conclusion Antimicrobial coatings are not supplementary; they are essential. Coated objects should offer a non-toxic, passive approach to mitigating microbial risk. A theoretical evaluation of its potential applications will inform targeted experimentation and design integration. This research positions antimicrobial strategies as foundational components of life support planning for sustainable space exploration.

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Gravity-adaptive *Wolffia* (water lentils) for bioregenerative life support systems: a three-year multi-g study and its application to terrestrial cultivation technology

Interplanetary travel is entering a new phase of deep-space exploration: the return to the Moon with Artemis III is envisioned not only as a scientific enterprise but as the proving ground for sustained habitation on other planets. The main bottleneck, among others, is logistics, because even the most capable launchers cannot deliver the mass of food, water, and oxygen required for autonomous settlements. Long-term success, therefore, hinges on bioregenerative life-support systems (BLSS), in which man-made ecosystems recycle air, water, and waste while producing fresh food. Within the European Space Agency's MELISSA project, we focus on identifying crops that maximise productivity and efficiency. Duckweeds (Lemnaceae), and especially the genus *Wolffia*, are compelling candidate species for BLSS. They propagate vegetatively (can double their biomass in ~48 h), require little resources, and provide a complete protein source that is comparable to soy and produce no waste. Yet their proposition as alternative food candidates for space exploration and deepening knowledge about their performance across different gravity levels had never been quantitatively evaluated. Our research activities have tested and validated the idea of *Wolffia* sp. as alternative crop species for space agriculture. Via a four-year research project (Superfood for

Space, ESA Contract No 4000133778), multiple accesses to the ESA Life Support and Physical Sciences Instrumentation Laboratory (LIS-Lab) (*Wolffia* Hyper-g ESA-CORA, ESA Contract No 4000142760) and through the utilization of multiple facilities, such as ESA's large-diameter centrifuge and random positioning machine (RPM), we have successfully characterized growth responses, protein content, and genetic expression of multiple *Wolffia* species under different gravity levels and different combinations of environmental factors. The great research achievement conducted within the framework of advancing space agriculture and the scientific know-how gathered during these years has been applied to a new technology-transfer project (LEDA). This platform couples *Wolffia*'s rapid growth with closed-loop resource use, simultaneously advancing terrestrial circular agriculture and the need for alternative protein sources. The project has now entered the final phase of testing the patent-pending modular photobioreactor, now in pilot operation at the University of Naples Federico II. LEDA is the first Italian example of space-agriculture knowledge translated into an Earth-based cultivation technology and was framed sharing ESA's goals to improve life on Earth.

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Lunar Agriculture Module Ground Test Demonstrator (LAM-GTD) an international effort to develop a full-scale testbed for bio-regenerative life support

The Artemis program aims to establish a sustained human presence on the surface of the Moon. Realizing this goal requires a reliable supply of food and other consumables like oxygen. To address this need in a sustainable way, traditional physico-chemical life support systems and food resupply missions from Earth cannot be the solution anymore. Instead, a closed-loop bio-regenerative life-support system (BLSS) will be needed to produce fresh food and oxygen in-situ. However, before going to the lunar surface, these technologies have to be developed, optimized and tested on Earth. To this end, an international consortium, led by the German Aerospace Center (DLR) and the Canadian Space Agency (CSA), was founded. The team currently works on the conceptual development of the so-called Lunar Agriculture Module Ground Test Demonstrator (LAM-GTD). This high-fidelity testbed would help to improve the technology readiness of the essential Controlled Environment Agriculture (CEA) subsystems. Furthermore, it would enable the simulation of crewed system operations and

refine our understanding of engineering requirements for the future space segment. To facilitate the development process, DLR and the CSA have divided the project into several work packages. At this point in time it is assumed that the German part of the consortium will focus on work packages related to the development of key subsystems including the Atmosphere Management System (AMS), Thermal Control System (TCS), Electrical Power Subsystem (EPS) and Data Handling & Control System (DHCS). In addition, DLR would develop adjacent mission elements like a Habitat Simulator (HabSim) and an Air Lock Module (ALM) that are needed to simulate the interactions and mass flows between a deep space habitat and the plant cultivation system. This paper will outline the current status of the LAM-GTD project as a whole, while also giving a detailed view on the mentioned plant cultivation subsystems. Additionally, it will show how LAM-GTD could be used to address the technical challenges related to lunar surface exploration missions.

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Urea hydrolysis, nitrification and COD removal of synthetic and human urine in a continuous packed-bed bioreactor with a defined microbial community at the MELiSSA Pilot Plant

Urine is a valuable source of water and nutrients in space-based life support systems. In Compartment 3 of the MELiSSA loop, a biological process is under development to enable urea hydrolysis (urea → ammonium), complete nitrification (ammonium → nitrite → nitrate) and Chemical Oxygen Demand (COD) removal (COD → CO₂) of urine. The objective is to produce a stable and safe nutrient solution suitable for the cultivation of plants and cyanobacteria. The influent of this ureolysis and nitrification compartment will be sourced directly from the crew urine, diluted to an appropriated concentration.

The bacterial consortium responsible for this process includes heterotrophic bacteria, for hydrolysing urea and COD removal, as well as nitrifying bacteria for ammonium oxidation to nitrate. As a preparatory step, ureolysis, nitrification and COD oxidation of synthetic urine was studied at the MELiSSA Pilot Plant to ensure feasibility under controlled conditions avoiding the variability of human urine. A six-bacteria consortium was tested using a defined medium in a 5.9 L packed bed bioreactor in continuous mode while maintaining axenic conditions. Nitrogen and COD loads were progressively increased by raising the synthetic urine influent concentration from 2.5% to 10% at a hydraulic residence time (HRT) of 20 hours. At these conditions, a maximum urea degradation rate of 180 mg N-L-1-d-1 was achieved at 10% urine (loading rate of 825 mg N-L-1-d-1). No accumulation of ammonium nor nitrite was observed. COD removal averaged 50%, with a maximum COD removal rate of 550 mg O₂-L-1-d-1 at 10% urine. To better characterize the potential of Compartment 3 as a nitrogen provider supporting

photosynthesis in downstream compartments, additional experiments were conducted under higher nitrogen loads. The nitrogen loading rate was progressively increased from 1000 to 1600 mg N-L-1-d-1, while decreasing the HRT from 7.1 to 3.5 hours. Under these intensified conditions, a maximum urea degradation rate of 546 mg N-L-1-d-1 was achieved at 3.5 h HRT (loading rate of 1600 mg N-L-1-d-1), again with no accumulation of ammonium or nitrite. The highest COD removal rate (932 mg O₂-L-1-d-1) was also obtained at 3.5h HRT, representing a 32% removal of the inlet COD. These results demonstrated the consortium capacity for full nitrification in axenic conditions. However, further optimization is required to improve the nitrogen recovery efficiency and maximize overall process performance. To further prepare for processing human urine at pilot scale, complementary experiments were conducted at bench scale using diluted human urine as influent. A 0.4 L packed-bed bioreactor was operated under sterile conditions and inoculated with the same defined six-bacteria consortium used in the pilot scale. This setup enabled evaluation of the consortium's performance when treating human urine under controlled conditions. These experiments provide a necessary intermediate step to understand system robustness and guide the transition from synthetic to human urine in the pilot bioreactor. Altogether, this study presents a significant step forward in developing a robust biological process for human urine treatment. The study provides a solid foundation for integrating urine treatment into the MELiSSA loop and constitute a key progress toward sustainable resource recovery.

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Advancements in ESA's ALISSE Tool: Development of Version 2 and Roadmap Toward a Comprehensive ECLSS Evaluation Framework

The ALISSE (Advanced Life Support System Evaluator) tool, developed within the framework of ESA's MELiSSA program, provides a multi-criteria methodology to evaluate Environmental Control and Life Support System (ECLSS) technologies for space missions. This presentation introduces the development of ALISSE Version 2 (V2), a significant milestone in the tool's evolution. V2 incorporates computational models for a targeted set of ECLSS technologies and selected ALISSE evaluation parameters, offering a functional yet scalable assessment platform. Key developments include the integration of improved analytical models, refined evaluation workflows, and a

structured data architecture aimed at enhancing the consistency and reproducibility of results. The tool currently supports assessment along core dimensions based on mass and power consumption. The presentation will outline the architecture of ALISSE V2 and demonstrate its capabilities through example use cases. A preview of a possible roadmap to extend ALISSE's capabilities is also pointed out: this includes expanding the library of modeled technologies, enhancing the accuracy and traceability of input data. These enhancements aim to align the tool more closely with the complex needs of long-duration and human-rated mission planning.

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Microclimate in Microgravity: Understanding Canopy-Level Environmental Conditions for the Growth of Soybean in Space

Sustainable food production is essential for long-term space exploration and requires a deep understanding of plant growth in partial or lack of gravity. Past experiments conducted aboard the International Space Station have demonstrated that while plants can complete their life cycle in microgravity, they often exhibit altered leaf and root development along with stress-induced behavior. While prior research on plant modeling in space has focused on individual leaf responses and the development of mechanistic growth models, less attention has been given to canopy-scale effects. However, in a plant canopy, the spatial arrangement of leaves may create a complex microclimate that can modify the local environment experienced by each leaf, and therefore its growth potential. Indeed, leaf photosynthesis and transpiration rates are functions of local irradiance and airflow velocity impact which are themselves impacted by plant morphology. This study presents preliminary results of simulation of the temperature, vapor pressure and

airflow velocity distribution within a Glycine max (soybean) canopy grown under partial gravity from 0 to 1g with a 0.1 step using Ansys Fluent for analysis.

Expected results suggest that the reduced airflow velocity within the canopy, due to the absence of buoyancy-driven convection, will lead to altered heat and mass exchange dynamics. This may result in higher leaf surface temperatures and ultimately reduced biomass production. Understanding these organ-level growth conditions can then inform the development of more accurate canopy-level models, which can be used to control key environmental parameters such as airflow and light intensity to optimize plant growth. This research aims to enhance predictive modeling capabilities for the design of more efficient controlled-agriculture chambers in extraterrestrial environments. This work was funded by a doctoral fellowship of CNES and the University of Clermont Auvergne.

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The use of the Plant Characterization Unit for investigating crop sub-optimal mineral nutrition.

Biogenerative Life Support Systems (BLSSs) will be essential in long-term space missions to reduce the requirement for supply from the Earth. Most of these BLSSs will include crops grown in hydroponics to generate oxygen (O₂), water (H₂O), and food needed by the astronauts, while capturing carbon dioxide (CO₂) from the atmosphere. The nutrient delivery to the plant will rely on recycled waste. However, providing optimal nutrient solutions to plants using recycled waste is a daunting challenge. Therefore, we need to study the impact of suboptimal plant nutrition on plant growth. To this end, we conducted four crop tests in the Plant Characterization Unit (PCU) located at the University of Naples Federico II, examining the impact of adding sodium chloride (NaCl) and reducing potassium (K) in the nutrient solutions on lettuce (*Lactuca sativa* L.). The control treatment with standard nutrient solution composition was run twice (Control_1 and Control_2) to investigate the reproducibility of the PCU crop tests. Plant growth during the experiment was monitored by measuring O₂ and H₂O production, projected leaf area, and canopy temperature throughout the crop tests. At harvest, the leaf, stem and roots were weighed, and selected parameters were collected, including the elemental composition of plant organs, and photosynthesis-related parameters (Fv/Fm, SPAD, stomatal

conductance, and transpiration rate). The assessment of impact of sub-optimal mineral nutrition on the plants was challenged by the high variability of plant growth in each test and by the low reproducibility of the tests, highlighted by the differences observed in plant growth in the two Control treatments. The projected leaf area and canopy temperature observed in each test were related to the position of the plant within the growth chamber. The comparison of our results to others suggested that Control_2 provided more realistic results than Control_1, which showed poor plant growth. The addition of 27 mM NaCl in the nutrient solution did not cause any significant decrease in biomass production or in photosynthesis-related parameters at harvest compared to Control_2. The net H₂O and O₂ productions were also comparable to those observed in Control_2. However, the projected leaf area showed retardation in plant development compared to Control_2. The reduction of K in the nutrient solution resulted in a retardation in development, captured by the projected leaf area, in a trend of reduction in biomass production, and in 18% less O₂ and H₂O production compared to Control_2. The high variability and low reproducibility of the crop tests are discussed in this paper to help improve the PCU so that it can be used reliably in the future to assess the impact of suboptimal crop nutrition regimes on plant performance.

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Future Foods for Future Space Flights: 3D Printing Solutions for Astronaut Meal Diversity to Combat Menu Fatigue

A common problem astronauts face while in space is malnutrition, which is often related to menu fatigue and lack of palatability that lead to lower calorie consumption. With the upcoming long-term missions to the Moon and Mars for 2030 and 2040, respectively, this becomes a more critical issue that needs to be addressed. This study aimed to develop more palatable and visually appealing foods for astronauts to develop using a 3D food printer while in the international space station (ISS) according to their daily cravings. Plant-based ingredients were adapted to the 3D printing consistency requirements to

develop food options with different designs and formulations. A low-cost and portable electronic nose (e-nose) developed by our research group was integrated to the 3D printer to measure the volatile compounds throughout the printing process. Machine learning models were created using e-nose data as inputs to predict the aroma development and oxidation of samples overtime. Findings from this study showed that 3D food printing is a potential solution for menu fatigue to offer more palatable options to enhance astronauts nutrition.

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Microbially Driven Electro-Filtration for recovery of energy, water and nutrients: Transforming Urine into Bio-Fertiliser for Growing Plants in Space Missions

This study explored the potential of using human urine as a source of energy, water and bio-fertiliser through the application of microbial fuel cells (MFCs). The success of urine to power MFCs in scaled-up systems on Earth underscores the importance of testing similar urine-based models in microgravity [1]. This research, which also explores the simultaneous generation of catholyte for recovering water and nutrients from urine, lays the foundation for developing these systems in space missions. Microbial fuel cells (MFCs) consist of an anode and a cathode. In the anode, bacteria consume organic matter, releasing electrons and protons. The electrons travel through an external circuit to the cathode, generating an electric current, while the cations move through a membrane towards the cathode, where they combine with electrons and oxygen. The movement of ions and water through the membrane, contributes to production of catholyte in this process. EF-MFCs have been developed to generate electricity from human urine and produce valuable byproducts such as catholyte recovering water through self-driven electro-filtration. The main study aim was to assess the suitability of catholyte, produced by electro-filtrating MFCs (EF-MFCs), for hydroponic plant cultivation of basil utilising human urine. This is to showcase the continuous production of catholyte, produced as a byproduct of electric current generation, as a processed nutrient-rich, clear liquid that is highly suitable for use as a bio-fertiliser for hydroponically grown basil. Experimental: Six MFCs were set up using ceramic cylinders and carbon electrodes. Synthetic urine was continuously supplied to the MFCs, and produced catholyte was collected for testing. Basil was grown hydroponically using various combinations of standard nutrient solutions and catholyte. Results and Discussion: A 60/40 mix of catholyte to standard nutrient solution was most effective for basil growth. The harvested plants showed the highest plant height of 19.7 cm (SD 1.2) in 60/40 condition while 80/20 reached 18.3 cm (SD 2.6), 90/10 reached 17 cm (SD 0.8) and the control condition 0/0 showed only 3.7 cm (SD 3.8), while the sole synthetic nutrient (100/0) reached 15.5 cm (SD 4.1), and 0/100 (pure catholyte) showed 4.17 cm (SD 2.7). This indicates that the catholyte addition to the nutrient solution has enabled growth in all mixed configurations and the highest growth was achieved in 60/40 where the catholyte supplementation was proportionally

the highest in the mixed conditions.

The results of 0/0 vs 0/100 seem comparable, however, looking at the data set in conjunction with the morphology of the plants, the control conditions 0/0 were chlorosed due to lack of nutrients, while full catholyte solution resulted in plants that developed a more vibrant green colouring. The visual difference in the distinctive pigmentation, highlights the nutrient value of the catholyte. In earlier study, the MFC catholyte is shown to exhibit antimicrobial properties [2]. Therefore, not only is the produced catholyte nutrient-rich but it also provides a bio-fertiliser that may also reduce the risk of pathogen transmission between crops. Pure catholyte might cause salt sensitivity issues in basil, suggesting the need for optimal mixing ratios. The study showcases the potential for using recovered catholyte for hydroponic cultivation. The results gained from using synthetic urine may differ when real urine is used as a substrate due to the presence of additional micronutrients and potential contaminants. Although sufficiently treated urine provide a source of nutrients, additional monitoring and supplementation are necessary due to high sodicity of the produced catholyte. Even so, the scaling up MFC technology could further support commercial viability of vertical farming and therefore contributing to sustainable agri-food production. Conclusion: This study highlights the potential of EF-MFCs in transforming human urine into a valuable bio-fertiliser for hydroponic cultivation. This approach supports resource recovery and circular economy initiatives, offering a sustainable alternative to conventional fertilisers. Future advancements in electro-filtrating MFCs (EF-MFCs), hold significant promise for space applications showing that urine filtration can operate in an energy-positive mode, making the system ideal for decentralised environments such as space missions. The ability to generate electricity while producing a nutrient-rich, treated liquid (catholyte) as a byproduct can support sustainable life support systems in space such as hydroponics, providing both power and bio-fertiliser for hydroponic plant cultivation.

This dual functionality improves waste-to-resource efficiency and sustainability in space habitats, advancing towards circularity and closed-loop systems.[1] A.P. Koehle, S.L. Brumwell, E.P. Seto, A.M. Lynch, C. Urbaniak, Microbial applications for sustainable space exploration beyond low Earth

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Passive Thermal Module for Space-Based Bioregenerative Life Support Systems As humanity moves toward long-term space habitation, the development of self-

sustaining ecological modules is critical for reducing reliance on Earth-based resupply missions. The Space-Oasis Project, developed in collaboration with The Spring Institute for Forests on the Moon, aims to design an autonomous bioregenerative life-support system for space stations by leveraging micro-ecosystems. This study builds upon previous work, including the PLANT-BCubeSat Terrarium mission, to upscale controlled ecological experiments from small-scale satellites to large orbital platforms. This paper presents an in-depth mechanical, thermal, and photobiological analysis of an advanced greenhouse module designed to support oxygen production, water recycling, and food cultivation in microgravity. The module employs a rotating hydroponic plant-growth system, optimizing root hydration and nutrient uptake. Thermal regulation is achieved through passive systems, including radiative cooling surfaces and selective optical coatings, ensuring stable internal conditions. A comprehensive sensitivity

analysis evaluates the impact of material choices, structural integrity, and orbital parameters on energy balance and system efficiency. Results indicate that a combination of polished aluminum for structural elements and radiation-protected glass for radiation management yields optimal temperature stability while maximizing natural light absorption. Additionally, simulations confirm that increasing water content within the system enhances thermal inertia, reducing extreme temperature fluctuations. These findings provide crucial insights for scaling up bioregenerative life-support technologies for future orbital habitats and lunar bases. The Space-Oasis module represents a significant step towards sustainable, closed-loop ecosystems in space, paving the way for long-duration crewed missions beyond Earth orbit. **Keywords:** Bioregenerative Life Support, Space Greenhouse, Thermal Analysis, Orbital Habitat, Space Agriculture, Closed Ecological System, Passive Thermal Management

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MELiSSA system study - Full loop model integration and what-if scenario simulations

The MELiSSA regenerative LSS implies a very complex framework and its modeling presents several challenges: establishing an adequate mathematical model for each compartment and its integration, control law definition and robustness, missing data for technology sizing and for ALiSSE criteria modelling and formulation. The VARSITY project fits in the framework of the MELiSSA system study activities and it touches all levels of life support system (LSS) developments: from process modelling up to control laws and from ground systems up to space architectures. The main objective of VARSITY is to investigate and advance various system aspects, ranging from independent processes up to the complete loop to facilitate a comprehensive understanding of the entire system ensuring progress towards

the primary objective of a fully interconnected network development. In fact, operating a regenerative LSS poses several challenges, mainly related to its complexity, efficiency, and reliability. A set of heterogeneous subsystems involving mechanical, chemical, biological, and energetic processes must be optimally coordinated to meet the requirements defined by the ALiSSE criteria. For the first time, a complete dynamical model including all the MELiSSA compartments connected on all the phases (solid, liquid, gas) was derived, simulated, and controlled by a supervisory Model Predictive Control algorithm. The design of such a controller follows a large set of requirements related to a potential space mission. Results on a set of what-if scenarios, which also includes system failure, will be presented.

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Integrating Microbial Radioresistance into the MELiSSA Loop: A Pathway Towards Microbiological Radiation Shielding for Deep Space Missions

Long-duration human space exploration beyond low Earth orbit, including on other planets, necessitates the development of integrated systems that ensure not only the sustainability of vital resources but also the protection of crew health against space-specific hazards such as cosmic radiation. Within the

MELiSSA (Micro-Ecological Life Support System Alternative) framework, substantial progress has been made in recycling waste into essential resources; however, the microbiological contributions of the loop to radiation shielding have yet to be fully explored. This study, performed in the context of an

internship within the Human Exploration Science Team (HRE-HS, ESTEC) on Radiation Microbiology, conducts a systematic review of microbial radioresistance mechanisms across bacterial taxa, including the model strains used by the MELiSSA loop, analysing molecular and physiological adaptations that confer exceptional survival under high ionizing radiation fluxes. Particular attention is given to DNA repair pathways, protective cellular compounds and structures, as well as antioxidant production. Furthermore, the potential inclusion of emerging radiation shielding systems within regenerative life-support architectures is assessed, including boron nitride nanotube (BNNTs)-like matrixes as biofilm support, or inoculated culture medium passive shielding. In alignment with MELiSSA's compartmentalized, deterministic design, we propose conceptual pathways to harness

radioresistant microbes within existing bioreactor compartments, thereby offering dual functionality: conventional waste recycling and passive or active radiation attenuation. This integration could strategically augment the system's resilience, reduce reliance on heavy passive shielding, and exploit in situ biological processes for crew protection. The study concludes by identifying key microbial candidates, outlining integration challenges, and proposing a research roadmap for the realization of biologically assisted radiation protection synergized with the MELiSSA loop. This approach directly aligns with MELiSSA's overarching goals of enhancing crew safety, optimizing resource autonomy, and expanding the functional capacities of closed-loop bioregenerative systems for deep-space missions.

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Potential for CO₂ Fixation and Novel Food Production in Purple Non-Sulphur Bacteria: Exploratory study in low-cost bag photobioreactors in controlled and space-analog conditions.

Despite global efforts to transition towards green industries and achieve net-zero emissions by 2050, a truly integrated solution capable of simultaneously mitigating greenhouse gas emissions, valorising organic waste, and addressing the growing demand for protein-rich food remains elusive. Traditionally explored for their heterotrophic capacities in wastewater and agrifood waste treatment (e.g., molasses), purple non-sulphur bacteria (PNSB) such as *Rhodospirillum rubrum* are also capable of autotrophic growth. This metabolic mode enables them to fix carbon dioxide (CO₂) while simultaneously assimilating waste-derived substrates such as volatile fatty acids (VFAs) and hydrogen (H₂). Moreover, PNSB biomass has demonstrated substantial nutritional potential, with high protein content and a suite of essential micronutrients, positioning it as a viable candidate for novel food applications. The present study focused on the design and experimental testing of low-cost, reusable bag photobioreactors engineered to support PNSB cultivation under photoheterotrophic (using VFAs such as butyrate) and photohydrogenotrophic (using H₂) metabolic regimes. Experiments were conducted in both resource-constrained space-analog environments (e.g., at MDRS and AATC) and under

optimal laboratory conditions to assess reactor robustness and metabolic flexibility under varying constraints. The study aimed to evaluate CO₂ assimilation kinetics, biomass nutritional profiles, and overall system efficiency in supporting carbon capture and utilization (CCU) while producing potentially edible microbial biomass.

Photohydrogenotrophy emerged as a particularly promising approach for CO₂ capture, while photoheterotrophy yielded higher biomass productivity and slightly elevated lipid content, likely due to enhanced polyhydroxybutyrate accumulation. The essential amino acid profile of PNSB biomass was shown to be comparable to that of beef protein, though the production rate of edible biomass remained insufficient to realistically meet the daily dietary needs of an adult. In conclusion, this work provides a complementary exploration into the integration of metabolically flexible microorganisms such as PNSB within closed-loop bioregenerative life support systems (BLSS). The low-cost bag photobioreactor architecture, combined with the dual capacity for CO₂ fixation and biomass valorisation, supports the potential deployment of such systems for both terrestrial circular bioeconomy initiatives and space-based BLSS strategies.

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Design and Experimental Evaluation of Photobioreactors for Cyanobacterium-Based Mars ISRU Applications

Bioleaching, diazotrophic, and photosynthetic bacteria, such as *Anabaena* sp. PCC 7938, have recently garnered significant interest for their potential application in in situ resource utilization (ISRU) of Martian regolith, water, and atmospheric gases. However, the updraft of fine regolith particles in leaching bioreactors leads to medium clouding, which obstructs light penetration and impairs photosynthesis. Moreover, integrating the liquid, gas, and solid phases of Martian resources within a bioreactor presents numerous operational challenges that restrict feasible reactor designs. This study contributes to the development of biological life support systems (BLSS) for

Martian ISRU by designing and evaluating photobioreactor (PBR) concepts optimized to address these operational challenges. The work focuses on adapting PBR configurations to the logistical and operational constraints specific to Mars, with the aim of cultivating *Anabaena* sp. PCC 7938 for air revitalization and biomass production through the ISRU of Martian resources (i.e., CO₂, N₂, and regolith-derived nutrients). Several photobioreactor prototypes were developed with a focus on geometric design, material compatibility, and operational protocols. More precisely, these systems were engineered to support chemical, thermal, or physical sterilization methods and

to enable controlled, sterile operations, including medium refilling, bacterial inoculation, gas bubbling (CO₂, N₂), and regolith handling for bioleaching purposes. Three designs were selected for experimental validation: a packed-bed reactor containing heat-sintered 0.5×0.5 mm cylindrical regolith pellets supported by a wire-mesh sieve; a horizontal drum reactor with a powdered regolith bed agitated by a pulsed bubble beam for regolith turnover; and heat-sealable polymer bags designed to maximize volumetric interaction with the regolith powder while

minimizing cost and equivalent system mass. Growth experiments were conducted in these three prototypes to evaluate their biomass productivity along with the growth dynamics of *Anabaena* sp. The results demonstrate the operational viability of all three systems under laboratory conditions and represent a foundational step toward the development of a full-scale biological Mars ISRU ground demonstrator.

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Altered gravity effects on pollen germination and tube growth: species selection for experimental models in the FLOS project

Ensuring efficient plant reproduction is a key challenge for the development of Bioregenerative Life Support Systems (BLSS), which are essential to sustain human life during long-duration space missions and in extraterrestrial habitats. Among the various stages of the plant life cycle, pollen germination and tube growth are particularly sensitive to environmental stresses. However, the specific role of gravity in these processes is still poorly understood. Improving this knowledge is crucial to enable reliable fruit and seed production in space-based agricultural systems. The FLOS project (FLOWers for Space: effects of altered gravity on flowers and pollen), funded by the Italian Space Agency (ASI), aims to address this knowledge gap by investigating how altered gravity conditions, such as simulated microgravity, lunar, and Martian gravity, affect flowering and pollen functionality. To this end, FLOS adopts a multidisciplinary approach that combines morphological, cytological, genetic, and molecular analyses. A key objective of the ongoing activities is the identification of suitable model species for experimental studies on pollen under altered gravity conditions. Selection criteria include factors such as ease of cultivation in controlled environments, flowers and pollen production, availability of established *in vitro* protocols, and diversity in reproductive biology. Ongoing experiments involve a panel of candidate species, including tomato,

strawberry, chickpea, pepper, basil, rice, borage, green bean, and rape. These species are being evaluated through *in vitro* pollen germination assays under simulated microgravity conditions achieved via 2D clinorotation, with static 1g controls for comparison. Key parameters such as pollen germination rate, tube elongation, and tube tortuosity are measured to assess their responsiveness to altered gravity. Preliminary results suggest that altered gravity can influence pollen functionality, with effects on germination and tube growth patterns. A general trend of reduced germination rates and increased tube tortuosity has been observed under simulated microgravity. However, the magnitude and nature of these alterations appear to vary among the species tested, highlighting species-specific responses to gravity conditions. Further investigations are underway to validate these observations and to explore the cellular mechanisms involved. By integrating morphological, physiological, and molecular data, the FLOS project will provide a comprehensive understanding of how gravity influences pollen functionality, supporting the development of reliable fruit and seed production strategies for future space agriculture. The outcomes will also be crucial for refining experimental strategies and for defining future *in-orbit* investigations within the FLOS project.

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Hydroponic crop production with high nutrient use efficiency from organic waste for space applications

The integration of crops as part of Bioregenerative Life Support Systems (BLSS) for extended space missions is considered by most space agencies to minimize the need for costly resupply of essential resources from Earth. Plants can provide food, clean water and oxygen to the crew, whilst simultaneously removing CO₂ from the atmosphere. The successful growth of plants in BLSS is dependent on the provision of optimal nutrient supply, which needs to be achieved through the recycling of nutrients from organic waste into water-soluble ionic forms. Insufficient or imbalanced nutrition can result in diminished crop yield, whilst excess nutrition can lead to the accumulation of unused nutrients within the BLSS, resulting in the inefficient use of scarce resources. The variable and heterogeneous nutrient

composition of waste sources, such as human urine and inedible plant parts, in conjunction with the presence of toxic elements to many plants such as sodium (Na) and chloride (Cl), poses a significant challenge in optimizing crop nutrition. The objective of this study is to ascertain whether it is feasible to formulate a nutrient-efficient solution from waste that does not compromise plant growth. To this end, a comparative analysis of the yield, nutrient balance and nutrient use efficiency of lettuce (*Lactuca sativa* var. Frillice) cultivated in hydroponics using a waste-based nutrient solution is undertaken, in contrast to a nutrient-efficient and balanced control solution. The waste-based solution, consisting of a blend of three waste sources derived from urine and inedible soybean parts, was optimized by the

least squares method to achieve the best possible approximation to the composition of the control solution. Finally, we will provide recommendations for the optimization of recycling

strategies in closed systems for testing the growth of different plant species with alternative waste sources.

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Towards cooking in space conditions : the development of an Innovative cooking processor to produce fresh food in space for long duration missions (the TasteInSpace project)

The TASTEInSPACE project ambioned to participate to the evolution of space food supply, going a step beyond the in-situ production of fresh ingredients, to the production of food products enjoyable by crew members on a daily basis. TASTEInSPACE allowed to select, demonstrate and to prototype the first specific hardware for space environment able to prepare and cook food preparation in space conditions while respecting the ALiSSE criteria (safe, minimum involvement of the crew, high cooking yields, low energy and water consumption, little waste, compatible with microgravity...) and designed to be compatible with the MELiSSA loop, to convert fresh *Limnospira indica* biomass to three palatable products of high nutritional density. Waffles, burgers and omelets were formulated with high fresh *Limnospira* contents and ohmic cooking was selected as

the most suitable cooking process. Ohmic heating, is a volumetric process based on the passage of an electric current through the food matrix resulting in a very rapid and non-destructive increase in temperature of the entire volume. In comparison, conventional heating methods depend on heat transfer from a hot surface by conduction or convection through the product. The technology proved to be extremely rapid, very efficient with high cooking yields, safe, rather simple to implement and energy-efficient. Indeed, TasteInSpace has open new opportunities to develop food production systems and give access to nutritious and palatable food in space in the context of future manned deep space missions and especially for planned Mars colonization and for a permanent lunar base.

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A Mathematical Framework for a Spaceborne Aquaponic Prototype

Long-duration human missions demand resilient, sustainable food-production solutions with minimal resupply. AquaLeap introduces a first-principles mathematical framework for an integrated aquaponic prototype aimed at supplementing one-quarter of the annual dietary requirements for a four-person crew in microgravity. Our model unites three interdependent subsystems fish cultivation, hydroponic crop growth, and microbial nutrient recycling through coupled mass- and energy-balance equations. Fish biomass dynamics are represented by ordinary differential equations incorporating specific growth rates, feed conversion ratios, and waste excretion. Plant growth is described by nutrient-dependent kinetics, employing a Michaelis-Menten formulation to relate biologically available

nitrate to biomass accumulation. A dedicated nitrification module captures the conversion of ammonia to nitrate by autotrophic bacteria under controlled environmental parameters. Volumetric and energetic constraints are integrated via design heuristics that link biomass density to tank volume, grow-bed area, and power consumption for lighting, circulation, and life-support. Sensitivity analysis protocols are outlined to identify critical parameters influencing system viability. This modeling groundwork establishes a rigorous platform for subsequent simulation, optimization, and experimental validation of closed-loop aquaponic life-support systems in space.

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Texture in Space: Exploring the Sensory Dimensions of Cream Cheese for Optimal Astronaut Nutrition

As long-term missions to the Moon and Mars approach, studying food perception in space versus Earth is critical to provide astronauts with varied, palatable options to counteract malnutrition and menu fatigue. Textures and mouthfeel, particularly in soft, moist products like cream cheese, remain

among the most understudied descriptors. This study assessed texture perception and biometrics under different immersive environments: a restaurant setting simulating on-Earth conditions and a view through the porthole of the International Space Station with a reclining chair simulating microgravity.

Four cream cheese samples with different fat content (13.7%, 21.8%, 21.9%, and 30.1%) were evaluated by 12 trained participants using the quantitative descriptive analysis method (15-cm non-structured scale) using the BioSensory® application. The principal components analysis (PCA) separated the samples tasted on Earth from those in space-simulated environments. All samples tasted on Earth were positively associated with fear, sadness, anger, and blood pressure. In contrast, samples with fat content within 13.7-21.9% tasted in a space-simulated environment were associated with spreadability, softness, smoothness, and smirk. While the 30.1% fat sample in space-

simulated environment was associated with mouthcoating intensity and lingering, surprise, smiling and laughing. These findings highlight the importance of texture perception and environment in astronauts' food experiences, showing that optimizing cream cheese textures can enhance palatability and emotional well-being in space. Additionally, incorporating similar textures into 3D-printed food from genetically modified plants grown in space offers an exciting opportunity to create enjoyable, nutritionally adequate meals, combating menu fatigue and supporting mental health during extended missions to space.

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PATRICK GROVE

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Simulation of a Constructed Wetland for Wastewater Treatment on the Moon or Mars

As plans coalesce for multiple permanent outposts on the moon and eventually beyond, the need to close the food and waste loops of a life support system becomes even more critical. For a base to be sustainable and safe, it must be able to indefinitely recycle life support material supplemented only with local resources and limited manufacturing capability. However, the state of the art ECLSS on the ISS does not address solid human waste and would be insufficient as the ECLSS in a long-term settlement. This presentation describes the design and simulation of a potential solution for waste treatment in any

setting with sufficient gravity - a constructed (waste treatment) wetland. This is a common method for treating sewage in remote communities on Earth, and this work builds on existing models to predict its performance in lunar gravity and with lunar regolith as the reactor substrate. The results indicate that the technique is feasible with a careful control scheme and sufficient buffer, informing efforts towards building a prototype device funded by the French space agency CNES (Centre national d'études spatiales).

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TICTACS: A Fresh Perspective on Closed Ecological Systems through Citizen Science

TICTACS (Tool for Investigating Closed Terrariums Assisted by Citizen Science) is a large-scale, distributed experiment designed to advance our understanding of closed ecological systems through standardized, low-cost, citizen-assembled terrariums equipped with automated sensor suites. Participants construct a multitrophic ecosystem within a closed container, which automatically collects and uploads environmental data and invites users to contribute manual observations over extended periods. By aggregating longitudinal data from similar ecosystems

with slightly different light/temperature environments, TICTACS enables robust analysis of closed ecosystem dynamics over long durations. This citizen science approach leverages the power of large quantities of data and parallel experimentation to address fundamental ecological questions about self-sustaining systems, alternative stable states, and the predictors of system collapse, while fostering public engagement with ecological research and the principles underlying closed life support systems.

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Studying the effects of mycorrhizal symbiosis in a simulated lunar environment under differing gravity levels

In recent years, the establishment of a long-term presence of humans on our moon has become ever more likely. Plants will play a pivotal role in any such mission for nutrition and nourishment, but also as a part of biological life support systems, and in their contribution to the psychological well-being of astronauts. One option for the cultivation of plants on the lunar surface is the in-situ resource utilisation of locally abundant lunar regolith. However, despite being easy to acquire on the lunar surface, lunar regolith is considered very stressful for plant

growth. One group of organisms that could potentially aid plant growth in this harsh environment is mycorrhizal fungi. On Earth, mycorrhizal fungi form a symbiotic relationship with a great number of host plants in diverse environments, binding to their roots to aid in the acquisition of water and nutrients, in exchange for photosynthesised carbohydrates from the plant. To assess the potential use of mycorrhizal symbiotes in supporting plant growth in lunar regolith, Red Romaine Lettuce (*Lactuca sativa* var. *outredgeous*) was grown in the LMS-1 lunar regolith

simulant on two distinct gravity simulation platforms at ESA's ESTEC facility in the Netherlands; a specialised random positioning machine to simulate lunar gravity conditions, and the Large-Diameter Centrifuge to expose plants to two levels of hypergravity (2g, 4g). Plants were cultivated for two weeks both with and without mycorrhizal symbiotes in custom-designed

hardware, after which their size, weight, and shape was measured, along with chlorophyll pigment, and carotenoid content. Despite their beneficial nature in terrestrial ecosystems, mycorrhizal fungi were found to have a negative effect on many aspects of their host plants, from germination to size and weight.

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Enhancing the conversion of organics in urine treatment with a synthetic community through genomic screening targeted for creatinine-degrading bacteria

Urine is a key waste stream in environmental control and life support systems for human space exploration. The regenerative approach of MELiSSA, the Micro-Ecological Life Support System Alternative developed by the European Space Agency (ESA), offers significant potential for resource-efficient urine treatment. Nitrification-based processes play a central role, not only enabling the safe treatment of urine but also yielding valuable outputs: clean water and inert nitrogen gas (N₂) for maintaining the atmosphere, as well as nitrate for producing oxygen (O₂), removing carbon dioxide (CO₂) and producing food. In MELiSSA, aerobic urine treatment occurs in Compartment 3 (C3), where ureolysis, nitrification, and the degradation of organic compounds (quantified as chemical oxygen demand, or COD) are integrated into a single process. To ensure biosafety, pathogen-free gnotobiotic bacterial consortia are typically employed. However, efficient COD removal remains a persistent challenge with the current MELiSSA C3 consortium based on *Acidovorax delafieldii*, *Cupriavidus necator*, *Comamonas testosteroni*, *Pseudomonas fluorescens*. We hypothesise that this synthetic microbial community lacks the metabolic capacity required for the complete COD degradation, and that expanding the community with additional candidate strains could improve its performance. This study presents a genome-informed, bottom-up approach to identify and select heterotrophic bacterial strains with the potential to enhance creatinine degradation under aerobic conditions. The methodology involved identifying key enzymes responsible for the complete aerobic degradation of creatinine to carbon dioxide, followed by genome-based screening of bacterial species harbouring the corresponding genes using KEGG and

BRENDA databases. Two successive filtering stages were applied: the first excluded pathogenic, spore-forming, or commercially unavailable strains, while the second refined candidates based on compatibility with the operational parameters of the MELiSSA C3 bioreactor (pH, salinity, temperature).

Additional selection criteria included proximity of culture collections, degradation potential of other urine-derived organics (e.g., urea, citric acid, hippuric acid). The environmental origin was also considered, favoring engineered or urine-associated habitats. Seven candidate strains were ultimately selected, predominantly extremophiles sourced from marine, industrial, or high-temperature environments. This bias reflects the availability of genomic and physiological data for organisms with biotechnological relevance. Two additional strains, namely *Pseudomonas aminophilus* and *Pseudomonas knackmussii*, were reinstated based on alignment with desired traits despite incomplete metadata. On the conference the results of ongoing experiments will be shown. Preliminary results has identified *Paracoccus aminophilus*, *Neotrabrizicola shimadae*, *Zobellella denitrificans* and *Marteella mediterranea* as promising candidates, due to their capacity to grow in the presence of creatinine. The subsequent experimental validation includes assessing the creatinine and urea degradation capabilities, biomass growth in a synthetic urine medium, and COD removal efficiency in both synthetic and natural human urine. A design-of-experiments guides the optimization of strain combinations to form a robust, high-performance consortium. The outcomes aim to improve the efficiency and feasibility of urine treatment for regenerative life support systems in Space, particularly enabling the recovery of water and nitrogen.

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Fast 2D NMR for deciphering lipidic extracts of microalgae

Food supply is a great challenge for long space mission as it needs a big storage space and is restricted in time. To overcome this challenge, the European Space Agency (ESA) created the MELiSSA programme (Micro Ecological Life Support System Alternative).

Microalgae as a source of food, are more and more studied [1]. Like a lot of plants, they contain proteins, carbohydrates and lipids [2] and are in fact, more and more cultivated for human consumption. *Limnospira indica* (LI), formerly called *Arthrospira platensis*, is a well-known super food usually taking the form of food supplements and called *Spirulina*, a cyanobacterium

chosen by the MELiSSA programme to be cultivated in space in the long term. The goal of the SPACE-ALG project is to develop an online control system of an LI cultivation in photobioreactor using flow benchtop NMR. The first step of this project is to identify and quantify all lipids in microalgal extract with high field NMR. Thus, to validate our NMR approach, a model microalga for lipid production : *Parachlorella kessleri* was considered. One sample was cultivated under standard growth conditions while three others were cultivated under different stages of nitrogen starvation which enhances the lipid production [1]. Then, the lipids were extracted with chloroform

and methanol (50/50). 1D NMR does not provide enough information to identify lipids in our complex samples due to many overlapping signals. Thus, we implemented more resolutive 2D experiments: 1H-1H TOCSY, 1H-13C HSQC and 1H-13C HMBC, performed at 400 MHz. The long time needed to perform those experiments was reduced with the use of fast 2D NMR methods such as NUS (Non-Uniform Sampling) [3] and NOAH (NMR by Ordered Acquisition using 1H-detection) [4]. Identification of several lipids was performed using literature [5]. On the sample studied in standard conditions (no starvation), storage lipids such as triacylglycerol (TAG) and diacylglycerol (DAG) were not observed while membrane lipids such as MGDG (monogalactosyldiacylglycerol), SQDG (sulfoquinovosyldiacylglycerol) and DGDG (digalactosyldiacylglycerol) were detected. The opposite situation was observed with the sample that was starved for the

longest time, as storage lipids were detected but no membrane lipids. Then for the two samples that has been less starved and can be thought of as an intermediate state, we could detect both storage and membrane lipids. This can be explained with the biosynthesis of those lipids: microalgae produce membrane lipids with DAGs, but when nitrogen starts to lack, the energy is redirected to produce the storage lipids TAG instead [1]. The continuation of this work will be to detect the same lipids on low field NMR. References [1] Klok, A. J et al., Trends Biotechnol. 2014, 32, 10, 521-528. [2] Becker, E. W; Biotechnol. Adv. 2007, 25, 2, 207-210. [3] D. GoBowicz et al., Prog. Nucl. Magn. Reson. Spectrosc. 2020; 116, 40-55. [4] Kupce, E.; Claridge, T. Angew. Chem. Int. Ed. 2017, 56, 11 779-11 783. [5] Bouillaud, D. 2020, Multiscale NMR analysis of the microalgae metabolism (PhD Thesis, Nantes Université)

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Fogponics in the Loop: Developing and Testing a Nutrient Delivery System for Bioregenerative Space Agriculture

Ensuring sustainable crop cultivation in lunar habitats presents critical challenges due to limited resources, constrained volume, and the need for closed-loop systems.

Efficient and reliable nutrient delivery is essential for supporting plant growth in such environments, where water must be conserved and infrastructure simplified. This research investigates the use of a fogponic nutrient delivery system an approach that generates a nutrient-rich mist using ultrasonic atomization as a viable alternative to conventional hydroponic and aeroponic systems for space-based plant production. The prototype is designed as a technology demonstrator for future lunar greenhouse applications. It operates by producing fine droplets in the range of 20-100 µm using ultrasonic foggers, allowing targeted delivery of nutrients directly to plant roots.

The physical fundamentals of this process such as droplet formation through acoustic cavitation, the influence of nutrient solution properties (e.g., surface tension and density), and the interaction between mist and root absorption form the basis for the experimental investigations. The research includes controlled experiments to study how fogging affects chemical properties of the nutrient solution (e.g., EC, pH), how solution parameters influence droplet size, and how fogponics compares biologically and resource-wise to traditional nutrient delivery systems. By advancing the understanding and readiness of this method, the study supports both extraterrestrial agriculture and the development of more water-efficient cultivation methods on Earth.

MARTIN HARTMANN

Senior Scientist in Microbial Ecology and Sustainable Agriculture - ETH Zurich, Switzerland

Circular Approaches in Terrestrial Applications and Management of Complex Microbial Communities

To achieve global sustainability, a fundamental shift from linear 'take-make-dispose' approaches to circular systems is needed in various sectors, particularly in agriculture. In this presentation, I will introduce the concept of circular agriculture by comparing conventional cropping systems with high external inputs and linear resource flows with organic cropping systems that focus on input reduction, resource recycling, and natural ecological processes for plant growth and health. The soil microbiome is crucial to this comparison, as it plays a key role in nutrient cycling, plant development and stress resistance.

Based on findings from the DOK long-term field trial in Switzerland, the world's oldest comparative study of organic and conventional farming systems, I will present results from four decades of research showing how management practices influence the soil microbiome and associated ecosystem functions. Organic and mixed cropping systems promote diverse microbiomes with improved decomposition and nutrient cycling capabilities, while conventional systems using synthetic

fertilizers select microbiomes that specialize in the uptake of inorganic nutrients and cellular resource allocation.

These differences lead to higher soil biodiversity, improved nutrient retention, increased carbon sequestration and reduced greenhouse gas emissions in organic systems, ultimately strengthening their circular economy and sustainability.

Building on these terrestrial observations, I will draw parallels to the stringent requirements of Bioregenerative Life Support Systems for space missions. I will explore how principles from circular agriculture can influence the development of modular components for extraterrestrial environments. Conversely, I will discuss how the stringent requirements for closed loops in space systems, such as the precise recycling of nutrients and the conversion of waste into resources through efficient biological consortia, can inspire innovations in terrestrial agriculture. This mutual exchange of knowledge offers a path to highly efficient, circular food systems on Earth and beyond.

BERAT HAZNEDAROGLU

Asst. Prof. Dr. - Bogazici University

Photosynthetic performance of two microalgae species tested during Ax-3 Mission: Physical and molecular dynamics under microgravity

Algal species have played major roles in increasing the oxygen levels and forming Earth's atmosphere suitable for the other living organisms due to their superior photosynthetic efficiencies. Having a wide umbrella of species diversity, microalgae can adapt harsh environmental conditions and be used for various life support systems and functions required in space stations and missions. In this study, we tested carbon dioxide capture efficiency and overall endurance of *Stichococcus* sp. and *Micractinium* sp. launched to The International Space Station (ISS) on January 2024 as part of Axiom-3 Private Astronaut Mission (PAM) supported under The Turkish Astronaut Science Mission (TABM) Project. Enclosed in a 2Ux2U experimental cube and installed on Columbus Module at ISS, *Stichococcus* sp. showed instantaneous carbon dioxide capture performance of 21 ppm whereas *Micractinium* sp. showed a much better performance and instantaneous carbon dioxide capture of 296 ppm. Output oxygen sensors recorded *Stichococcus* sp. with an average of 22.28%, whereas *Micractinium* sp. showed an

average of 22.24%. In the second part of the mission, an RNA-seq based de novo transcriptomics analysis have been performed to investigate differential gene expression comparing in-flight preserved samples exposed to microgravity conditions for 10 days to pre-launch samples preserved at Kennedy Space Center. Preliminary bioinformatics analyses were completed and differential gene expression (DGE) levels were examined in *Micractinium* sp. in which a total of 14,042 gene features were found to have statistically significant differences in microgravity conditions (FDR < 0.05). Of this, 11,884 gene features were determined to be up-regulated whereas 2,158 were found to be down regulated under microgravity conditions. According to the Gene Set Enrichment Analysis (GSEA), it was determined that microalgal cellular activity and biosynthetic mechanisms were higher under microgravity conditions. Integration into metabolic pathway analysis carried out using the Kyoto Encyclopedia of Genes and Genomes (KEGG) databases, a total of 154 metabolic pathways with DGE were generated.

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The Space Omics spaceflight related results and Simulated Microgravity facilities provided to the Space Biology, Life Support Systems and Astrobiology communities in Spain

Since the completion of the International Space Station assembly by 2001, our lab has been working in the study of animals and plants adaptation strategies at the gene expression level using the whole genome analyses tools available at any time. In this talk we will summarize the achievements of the *Drosophila* GENE experiment performed by Pedro Duque at the ISS during the Spanish Cervantes Mission (2003) and the Seedling Growth series of spaceflights (2012-2018) including a complex set of environments and plant mutants. In addition, we will elaborate on the simulation facilities projects coordinated from Madrid (SEMGMSPE_Ph1, GIA1, GIA2) or participated (ROOTROPS). During these works significant achievements have been reached, for example the large impact of suboptimal environmental conditions in the -omics response to spaceflight (1), recommendations for the use of simulation facilities on ground (2) as well as the development of partial gravity paradigms on Earth (3,4). In the case of plants, we have been able to dissect the transcriptional response to microgravity in cell cultures so different Gene Ontologies are affected at different cell cycle subpopulations (5). On the other hand, adaptation to spaceflight conditions in seedlings depends on the photostimulation and it is particularly stressful at low gravity levels in the 0.1g range (6) in the wild type samples. Thousand of

seedlings including mutants have been also included in the SG experiment and those results will be disclosed here. Finally, we will present our portfolio of simulation facilities available at CIB Margarita Salas (CSIC) including cell culture and petri dishes clinostats and the new 3-axis rotating facility U- Grav. The Spanish research community interested into simulate microgravity on Earth can apply for simulation time as a first step to prepare a flight experiment to be performed at orbital stations or the Moon surface, and also as a complement to validate spaceflight experiments results. Acknowledgments: We acknowledge the Spanish Government (AEI) funding of the PID2023-150842OB-I00 project, ESA funding of the Space Omics Topical Team by contract 4000131202/20/NL/Pg/pt and three GBF projects (ESA contracts 4200022650, 4000105761 & 4000130341/20/NL/Pg/pt) and the CIB Margarita Salas (CSIC) staff supporting its management. References: 1. Herranz et al. (2010) *Molecular Ecology* 19 (19), 4255-4264. 2. Herranz et al. (2013) *Astrobiology* 13 (1), 1-17. 3. Manzano et al. (2018) *NPJ Microgravity* 4 (1), 9. 4. Herranz et al. (2012) *BMC genomics* 13 (1), 1-14. 5. Kamal et al. (2018) *Plant, cell & environment* 42 (2), 480-494. 6. Herranz et al. (2019) *Frontiers in Plant Science* 10, 1529.

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Operation of Biolab for Human Spaceflight Applications at the Microgravity User Support Center (MUSC)

The Microgravity User Support Center (MUSC) in Cologne, Germany, supports a significant variety of scientific experiments aboard the International Space Station (ISS) by being the responsible center (FRC) for several material- and life sciences-payloads. One of these payload facilities, Biolab, offers scientists the ability to conduct biological research in microgravity and simulated gravity environments. Biolab provides an incubator with centrifuges that can house experiment containers which are developed for specific experiments. These experiments can last from a day to several months and cover various biological topics, including cellular behavior, photosynthesis, and immune response. Biolab can be operated in an unattended manner, allowing for flexible and continuous experimentation especially for long-running experiments, such as Arthrospira-C. Throughout the whole experiment execution, the facility as well as experiment hardware specific telemetry is automatically monitored. In case out-of-limit values are detected, operators are notified and will respond within maximum two hours to eventually occurring anomalies. Simultaneously, a remote

monitoring of all telemetry is possible by the flight control team, by the scientists as well as by the supporting developmental engineers. This is realized within a browser-based software environment. An important factor in the experiment preparation is thorough testing.

From an operational point of view, the experiment sequence test (EST) is the most important milestone prior to execution of the experiment on orbit. In this test series, all operational products are validated in an integrated test including flight hardware and scientific samples at the Biolab Engineering Model facility in a laboratory at MUSC. Experience has shown that getting the whole experiment community involved and familiarized with the available monitoring tools and complex operational environment can lay a good foundation for a successful experiment execution. This presentation provides an overview of the operations and developments at MUSC and offers a glimpse into the planned future operational scenarios as part of the ongoing utilization of the ISS for scientific exploration and could form the basis for post-ISS space operations.

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Research Progress on Bioregenerative Life Support System (BLSS) by “Lunar Palace-1” Team

The “Lunar Palace 365” mission, conducted in the ground-based Lunar Palace 1 (LP1) facility, represents one of the longest and most integrated experiments in bioregenerative life support system (BLSS) research. This 370-day mission aimed to simulate a lunar base environment with high closure and crew rotation, involving eight volunteers divided into two groups. The system achieved 100% regeneration of oxygen and water, 73% food regeneration (dry weight), and 98.2% overall material closure. Key subsystems included plant cultivation, waste processing, water recovery, and atmospheric management. The BLSS demonstrated remarkable stability under prolonged operation and crew shifts. Atmospheric O₂ and CO₂ levels were maintained within NASA-recommended ranges through biological regulation strategies involving soybean photoperiod adjustment and solid waste reactor activity. The system efficiently recovered 99.7% of urine and 67% of solid waste, with improved degradation rates over previous missions. Crew health and psychological states were closely monitored throughout the mission. No psychological distress was reported, attributed to the presence of plants, structured routines, and a high-fiber, plant-based diet. Multi-omics analyses revealed significant correlations between gut microbiota (e.g., *Bacteroides uniformis*, *Faecalibacterium prausnitzii*) and positive mood, suggesting a role of

psychobiotics in mental health maintenance. Salivary microbiota and cytokine levels remained stable, indicating no persistent immune dysregulation. Energy consumption was monitored, and gender-based differences in metabolic and psychological responses were noted. The mission also included simulated emergencies (e.g., power failures, extended isolation), to which the system and crew adapted effectively. These results validate the feasibility of long-term BLSS operation for future lunar or Martian habitats. The integration of biological, psychological, and environmental monitoring provides a holistic model for future space life support systems and offers insights into human adaptation to isolated, confined environments.

Currently, the “Lunar Palace” team is developing “Lunar Palace 2.0” to simulate extreme extraterrestrial conditions such as low gravity, strong ionizing radiation, and weak magnetic fields. In the future, we will conduct experiments on ecosystems composed of single and multiple organisms aboard the China Space Station and in ground-based simulation platforms designed for extreme lunar environments. We warmly welcome international collaborators to discuss partnership opportunities and jointly advance the application of bioregenerative life support system technologies for deep space exploration.

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Lettuce cultivation based on urine-derived fertilizer in-situ resource utilization of calcium oxide for pH control and calcium supplementation in hydroponics

Resource recycling and integration of in-situ resource utilization (ISRU) represents a key step towards plant cultivation on the Moon or Mars. Bio-regenerative life support systems can repurpose human urine as a valuable source of nitrogen and other essential plant nutrients. Processed and mineralized urine does however present some challenges, such as a high ammonium (NH_4^+) to nitrate (NO_3^-) ratio which is suboptimal for plants. Additionally, urine lacks certain essential nutrients, particularly calcium (Ca^{2+}) and magnesium (Mg^{2+}), which are essential for plant growth. Adding these cations require counterions such as SO_4^{2-} or Cl^- , which may accumulate in the nutrient solution to levels causing suboptimal conditions for plant development. Hydroponic plant cultivation requires the pH of the nutrient solution to be maintained within a certain range to ensure high crop productivity. When supplied with urine-derived solutions containing high levels of NH_4^+ , the plants typical preference of taking up NH_4^+ over NO_3^- , leads to an increased excretion of H^+ and thereby a decrease in pH. This decrease in nutrient solution pH can be counteracted by anion supplementation, e.g. addition of OH^- . When adding OH^- ions to increase the alkalinity, they are typically coupled to Na^+ or K^+ . In a closed-loop system, however, these cations may accumulate over time and cause nutrient imbalance in the solution. By adding calcium oxide (CaO) to the urine-derived nutrient solution, it forms $\text{Ca}(\text{OH})_2$, which makes Ca^{2+} available to the plants and adds OH^- ions to the solution. Thereby, CaO can

contribute to alleviate both the challenge of Ca^{2+} deficiency and the need for alkalinity. CaO can be found on the Moon and Mars and can be extracted as a by-product of regolith processing for oxygen and silicon production. The dual use of calcium oxide, as both a nutrient and pH regulator, was tested in deep-water cultivation of lettuce (*Lactuca Sativa* L. Frillice) in a controlled environment. CaO in solid form was automatically dosed into the nutrient solution by a custom-built system, operated based on a feedback loop from real-time pH measurements. Results show that the strategy of supplementing mineralized urine solutions with CaO increased the concentration of Ca^{2+} available to the plants, while simultaneously providing the required alkalinity for pH control. Further, it was found that the plants preferred to take up NH_4^+ over NO_3^- at pH levels from 5.5 to 7.0. To avoid calcium accumulation from added CaO, which was observed at pH 5.5 and 7.0, the pH was lowered to 4.0. Due to an increased uptake of NO_3^- over NH_4^+ at this pH level, less alkalinity was required, leading to a constant calcium concentration, while there were no noticeable negative effects on plant biomass. This regulation strategy offers a means to control the need for OH^- ions and thereby optimize both Ca^{2+} supplementation and pH control. These findings highlight the potential of combining the use of mineralized human urine with locally sourced metal oxides (ISRU) for cultivation of plants on Moon and Mars, supporting the broader goal of a near-closed-loop life support system for long-term space missions.

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Toward a more representative synthetic urine: Inclusion of organic compounds and validation of their conversion during anaerobic storage

Synthetic urine is widely used in both terrestrial and space-related research on nutrient recovery. Access to real urine can be limited due to ethical constraints or insufficient infrastructure, and certain experimental setups may require a stable influent composition, which is hardly possible with real urine due to its variability. Despite its broad application, there is currently no widely accepted synthetic urine formulation that has been thoroughly validated. Furthermore, most existing recipes only add urea as an organic compound, while organics representing chemical oxygen demand (COD) are rarely added or largely oversimplified, which can lead to discrepancies in experimental outcomes. For instance, studies have shown that synthetic urine often yields more favourable results than real urine in experiments on the adsorption of pharmaceuticals. In this study, a synthetic urine recipe including organic compounds is proposed and validated with an anaerobic storage experiment comparing it with real urine. A recipe was developed for average concentrations in urine collected over a 24-hour period as well as average concentrations in urine collected in an office building, referred to as day urine. During the development of the synthetic urine recipe, alkalinity was carefully considered, resulting in a synthetic urine solution that does not require pH adjustment. Furthermore, the chemical oxygen demand (COD) in real urine, which typically consists of over 90 different organic compounds,

is simplified in the synthetic recipe. Two distinct recipes are proposed, where the COD is represented either by three or nine selected compounds, with their concentrations proportionally scaled to match the total COD of real urine. Both recipes included creatinine, hippuric acid, and citric acid as the most abundant compounds and the more detailed recipe was extended by the addition of formic acid, trigonelline, glycine, creatine, lactic acid, and alanine. Both recipes were validated for anaerobic storage by comparing the pH increase, phosphorus precipitation and organic conversion of the synthetic solution to the real urine. For anaerobic storage, two versions of the synthetic urine recipe, one with three and one with nine organic compounds, were inoculated with anaerobic sludge from the urine storage tanks at Eawag (Switzerland), and the organic composition was measured by ^1H NMR. The proposed synthetic urine formulations had an initial pH of 6.2 prior to inoculation. Within a few hours, the pH rose to 9.3, aligning with both expectations and measurements observed in real urine. During the first six hours, approximately 25% of the phosphorus precipitated, which is also consistent with behaviour seen in real urine samples. Furthermore, organic compound transformations were monitored using ^1H NMR, revealing conversion pathways similar to those in real urine. Organic degradation started after 24 hours, after the pH had exceeded 9 and urea had been fully

hydrolysed. Firstly, hippuric acid was converted to benzoic acid. After 30 hours, a decline in creatinine concentration was observed, accompanied by an increase in acetate levels. By day six, creatinine, hippuric acid, and citric acid were no longer detectable. The organic profile at this stage was dominated by acetic acid, benzoic acid, propionic acid, and methylamine, which was also observed in real urine. After 22 days, all additional compounds in the nine-organic-compound recipe variant had also been degraded. The final composition of the synthetic urine closely resembled that of naturally stored urine. However, the concentration of benzoic acid remained higher by a factor of three in the synthetic samples. This discrepancy is attributed to higher hippuric acid concentrations in the

synthetic urine due to the scaling of compounds to represent the total measured COD in real urine. In this study, it could be shown that the proposed synthetic fresh urine solution undergoes similar biological transformations as real fresh urine when stored anaerobically. We therefore conclude that the proposed simple recipe is well suited to produce a representative urine solution. Despite the utilization of a substantially reduced set of compounds - typically more than 90 in real urine - only minor deviations in the final organic conversion product were observed. Overall, this synthetic urine recipe enhances the comparability across studies and can improve the representativeness of experimental results relative to real urine.

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LunarPlant: Human waste utilization in hydroponic systems for cultivation of leafy greens in Space

Future establishment of long-term, or even permanent, human bases on the Moon or Mars calls for a strategy with Bioregenerative Life Support Systems (BLSS) and technology to ensure sufficient food production and utilization of waste. Plants will serve as one of the main food sources, generate O₂ and remove CO₂, and contribute to wastewater recycling. Through the LunarPlant project, funded by the Research Council of Norway, researchers from Norway and Finland have developed and investigated strategies and concepts for hydroponic cultivation of leafy greens for food production and human waste utilization during long duration space missions. Researchers from SINTEF and VTT developed and optimized novel cellulose-based root substrates by combining different straw and wood pulp materials, forming them into growth media, and testing their performance. There were challenges related to microbial growth on the substrates, and various mitigation strategies were tested. The substrates provided essential functions such as mechanical support, water retention and nutrient delivery to the roots, and were shown to support growth of healthy plants. CIRiS emphasizes characterization, tuning, and effects of human waste-based fertilizers in hydroponic cultivation of leafy greens. As part of this work, different types of nutrient solutions were used, such as commercially available human urine-based fertilizer (Aurin) and synthetic urine. In addition, standard nutrient solutions with or without sodium chloride

supplementation were used to test effects of salt stress. Effects of these nutrient solutions on plant growth were investigated and nutrient solutions based on supplemented Aurin were designed and tested in multi-crop-cycles. The results provide ranges of plant tolerance to sodium chloride concentration and various imbalanced nutrient solutions. In addition, the dynamic changes of nutrient elements in the medium over multi-crop-cycles were mapped as an input to new fertilizer utilization strategies for long-term cultivation in recycling systems. The survival and growth of selected pathogens in urine-based nutrient solutions, such as Aurin, under relevant crop cultivation conditions have been investigated by researchers at NTNU. The results demonstrated that foodborne pathogens could survive in the nutrient solution cultivation of lettuce and possibility contaminate the food product. The results from LunarPlant show promising concepts and strategies for utilization of human waste as a nutrient source in hydroponic production of leafy greens for future space missions. This includes innovative plant cellulose substrates and supplementation strategies for nutrient solutions based on processed human urine. The project provides new knowledge on plant tolerances when using suboptimal nutrient solutions and the risk of unforeseen growth of microorganisms that may impact both crop production and food safety.

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MELISSA Feeder: Biomass Harvesting system for food preparation

Within the MELISSA framework for closed-loop space life support, *Limnospira indica* serves as a key microbial component due to its high photosynthetic efficiency, rich nutritional content, and resilience to space-relevant conditions. However, current

bioreactors, including the MELISSA Pilot Plant, lack automated biomass recovery systems optimized for zero-gravity conditions. This project presents the design, integration, and validation of an automated hardware demonstrator for harvesting *L. indica*

high biomass concentrations. The system integrates a vibrating sieve and pulse- regime microfiltration, controlled through a unified panel that enhances user efficiency and operational safety. Subsystem testing, followed by full integration within the MELISSA loop, confirmed reliability, energy efficiency, and adaptability. Performance metrics such as filtration efficiency, regrowth dynamics, and energy consumption were evaluated across multiple test phases, demonstrating successful regrowth of cell culture post-harvest within 11-14 days depending on bioreactor conditions. All system components are mounted on a mobile, corrosion-resistant stainless-steel skid. Electronics are housed in an IP65-rated enclosure with Siemens Simatic S7-1200 PLC control, integrated sensors, and modular

relay and power distribution architecture. Shielded 4-20 mA analog signal wiring and IPx4-rated sensors/actuators ensure resilience in wet environments. The system's automated detection and visualization features enhance safety by minimizing failure rates and user interventions. Testing showed the feasibility of partial filtration in pulse mode, allowing regrowth cycles within the bioreactor. Significant reductions in user time, enhanced process safety through visualization and fault detection, and compatibility with closed-loop operation were achieved. This harvesting solution represents a critical step toward autonomous biomass recovery in future space missions and terrestrial MELISSA spin-offs.

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Limnospira - Lipidomics and Pathways

In the context of the MELISSA project, understanding the biochemical potential of *Limnospira indica* (PCC8005) is essential to fully leverage its role in bioregenerative life support. This study focused on the targeted lipidomic profiling of *Limnospira* biomass, aiming to characterize key metabolites and link them to metabolic pathways relevant for human nutrition and system efficiency. *Limnospira* has been chosen for its light energy conversion efficiency, oxygen production, and mainly, its high nutritional value. This simple microorganism is also genetically robust and able to adapt to a wide range of culture conditions, including space radiation. The project began with method development using standard samples to optimize biomass disintegration and lipid extraction techniques. Lipid extraction protocols were optimized across six batches using isopropanol:n-hexane (3:2) and a variety of disintegration techniques. Soxhlet extraction emerged as the most reliable and reproducible method, outperforming pressure homogenization, which showed lower free fatty acid yields potentially due to

enzyme activation at elevated temperatures. Following this, lipid compounds were identified and grouped into categories such as pigments, fatty acids, and other metabolites. Metabolite screening techniques included GC, GC-MS, and LC-MS analyses, supported by flash chromatography and TLC for purification. While several MS platforms (e.g., TOF, Orbitrap, DART-MS) were employed, full structural elucidation of glycolipids was achieved via NMR spectroscopy following sample concentration and purity optimization. In the final stage, annotated data were compared with known metabolic databases, and biosynthetic pathways were reconstructed. Bioinformatics analyses, conducted in collaboration with Comenius University, enabled annotation of lipid metabolic pathways and identification of associated genes in the *Limnospira* PCC8005 genome. These results provide foundational insights into lipid metabolism in *L. indica*, supporting its use in bioregenerative systems and future biotechnological applications in space.

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An Integrated System for Water and Nutrient Recovery to Enable Sustainable Space Habitation

Developing sustainable life support systems is essential for the viability of long-term space missions, particularly for commercial space stations. The primary goal of the Hydromars system is to recover water and nutrients efficiently, addressing critical needs for long-term space missions. Our system utilizes membrane distillation (MD) combined with a crystallization process to achieve near-total recovery of water and essential nutrients, including nitrogen (N), phosphorus (P), and potassium (K), from spacecraft feed water streams. It produces high-quality water to support crew health and research activities while recovering

nutrients essential for in-situ food production, ensuring resource sustainability and self-sufficiency. By simplifying process flows and reducing operational steps compared to the state of the art, the system significantly enhances reliability and efficiency. Hydromars is currently exploring the adaptation of this system for ground-based testing to validate its performance in a controlled closed-loop environment. This effort aims to increase European competitiveness in the global life support system market while advancing the development of regenerative technologies for sustainable human habitation in space.

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MVIPER The Magnetohydrodynamic Vortex-Inducing Photobioreactor Experiment

With ambitions to send astronauts on missions of increasing duration and distance from Earth, biological life support systems based on microalgae are currently being researched for air revitalization, food production, and wastewater treatment. Under microgravity conditions in space, microalgae have so far been cultivated in closed-loop photobioreactors, requiring mechanical pumps to drive a membrane phase separation system. However, these designs suffer from high mechanical stress on the algae, biofilm buildup, and a limitation by the diffusion coefficient. Furthermore, both membranes and pumps require spare parts, further limiting the long-term feasibility of these reactor concepts. This presentation introduces MVIPEr, the Magnetohydrodynamic Vortex-Inducing Photobioreactor Experiment, a parabolic flight experiment designed for the ESA Academy Experiments Programme 24/25 to test a novel

photobioreactor concept for microalgae cultivation in space. This design uses AC electric and magnetic fields acting on the algae-compatible nutrient solution to create a vortex in a small-scale circular reactor through the Lorentz force. In this geometry, this vortex creates a centrifugal force, driving liquid to the outside and bubbles to the inside of the reactor, where a liquid-gas boundary forms. The experiment aims to demonstrate this technology by observing the flow characteristics and phase separation capability of the vortex in microgravity. Overall, this magnetohydrodynamic photobioreactor design aims to remove mechanical pumps and the membrane phase separation system, relying solely on magnetohydrodynamic pumping and phase separation by the vortex, increasing the overall reliability of microalgae photobioreactors for use in future long-term biological life support systems.

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Results from ESA OSIP activity: Deep-Space food production based on single-cell protein production by means of gas fermentation

In the quest for sustainable human presence beyond Earth, closed life support systems are essential for recycling resources and ensuring long-term autonomy. A key component of these systems is autonomous food production that is automated, recycles waste streams, and outputs high quality nutrition. At Solar Foods, we address this need through a novel microbial protein technology that earned us victory in the NASA Deep Space Food Challenge (DSFC) out of more than 300 companies. Our system cultivates edible microbial biomass called Solein® needing just carbon dioxide, water and electricity as the main inputs enabling food production in isolated, resource-constrained environments. This presentation explores the results

of a completed ESA OSIP study into gas fermentation as a method for deep space food production. In the study, we established preliminary system requirements, carried out preliminary design for an operational-scale Solein® bioprocess, breadboarded and demonstrated a cross-flow filtration system for downstream processing, carried out a risk assessment and assessed potential waste treatment methods for nitrogen and mineral recovery. Key outcomes include a CAD model of the process fitted inside an International Standard Payload Rack, confirmation of diafiltration as a method for washing out unused media components, and indications of flux for chilled Solein® product using a ceramic cross-flow membrane.

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Heat and mass transfer studies using leaf replicas, for future space plant systems. Effects of angle, airflow, and gravity.

Future space missions aiming for long-term sustainability will rely on bioregenerative life support systems in which higher plants play a central role in food production, air revitalization, and water recycling. Photosynthesis efficiency is highly dependent on heat and mass transfer at the leaf surface, which is altered in reduced gravity. To isolate and better understand the underlying physics of heat and mass transfer, this research employs leaf replicas that mimic stomatal-driven evaporation without the influence of biological processes and variability. This presentation includes findings from two experiments: a ground reference experiment and a parabolic flight experiment. In both experiments, the replicas were tested for multiple airflow regimes and inclination angles to simulate a range of realistic canopy conditions and convective scenarios. The ground

reference experiment was conducted in the MELiSSA Pilot Plant (MPP) Higher Plant Chamber (HPC) and provided baseline data by evaluating thermal and evaporative responses of the replicas in steady state under different temperatures in Earth gravity. The parabolic flight experiment captured transient heat and mass transfer responses into micro- and hypergravity environments. Both airflow velocity and leaf orientation significantly influenced thermal response in each experiment. For the Ground Reference Experiment, inclined surfaces exhibited lower steady-state temperatures compared to horizontally aligned configurations, indicating enhanced convective cooling efficiency in the angled position. In the parabolic flight experiment angled surface led to a lower temperature increase during the microgravity phase and to a

different ratio between convection-driven heat transfer to the total heat transfer. These results show that leaf orientation can significantly influence the development of boundary layers and convective heat exchange, affecting the plant's ability to transfer mass and energy with its environment plants. This study allows

more precise modelling of physical processes at the leaf surface and will be coupled with biological models of plant growth. This work was funded by a doctoral fellowship from CNES and the MELISSA Foundation.

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SOPHIE LABONNOTE-WEBER

Research Manager - NTNU Samfunnsforskning

From plant biomass and sidestreams to tissue engineering and biocomposite production

In the context of space exploration, ISRU is paramount for future endeavours like Mars transit habitats. Plant biomass, rich in cellulose, is such a versatile reusable resource for various applications, including the creation of inks for 3D printing scaffolds for cell culturing. On the other hand, nanocellulose fibres from biomass streams can offer unprecedented improvements of recycled thermoplastics when combined into biocomposites, providing enhanced flowability during melt-processing and mechanical strength of tailored 3D-printed spare parts. Also, the extraction of nanocellulose and other biopolymers and active compounds from plant or side-stream biomass holds the potential to produce hydrogels for applications like 3D bioprinting and tissue engineering. Accordingly, MELISSA lignocellulosic biomasses were identified and evaluated for nanocellulose gel production, focusing on cellulose content and availability. From the estimations, the

most promising biomasses for (nano)cellulose production in a surface mission, and in a transit mission scenario were identified, and are used as case studies for identifying and assessing processing technologies, focusing on versatility potential. Considering spirulina as a functionalization agent of the nanocellulose for antioxidant, anti-inflammatory, and antimicrobial properties is assessed in terms of application benefits vs technological implementation. Another available resource to be considered for recycling and repurposing strategies is packaging materials. Upgrading plastic-based packaging materials with cellulose for biocomposite production is a potential route. In this context, due to temperature constraints, packaging materials consisting of polyolefins, or a mixture with other polymers with similar processing temperature (

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Liquid Management in Space (LiMiS): Innovations in Microgravity Food Production

In the context of long-term human space exploration, in-situ crop cultivation is essential to provide the crew with vital nutrients and vitamins, as current space-food systems, based on supply of pre-packed food from Earth, fall short due to limited shelf-life and nutrient degradation over time. On board food production is hence crucial for enhancing crew health and well-being. With the broader ambition to develop a stand-alone leafy greens production payload to supplement crew diets with fresh vitamins during long-duration space missions, the Liquid Management in Space (LiMiS) project addresses one of the most challenging aspects of plant cultivation in microgravity: managing liquid in the plant root system. Starting with a

comprehensive system study at the payload level, the project aims to develop and test a Plant Root Substrate (PRS) breadboard that ensures efficient aeration, water and nutrient delivery to the root system in microgravity environments. In parallel, modifying and testing a commercial microgreen production system in a space habitat simulator will provide valuable insights into operational activities and human-system interactions. A preliminary payload concept will be presented, and the outcomes of the LiMiS project will significantly contribute to de-risking future manned missions and developing sustainable life support systems for deep space exploration.

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VALÉRIE LEGUÉ

Professor - Université Clermont Auvergne

Can Plants Grow Upright in Space? Mechanisms Underlying Stability and Root Anchorage

On Earth, plants are continuously exposed to mechanical stimuli such as wind and gravity. These factors influence their growth and posture, both of which are essential to ensure autotrophy and development. In a lunar or microgravity environment, the absence of wind, combined with the constraints of closed

cultivation systems, could lead to reduced growth and altered fruit production capacity due to the lack of weight sensation and airflow. One of the major challenges in space environments is therefore to promote an upright plant habit and maintain effective root anchorage. In this talk, I will discuss ground-based

studies that have improved our understanding of gravity perception and proprioception in plants. I will highlight the respective roles of wind and gravity in shoot development and root anchorage. Results from microgravity experiments will be presented along with future research directions. I will emphasize the importance of identifying the molecular and cellular players involved in the formation of supporting tissues and the

responses of the root system. I will illustrate how phenotyping of agronomically relevant plants, either in the absence of or in response to mechanical stimuli under reduced gravity conditions, can help to identify valuable ideotypes for space cultivation. Finally, we will discuss the conditions required to maintain plant growth and posture in spatial conditions.

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NATALIE LEYS

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Running a photobioreactor in space for the production of oxygen and edible spirulina biomass

Microbially produced oxygen and microbial edible biomass are very interesting sustainable resources for future space travelers. Arthrospira-C (ArtC) is a spacebiotechnology flight experiment, to test as a proof of principle a cyanobacterial oxygenic photosynthetic bioprocess in space. ArtC is the followup and step-up of the pioneering ArtB flight experiment which flew 7 years earlier to the ISS, this time allowing continuous culturing and variable light settings. A space compatible photobioreactor was built, allowing online measurements of both bio-oxygen production rate and microbial growth rate in space. The photobioreactor contained the cyanobacterium *Limnospira indica* PCC8005 (aka *Arthrospira* sp. PCC8005, or under the commercial name *Spirulina*). Four of such bioreactors were integrated in the ISS Columbus Biolab incubator and operated in turbidostat mode by semi-continuous feeding for a duration of 2 months, to test production kinetics at 4 different light intensities. The experiment was performed in parallel on ISS and on ground. The bioprocess was remotely monitored and steered, using space bioreactor communication software systems and a dedicated model for the growth of *Limnospira* in membrane photobioreactors. The space grown biomass was analysed for its biomolecular and biochemical composition and nutritive value in detail postflight. In this presentation we will update you on how following challenges were addressed : (1) to define detailed requirements for, to test and to validate a space-

compatible photobioreactor, with special attention to liquid mixing and Computational Fluid Dynamic modelling of reactor designs and operation states, (2) to restart the cyanobacterial cultures in the bioreactor in the ISS after a period of dormant storage and upload, (3) to maintain a photosynthetic active culture in the bioreactor, under space conditions (including exposure to space reduced gravity and space increased ionising radiation), (4) to implement successfully ground commands to adapt bioreactor conditions and allow several crew interventions, (5) to remotely monitor live oxygen and biomass production of the culture, and the fitting to the predictive simulation, and (6) to maximise quantity and quality of the produced biomass for its application as potential dietary supplement in future space flight. We will also update you on the current scientific outcome of the ArtC flight experiment. We will present data of the significant amount of pre-flight testing that was performed on ground to obtain reference data and prepare for operation in space. We will present the preliminary data obtained from the actual ArtC flight experiment (launched on 4th of Nov. 2024) online data and samples, which are currently being processed. Our research shows that it is very challenging but feasible to send, start, and operate a space-compatible continuous microbial (photo)bioreactor in the ISS in space, with remote control and monitoring of the bioprocess from ground.

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CESARE LOBASCIO

Space Exploration and Science Innovation Lead - Thales Alenia Space, Italy

From Low Earth Orbit to the Moon and Mars Horizons: The Evolution of Habitats and Life Support System

Europe has long played a crucial role in the advancement of space life support technologies, with Thales Alenia Space serving as a leader in the design and implementation of mission-critical systems. Drawing on decades of experience, the company has significantly contributed to the International Space Station (ISS) through both permanent and cargo transportation elements such as the Multi-Purpose Logistics Modules, Columbus, Node 2 and Node 3, Cygnus. These pressurized modules have set benchmarks in modular habitat design, environmental control, and integrated life support, enabling sustained human presence in low Earth orbit.

A key aspect of this heritage is Europe's expertise in potable water management, prominently demonstrated by the Automated Transfer Vehicle, which regularly delivered vital

water supplies for the ISS crews. The operational know-how gained from dealing with water resupply, storage, and quality assurance has paved the way for the development of new water storage (Orion) and recycling systems, essential for future long-duration missions.

Looking forward, Thales Alenia Space remains at the center of Europe's ambitions for Low Earth Orbit (LEO) and deep space transportation and habitation. For a post-ISS scenario, we support new commercial LEO stations being developed by private companies such as Axiom and Blue Origin, and the European cargo vehicle which will evolve towards crew transportation, with new challenges concerning habitability and life support, comfort and affordability.

Beyond LEO, the contributions to the Artemis program via the

Lunar Orbital Outpost Gateway —with the International Habitation Module and the Airlock— extend proven approaches in life support into the more demanding deep space environment. In anticipation of even greater autonomy from Earth, the company is actively engaging with the challenges of planetary surface habitation and mobility through the Multi Purpose Habitat.

In this context, beyond water recycling, food production is recognized as a cornerstone for sustainable long-duration missions to the moon and Mars. European initiatives such as the ESA's Precursor of Food Production Unit (PFPU) and ASI's Microx2 experiments, both developed by Thales Alenia Space and

academia, are validating technologies for crop cultivation, resource utilization, and bioregenerative systems in space. The target missions for such technologies are the Mars Transit Habitat and long-term surface bases.

These evolving efforts illustrate a profound shift towards increasingly self-sufficient habitats, incorporating lessons learned from the ISS and leveraging new advances in autonomy, recycling, and food production. With an enduring focus on modularity, flexibility, and international collaboration, Thales Alenia Space is shaping the future of European leadership in human space exploration—bridging a proven legacy with the needs of upcoming lunar and planetary missions.

ELENA LUCIANI

Student - Università Campus Bio-Medico

Noble Rot Wine Pills

Creating wine pills for space involves overcoming several challenges, such as ensuring the preservation of taste and quality in a compact form, addressing potential health implications, and considering the effects of microgravity. While it's a complex idea, advancements in space food technology are continually being explored. While the importance of wine in space might be subjective, providing astronauts with a variety of enjoyable and culturally familiar foods, including beverages

like wine, can contribute to their well-being during long missions. Noble rot wines are peculiar wines: they include a vast range of bioactive molecules (antioxidants, antiviral and anticancer). Creating ways to enjoy familiar pleasures in space can have positive psychological effects and help maintain mental-physical health during extended periods away from Earth.

ØYVIND M. JAKOBSEN

Research manager - CIRIS, NTNU Samfunnsforskning AS

Real-time thermal imaging of leaf temperature to explore plant transpiration and leaf boundary layer effects on ground and in microgravity

Transpiration is a key physiological process in plants, influencing water and nutrient transport, temperature regulation, and photosynthesis. It is governed by both biological mechanisms such as stomatal behavior and physical factors like the gas boundary layer surrounding leaves. Understanding how these factors interact is essential for modeling plant growth and optimizing cultivation in both terrestrial and space environments. The Water Across Plants (WAPS) project, funded by the European Space Agency, aims to disentangle the direct effects of microgravity on plant function from indirect effects caused by altered boundary layer dynamics. The project investigates how reduced buoyancy-driven convection in microgravity thickens the boundary layer, potentially suppressing transpiration. To achieve this, mung bean (*Vigna radiata*) plants are grown under different gravity conditions aboard the International Space Station, using custom-designed Experiment Containers, essentially miniature greenhouses with climate control and integrated thermal cameras. For WAPS, robust 1g control is a key element to interpret effects of microgravity, but boundary layer effects are challenging to study at this condition given the presence of natural convection. This study presents results from extensive ground-based experiments to refine, verify, and exploit the experimental setup. Specifically, we demonstrate that the thermal imaging system can detect subtle changes in leaf temperature, linked to transpiration and boundary layer dynamics, even under Earth gravity and within short timeframes. Verification of this capability, given the size and boundary conditions of the flight hardware, represents a significant milestone for WAPS, and holds relevance also for similar studies. This presentation outlines how thermal imaging was refined through automated Flat Field Correction and the inclusion of a reference object with known temperature,

reducing variances and inaccuracies in absolute temperature from up to 4°C to negligible levels. Verification experiments using an artificial leaf illustrated differences between effects of biological transpiration and environmental influences such as light and airflow. For example, cycling of airflow on and off resulted in leaf temperature changes up to 1.6°C, while the artificial leaf showed only 0.4°C variation. Similarly, light on/off cycles revealed differences in thermal response between real and artificial leaves, indicating the involvement of stomatal regulation mechanisms. Based on refined strategies and setup, several ground experiments were performed to track plant development from seed to early leaf stages, with repeated airflow perturbations. Leaf temperature monitoring by thermal imaging demonstrated effects of air velocity cycling and changes in plant transpiration across time of day. These patterns confirmed that the WAPS system can resolve dynamic transpiration behavior across daily cycles, developmental phases and environmental conditions. More specifically, the results demonstrate that measurements of changing leaf temperature can be made within minutes, even under 1g conditions, to detect and quantify boundary layer and transpiration effects in real time. The presented strategies hold relevance for ground-based thermal imaging studies, for example in investigating plant transpiration for applications in Controlled Environment Agriculture, such as vertical farms benefiting from fine-tuned ventilation. Additionally, the demonstrated capabilities lay a solid foundation for future experiments in microgravity, where boundary layer dynamics and transpiration effects remain poorly understood but are expected to be more pronounced and therefore accessible using the presented strategies.

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ØYVIND M. JAKOBSEN

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From mineralized urine to balanced nutrient solution for crop cultivation: Long-term supplementation strategies and nutrient solution dynamics

Recycling organic waste as plant nutrients supports both Bioregenerative Life Support Systems for space and sustainable agriculture on Earth, promoting a shift from linear production to nutrient recycling and circular economy. This study explored strategies to convert mineralized human urine into a balanced nutrient solution for hydroponic crops, addressing long-term challenges in nutrient supplementation and pH control in closed systems. Mineralized waste fractions often contain imbalanced nutrient levels and excess non-nutrients. While short-term supplementation is manageable, long-term nutrient solution maintenance in closed hydroponics is complex. Nutrients must be individually controlled to prevent depletion or accumulation. Even with optimized supplementation and pH control, counterions used for charge balancing may accumulate over time, increasing salinity and thereby reducing crop productivity. This presentation summarizes results from a considerable number of crop cultivations using mineralized human urine and various nutrient supplementation strategies differing in macronutrient ratios and levels. Emphasis is placed on macronutrient dynamics, monitoring individual nutrients over time to explore how different nutrient solution supplementation and maintenance strategies can minimize nutrient imbalance and salinity increase, thereby allowing multiple crop cycles without discarding the nutrient solution. Lettuce (*Lactuca sativa* L. Frillice) was grown hydroponically using partially nitrified human urine (Aurin fertilizer) and a Hoagland-like control. After harvesting 5-week-old plants, remaining nutrient solutions were replenished according to various supplementation strategies and used for the next crop. This reuse strategy was repeated for a total of 4 cultivation cycles, each time restoring the total nitrogen concentration and the liquid volume. As predicted, using only mineralized urine in deionized water led to poor growth due to

low levels of essential nutrients like Ca^{2+} and Mg^{2+} . In contrast, considerable biomass production was observed when preparing the initial nutrient solution with tap water, illustrating the effect of even minimal nutrient supplementations. However, sustained productivity required further supplementation. To ensure macronutrient levels over time, mineralized urine was supplemented with all macronutrients beyond nitrogen to match the control solution with respect to ratios relative to nitrogen. The required supplementation of cations (K^+ , Ca^{2+} , Mg^{2+}) exceeded that of anions (PO_4^{2-} , SO_4^{2-}) by a 3-factor, requiring the use of excessive SO_4^{2-} and Cl^- for charge balancing. While these supplementation strategies successfully provided required nutrients and allowed biomass production similar to the control, charge-balancing ions accumulated over time and illustrated challenges of nutrient solution maintenance in closed systems. The nutrient supplementation strategies were later refined based on detailed nutrient profiling of previous crop cultivations, resulting in improved nutrient solution stability and reduced salinity increase. Furthermore, the coupling of cation supplementation (Ca^{2+} , Mg^{2+}) to the required addition of alkalinity (OH^-) for pH control further improved the nutrient supplementation strategy, reducing the need for charge-balancing ions for alkalinity and cation supplementation and illustrating a key concept for nutrient supplementation of partially nitrified urine. The presented work fertilization strategies using mineralized urine that mitigate ion accumulation and salinity increase in closed hydroponic systems over time. The results hold relevance not only for the process control of crop cultivation in a space perspective, but also for the use of mineralized organic waste for terrestrial food production.

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KAIA MACLEOD

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Stress Response of Hydroponically Cultivated Kale (*Brassica oleracea*) to Sodium Chloride and the Potential Mitigation Effect of Co-cultivation with Saltwort (*Salsola komarovii*)

Sustainable food production is a critical component of Bioregenerative Life Support Systems (BLSS) for long-term space missions. One key challenge in hydroponic systems using recycled wastewater is the gradual accumulation of sodium chloride (NaCl), which can negatively impact plant growth. This study investigates the effects of increasing NaCl concentrations on the growth and nutritional quality of kale (*Brassica oleracea*), as well as the potential mitigating role of co-cultivation with a salt-tolerant halophyte, saltwort (*Salsola komarovii*). Two experiments were conducted. The first examined the growth and physiological responses of kale to NaCl levels ranging from 1 to 120 mM. Results showed significant growth inhibition at high concentrations (80–120 mM, 54–96% reduction), while

moderate concentrations (40–60 mM) had a limited impact (0.02–7.23% reduction). Antioxidant activity increased by 12% up to 100 mM NaCl . Additionally, the Na^+/K^+ ratio increased with salinity, indicating osmotic and ionic stress, with a critical threshold around 80 mM NaCl . The second experiment assessed co-cultivation of kale with saltwort at 100 mM NaCl . While shoot growth of kale was not significantly altered by the presence of saltwort, Na^+ accumulation in kale was reduced. However, co-cultivation also led to a decrease in nitrogen content, suggesting potential competition for nutrients. These findings suggest that co-cultivation with halophytes may partially mitigate salt stress in glycophytic crops and could be a useful strategy for optimizing BLSS crop systems.

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FABIO MAGRASSI & RICCARDO CAPOLLA

Energy and Sustainability Business Area Manager & Energy and Sustainability Project Manager - STAM srl, Italy

From orbit to Earth: Bioregenerative systems for sustainable food and water in space and on Earth

Long-duration space missions demand innovative solutions for self-sufficiency and resource optimization. The TRANSFORM project pioneers the integration of a smart, modular greenhouse, developed by SPACE-V, with a microalgae-based water treatment system, developed by STAM, to establish a fully circular and biocompatible food production unit. Initially designed for terrestrial use, the system is being explored for its viability in orbital and planetary applications, offering volume and energy savings, adaptive shelf management, and in-situ production of oxygen, clean water, and fertilizers. Complementing this effort, a second demonstrator focuses on the treatment of grey and yellow waters within a mobile containerised system, adapted from technologies developed

under ESA's MELiSSA programme. This container implements a pre-treatment and microalgae bioreactor sequence for nutrient removal and biomass valorisation, with the aim of supporting closed-loop water and nutrient cycles in isolated and space-constrained environments. Together, these solutions highlight a pathway towards bioregenerative life support systems applicable to both space habitats and Earth-based infrastructures, enabling sustainability in extreme or resource-limited environments through the intelligent reuse of waste streams. The presentation will share progress on both systems, their ESA heritage, and their potential as future life support technologies for lunar and Martian settlements as well as terrestrial off-grid scenarios.

ELENA MARIA CAMPANARO

ESA Graduate Trainee in Environmental Control and Life Support Systems Engineering - European Space Agency

Inspiring the Next Generation: Life Support Systems Solution from Space for Earth Training Course

The Life Support Systems Solutions from Space for Earth Training Course is the inaugural course of the Space for Earth series, first held in 2025. It ran for two weeks the first at ESA Academy's Training and Learning Facility at ESA's ESEC Galaxia site in Belgium, and the second online. Developed by ESA's Education Office and the Life Support and Physical Sciences Instrumentation Section at ESTEC, the course focuses on research into space technologies for circular, autonomous life support systems, and their applications on Earth with a business-oriented approach, within the context of the MELiSSA Project. Delivered by experts from ESA and/or involved with the MELiSSA Project, the course consisted of fourteen lectures, two workshops - one focusing on

closed-loop systems, the other on ideation - and both onsite and online working sessions. These sessions aimed to allow students to put into practice the acquired knowledge to build and validate their business case. The course brought together 30 students from 15 different nationalities and a wide range of academic and professional backgrounds from aerospace engineering, earth physics, ecology, bioengineering, computer science, and business. The presentation will focus on the approach taken to structure the course and its performance, as well as the overall achievements and future improvements to be taken.

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ERIK MAZZOLENI

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Oxygen Separation Technology for the PaCMan PCU - overview and future perspectives

The Plant Characterization Unit (PCU) is a ground-based research facility located at the University of Naples Federico II, which enables data collection for modelling purposes and allows the MELiSSA community to perform scientific experimentation on crops. The PCU was engineered, manufactured and tested by EnginSoft and other partners in the PaCMan (Plant Characterization unit for closed life support system engineering, Manufacturing & testing) project. As part of the scientific experimentations, three different leafy green species were grown in the PCU. The results demonstrated that they produce different total amounts of oxygen during life tests on a 28-days span. In each of these tests, the concentration of oxygen in the PCU increased above the fire limit threshold level, posing a potential hazard. In response to these elevated oxygen concentrations, a predefined safety protocol based on a venting procedure was activated. This procedure necessitates the

opening of the PCU's closed atmospheric loop, allowing for the exchange of the internal atmosphere with ambient air. While this procedure is a necessary safety measure, it is suboptimal for several reasons. Therefore, alternative approaches to regulate the oxygen concentration within the PCU need to be explored. Specifically, the objective is to identify a state-of-the-art technology that can effectively remove excess oxygen from the system, ensuring that its concentration always remains well below the safety hazard threshold. This presentation will outline the motivation behind this upgrade, give an overview of the possible commercial technologies for oxygen separation and explore the engineering design and manufacturing of a breadboard which will evaluate the performance of the selected technology. Moreover, perspectives for its implementation in the PCU will be highlighted.

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ORSOLYA MEIER

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Research towards a novel biomanfactory-based blss system using giant reed (*Arundo donax*) as a multifunctional clonal propagated biotech space-plant candidate

The demand for sustained economy generates challenge for basic science and technology in the 21st century. This demanding environment stimulated the rapid development of plant biotechnology utilizing more and more cutting edge science. The data shows the upward trend in Europe toward the generation of new green industries grouped under the biorefinery concept. Developing Bioregenerative Life Support Systems (BLSS) is critical for achieving sustainable human habitation in space, too. The BLSS is a highly promising way of addressing this limitation, even more so if it can be combined with in situ resource utilization (ISRU). The ISRU approach aims to reduce terrestrial input into a BLSS by using native regoliths and recycled organic waste as primary resources. The combination of BLSS and ISRU may allow sustainable food production on the Moon and Mars. This task poses several challenges, including the effects of partial gravity, the limited availability of oxygen and water, and the self-sustaining management of resources. Lunar and Martian BLSS will, therefore, most likely include plants, which are necessary for food production. In addition, they provide air revitalization and water purification capabilities, and could be used for other functions including, for instance, pharmaceutical production, among many others. *Arundo donax* is a robust, sterile, clonally propagated decaploid monocot grass species which has been introduced around the world by humans as an ornamental and economic plant.

Arundo donax is well known for its ability to adapt to a wide range of marginal environmental conditions from wetlands to the semi-desert, high or low pH, high salinity, halogenated organics or heavy metal contaminated soils, from continental to tropical climate. The mechanism and stability of this wide range

of adaptation abilities have great biological potential, especially for the future space agriculture. Giant reed (*Arundo donax* L.) is one of the most well-studied perennial biomass crops because of its high productivity and potential to store carbon. The massive availability of biomass generated by this crop motivates the search for its possible biomanfactory use for the generation of high added-value products through implementing the biorefinery approach. In view of its importance, the University of Debrecen started the scientific foundation of the first Hungarian BLSS and Biomanfactory research program, focusing on giant reed biotechnology in 2009. As part of this initiative, a special biological research and experimental plant cultivation space, the BIODROME Debrecen, was constructed. According to our best knowledge, *Arundo* has not been included so far in the ISS space plant programs, nor in the plans for Mars and Moon space agriculture research. Despite the fact that *Arundo* is one of the most important model plant species for the terrestrial biomass-based biomanfactory industry and green chemistry. Hence, our research team has launched the 'Terraphon' research programme to investigate giant reed as a novel candidate space plant, exploiting its exceptional multifunctionality in biomanufacturing as the following: *Arundo donax* is a robust, decaploid grass species. The genetic variation within this species is very limited due to the lack of sexual reproduction. The investigation of polymorphism of the DNA of American, Australian, and Eurasian populations revealed a high level of relatedness and pointed to founder effects. However, a wide range of well-adapted ecotypes is known to grow under very different environmental conditions, and the mechanism of these adaptations remains a very exciting scientific question

JAN MIKOLAJCZYK

Student - University of Warsaw

Snow s Eye Measurement Suite: a modular acoustic-radar spectroscopic payload for quantitative and compositional water-ice reconnaissance in support of MELiSSA life-support and ISRU systems

Snow s Eye Measurement Suite (SEMS) is a lightweight, self-contained payload that turns raw echo data into engineering-grade knowledge of both the quantity and quality of extraterrestrial water resources, thereby providing the hard numbers required to close MELiSSA life-support loops and to design reliable ISRU chains. At its core is a miniaturised acoustic sounder derived from the award-winning Snow s Eye system: a low-power broadband loudspeaker and a 24-element MEMS microphone array transmit a 20 Hz 20 kHz chirp and through on-board FPGA processing deliver centimetric ice- thickness ranging and direct computation of Snow-Water Equivalent (SWE). Because SWE scales linearly with mass, SEMS outputs geo-referenced kilogram budgets of H₂O with an uncertainty of roughly $\pm 5\%$, allowing mission planners to size water, oxygen and nutrient stores instead of relying on indirect proxies. For airless environments such as the Moon, where acoustic propagation is impossible, the sounder is complemented by a compact 200 900 MHz ground-penetrating radar that penetrates up to five metres of desiccated regolith, adding redundancy and extending reach into heterogeneous strata. A current upgrade couples the sounder with an ultra-miniature SWIR spectrometer

and a Raman/LIBS micro-probe, enabling in-situ determination of ice chemistry, isotopic signatures, hydrated mineral phases and contaminants such as perchlorates; this dual- modality approach provides not only how much water is available but also how clean it is for MELiSSA reactors. All electronics, firmware and algorithms have been demonstrated on Earth: two prototype UAVs carrying SEMS completed 60 °C / 100 mbar chamber trials and autonomous Svalbard campaigns, confirming data fidelity, environmental robustness and fully automated operation under harsh polar conditions, thereby establishing TRL 3 4 for the sensor physics. The project is now advancing toward TRL 4 5 through pressure-vessel acoustics for the martian 6 mbar CO₂ regime, joint acoustic-radar firmware, radiation-hardened power and data buses and GNSS- independent navigation. Crucially, SEMS is platform-agnostic: the 2.5 kg module draws a single 24 V \times 4 A line, publishes processed water maps over CAN or SpaceWire and can be bolted onto rovers, hoppers, skid-mounted drill rigs, tripod towers or future UAVs, making it equally valuable for rapid pre-landing reconnaissance and for continuous habitat monitoring. By delivering fast, precise and chemically resolved inventories in a

rugged, power-efficient package, Snow s Eye Measurement Suite lowers mission mass, risk and cost, establishing a direct bridge

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between robotic prospecting and closed-loop bioregenerative utilisation for sustainable human exploration beyond Earth.

KATARÍNA MOLNÁROVÁ

PhD Student - Mendel University in Brno

Exploring Perchlorate Tolerance in Freshwater Microalgae for Martian Applications

Microalgae are promising organisms for space-based biotechnologies due to their roles in oxygen production, nutrient recycling, and potential food generation. Their use in bioregenerative life support systems is of particular interest for future long-term space missions, including those targeting Mars. While cyanobacteria have been widely studied for such applications, the tolerance of freshwater eukaryotic microalgae to Martian conditions remains poorly understood. A major obstacle to the biological utilization of Martian regolith is the presence of perchlorates chaotropic, highly oxidizing salts detected in concentrations up to 2% by weight. These compounds can disrupt cellular homeostasis, generate reactive oxygen species (ROS), and impair photosynthetic and antioxidant functions in terrestrial organisms. Although previous studies have examined the effects of perchlorates on

cyanobacteria and some model algae, little is known about their impact on diverse eukaryotic microalgae, which could serve as viable components of closed-loop ecosystems on Mars. This study investigates the growth, pigment profiles, and oxidative stress responses of multiple green microalgal species exposed to magnesium perchlorate at concentrations analogous to Martian regolith (0.25%, 0.5%, and 1%). By assessing photosynthetic pigments, antioxidant capacity, ROS accumulation, and growth under both liquid and solid culture conditions, this work identifies key physiological traits associated with perchlorate resilience. These insights contribute to the selection of robust microalgal candidates for extraterrestrial cultivation and help advance strategies for in-situ resource utilization (ISRU) and sustainable biological life support in extreme environments.

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ANTONIELLE MONCLARO

Postdoctoral Researcher - CMET/UGent

INCITE - Innovative Ionic Liquid-enzyme tandems for enhanced biomass degradation

The MELISSA loop is a closed-loop life support system consisting of interconnected compartments for the recycling of waste into oxygen, water, and food. The Higher Plant Compartment, one of the most complex and important, hosts various crops that simultaneously support air regeneration, water purification, and food production, though it also generates waste, such as inedible plant material, which may accumulate over time. This waste, rich in lignocellulose is hard to degrade due to the complex and recalcitrant nature of the plant cell wall. In this contribution, we present an alternative method to overcome current bottlenecks of biomass conversion by developing an advanced pretreatment platform combining the selective solubilization capacity of ionic liquids (ILs) with the robustness of extremophilic enzymes. This integrated strategy addresses key challenges in biomass fractionation, such as the need for milder, more efficient, and environmentally compatible processing routes. ILs, specifically [EMIM]Cl, [EMIM]TfO, and [Chol]OAc, have been selected for their tunable physicochemical properties and solubilization performance. These ILs have been evaluated in combination with enzyme cocktails derived from six fungal species: the thermophilic *Thermomyces lanuginosus*, *Thermoascus aurantiacus*, *Myceliophthora thermophila*, and *Thermothelomyces guttulatus*; and the mesophilic *Trametes*

versicolor and *Aspergillus oryzae*. These fungi were cultivated on wheat straw, maize straw, and ryegrass, and enzyme induction curves were established to identify peak activity days for harvesting optimal enzymatic pools. The following synthetic substrates were used to assess enzymatic activities: microcrystalline cellulose, carboxymethylcellulose, beechwood xylan, locust bean gum mannan, and citrus pectin. Distinct induction profiles were observed across the fungi tested. Notably, *M. thermophila* and *A. oryzae* reached high enzymatic activities within 3 days, while *T. lanuginosa* and *T. versicolor* exhibited a slower response, requiring longer incubation periods to reach peak activity. These time points guided subsequent sampling, which led to the identification of enzyme pools with high tolerance to ionic liquids at 50/ °C. Several of these enzymes maintained substantial hydrolytic activity in the presence of 5% (v/v) ILs, particularly [Chol]OAc. These findings highlight the potential for integrating enzymatic and chemical pretreatment into a simplified single-vessel process, offering a resource-efficient strategy for biomass decomposition in closed-loop life support systems. This approach holds also vast potential for the production of fermentable sugars and other value-added products that can be used in terrestrial applications.

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HRISTO NAJDENSKI

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Microbial Degradation of Cellulose Containing Waste a Key Process in Life Support System at Earth and Long Term Space Missions

Valorising various organic wastes onboard the manned spacecraft is an actual problem of modern and future interplanetary and other long-duration space missions. Therefore, aims of our study were to achieve maximum cellulose biodegradation in laboratory conditions and useful laboratory model with potential for implementation in the waste management and environmental protection, which would assist the implementation of Compartment I of MELISSA project. Bacterial consortia and individual strains isolated from partly destroyed wood (PDW), goat feces (GF) and bioreactor (BR) were evaluated for cellulose degradation capacity by using toilet or filter paper as cellulose substrates. The cellulose biodegradation of mesophilic (37 °C) bacterial population from methanogenic BR, GF and PDW varied from 65% to 74% at days 9 and 21 under aerobic, microaerophilic and anaerobic cultivation. The metagenomic profiling of the bacterial consortium from BR showed the highest presence of species from the genera Clostridium, Bacteroides, Ruminiclostridium. Others genera like

Lysinibacillus, Bacillus, Achromobacter, Dendrosporobacter, Ruminiclostridium, Acetanaerobacterium, Alkalitalea and Treponema were detected in the PDW and GF consortia. Further, six anaerobic strains with proved cellulolytic activity were isolated and their species identification was done by 16S rDNA sequencing and MALDI-TOF. Studies on the microgravity effect reveal the highest values of cellulose biodegradation in the simulated microgravity conditions at 2D clinostat (10⁻⁶ g) with GF consortium. By scanning electron microscopy bacterial diversity, ultrastructural changes, adhesion to the cellulose matrix and its decomposition are demonstrated, both in samples cultivated under the Earth's gravity conditions and simulated microgravity at 2D clinostat. Under the simulated microgravity condition at 3D Random Positioning Machine (10⁻³ g) a slight decrease in cellulolytic activity is confirmed by the expression of the *exgS* gene and could be due to the lack of highly sensitive structures and organelles in bacterial cells which to accept and respond to various environmental factors.

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LYDIA ONG

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Perception of green juice under simulated immersive Earth and space environments for the design of palatable space compatible beverages

Water spinach (*Ipomoea aquatica*) is a semi aquatic plant with favorable nutritional properties that can provide fiber, phytochemical compounds, minerals, vitamins, antioxidants and all essential amino acids. Due to its fast growth and nutritional properties, water spinach is considered a potential candidate for growth in vertical farms to enable deep space exploration. Long-term missions to the Moon and Mars planned in the next twenty years create a need for reliable space farming and in situ food production, as astronauts cannot rely on re-supply. The feasibility of producing minimally processed foods with different flavors from water spinach, however, is not known. The sensory perception of such foods in space is also unknown and the extent to which sensory properties change between Earth and space environments unclear, yet this information could help to inform product research and processing routes selected for development. This study seeks to address these questions by producing water spinach juice with differing flavor and assessing their sensory perception using immersive environments that simulate space or Earth, providing insight into the potential use of water spinach in space diets. Water spinach juice was produced by twin-screw cold pressing and process variables and formulations systematically altered to provide different flavored juice products. Sensory perception and biometric responses to the juice were measured using a simulated microgravity seating position and effect of sweetness and acidity on panel responses assessed. Twelve trained panelists each assessed 10 water spinach juice samples using a Quantitative Descriptive Analysis (QDA[®]) with a non-structured

scale displayed in BioSensory[®] application. This application also captured the biometric responses of the participants, including the emotional and physiological responses. The juice samples were palatable and aroma and taste descriptors selected by the panel included terms commonly used as descriptors for commercial fruit and vegetable juices, such as grassy green, banana, cucumber, green apple or mushroom, depending on the processing and formulation variables employed. Principle component analysis showed how differences in flavor, due to sugar and citric acid or heat treatment could be detected in each of the simulated environments. Importantly, the perception of juice differed between the immersive space and Earth environments. Bitterness perception was significantly lowered in the simulated space environment, together with the perceived intensity of aromas and mouthfeel. The positive interaction between sugar and citric acid was particularly enhanced in the space setting, as well as physiological responses, such as heart rate and diastolic pressure. This study shows that water spinach can be successfully used to produce palatable juices with tailored flavors. The reduction in bitterness perception in simulated space environment may assist the development of plant-based diets, as bitterness is common in plant-based foods. Other changes in flavor intensity and changes of physiological response may require both consideration and optimization during product development. Overall, insights into the perception of green juice in simulated space conditions will help in the design of palatable beverages for astronauts to combat menu fatigue during long-term space missions.

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SONIA PALOMO

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Málaga TechPark: A Unique Innovation Ecosystem Accelerating Sustainability

Málaga TechPark, founded in 1992 as a public-private initiative, has become one of Spain's leading hubs for innovation and technology, with more than 700 companies, 28,000 professionals, and a contribution of 22% to Málaga's GDP. With a strong international profile, the park hosts over 70 multinational firms representing 23 nationalities, creating a dynamic environment for business and research collaboration. Its alliance with the University of Málaga strengthens talent generation and research capacity, with joint laboratories, research centers, and innovation initiatives that position the park as a reference point in Southern Europe.

The park's growth strategy includes expanding infrastructure to host 1,500 new companies and 40,000 professionals, while

developing strategic hubs such as The Green Ray, Soho CaixaBank Theatre, and the Port of Málaga. Málaga TechPark also serves as a facilitator of international working groups in sustainability, circular economy, and bioeconomy, actively engaging with global networks like IASP. Flagship projects such as eCityMálaga, supported by major partners, aim to transform the park into a sustainable and smart city model by 2027, anticipating global climate targets.

Additionally, initiatives like AgriFood4Future and Agrobotics-DiTwins highlight the park's commitment to digital transformation, sustainability, and innovation across key industries

ANTONIO PANNICO

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Enhancing performance, stability, and resilience of lunar Bioregenerative Life Support Systems through intercropping strategies

Prolonged human presence on the Moon will constitute a historic milestone and will be crucial for validating the technologies and operations needed to undertake longer and more distant exploration missions. The Moon is particularly well suited as an experimental laboratory due to its proximity to Earth, allowing testing and preparation of missions aimed at Mars colonization and exploration of more remote parts of our Solar System. The sustainability of such missions depends critically on the development of highly efficient Bioregenerative Life Support Systems (BLSSs). One of the challenges in using BLSSs is identifying operational conditions that optimize mass and energy flows, maximizing resource-use efficiency while minimizing the imbalances in consumption and production rates of various resources across different compartments. Plants play a central role in life support systems because they provide oxygen through photosynthesis, purify water via transpiration, recycle waste products through mineral nutrition, and supply food for the crew. Currently, the predominant approach in plant compartments for BLSSs consists of cultivating single-crop systems under optimal environmental parameters for the selected species, thereby simplifying system management and ensuring maximum productivity. However, in the context of long-term space activities, the plant compartment should serve not only for intensive food production but also as a vital buffer within the broader BLSS ecosystem, similar to terrestrial biosphere. In this respect, ecological research has demonstrated that stability increases with biodiversity; therefore, single-crop plant compartments are likely to exhibit significantly lower adaptability and resilience to perturbations compared to multi-crop systems. While simplified systems remain a rational solution for short-term space missions, long-term planetary missions, involving permanent bases with fully enclosed BLSSs, will require more complex ecosystems, analogous to terrestrial agricultural systems, which offer higher stability and resilience. In this context, as part of the 'Plant Biology and Artificial Intelligence for Bio-Regenerative Life Support on the Moon'

(BIOLUNA) project, funded by the Italian Space Agency (ASI), a growth chamber experiment involving the simultaneous cultivation of multiple crops in association (intercropping) was conducted at the Department of Agricultural Sciences, University of Naples Federico II (Italy). The experiment consisted of a closed-loop hydroponic cultivation of a salad crop (lettuce, *Lactuca sativa* L.) and two staple crops (broad bean, *Vicia faba* L.; rice, *Oryza sativa* L.) tested in two different configurations: (i) independent nutrient recirculation per species, and (ii) a shared nutrient solution among species.

Throughout the experiment, the recirculating nutrient solution was never renewed; its electrical conductivity (maintained at 1.6 dS m⁻¹) and pH (kept at 5.5) were regulated constantly via four independent fertigation systems (three dedicated to monocultures and one to intercropping). The trial lasted four months, involving one growth cycle of rice and broad bean (from transplanting to seed production) and four consecutive cycles of lettuce. Morpho-physiological measurements were conducted during the growth cycle, and biometric and qualitative analyses were performed on harvested crops. Preliminary results showed significantly higher edible biomass in the shared hydroponic system compared to the independent system for all species (lettuce +14%, broad bean +62%, rice +310%). Similarly, all three crops cultivated in the shared nutrient solution exhibited significantly higher photosynthetic rates (lettuce +54%, broad bean +17%, rice +110%) compared to those grown independently. These findings demonstrate that intercropping using a shared nutrient solution significantly improves overall crop performance, enhances resource-use efficiency, prolongs nutrient solution utilization, and consequently reduces waste production. The experimental data obtained will contribute to developing models for accurate estimation of mass and energy balances in advanced multi-crop systems, which are essential for optimizing the integration of different BLSS compartments.

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MARTIN PERSSON

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Power-2-Food using Green Acetate and Microorganisms

Novonosis was founded when the two Danish companies Novozymes and Christian Hansen were merged in 2024. Novozymes in its turn was formed after a spin out of the Enzyme Business from Novo Nordisk in 2000. Novonosis has a more than 70-year history in the production of industrial enzymes using microbial expression. Initially, this was enabled by classical strain development approaches combined with fermentation and downstream process development.

With the emergence of recombinant DNA technology, gene technology was added to the classical strain development toolbox and Molecular biology methods were developed to utilize some of the production strains already used in production for recombinant expression. Novonosis launched a lipase product produced by a recombinant *Aspergillus oryzae* strain and we have been improving the *Aspergillus* cell factories for protein production since that time. All available technologies including classical mutagenesis, adaptive evolution, metabolic engineering, optimization of promoters, signal peptides, DNA integration methods etc. have been applied. Strain development has focused on improvement of titer (g product/l fermentation broth), yield (g product/g carbon source) and productivity (g product/l fermentation broth/hr fermentation time) to secure production economy for the fermentation industry.

All of Novonosis expression systems are currently based on and optimized for growth and production on carbohydrate substrates such as glucose, maltodextrin or starch.

Power-to-X (P2X) is a potentially disrupting concept for the production of chemicals and fuels by utilizing excess power from e.g., solar and wind. In a first step, hydrogen is produced by

electrolysis of water, and later that hydrogen can be used to produce e.g. methanol and ammonia that can be used to fuel the shipping industry. Methanol can also be used in biological processes and a *Pichia* system that can grow and produce protein on methanol has been optimized by others¹.

However, utilizing C1 compounds (formic acid and methanol) as carbon source for microbial fermentation poses a significant biological challenge. As P2X methods for producing C2 compounds (ethanol and acetic acid) from CO₂ and hydrogen are also available and have been recently optimized, we hypothesized that it would be more feasible to develop *Aspergillus* expression systems based on these carbon sources rather than the C1 carbon sources.

For the last two years, Novonosis has been part of the "Acetate Consortium" funded by the Gates Foundation and the Novo Nordisk Foundation. Focus for this consortium is P-2-Food, more specifically, producing green acetate using P2X and converting that to protein using microorganisms. Green ammonia, produced using P2X, can act as a nitrogen source, as well as oxygen from the electrolysis of water can act as a terminal electron acceptor in the respiratory chain. At Novonosis, two tracks are being pursued. The first is to develop *Aspergillus* cell factories to produce precision proteins and approaches towards adapting *Aspergillus oryzae* and *Aspergillus niger* to grow on acetic acid and produce protein. Secondly, we are screening microbes that, using acetic acid as carbon source, can grow to high biomass concentrations with a high protein content and therefore could be used as single cell proteins.

CO-AUTHOR :Domen Zavec, Christina Troyer, Daniel Maresch, Friedrich Altmann, Stephan Hann, Brigitte Gasser, Diethard Mattanovich, Beyond alcohol oxidase: the methylotrophic yeast *Komagataella phaffii* utilizes methanol also with its native alcohol dehydrogenase *Adh2*, FEMS Yeast Research, Volume 21, Issue 2, March 2021, foab009, <https://doi.org/10.1093/femsyr/foab009>

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A Quantitative water system model for local decision making and circularity : the Caux Seine territory case study.

The MELISSA approach applies deterministic modeling to multiphase circular systems, with over 30 years of experience in designing predictive, closed-loop life support models. Originally space-oriented, it now shows strong potential for terrestrial applications requiring system-wide resource circularity. Local territories face long-term challenges in managing water sustainability, but often lack integrated, system-wide decision-making tools and solutions. Despite growing concern over water circularity, interest remains heterogeneous. We present a static, territory-scale water system model combining functional and material flow analysis, applied to the Caux Seine region (France), a coastal and river-adjacent area with industrial, domestic and sanitation water networks. The model maps average daily flows, stocks, and infrastructure capacities, using data from local

authorities, research, and extrapolations over the 2020s. Its boundary includes the territory, atmosphere, and soil. It displays water flows and storage, leveraging physics balance throughout the territory and its sub-systems. As a result, this project provides local authorities with a plug-in tool, where flows, infrastructure capacities and KPIs displayed allow for an operational diagnostic and support better circularity design at the local scale. Sanity checks confirm its initial validity and this generic tool is adaptable to other territories. For future developments, we look next to i) extending validation, ii) improving spatial, temporal and data granularities, iii) developing a dynamic model, iv) aggregating external drivers in scenarios, and input them into the model to identify criticalities and enhance circularity.

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GIORGIA PONTETTI

CEO - G & A Engineering S.r.l.

SELENE

We have grown in hydroponic technology inside a 'ground demonstrator' greenhouse of a space greenhouse. We have grown both using an inert vegetal substrate, and grown using homologues of lunar regolith (highlands and seas). We have used the same methods and the same cultivation recipe that we

typically use for the CEA cultivation of microgreens. We have successfully grown 5 consecutive times microgreens of turnip tops. The samples taken will be analyzed by the University of Camerino for edibility checks.

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GIORGIA PONTETTI

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Lunar Rapa

This internal research project explores the feasibility of cultivating turnip greens (*Brassica rapa* subsp. *rapa*) on two types of simulated lunar regolith Mare (Sea) and Highland as part of a broader investigation into onboard food production, preparation, and in-situ resource utilization (ISRU) for long-duration lunar missions. The study examines early plant responses, growth characteristics, and water management strategies under hydroponic conditions using both regolith simulants as organic substrates. Our results demonstrate that both types of regolith can support plant growth, though they exhibit distinct physical properties affecting hydration dynamics

and overall biomass development, nor plant antioxidant and polyphenol contents. In addition to cultivation, the project assesses food preparation techniques using both fresh and dehydrated greens with a 3D food printer, aiming to develop nutraceutical-rich foods tailored for astronaut health and performance. The outcomes underscore the potential of simulated regolith for supporting sustainable agriculture on the Moon and highlight the importance of substrate-specific management in future extraterrestrial crop planning, as well as the possibilities offered by combining hydroponic growing and 3D printing of personalized food.

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LUCIE POULET

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Design options for a lunar greenhouse module using the SERENITY methodology Food production is one central function of bioregenerative life-support systems for

distant and long-duration space exploration missions. Current food production systems have been designed based on spacecraft volume limitations and fail to adequately meet the challenges of sustaining astronauts with food in space. On the other hand, oversized engineering concepts for planetary missions fail to consider specific environmental constraints and mission scenario specificities. To address this, the SERENITY methodology was developed to allow efficient design of space greenhouse modules.

Based on multi-criteria optimization, users can generate and compare different design solutions, with constraints from the mission scenario (i.e., location, duration, crew size, nutritional targets, available utilities) and requirements on the system's mass, energy, crew time, efficiency, reliability, sustainability, and risk for humans. These solutions are evaluated using the multi-objective optimization platform for energy systems, OSMOSE (Optimisation Multi-Objectifs de Systèmes Énergétiques) developed at the laboratory of Industrial Processes and Energy Systems Engineering (IPESE) at the École Polytechnique Fédérale de Lausanne (EPFL). To illustrate this methodology, a case study

of a greenhouse module on the Lunar Shackleton Ridge is presented. The Life-Support Baseline Values and Assumptions Document (BVAD) is used for the plant database and for assumptions on human caloric requirements and mass of the different elements.

The solution space of plant combinations is explored using Sobol sequences and optimized design are proposed for key subsystems, with the objective of reducing the overall system mass. Not only does the total caloric requirement influence the system's dimensions, mass and energy requirements, but the nutritional composition of the diet also plays a crucial role. This type of approach will be necessary for serious planning of local food production in space missions. This methodology holds the potential to unlock efficient systematic design for greenhouse modules for space, and it could be expanded to other life-support system components. This project has received funding from the European Union's Horizon Europe research and innovation programme under the Marie Skłodowska-Curie grant agreement N° 101067017.

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SEBASTIÀ PUIG

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Microbial electrochemistry for wastewater remediation on Earth and in space

The persistence of pollutants in wastewater is often linked to limitations in electron transfer, where the absence of suitable donors or acceptors constrains microbial metabolism and slows biodegradation. Conventional treatment technologies struggle to address this bottleneck, particularly for contaminants such as nitrate and microplastics, which pose serious ecological and health risks. Microbial electrochemical technologies (METs) represent a promising alternative by directly regulating electron flow, thereby enhancing pollutant removal while reducing energy demand and reliance on chemical additives. Electro-bioremediation thus provides a flexible and decentralized approach to wastewater treatment, complementing or replacing

conventional infrastructure in settings where it is unavailable, impractical, or energy-intensive.

Beyond terrestrial applications, wastewater management is also a cornerstone of life support systems in space. Within the MELISSA loop, bioelectrochemistry enables controlled removal of contaminants via current-driven processes, contributing to resource recovery and closed-loop water management essential for long-duration missions. Recent studies at laboratory and pilot scales demonstrate the applicability of METs for treating priority pollutants in wastewater, highlighting their potential as sustainable technologies that support both terrestrial needs and the unique challenges of space exploration.

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KORNEEL RABAEY

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Removal of organic acids for life support systems in space using a synthetic microbial community in a microbial electrolysis cell

To enable astronauts to survive in space, a life support system is essential to maintain good air quality, provide potable water and food, and deal with wastes, be they solid, liquid or gaseous. As missions of increased duration are envisaged for the future, the life support system needs to become increasingly efficient and consider all waste as a resource to be recycled. The European Space Agency has developed the so-called MELISSA loop to create almost full circularity. In this loop, the fecal matter, food residues and other (semi)solid wastes such as unused plant material are first fermented to liquefy the waste while also unlocking already some 15% of the carbon as CO₂. The filtrate from this stage contains some 10 g/L of unused organics, mainly organic acids.

We have developed in the past 10 years a microbial electrolysis cell (MEC) able to treat this stream by oxidizing the organics at the anode while creating needed alkalinity at the cathode. In the initial project, we observed that while removing the organic acids well (>90% overall), the microbial community fluctuated over time giving rise to, e.g., unwanted methanogenesis. Given the need for control, a second generation system was designed (breadboard) in which the MEC can be sterilized via steaming in place (SIP) and in which the fermenter effluent is brought after membrane separation to be treated by a synthetic community. The synthetic community was selected based on the need to remove all organic acids from C₁ to C₆, and consisted of 6 species including *Geobacter* and *Shewanella* species along with a facultative aerobe (in part to scavenge oxygen) and syntrophs. In a laboratory set-up (100 cm² anode) the community removed all organic acids tested albeit at different extents, from synthetic

and real feeds. Subsequently, the community was used to inoculate the breadboard system which was operated with a PVDF membrane separating the two anodes from the cathode. This configuration allowed influent flow to the anode with fluid passing through the membrane towards the cathode where OH⁻ production was targeted along with H₂. The pH was controlled in the anode compartment by transfer of part of the basic cathode effluent. After steam sterilization and verification, the system was initially fed with a batch of fermenter effluent. Upon the emergence of microbially-induced electrical current the system was switched to continuous mode with increasing loadings for an operation over several months. Whereas initial organic acids removal followed supply quite well, in time organic acids accumulated. Analysis of the microbial community revealed that over time the synthetic community could not be maintained and re-inoculation to boost the target community could not lead to higher performance. Interestingly, whereas the cathode was steam sterilized and separated from the anode by a PVDF membrane not allowing any microbial transfer, over time a community developed dominated by *Sporomusa* sp. (which was not part of the inoculum) which caused acetate concentrations in the cathode to decrease. This finding was not only surprising, but also shows the resilience that even such strict anaerobes can exhibit. Even though we were able to show that a synthetic community can remove all organic acids from complex waste, a main conclusion is that even with all precautions maintaining a synthetic, defined community over longer time durations is likely not realistic.

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JONATHAN RAECKE

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Advanced Control as a Key to Efficient Multitrophic Food Production From Earth to Space

Multitrophic food production systems integrate species from different trophic levels into a single, interlinked operation. In space they pave the way for closing the loop of BLSS. On Earth, they can contribute to the circular bio-economy by valorizing waste streams on-site such as biomass residues, CO₂ emissions, heat or waste water. Despite their potential, there is limited operational knowledge on how to effectively manage the resource flow in such complex systems to fully exploit their inherent synergies. To address this, we developed a mechanistic model (ordinary differential equations) of a coupled production system involving plants, fish and insects. The model comprises three production systems and the interconnection system that governs the flow of resources between them. Furthermore, we implemented optimal control for the interconnection system, aiming to minimize external inputs of resources and energy,

while ensuring each module's resource demands are met. We then analyzed resource utilization across several scenarios, varying the degree of system knowledge available to each production module. The results revealed substantial differences in the efficiency of waste stream usage. Most notably, venting process air from insect and fish modules into the plant module had outcomes ranging from negative to highly beneficial, depending on the level of cross-system information integration. These findings underscore the importance of incorporating model-based system knowledge not only into the control of the central interconnection system but also into the local control strategies of individual production units. This approach is essential to unlocking the full synergistic potential of multitrophic food production systems.

CÉCILE RENAUD

PhD Student - Umons

Effect of biostimulation from *Limnospira indica* on microbiome modulation and plant resilience.

Abiotic stress, threatens food security under challenging environmental conditions, such as Space. Cyanobacteria-based biostimulants, such as *Limnospira indica*, have emerged as promising tools for enhancing plant resilience. This study investigates how *L. indica*-based biostimulants influence *Solanum lycopersicum* performance under hydric stress and their impact on the rhizosphere microbiome. This research is conducted within the framework of ESA's MELiSSA (Micro-Ecological Life Support System Alternative) project, which aims to develop regenerative life support systems for long-term space missions. We aimed to evaluate the physiological responses of tomato plants treated with *L. indica* under normal and under hydric stress conditions, characterise the influence of *L. indica* on plant-microbe interactions, and analyse shifts in the rhizospheric/endospheric microbial community induced by the biostimulant treatment. Physiological data revealed that biostimulant-treated plants exhibited improved flower

production and a better resistance in case of hydric stress. Genomic analysis highlighted key plant growth promoting bacteria development in presence of *L. indica*-based biostimulants which may contribute to plant growth improvement and better stress tolerance. Our findings demonstrate that *L. indica*-based biostimulants increased the number of flower produced by *S. lycopersicum* and confer a hydric stress resilience. This study provides novel insights into the functional role of *L. indica* polysaccharides in plant-microbe interactions, supporting its potential application in sustainable agriculture and controlled-environment for space missions. The integration of these results into the MELiSSA framework highlights the feasibility of using cyanobacteria-based biostimulants in closed-loop ecosystems designed for long-duration space travel while cyanobacteria are also present for their food and oxygen supply to the station.

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DR. EVA REYNAERT

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Risk-based treatment and monitoring of on-site water reuse on Earth

Water is a limited resource during space missions, making recycling essential. However, water reuse is also becoming increasingly important on Earth, as traditional water sources are facing growing pressure. This has prompted many countries and regions to turn to water reuse as a strategy to address decreasing availability and rising demand. Among the various approaches, on-site water reuse, e.g., at the scale of individual households or buildings, offers an opportunity to supply reliable quantities of water for non-potable applications without the need for large-scale centralized infrastructure to collect wastewater or

distribute reclaimed water.

However, the widespread implementation of on-site water reuse is currently impeded by the difficulty of safeguarding human health through appropriate design and long-term operation of such systems. Experience with centralized water reuse is not directly applicable to on-site systems, as pathogen dynamics and water usage patterns in on-site systems differ fundamentally from those in centralized systems. In particular, on-site systems are characterized by intermittent pathogen occurrence and intermittent use of reclaimed water. Especially

small systems, for instance at the scale of individual households, experience low pathogen occurrences throughout the year, but can have high concentrations when pathogens are present. These differences have important implications for risk-based treatment and monitoring. In my presentation, I will discuss recent advances in quantitative microbial risk assessment that support the development of fit-for-purpose water quality targets and monitoring requirements

for on-site water reuse. I will also explore innovative operational strategies and real-time monitoring approaches, including the use of online sensors, to address these challenges. Finally, I will extend the discussion to applications in Space, examining how these findings can inform water reuse systems in Space applications and highlighting the key differences between Earth-based and Space-based water reuse needs.

MICHEL RIECHMANN

Project Coordinator Eawag / Co-founder and CEO Ogmo - Eawag / Ogmo

Technology transfer of the Nutrient Harvester: from research to an on-site urine resource recovery product

Non-sewered sanitation systems on Earth and sanitation systems for space applications will have to be small, robust and, at least on Earth, cheap. To achieve these goals, these technologies must be developed in an industry logic, which is very different to the civil engineering logic used for current centralized wastewater treatment plants. At the Swiss Federal Institute for Aquatic Science and Technology Eawag, we are using such approaches to develop a modular unit for urine treatment, the 'Nutrient Harvester'. The module is installed near the user interface, e.g. a urinal or urine-separating toilet and processes the urine on site, producing clean water and a solid, nutrient-rich fertilizer that contains urea-nitrogen, phosphorus and potassium. During the research phase, we continuously engaged with a several industrial partners to facilitate the technology transfer from research into practice after the successful process development.

However, we entered a phase where the applied process development was reaching its limits at the research institute Eawag but had not yet reached a technology readiness level (TRL) attractive for industry adoption highlighting the so-called valley of death in technology transfer. To bridge this gap between TRL 6 and 7, we established a spin-of company, offering the flexibility to explore new business models that do not need to be optimized for immediate maximum profitability. Through

strategical partnerships, a supply chain was established and a range of potential primary target markets explored. A market analysis considering demand, financial viability, and entry barriers guided the focus on few specific niche markets in Central Europe. A market entry strategy was developed based on a minimal viable product and subsequent continuous improvement following market feedback. The spin-of's key offer was to depart from the conventional path of project-based time- and resource-intensive planning framework and deliver prefabricated technological units early on. Due to its modular design, this appliance-type technology still offers the possibility for customization to different use cases.

Accordingly, emphasis was placed on design for manufacturing and assembly, with scalability achieved through increased unit numbers rather than system size. By calculating the associated cost reduction potential broken down to single product parts, we were able to estimate the pricing at various production capacities. Once economies of scale reduce the production costs, we will be able to return to our initial target market, people in low- and middle-income countries lacking sanitation. This will further unlock new business opportunities through resource-oriented sanitation infrastructure that generates value from waste.

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MICHEL RIECHMANN

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On-site resource recovery from urine with zero-waste discharge: Challenges on the way from a process to a product

In space as well as in terrestrial applications urine not only presents a challenge but also an opportunity. It mainly consists of water and is thus of high value in space. In addition, urine contains most of the excreted nitrogen (N), phosphorus (P), and potassium (K).

Nutrients crucial for sustaining circular food systems, particularly during long-term space missions or stations on Moon and Mars. In terrestrial applications, urine contributes less than 1% to the volume of urban wastewater, but contributes around 70% of its nutrient load, in this context with a polluting effect. At the Swiss Federal Institute for Aquatic Science and Technology Eawag, we have developed a process that tackles these challenges with a zero-waste producing approach. In proximity to the toilet our modular designed treatment unit Nutrient Harvester processes the urine on site and produces clean water and a solid, nutrient-rich fertilizer. Through stabilization of the fresh urine, pathogens

are inactivated, and nitrogen is preserved in its organic urea form. This process has two major advantages: (1) it minimizes the formation and subsequent loss of volatile ammonia, and (2) promotes a lower-emission fertilizer component compared to direct ammonia fertilizer. After stabilization, forced ambient air evaporation reduces the water content of the solution by 95%, leaving a concentrate containing all major nutrients such as N, P and K but also several micronutrients and trace elements essential for plant growth. A mass balance over the nutrient flows of the 4th version of the Nutrient Harvester showed recovery rates of 97% N and 100% for P and K in a multi-nutrient. In a subsequent step, the water is recovered from the air stream by a dehumidification unit. This allows a completely closed air circulation within the treatment unit, creating flexibility and independence from any exhaust pipes. By encapsulating the air flow, the system also provides a barrier for odor emissions.

Finally, a heat pump uses the excess heat that is produced during dehumidification to reheat the incoming air of the evaporation reactor, making the process more energy efficient. Tests showed that a small fraction of ammonia in the air can be found in the condensation water. This amount corresponds to

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the around 5% nitrogen, which is excreted with urine as ammonia. The ammonia found in the condensate can be stripped in a membrane contactor. To ensure a high fertilizer quality, the nutrient concentrate is further dried and granulated in a last process step.

LUKE ROBERSON

Principal Investigator for Flight Research - NASA

BLiSS for Surface System Habitats

Closed Bioregenerative Life Support Systems (BLiSS) enable sustainable life support for lunar and Martian surface habitats. The regenerative ISS Environmental Control and Life Support System (ECLSS) supports short duration (30-day) missions and represents a survival stage of life support requiring frequent resupply that is not viable for long- duration exploration missions. The ISS on-orbit ECLS can be modified in stages of maturity to achieve increasing degrees of system closure and sustainability. Currently, the ISS ECLS is being modified at KSC to include bioreactors to process urine, hygiene/laundry water,

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metabolic waste slurries (i.e., feces, food waste, etc.), to produce potable water and recover essential resources (i.e., fertilizer, CH₄, CO₂, and N₂). Although further stages of closure are needed for implementing a fully closed BLiSS, these ground-based tests are a first step towards integrating physical-chemical and biological technologies for demonstrating sustainable life support. This effort will be useful for characterizing the quality and production rates of recycled feedstocks from these bioreactors, as well as for developing novel integrated systems that can be used in Earth-based circular economies.

ÁLVARO ROPERO LÓPEZ

Bioinformatician - The Spring Institute for Forest on the Moon

DRAFT: Dynamic Regolith Air Filtration Technology

One of the critical challenges in environmental control and life support systems is the effective removal of volatile organic compounds (VOCs) from a habitat's atmosphere. State of the art air filtration systems, like the Trace Contaminant Control System (TCCS) on the International Space Station, are effective in removing a limited number of potentially harmful gasses, but do not address the majority of the broad spectrum of potential VOCs produced by human activities. Moreover, this system relies on resupplies of consumables including chemical reagents and filter matrices that cannot be easily fabricated in-situ. This study explored a bioregenerative alternative technology, soil air filters,

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which have previously been used to great effect for VOC removal in closed environments such as Biosphere 2. This experiment builds on that work by comparing conventional soil air filters with bioremediated lunar regolith simulant-derived soil air filters, reducing the need for resupplies and facilitating self-sustaining lunar settlements by adopting an ISRU approach. Data was collected over the two week ASCLEPIOS V analog astronaut mission in Switzerland, demonstrating the effectiveness of regolith-based soil air filters in a realistic environment.

CARLA RUIZ GONZÁLEZ

PhD student - The University of Edinburgh and The Scottish Association for Marine Science

Snow Algae Plasticity and Metabolic Shifts Under Simulated Lunar Light Cycles and Gravity Conditions: Implications for Biological Life Support Systems

The development of Biological Life Support Systems (BLSS) is critical for sustaining long- term human space exploration. Microalgae, with their ability to produce oxygen, recycle carbon dioxide, and generate nutritious biomass, are integral to these systems. While most research has focused on model species like *Chlorella* and *Chlamydomonas*, the potential of extremophilic species, such as snow algae, remains underexplored. Snow algae thrive in harsh environments, such as Antarctica, where they endure prolonged light-dark cycles, subfreezing temperatures, and nutrient limitations. Their exceptional

metabolic plasticity and stress tolerance make them promising candidates for extraterrestrial applications. This study investigates the physiological and metabolic responses of two snow algae species; the psychrophilic *Limnomonas* sp. and the psychrotolerant *Chloromonas* sp., to simulated Lunar light cycles (14 Earth days of light followed by 14 Earth days of darkness) and to a 3D clinostat-simulated microgravity and partial gravity conditions. The goal is to assess their adaptability and suitability for cultivation in Lunar and Martian habitats under axenic and xenic conditions. Growth performance and

metabolic plasticity were assessed in both axenic and xenic cultures to address critical questions for space-based bioreactor systems: Can axenic cultures maintain long-term stability without contamination? Are xenic cultures more resilient due to microbial interactions, or are they vulnerable to microbial competition and pathogenic blooms? Key findings indicate that both species successfully adapted to the simulated Lunar light cycle, with notable differences in growth performance and metabolic strategies. *Chloromonas* sp. grew densest in xenic cultures, highlighting its reliance on microbial interactions, whereas *Limnomonas* sp. performed better axenically, indicating a more self-sufficient growth strategy. Interestingly, *Chloromonas* sp. exhibited greater overall growth during the Lunar cycle, though the *Limnomonas* sp. control group achieved the highest density. These results suggest that *Chloromonas* sp. benefits from its associated microbial community, while *Limnomonas* sp. demonstrates a more metabolically plastic response. Metabolomic analyses revealed significant differences between the isolates. *Limnomonas* sp. synthesized stress-

related metabolites such as D-Trehalose, D-(+)-Gluconolactone, and D-Allose, which aid in oxidative stress resistance and energy storage, enabling resilience during the intense and prolonged Lunar day. Both species also produced bioactive metabolites, including Xanthine, Uridine, and D-Myo-Inositol Phosphate, which contribute to cellular repair, membrane stabilization, and anti-inflammatory responses traits advantageous for withstanding harsh extraterrestrial conditions. Additionally, both species synthesized essential amino acids (e.g., L-Aspartic Acid, L-Ornithine, L-Threonine) and omega-3 fatty acids (\pm -Linolenic acid and Docosaehaenoic acid), highlighting their potential to support human nutrition in space. These findings demonstrate the remarkable plasticity of snow algae and their potential role in BLSS. By coupling light cycle simulations with clinostat experiments, this study provides insights into the adaptive mechanisms of snow algae under extraterrestrial conditions, offering critical information for designing sustainable life support systems for future Lunar and Martian habitats.

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ARNAUD RUNGE

ESA/ESTEC, the Netherlands

ESA BASS Programme Opportunities

PIERO SANTORO

CEO & Technical Director - MEG Science

Custom Light Engines for the PaCMan Plant Characterization Unit: a replicable design pathway for upgrading photobiological systems in space research

The Plant Characterization Unit (PCU) is a ground-based research facility located at the University of Naples Federico II, which enables data collection for modelling purposes and allows the MELISSA community to perform scientific experimentation on crops. The PCU was engineered, manufactured and tested by EnginSoft and other partners in the PaCMan (Plant Characterization unit for closed life support system engineering, Manufacturing & testing) - phase 1 project. The PaCMan project is currently in phase 3. In 2024, PaCMan underwent a major functional revamp coordinated by EnginSoft, who was tasked with upgrading several key subsystems. This presentation focuses on the design and supply of a new lighting system, capable of significantly improving the photobiological performance of the PCU while preserving its mechanical integrity and pre-existing control infrastructure. MEG Science was responsible for this upgrade, carried out through a structured and multidisciplinary design process focused on the development of fully custom Light Engines, from concept to validation. Particular emphasis was placed on achieving optimal integration between plant physiology needs, optoelectronic design, thermal management, and system-level constraints. The project began with a requirements definition phase, where the target performance envelope including spectral composition, photon flux density, uniformity, and thermal footprint was shaped through close interaction with EnginSoft and the PaCMan research team.

Their experimental needs guided the optoelectronic architecture design and emission strategy of the new lighting system. The core hardware consists of two modular optoelectronic units, each hosting two custom-designed PCBA boards, that support

multi-channel spectral emission and were engineered for continuous-use, stability and field-replaceable mechanical integration. A key role in this phase was played by dedicated ray-tracing optical simulations, which allowed precise modeling of beam geometry, minimization of shadowing effects, and accurate prediction of PPFD distribution across the growth chamber. The result is a drop-in light engine upgrade that drastically improves spectral quantum distribution accuracy, irradiance uniformity, and photon delivery efficiency, enabling more precise and reproducible plant growth experiments. Thermal and mechanical aspects were co-designed to ensure safe active dissipation without introducing turbulence or mechanical stress into the cultivation environment. The electrical interface was implemented with full compatibility to the existing communication protocols of the PaCMan system, avoiding disruptive changes to software or supervisory controls. Additionally, redundancy features were integrated both at the hardware and software level, ensuring experimental continuity even in the event of partial LED system faults an essential requirement for long-duration trials in sealed environments. This project demonstrates how a targeted retrofit, developed through a rigorous and collaborative design process, can significantly improve the equipment performances and satisfy specific needs of the researchers. MEG Science's contribution, while focused on a single subsystem, exemplifies a broader approach to photobiological system engineering that is both high-performance and replicable.

Ultimately, it reinforces the importance of lighting management as a foundational driver for biological performance and technological reliability.

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A computational study and biosafety assessment of a hybrid microbial fuel cell and compost heat recovery system for decentralized rural energy applications

The transition to circular and autonomous energy systems in rural or isolated areas requires innovative approaches that combine waste utilization with low-cost energy production. This study, based on the MELISSA Project and Europe's goals for eco-friendly systems, suggests a combined approach that uses microbial fuel cells (MFC) and compost to capture heat, with the goal of turning food and organic waste into electrical and thermal energy. A computational model is developed to simulate the electrochemical and thermal performance of the system using waste load data from real communities. The electrical performance of the MFC is determined by the type of material

used, the behavior of the microbes and the reactor design, while the heat generated by the compost decomposition is simulated to see how much it can help in heating the spaces. A parallel medical safety analysis is taken into account for the biohazards associated with exposure to microbial and pathogenic agents during waste management and system maintenance. The aim of this research is to demonstrate the possibility of daily electricity recovery and to offer a sustainable and decentralized solution for off-grid energy production with robust applications in climate-vulnerable communities or with limited infrastructure.

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PETER SCHEER

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Waterneutral and sewageless buildings

A water cycle system innovation has been implemented in the new, highly sustainable residential area of Heuvelstraat in the Dutch village Silvolde for 13 houses. The project aims to gain experience with local circular water systems and to collect data for optimising and scaling up the implemented systems for future projects. Partnering stakeholders include drinking water company Vitens, housing corporation Wonion, municipality Oude IJsselstreek, Water authority Rijn en IJssel and Nijhuis Saur Industries. The common interest of the partners is to develop new solutions to address increasing societal water challenges due to climate change and expected demographic developments. The circular approach consists of targeting rainwater as the sole source for producing drinking water quality for all household uses except toilet flush water.

Secondly, consumption of water with drinking quality is reduced by using 2nd quality water for toilet flushing. Thirdly, after use, water is purified and given to the local water system by soil

infiltration. To this purpose, drinking water is produced on residential area level from rainwater collected from roofs, with nanofiltration and UV as key barriers for contaminants. In addition, shower wastewater is purified on individual home level with the a grey water recycling system and used as toilet flushing water, reducing water consumption by as much as 30%. Collected sewage water is purified on residential area level with a membrane bioreactor, activated carbon and UV, and the purified water will be infiltrated into the soil. As this is an early innovation effort, back-up systems are in place (network drinking water connection, sewer connection). Legal aspects of the drinking water production and soil infiltration will be evaluated with stakeholders within and outside of the project. In the coming years, the water cycle approach in the Heuvelstraat will be closely monitored for water quantity and quality. Results for the first half year are presented.

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MONA SCHIEFLOE

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Lettuce cultivation in a urine-fertilizer scenario: exploring sodium tolerance and acclimation

Plant food production on long-term spaceflights or extraterrestrial bases require reliable access to water and nutrients. In this context, processed human urine represents a valuable resource that can provide both water and nutrients for crop cultivation. The LunarPlant project, funded by the Research Council of Norway, aims to study complex plant responses to nutrient sources made of human waste and develop infrastructure for sustainable hydroponic plant cultivation on a lunar base. A key objective is to increase fundamental knowledge on plant responses to human waste-derived fertilizers in hydroponic cultivation, as a step towards enhanced water and nutrient recycling in space-based agriculture. Additionally, the results provide valuable input toward a more advanced circular economy and sustainable food production practices on Earth.

Human urine-derived fertilizers present several challenges, including imbalanced element ratios, element deficiencies, and excess sodium, which is non-essential for most plants. In a closed-loop space life support system utilizing a recirculating urine-derived nutrient solution, sodium may accumulate over time, negatively affecting crops and yield. This presentation summarizes results from a series of deep-water culture experiments on lettuce (*Lactuca sativa* L. Frillice) grown in a controlled environment, investigating the effects of sodium chloride (NaCl) concentrations ranging from 0 to 240 mM. Plants were exposed to various NaCl concentrations at different growth stages, as well as gradually increasing exposure throughout the growth cycle. A standardized nutrient solution, differing only in NaCl content, and thereby EC, was used in four different

experiments with up to 12 treatments in each setup. Lettuce was grown from seeds to maturity over 5 weeks, with destructive sampling after three, four and five weeks, for characterization of biometrics and biomass properties throughout the growth cycles. The results include comparisons of biomass, relative growth rates, chlorophyll content, chlorophyll fluorescence and leaf proline content, as a stress response indicator. Concentrations up to 30 mM showed no significant effects on measured parameters, aside from a slight increase in biomass in some instances. A threshold for negative impacts on physiological parameters was identified between 40 and 80 mM NaCl. At 80 mM, plant growth was significantly reduced, with early-stage exposure resulting in more pronounced growth reduction than exposure at later growth stages. Concentrations of 160-240 mM NaCl severely affected plant growth, chlorophyll content, and reduced the operating- and maximum quantum

efficiencies of photosystem II. Gradual increases of NaCl concentrations up to 160 mM over several weeks showed positive effects with regards to final biomass and growth rates compared to abrupt exposures to high salinity levels, indicating the ability of the plants to acclimate over time. Exposure to NaCl concentrations exceeding 80 mM initially triggered significant increases in leaf proline content and reductions in both transpiration and growth rates, however these stress responses showed signs of acclimation after two weeks. The findings hold relevance for crop cultivation using recycling urine-derived nutrient solutions, where salt accumulation may pose challenges. In broader context, they enhance our understanding of plant responses and acclimation to NaCl and may serve as input to development of more resilient cultivation strategies for using urine-based nutrients, other saline waste streams or saline water, both in space systems and terrestrial agriculture.

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■ DYLAN SHUN IZUMA

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Development Status and Test Results of JAXA's Plant Growth Unit for Advanced Cultivation Experiments.

The development of plant cultivation technology within the closed environment of spacecraft is being pursued by various nations, with a focus on securing fresh food supplies, providing mental support for astronauts, and advancing future life support systems. The Japan Aerospace Exploration Agency (JAXA) has conducted plant biology experiments under microgravity (¼ G) conditions, significantly enhancing the understanding of fundamental plant biology, including morphogenesis, hydrotropic responses, and seed-to-seed life cycles. However, earlier experiments were constrained by limited cultivation volumes, short durations, and a narrow range of plant species, relying on pre-existing devices. While earlier research focused on cultivation model plants, future systems must address broader needs, such as supporting food production during interplanetary missions and accommodating diversified experimental requirements. Increasing the cultivation volume and optimizing environmental conditions is necessary for expanding the range of cultivable plant species. Additionally, achieving long-term cultivation capabilities is essential for sustained food production. Key challenges in optimizing the environment include achieving uniform temperature and humidity distribution in the absence of convection and ensuring efficient nutrient solution delivery to the plant roots. Additionally, water usage, algae and mold growth, and maintaining nutrient balance will be a major issue for long-term cultivation. To address these issues, JAXA has focused on three core challenges, temperature and humidity control, nutrient solution

management, and water resource efficiency. A breadboard model has been developed for subsystem-level evaluations. To secure large cultivation volume, the system is designed for deployment in the MPSR Rack's work volume or the EXPRESS Rack Double Mid-Deck Locker Size aboard the ISS. A prototype dehumidifier was developed to regulate temperature and humidity. Using a mechanism that cools metal slits with a Peltier device, this dehumidifier achieves both dehumidification and air cooling efficiently within a compact size. To ensure uniform temperature distribution without convection, air circulation ducts were incorporated, enabling parallel airflow and achieving stable environmental conditions within the unit. For nutrient solution management, a substrate box was developed to store and water the substrates. Even distribution of nutrient solutions across the substrate is a critical factor for successful cultivation. Accordingly, multiple substrate types were selected and evaluated for their plant cultivation potential and nutrient solution delivery performance. To minimize water usage, a prototype dehumidifier was also created to recycle transpired water from plants. Water condensed on cooled metal slits was absorbed into sponges and extracted using syringe needles, allowing water recovery without relying on gravity. This system is being prepared for validation aboard the ISS. After successful demonstration, interface designs will be developed to ensure compatibility with future commercial space stations. Detailed results from these evaluations and integrated subsystem tests will be presented during the session.

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■ MICHAEL T. FLYNN

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Integration of Biological Life Support Systems into Future Human Space Missions

The National Academies' 2023 NASA Decadal Survey recommends that NASA place greater emphasis on Bioregenerative Life Support Systems and in-space manufacturing. This landmark report highlights the critical scientific questions, research priorities, and ambitious initiatives needed to advance

human space exploration while deepening our understanding of the universe. Similarly, NASA's 2023 Space Sustainability Strategy identifies in-space biotechnology as an essential element for creating a sustainable future in space. This presentation will review past and ongoing efforts in biomimetic technologies,

biologically based life support systems, and bio-inspired mission architectures that align with these directives. Topics will include innovations such as living membranes, aerobic and anaerobic systems for water and waste treatment, bioregenerative food

production, and living habitat concepts. Together, these projects demonstrate how biology can be integrated across component, subsystem, and system levels to support future spaceflight mission architectures.

■ CATHERINE THANNIPPILLY ALEX

Food systems innovator - Infinite roots

Exploring the Efficacy of Bioregenerative Life Support Systems: Space-Derived Innovations for Climate-Resilient Agri-Food Systems on Earth

The paper Exploring the Efficacy of Bioregenerative Life Support Systems: Space-Derived Innovations for Climate-Resilient Agri-Food Systems on Earth aims to capture the essence of value creation avenues of space innovations for applications back on Earth. It undertakes a comprehensive analysis of space-based food systems drawing upon Bioregenerative life support systems studies. It applies key management concepts to create a synergistic integration connecting the domains of agriculture, space, technological innovation, value creation, and sustainability to catalyze cross-sectoral value creation benefits specifically targeting terrestrial system enhancement for advancing the United Nations 2030 Sustainable development

goals. The study employs qualitative research methodologies like stakeholder interviews, focus groups, and case studies to establish a triangulated methodological framework and generate key deliverables spanning from interview reflection, thematic analysis, and roadmap. Along this intricate process to identify state-of-the-art inventions with spin-of transferability for revitalizing climate-resilient agri-food systems, the research also delves into sub-themes pinpointing the role of eco-innovation, global collaboration, and open innovation in the era of Industry 5.0 innovations aiming to address global challenges like food security in the light of climate change.

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New Space Economy: Value Creation Avenues for the Agri-Food Industry

This paper explores how space-derived technologies, with a focus on Bioregenerative Life Support Systems (BLSS), can create, deliver, and capture value when transferred to terrestrial agri-food systems, positioning them as catalysts for multi-sectoral innovation within the New Space Economy (NSE). The research is guided by the core question: How can space technologies be seamlessly translated into Earth-based food systems to drive value across economic, environmental, and societal dimensions? Framed through the lens of innovation ecosystem theory and sustainable value creation, the study emphasizes the role of technological co-creation, stakeholder engagement, and system-level integration in fostering climate-resilient, resource-efficient food production. Employing qualitative methods including semi-structured interviews and focus group discussions with stakeholders from academia, space agencies, startups, and analogue astronaut

missions the study maps value creation mechanisms within real-world spin-of initiatives. Findings show that technologies originally designed for space, such as closed-loop agriculture, precision cultivation, and microbial protein systems, not only solve extraterrestrial challenges but also unlock high-impact terrestrial benefits. These include circular economy applications, enhanced food system resilience, and inclusive nutrition strategies. The research concludes that value in the NSE is generated not merely through innovation, but through its effective orchestration, where actor networks, policy frameworks, and societal demand converge to scale solutions. By embedding space spin-offs into Earth's agri-food ecosystems, this study positions space-derived technologies as enablers of systemic value creation aligned with the UN 2030 Sustainable Development Goals (SDGs), offering a blueprint for sustainable transformation beyond the space sector.

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■ LUCIE THIBAUD

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Adaptive laboratory evolution of cyanobacteria for perchlorate resistance in the context of Martian ISRU.

Anabaena sp. PCC 7938, a filamentous, lithotrophic, and diazotrophic cyanobacterium, has emerged as a promising organism for the development of biological life support systems (BLSS) on Mars. Its ability to grow on Martian regolith simulants, to fix atmospheric nitrogen and to assimilate atmospheric CO₂

positions it as a key player for the in situ production of oxygen, food, and biomass. However, Martian regolith also contains perchlorates (chaotropic, oxidative, and osmotically disruptive salts) which severely limit microbial growth and viability. To explore whether this tolerance can be improved and to gain

insight into the mechanisms mediating perchlorate resistance, we subjected three independent populations of *Anabaena* sp. to adaptive laboratory evolution (ALE) in BG110 medium supplemented with incrementally increasing concentrations of calcium perchlorate, from 15 mM to 55mM. Over three years, these populations have evolved under selective pressure, for more than 140 generations. The evolved strains exhibited enhanced growth compared to the wild type at low and intermediate concentrations (15 and 35 mM) and maintained survival at a high concentration (55 mM), inhibitory to non-evolved strains. These enhanced phenotypes were stable across

successive subcultures. To uncover the molecular basis of this adaptation, we performed a multi-omics analysis combining whole-proteome profiling, metabolomics, whole-genome sequencing, and microscopy. This study demonstrates that filamentous cyanobacteria can be evolved to tolerate extreme chemical stress.

Beyond uncovering stress response mechanisms, our study demonstrates the value of ALE as a tool for both exploring resistance pathways and adapting microbial strains to harsh cultivation conditions. This study contributes to the development of life support systems in the context of resource production on Mars.

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Watermeal as a resilient nutrient source for space farming: Omics-based insights into gravity-driven adaptation for closed life support systems

Future long-duration space missions require bioregenerative life support systems capable of producing fresh, nutrient-rich food in confined, resource-limited environments. *Wolffia globosa* (watermeal), the smallest known flowering plant, offers exceptional potential for such systems due to its rapid growth, minimal cultivation requirements, and high nutritional value. This study investigated the biochemical adaptation of Watermeal under prolonged hyper-gravity (20g) using a Large Diameter Centrifuge (LDC) at ESTEC, European Space Agency. By integrating high-resolution metabolomics, lipidomics, and proteomics, we characterized key metabolic adjustments associated with gravitational stress. Our findings revealed that hyper-gravity significantly enhanced the phenylpropanoid biosynthesis pathway, with increased accumulation of stress-related metabolites such as tyrosine and 2-hydroxycinnamic acid. Lipidomic analysis showed a marked increase in membrane-associated lipids, particularly saccharolipids and

phosphatidylethanolamine, suggesting membrane remodeling to maintain cellular integrity. Additionally, triacylglycerol accumulation indicated energy storage adjustments, potentially linked to oxidative stress defense mechanisms. Proteomic profiling highlighted the upregulation of calmodulin-related proteins, supporting the role of calcium signaling in gravity sensing and metabolic regulation. These results demonstrate that watermeal possesses robust biochemical flexibility, enabling it to adapt to extreme mechanical stress while maintaining metabolic function. The observed mechanisms are likely beneficial not only under hyper-gravity but also in low-gravity environments such as the Moon, Mars, or microgravity. This work supports the strategic inclusion of watermeal in closed-loop life support systems, contributing to sustainable food production, oxygen regeneration, and resource recycling for future space exploration.

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Benchmarking ALSSAT with ALiSSE: Aligning Life Support System Optimization with Ecosystem Efficiency and Closed-Loop Sustainability

The integration of In-Situ Resource Utilization (ISRU) data into NASA's Advanced Life Support Systems Assessment Tool (ALSSAT) is crucial for enhancing life support system (LSS) optimization for long-duration space missions. This paper explores the application of the ALiSSE criteria developed by the MELISSA project as a benchmarking framework to evaluate the effectiveness of these updates in ALSSAT. The ALiSSE criteria prioritize ecosystem efficiency, closed-loop sustainability, and resource recycling, which are essential for ensuring that life support systems can support sustained human presence on the Moon, Mars, or beyond. We propose a detailed methodology for using ALiSSE to evaluate ALSSAT's performance, focusing on how the tool can adapt to dynamic resource availability, optimize system mass, and enhance energy efficiency through

real-time ISRU data integration. This paper examines how specific ALiSSE metrics such as resource utilization rates, recycling efficiency, and system adaptability are applied to measure the success of ALSSAT's updates. Additionally, it discusses how the integration of ISRU data shifts the priorities of ALSSAT's optimization, with a focus on sustainability over the long term. By employing ALiSSE as a benchmark, this paper provides a framework for evaluating ALSSAT's alignment with sustainable life support system standards, offering insights into the prioritization of ecosystem efficiency and resource autonomy in the context of space exploration. The findings will help guide future efforts to develop robust, adaptable life support systems that can operate in closed-loop environments, ensuring the viability of human exploration and habitation in deep space.

CYPRIEN VERSEUX

Research Group Leader - ZARM, University of Bremen

Influence of atmospheric pressure, and of the partial pressures of carbon dioxide and dinitrogen, on the productivity and mass-efficiency of biological ISRU systems based on diazotrophic cyanobacteria.

While in situ resource utilization systems based on cyanobacteria could support the sustainability of crewed missions to Mars, their resource-efficiency will depend on the extent to which gases from the Martian atmosphere must be processed to support cyanobacterial growth. In the work discussed here, we investigated the impact of changes in atmospheric conditions on the photoautotrophic, diazotrophic growth of the cyanobacterium *Anabaena* sp. PCC 7938. Lowering atmospheric pressure from 1 bar down to 80 mbar, without changing the partial pressures of metabolizable gases, proved not to reduce growth rates. The partial pressures of carbon dioxide and

dinitrogen, on the other hand, modulated growth rates in a way which can be described with equations analogous to Monod's, for which we provide parameters. These equations can be used to determine the relationships between atmospheric pressure and composition, the minimal mass of a photobioreactor's outer walls (which is dependent on the inner-outer pressure difference), and growth rates. Relying on these relationships, we will quantitatively describe how the equivalent system mass of a cyanobacterium-based ISRU system on Mars can be lowered by optimizing atmospheric conditions.

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SIEGFRIED VLAEMINCK

Full professor - University of Antwerp

Nitrify for life: Sustainable solutions for Space and Earth

This presentation outlines the research and development trajectory, scientific rationale, key achievements, and future outlook of nitrification-based processes within the Micro-Ecological Life Support System Alternative (MELiSSA) developed by the European Space Agency (ESA). Also related terrestrial applications will be highlighted. Nitrification is a cornerstone of nitrogen (N) conversion in regenerative life support systems. Each crew member typically consumes 9.19 g of protein-N per day, excreted primarily in the form of urea in urine (7.16 g N/day). Nitrification-based processes are essential not only for the safe treatment of these waste streams but also for the production of valuable outputs: clean water, inert nitrogen gas (N_2), and nitrate for food production. The biological conversion begins with urea hydrolysis by ureolytic heterotrophic bacteria, generating ammonium (NH_4^+). Subsequent steps involve aerobic ammonia-oxidizing bacteria (AOB), and - depending on the target product - either nitrite-oxidizing bacteria (NOB) for nitrate production or anoxic ammonium-oxidizing or anammox bacteria (AnAOB) in partnership with AOB for nitrogen gas production via partial nitritation/anammox. In parallel, heterotrophs convert organic matter to CO_2 , preventing downstream biofouling and enabling carbon recovery. The overarching objective is to establish a reliable and predictable bioprocess under dynamic operational conditions, integrated with upstream and downstream treatment steps. For nitrate production, ground-based research and demonstration activities have spanned microbial physiology studies, bioreactor implementation, mathematical modelling, and control systems development. Functional nitrification has been demonstrated using both undefined microbial consortia and defined gnotobiotic communities, the latter enhancing safety and process predictability. For the synthetic communities, the latest updates on expanding the heterotrophic members will be shared, aiming to increase the conversion efficiency of the organics. The process was shown to enable conversion, refinery

and concentration of nutrients from urine, as well as downstream water recovery from combined urine, condensate and grey water streams. Technological progress is being made in aeration systems, transitioning from conventional gas sparging to gravity-independent membrane aeration, which is more suitable for space applications. The influence of key parameters on microbial community structure and performance has been characterized, and predictive control strategies have been validated. Long-term stability has been successfully demonstrated, including at the MELiSSA Pilot Plant (Universitat Autònoma de Barcelona). Additional innovations include electrochemical stabilization and advanced pH control. Products from the process(es) have proven suitable for the cultivation of both microalgae and crops. For nitrogen gas production, a proof-of-concept bioreactor treating real urine has been successfully demonstrated, even on undiluted urine, with space-proof membrane-based aeration and gas extraction. To date, two spaceflight experiments have validated the feasibility of nitrification-based processes in microgravity. These studies confirmed the reactivation potential of critical microbial strains after exposure to launch logistics and elevated radiation levels. Post-flight performance was promising across all microbial groups involved. The next mission is currently in preparation, aiming to demonstrate in-flight microbial activity aboard the International Space Station (ISS). Finally, MELiSSA's research and development portfolio on nitrification and partial nitritation/anammox has led to several terrestrial applications, both operational and in development. These examples highlight the broader relevance and transferability of space biotechnology to Earth-based sustainability challenges. Notable cases include the production of nitrate-rich liquid fertilisers from solid organic waste for hydroponic systems, and the conversion of cow urine into nitrogen gas, reducing reactive nitrogen emissions from dairy farming.

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VINCENT VRAKING

System Engineer - German Aerospace Center

EDEN LUNA - Science and Technology Demonstration Platform

The DLR-ESA analogue facility LUNA will provide an environment to simulate future lunar habitats and surface exploration missions. A number of external modules will be attached to the main LUNA hall, including the semi-closed plant production module EDEN LUNA Mobile Test Facility (MTF). Building on the heritage from the successful EDEN ISS project, EDEN LUNA aims to provide a state-of-the-art platform for scientific investigations and technology demonstrations related to controlled environment agriculture (CEA) and bio-regenerative life support systems (BLSS). Upgrades to the existing Mobile Test Facility consist of improvements to the CEA systems, as well as the

incorporation of the EVE robotic payload, and the C.R.O.P.[®] biofilter for urine processing. EDEN LUNA is currently in the Assembly, Integration and Verification (AIV) phase, having successfully passed a Critical Design Review (CDR) in October 2024, and is scheduled to begin operations at the LUNA facility in Cologne, Germany in 2026. An extensive campaign is being planned for the first years of operation of the EDEN LUNA MTF. This presentation provides an overview of the EDEN LUNA plant production module, the initial operations phases and potential areas for collaboration with interested parties.

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LAIA VULART

PhD student - Universitat Autònoma de Barcelona

Effect of feeding regime and pH on the first compartment of the MELiSSA loop

The first compartment of the MELiSSA loop is responsible for the biodegradation of the waste generated by the crew via mixed-culture thermophilic fermentation. The process conditions determine which microorganisms and metabolic routes are active, and which products are obtained. Despite the progress previously made in the development of this compartment, the understanding of the process remains limited. Here, the effects of feeding regime (continuous feeding and sequential batch reactor (SBR)) and pH (5.3, 5.8 and 6.3) on the C1 compartment were assessed. The results showed that a mix of volatile fatty acids (VFAs) were the main product under continuous feeding, with *Caproiciproducens* being the dominant genus in the reactor. At pH 5.3, the total concentration of VFA was 3593 ± 335 mg COD-L-1, with acetic (1742 ± 173 mg COD-L-1) and butyric acid (1077 ± 208 mg COD-L-1) being the main products. The increase in pH to 5.8 led to a higher VFAs production, with the

total VFAs concentration reaching 5805 ± 376 mg COD-L-1. This increase can be correlated to a higher hydrolysis rate and/or a decrease on VFA toxicity. After the pH was changed to 6.3, there was an increase in the total amount of VFA, reaching concentrations of 8617 ± 153 mg COD-L-1. However, biogas containing 63 ± 2 % of methane was produced after 33 days at this pH, underscoring the role of low pH in inhibiting methanogenic activity. Conversely, lactic acid was the main product in the reactor operated as a SBR at both pH 5.3 (3359 ± 236 mg COD-L-1) and 5.8 (4360 ± 260 mg COD-L-1). The microbial community was dominated by *Bacillus* and other genera from the Bacillaceae family. Overall, this research underscores the importance of the operational parameters in the selection of microbial communities and product profile in the first compartment of the MELiSSA loop.

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MICHAELA WALSH

PhD Researcher - University College Dublin

Radiobiome: Host-Gut Microbiome Functional Resilience to Radiation

The gut microbiome is involved in functions important for human health, and is implicated in cancer therapy effectiveness [1], and side-effects [2]. It is also implicated in astronaut health, with mice displaying gut microbiome dysfunction during spaceflight

[3] potentially underlying spaceflight pathology. Radiotherapy patients and astronauts are exposed to harmful levels of radiation, and the effect this has on the gut microbiome is poorly understood. This study characterises the resilience of the gut microbiome to radiation by investigating the radiosensitivity of

exemplar gut bacteria species. Bacterial samples were exposed to radiation doses representative of radiotherapy and spaceflight scenarios using a LINAC with bacterial growth and functionality subsequently characterised. This functional response to radiation will be explored using super-resolution microscopic imaging, a panel of microplate enzymatic assays, and multiomics approaches. Initial studies using the strain 'Lactobacillus acidophilus' have shown it to be affected by exposure to doses from 2 Gy to 50 Gy. Bacterial growth rate was reduced, and statistically significant decreases (t-test, p

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DAVID WEISSBRODT

Full Professor - Norwegian University of Science and Technology

Modelling nutrient dynamics in hydroponic lettuce production using source-separated urine

Hydroponic systems enable circular crop production in bioregenerative life support systems (BLSS) designed for closed environments such as space missions and remote terrestrial applications, where recycling and food self-sufficiency are essential. Our research focuses on soilless cultivations of edible leafy greens like lettuce (*Lactuca sativa*), grown over multiple cycles in batch or closed-loop deep water cultures fed with human-derived fertilisers such as source-separated urine. However, unlike conventional nutrient solutions, mineralised urine presents an unbalanced nutrient profile and must be supplemented to meet the nutritional demands of crop cultivation. The concentrations of essential nutrients like nitrogen, phosphorus, potassium, magnesium and calcium are dynamic in the cultivation medium, leading to nutrient shifts that affect plant growth and development. Unravelling the mechanisms underlying nutrient dynamics is crucial to control the nutrient solution and availability, and thereby ensure plant productivity. Our work aims to unravel these interactions, contributing to the development of robust, resource-efficient hydroponic strategies in BLSS. We developed a mathematical model to simulate nutrient dynamics and assess their effects over successive lettuce cultivation cycles using mineralised urine (Aurin™), compared to conventional nutrient solutions. Nutrient

supplementation strategies were evaluated to identify approaches for sustaining plant growth. Model development and calibration were supported by data from a previous multi-cycle experiment. The 0-D model consisted of a system of equations describing nutrient flows and transformations across multiple cultivation cycles. Plant growth stoichiometry and Monod kinetics were incorporated, in analogy to black-box growth models. Model parameters (including biomass yields, growth rates, nutrient affinity, and inhibition constants) were calculated and calibrated using data obtained on the reference nutrient solution. When applying the model to cultivations with mineralised urine, potassium evolutions were poorly predicted. A non-competitive inhibition term by salt (as measured by electrical conductivity) was included, leading to a more accurate description of potassium uptake. The model reliably reproduced observed data across the different cultivation strategies. When applied over the long term, it illustrates a strategy to forecast the behaviour of critical nutrients across multiple plant cultivation cycles. Combined with pilot studies, models as the ones developed and future refined versions can enhance our ability to control the productivity of the higher plant compartment in closed-loop hydroponic systems using recycled human-derived fertilizers.

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DAVID WEISSBRODT

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Persistence of foodborne pathogens in hydroponic lettuce cultivated on urine-derived nutrients

Recycling nutrients from human-derived fertilisers fosters food production in bioregenerative life support systems (BLSS) in space and on Earth. Urine-based fertilizers, such as Aurin™, offer a low-cost alternative to synthetic nutrients. However, food safety remains a concern regarding the potential for nutrient solutions to support foodborne pathogens. We assessed the persistence of selected foodborne pathogens through their growth and survival in defined mineral and urine-derived nutrient solutions, by conducting in vitro challenges and hydroponic lettuce cultivations in a class 2 biosafety lab. The growth of 18 pathogens among bacterial strains of *Listeria monocytogenes*, *Escherichia coli*, *Salmonella enterica*, *Staphylococcus aureus*, *Bacillus subtilis* and *Bacillus cereus* was first tested in Luria Broth (LB) under varying stress conditions relevant to hydroponic systems (pH 4-7, 0-250 mM NH₄⁺, 0-1.7 M NaCl; each tested at seven levels) in 96-well plates at 22 °C. From spectrophotometric measurements at 600 nm (OD₆₀₀) taken every 2 h over 2 days and extended after 4 and 10 days, most strains grew well in LB under the various stresses, except at pH 4. Pathogens growth was then monitored over 14 days in one defined mineral solution and six single or supplemented Aurin-based solutions (either fresh, used sterilised or used non-sterile). No growth occurred in any nutrient solution, likely due to limited carbon availability. However, survival testing by plate counts after 28 days highlighted that several strains persisted in all nutrient solutions, with some differences between populations. Worst-case conditions were simulated to assess the potential food safety risk in hydroponic

systems. Five pathogens (*L. monocytogenes*, *S. aureus*, *S. enterica*, *E. coli* and *B. cereus*) were inoculated at ca. 10⁴ CFU mL⁻¹ into nutrient solutions of deep-water culture systems (one pathogen per culture) each growing five plants of crispy lettuce (*Lactuca crispis*) at 22-24 °C, pH 5.0-5.6, and 33-49 % RH under an airflow of 1 L min⁻¹ after germinating plant seeds over one week. Microbial growth and survival were measured by plate count from solutions during plant cultivation and from leaves and roots at harvest. Over time, all pathogens declined below detection limits, except *S. enterica* which accumulated to 10⁵ CFU mL⁻¹ in liquid. *L. monocytogenes* and *E. coli* were still detected in plant roots at harvest. *S. enterica* was detected in all harvested sample types, the only pathogen detected on plant leaves. A follow-up hydroponic experiment confirmed *S. enterica* growth in both urine-based and defined mineral nutrient solutions, with no significant difference in plate counts between nutrient sources and between solutions (unspiked with pathogens). Cross-contamination is a risk. Hydroponic nutrient solutions, regardless of their urine-based or defined mineral origin, can serve as reservoirs for foodborne pathogens such as *S. enterica*. This pathogen can threaten the food safety of hydroponic crops, in the event of a contamination of the nutrient solution. Preventive measures must safeguard the feed solutions, avoid cross-contamination to edible plant parts, maintain the indoor environment of the Higher Plant Compartment in BLSS and vertical farming systems, for protecting the health of the crew and plant consumers.

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HEATHER WRAY

Senior Scientist - TNO

Closing the loop: innovations in waste valorization for circular by design materials and products

Waste valorization plays a pivotal role in advancing the transition away from fossil-based energy and materials. This talk will showcase TNO's (Dutch Organization for Applied Scientific Research) circular-by-design strategies, supporting the shift to a more sustainable and resource-efficient economy. This includes the development of innovative, bio-based materials and products from (often challenging) waste streams and end-of-life considerations, including technologies and processes to facilitate re-use and recycling. Recent research on the development of

innovative materials, including printed electronics, and biobased epoxies and polymers will be presented. These solutions are designed to not only reduce reliance on virgin raw materials, but also to support resource independence and closed-loop systems. While primarily applied in terrestrial contexts, the principles and technologies developed hold potential for future use in resource-constrained environments, including space. Through this lens we explore how circular design and waste valorization can contribute to resilient material and energy systems now and in the future.

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SOLÈNE WURTZ PRA

Engineer - Université Clermont Auvergne

Hardware development for the BASIC ISS experiment and planned in-flight and post-flight data collection

The BASIC (Boundary Layer Studies in Microgravity) experiment aims at studying heat exchange between the surface of a leaf replica and the surrounding air in microgravity conditions. The main research question is: how do the combined effects of microgravity, forced convection and surface orientation influence heat transfer rates at the surface of a leaf replica? A flight model will be sent to the International Space Station (ISS) and a control experiment will be performed in the ground model, using a leaf replica and using real plants. This presentation details the approach and the different milestones in hardware design, manufacturing and testing until flight readiness for the ISS, and details these sets of measurements that will be performed in-flight and post-flight. The hardware will be placed within the ICE Cubes facility in the Columbus module and implemented via the ICE Cubes Service. To investigate the influence of various forced convection conditions on heat transfer, the leaf replica is placed inside a wind tunnel and an exhaust fan generates laminar airflow. The leaf replica is mounted on a stepper motor, allowing precise control of its angle of inclination. CAD models and CFD simulations were used to design the hardware and adequately place the replica within the wind tunnel. The variable of interest is the leaf

replica surface temperature evolution versus time, which will be measured using a surface-evaporated platinum resistor, as well as an infrared camera placed above it. Multiple combinations of airflow velocity and angle will be tested. For high air velocities (> 0.5 m/s), no difference is expected on the leaf surface temperature, between ISS and ground results. For low air velocities (< 0.15 m/s), the boundary layer on the leaf replica is expected to be larger in microgravity than in 1g, translating into a significantly higher surface temperature on ISS than the one in the ground experiment. The results will serve to validate heat transfer models in steady-state microgravity at the surface of a leaf (not accounting for the biological part). The ground experiment using real plants (three crop species to be selected among space crop candidates) will investigate differences and similarities in the results between the leaf replica and the real leaves, based on morphological, anatomical and physiological parameters (e.g., leaf temperature, leaf area, leaf epidermis roughness, stomatal traits and conductance). These results will serve to improve the heat and mass transfer models at the leaf surface in terrestrial gravity. This experiment is funded by ESA via the Reserve Pool of Activities program.

CO-AUTHOR : Solène Wurtz Pra, Joanna Kuzma, Louise Fleischer, Jean-Pierre Fontaine, Claude-Gilles Dussap, Giovanna Aronne, Luigi Gennaro Izzo, Leone Ermes Romano, Øyvind Mejdell Jakobsen, Ann-Iren Kittang Jost, Francesc Godia, Stanislaus Schymanski, Cesar Pascual Garcia, Divya Balakrishnan, Leonardo Surdo, Lucie Poulet.

IZABELA ZWICA

PhD student - University Warmia and Mazury in Olsztyn

Use of microalgae and cyanobacteria from BLSS as fertilisers for lunar and Martian regolith simulants

Bioregenerative Life Support Systems (BLSS) play a crucial role in the planning of long-duration space missions. Among their key components are microalgae and cyanobacteria, which, in addition to producing oxygen and biomass, can serve as natural fertilisers in extraterrestrial agriculture. This study investigates the potential use of biomass from microalgae (*Chlorella vulgaris*) and cyanobacteria (*Arthrospira platensis*), cultivated under conditions simulating BLSS, as a source of nutrients for plant cultivation on lunar and Martian regolith simulants. I used several variants of JSC-1A (recycled, after magnetic

separation), the Polish lunar simulant AGK-2010 and two Martian regolith simulators: MMS-1 and MGS-1. The experiments include the evaluation of various doses of microalgae and cyanobacteria biomass, the analysis of nutrient dynamics in the biofertiliser, and the yield of the indicator plant- lettuce (*Lactuca sativa* L.). The research aims to develop a model of organic matter cycling within closed-loop systems in line with the concept of In-Situ Resource Utilisation (ISRU), and to explore the potential of microalgae and cyanobacteria in integrated plant cultivation systems beyond Earth.

POSTER PRESENTATIONS

ABSTRACTS

IAN ALVAREZ

Veterinary Student - The Royal Veterinary College

Integrating Veterinary Insight into Bioregenerative Life Support: A Student Perspective on Animal Health Monitoring in Closed Ecosystems

Bioregenerative life support systems (BLSS) are vital for sustaining astronauts during long-duration space missions, yet veterinary contributions remain largely unexplored. As a final-year veterinary student at the Royal Veterinary College, I recently completed a six-week externship at NASA, contributing to animal welfare and monitoring protocols for rodent spaceflight missions (MHU9, RR26, RR27). Drawing from these experiences, I

propose that integrating veterinary perspectives such as ethical animal welfare protocols, sentinel species monitoring, comparative physiology frameworks, and clinical biomarker identification could significantly enhance BLSS sustainability. This student perspective aims to foster interdisciplinary collaboration and highlight the unique value veterinary medicine brings to regenerative life support systems.

NOUHAILA BOUHADI

PhD student - University Chouaib Doukkali, Faculty of Sciences

Hybrid Optimization of Resource Recycling Systems Using Machine Learning for Space and Earth Applications

Closed-loop life support systems (LSS) are critical for sustaining human presence in space by enabling efficient resource recycling, including waste management, air revitalization, and water treatment. This research focuses on the application of advanced machine learning techniques, particularly Physics-Informed Neural Networks (PINNs) and Explainable AI (XAI), to optimize the modeling, simulation, and control of closed-loop recycling systems. By integrating physical principles with data-driven models, this hybrid framework ensures higher reliability, efficiency, and adaptability in resource management. The proposed approach is validated using experimental data from ground analogues simulating waste and water recycling processes under controlled conditions. Preliminary results show

significant improvements in the efficiency of waste-to-resource conversion and predictive accuracy for system behaviour under varying conditions. Additionally, the integration of XAI techniques enhances the interpretability of optimization models, making the systems safer and more robust for both space and terrestrial applications. This contribution bridges space and Earth applications by showcasing how advancements in LSS for space missions can inform sustainable practices on Earth, particularly in the areas of waste recycling and circular systems design. The interdisciplinary nature of this work aligns with the core objectives of the MELISSA program, fostering collaboration among researchers, engineers, and public-private stakeholders.

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EUGÉNIE CARNERO DIAZ

Associate Professor - Space Biology Group Lead - Sorbonne Université - Institut de Systématique, Evolution, Biodiversité (ISYEB - MNHN/SU/CNRS/EPHE/UA)

Red light and altered gravity: integrated transcriptional and morphological analysis of the early adaptive response of *Arabidopsis thaliana* to space environments

The integration of plants into space-based Bioregenerative Life Support Systems (BLSS) requires a comprehensive understanding of how plants adapt to unavoidable space-related stress factors, including microgravity and artificial light. In this context, we combined morphometric and transcriptomic analyses of *Arabidopsis thaliana* wild-type and auxin-signaling mutants (*aux1.7*, *eir1.1*, *tir1.1*), grown under different gravity levels (0g, 0.3g, 0.65g, 1g) and two light regimes (darkness vs. directional red light) during the Seedling Growth 3 (SG3) mission on board the International Space Station (ISS) using the European Modular Cultivation System. Morphometric data revealed that microgravity alone had little effect on hypocotyl or root growth, but red light significantly enhanced shoot elongation, even in 0g. In auxin mutants, disruption of polar auxin transport (PAT) or perception altered this response; supporting the hypothesis that red light acts through auxin-dependent pathways to promote aerial growth. Interestingly, plant responses at 0.3g (Martian gravity) were not intermediate

between 0g and 1g but rather showed distinct morphological profiles, suggesting specific signaling pathways are engaged in a different manner at this gravity level. At the molecular level, red light had a strong attenuating effect on gravity-induced transcriptional changes. The number of differentially expressed genes (DEGs) between gravity conditions was reduced under red light compared to darkness. This transcriptional response was reduced in auxin mutants, suggesting that a protective role of red light depends on functional auxin signaling. Gene expression profiles across 0.3g and 0.65g conditions revealed specific, non-linear responses, consistent with the morphological data. Together, these multiscale data support a model in which auxin functions as an integrator of gravity and light signals, coordinating adaptive growth responses through polar auxin transport and perception mechanisms. Red light emerges as a powerful modulatory tool, capable of mitigating microgravity effects at the transcriptomic level while selectively enhancing shoot development. This study highlights the combined utility

of red light and auxin regulation in mitigating the physiological impacts of altered gravity. These insights are essential for optimizing plant cultivation strategies in future space missions,

including Martian greenhouses and long-term orbital crop production.

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MARTIN CERFF

Project engineer biological life support systems - Blue Horizon SarL

BIORAT 2: Conceptual Design of the Urine Nitrification On-Board Demonstrator for MELISSA and first results from lab testing

Technologies for regenerative life support systems are required for long-distance deep space exploration such as Mars transit missions. Among others, they are investigated in the Micro-Ecological Life Support System Alternative (MELISSA) project, which is developed by ESA and partners. Their fundamental role is to support the crew with their physiological daily needs during the mission by managing and valorizing generated wastes and hence reducing the needed upload mass. Fundamental needs are the production of oxygen provided through recycling of carbon dioxide, of water and of fresh food, mainly by means of plants and cyanobacteria/algae. They require water and nutrients for optimal growth. Both can be derived by step-wise degradation and recycling of the waste streams. To gain a deeper understanding of waste stream conversion into nutrients under reduced gravity, flight precursor studies such as Arthrospira-B and URINIS were already successfully conducted on-board the International Space Station (ISS) or are currently under preparation. Growth of respective microorganisms was demonstrated under reduced gravity and increased radiation. Besides carbon, nitrogen is among the most important nutrients for algae and plants and is mainly found in urine. Currently, urinary nitrogen is neither recycled nor reutilized on-board the ISS. The BIORAT-2 project is aiming to perform the technological demonstration of urine nitrification on board the ISS by means of an on-board demonstrator (OBD). Within its final function the BIORAT-2 OBD shall convert urine into a nitrate-rich feed medium with the goal to enable plant and algae growth and facilitate the removal of nitrogenous compounds from wastewater. Based on the current state of the art, as reported by (Cruvellier et al., 2017; Garcia-

Gragera et al., 2021; De Paepe et al., 2020) and others, the initial conceptual design of the OBD was elaborated. As the core part of the solution we selected a membrane-aerated biofilm reactor (MABR) to provide bubble-free aeration. Here, we report on the preliminary results of the lab phase. During lab testing we demonstrated the suitability of the MABR design to sufficiently supply selected nitrifying organisms *Nitrosomonas europaea* (ATCC 25978) and *Nitrobacter winogradskyi* (ATCC 25391) with oxygen to convert simplified artificial urine into nitrate. Ammonium was utilized as sole nitrogen source. Preliminary results revealed that a quasi-steady state was reached close to full nitrification and maintained for approx. three weeks at a load of 0.4 g N-NH₄⁺ per L per d at 30-32 °C in the pH range 6.7-8.7. Full N-conversion of similar and higher loading rates were reported in the literature for a fixed-bed column setup (Cruvellier et al., 2017). In future, higher nitrogen loads should be tested over an extended period of time to demonstrate the flexibility of the system, as well as the extension of the microbial consortium towards real urine nitrification. Cruvellier N, Poughon L, Creuly C, Dussap CG, Lasseur C. 2017. High ammonium loading and nitrification modelling in a fixed-bed bioreactor. *J. Water Process Eng.* 20:90-96. Garcia-Gragera D, Arnau C, Peiro E, Dussap C-G, Godia F. 2021. Integration of Nitrifying, Photosynthetic and Animal Compartments at the MELISSA Pilot. *Front. Astron. Sp. Sci.* 8:1-16. De Paepe J, De Paepe K, Godia F, Rabaey K, Vlaeminck SE, Clauwaert P. 2020. Bio-electrochemical COD removal for energy-efficient, maximum and robust nitrogen recovery from urine through membrane aerated nitrification. *Water Res.* 185:1-12.

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DORIAN DOHOGNE

PhD Student - University of Liege - Gembloux Agro-Bio Tech

Conception of a fresh bacterial biomass based dessert for space traveling cooked in an ohmic oven

In a rapidly evolving space context, where greater autonomy in food supply is being pursued, this study aims to develop a first food product that could be partially prepared in space using fresh microbial biomass. To enhance astronaut well-being, the chosen concept is a familiar dessert the waffle combined with an innovative cooking method adapted to space conditions: ohmic heating. The main objective of this study is to design a dessert based on fresh microbial biomass (spirulina) that is compatible with the specific constraints of space environments.

The experimental approach consisted first in adapting a traditional waffle recipe by replacing all fresh ingredients with dry components, rehydrated either with water or with fresh spirulina, in order to compare both formulations. Waffles were then cooked using a lab ohmic oven. Several parameters were assessed: texture, taste, cooking dynamics, nutritional composition, and visual appearance. The results show that ohmic heating is faster than conventional heating, improved moisture retention, and produces a light and pleasant texture.

The final product has an acceptable taste and a better nutritional profile for astronauts needs. This experiment highlights the potential of ohmic heating and the integration of fresh microbial

biomass in the development of food products adapted to space missions. Further studies are required to confirm the feasibility of this concept under real space conditions.

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NICOLAS DROUGARD

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AI tools for monitoring plant growth and optimizing cropping systems in BLSS

Plant growth is an essential aspect of bioregenerative life support systems, which must be achieved while managing resource use. In their future missions, astronauts will have limited time for crop cultivation, as their main focus will be on completing mission objectives. Therefore, upcoming space crop systems must be designed to require less crew involvement than current systems, incorporating more automation. Monitoring the growth and health of plants throughout their life cycle is essential, not least to guarantee the safety of the food consumed by astronauts during these missions. Advanced imaging techniques can gather essential data enabling non-invasive and automated assessments of plant state that require minimal crew involvement. Swiftly identifying signs of nutrient deficiencies, drought or infections enables early response, ultimately improving the success of long-term space missions. With the support of Innovspace (ISAE-SUPAERO's Fablab), the ALICE (Artificial Intelligence for Life In spaCE) project is contributing, with student research projects, to the development of AI methods to improve not only the perception functions of plant growing systems, but also the associated decision-making and design. In terms of perception, computer vision models were trained for crop segmentation and leaf area estimation. These models provide crucial information, since their outputs are linked to photosynthesis, evapotranspiration and the development level of plants. The challenging context of hydroponic systems has been tackled by using an appropriate selection of labeled datasets freely available online. Perspectives in this field are the estimations of other essential information about the crop, using computer vision alone or coupled with

other sensors outputs. With regard to decision-making and the operation of plant cultivation systems, Classical Planning algorithms have been used to compute optimal strategies: given specific tasks to be carried out on several plants in different locations, these algorithms were used to compute a sequence of actions that minimized the energy consumed. Constraints respecting the physics of the problem were added to the model to enable optimal strategies to be computed in a reasonable time for a large number of plants. The next steps in this field concern the development of more faithful and richer models, including, for example, the control of light or nutrient use in a hydroponic system.

Accurate simulators could be very useful here too, to benefit from the results of powerful reinforcement learning algorithms. Finally, some interesting work on Multi-Disciplinary Optimization for the design of hydroponic systems has been initiated. A model taking into account, among other things, the plants grown, their lighting, as well as the dimensions of the system, was developed. Optimal designs for hydroponic systems based on this model were computed, minimizing space and energy with different weightings. This work inspires the development of a general framework in which the link between perception and decision would be addressed through the optimization of a partially observable decision process, constraints and objectives would be appropriately considered in this multi-criteria context, and finally data-driven plant growth monitoring would be properly integrated with model-based robotic planning.

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Development and implementation of a simulated microgravity setup for edible cyanobacteria

Regenerative life support systems for space crews recycle waste into water, food, and oxygen using different organisms. The European Space Agency's MELISSA program uses the cyanobacterium *Limnospira indica* PCC8005 for air revitalization and food production. Before space use, components compatibility with reduced gravity was tested. This study introduced a ground analog for microgravity experiments with oxygenic cyanobacteria under continuous illumination, using a random positioning machine (RPM) setup. *L. indica* PCC8005 grew slower under low-shear simulated microgravity, with

whole proteome analysis revealing downregulation of ribosomal proteins, glutamine synthase, and nitrate uptake transporters, and upregulation of gas vesicle, photosystem I and II, and arboxysome proteins. Results suggested inhibition due to high oxygen partial pressure, causing carbon limitation when cultivated in low-shear simulated microgravity. A thicker stagnant fluid boundary layer reducing oxygen release in simulated microgravity was observed. These findings validate this RPM setup for testing the effects of non-terrestrial gravity on photosynthetic microorganisms.

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ENZO FERREC

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Orbital Farming Infrastructure Design for Sustainable Space Exploration

This proposal will investigate the concept, feasibility, and scientific potential of an autonomous (or semi-autonomous) orbital farm designed to operate with minimal human intervention. The primary objective is to develop a sustainable system capable of producing food and recycling resources while minimizing reliance on Earth-based inputs and crew-based management. The study focuses on the architectural, technological, and biological requirements for maintaining such a system in Low Earth Orbit (LEO) or aboard future space habitats. Key aspects include autonomous environmental control, robotics for crop management and system maintenance, and artificial intelligence for monitoring and optimization. By exploring such an infrastructure, the project aims to address critical challenges in long-duration space exploration, including food production, and environmental sustainability. Furthermore,

it serves as a testbed for understanding plant growth, microbiomes, and ecosystem dynamics in microgravity. Minimizing human presence will reduce operational requirements and decrease the workload on astronauts during their routine activities. The outcomes of this research could have profound implications for future missions to the Moon, Mars, and beyond, where self-sufficiency and automation will be essential. Additionally, spin-offs from the development of autonomous farming technologies may benefit Earth-based agriculture, particularly in remote or extreme environments. This abstract proposes a multidisciplinary approach combining aerospace engineering, biology, robotics, and systems science to explore how orbital farms can become pivotal infrastructures in the next era of space exploration.

ENZO FERREC

Student - ISAE-SUPAERO

Intelligent Systems Engineering for Life Support: Leveraging AI and Digital Twin Technologies

The engineering of Life Support Systems (LSS) presents a complex systems challenge, requiring the integration of multidisciplinary subsystems under strict constraints of autonomy, safety, and sustainability. As mission durations increase and human presence expands beyond low Earth orbit, the limitations of conventional engineering approaches become more evident. This abstract proposes to explore how emerging digital technologies (specifically artificial intelligence, digital twins, and generative design tools) can enhance the system-level design and lifecycle management of LSS. Digital twins enable the creation of dynamic, data-driven models that reflect the real-time state of critical life support subsystems. Their integration with AI techniques allows for continuous system monitoring, fault prediction, and adaptive control, facilitating the transition from static, rule-based designs to intelligent,

responsive architectures. In parallel, AI-driven optimization and simulation methods can support early-phase trade studies, accelerate system validation, and enable the co-design of hardware and control strategies. This approach aligns with current trends in systems engineering that emphasize model-based design, autonomy, and resilience. By embedding intelligence directly into the design and operational phases, engineers can better manage complexity, reduce development cycles, and improve the reliability of mission-critical infrastructure. The proposed contribution aims to initiate a structured discussion on the role of intelligent digital technologies in the future of space systems engineering. It positions these tools not as supplementary aids, but as integral components of next-generation engineering methodologies for life support in extreme environments.

INÊS FIGUEIREDO

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Myco Space solutions: Fungal Biotechnology for Sustainable Space Resource Exploration - an ESA supported project

As we move towards a permanent human presence on the Moon and Mars, the need for Sustainable Life Support Systems becomes critical, and integrating biotechnology techniques that rely on utilizing locally available resources is crucial to enhance self-sufficiency and resilience in long-term space missions. Using filamentous fungi that can thrive on locally available nutrients is a promising approach. These have proven their value on Earth, by contributing significantly to resource exploration and a circular bioeconomy [1]. Their potential for space applications has also been demonstrated, with species successfully performing biomining on lunar regolith simulants and meteoritic material, including aboard the ISS [2], and thrive in space microgravity conditions [3], making them excellent candidates for space biotechnology applications. In this ESA-funded project, we focus on studying the recycling of two major space mission-relevant waste streams: hygiene wastewaters

and plastic waste. Hygiene wastewaters will become a significant component of the water recycling systems on long-term missions, and their reuse is critical to reclaim water and for closed-loop life support systems. Similarly, the recycling and upcycling of plastic waste is critical, both in space and on Earth, offering the dual benefits of reducing waste while at the same time providing feedstock for manufacturing. Some plastics can be biodegraded into nutrients by microorganisms, including fungi, though little is known about the mechanisms behind the process. Basing on formulations used to test ISS water recycling systems [4,5], we developed a synthetic hygiene wastewater (SHW) medium to evaluate whether fungal species can utilize this nutrient-poor waste source to sustain its growth for biotechnological processes. The first results indicate fungal growth in SHW medium, although lower compared to standard rich medium. This positive data poses the basis for the

optimisation of wastewater media for biotech applications. We are also evaluating the fungal production of biotech-relevant secondary metabolites in SHW, such as organic acids for biomining and bioremediation processes in space.

Additionally, we performed a fieldtrip in Iceland to investigate the microbiome associated with plastic debris collected from extreme environments. Fungal species with plastic-degrading capabilities isolated from these locations may be well suited to produce biotech-relevant compounds under the harsh conditions of space. In both lines of research, we are investigating the potential correlation between fungal morphology and metabolite production under resource-limited conditions from waste sources. To support this, we quantitatively analysed fungal morphology using the morphology number and image analysis techniques, as described in [6]. In conclusion, this ESA-funded ongoing project is demonstrating the promising potential of filamentous fungi as key biotechnological agents in future space missions, particularly for recycling hygiene wastewater and plastic waste. Our findings highlight the viability of integrating fungal biotechnology into the MELISSA closed-loop system, and its potential to reduce waste and enhance self-sufficiency in future human space missions, as well as supporting circular economy on Earth.[1] Meyer, V., Basenko,

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Beyond Earth: The Design and Operation of a Fermentation Membrane Bioreactor for Sustainable Biomanufacturing for Early Planetary Habitats

Long-duration space missions and early planetary colonization necessitate the development of innovative biomanufacturing technologies to ensure sustainable resource utilization that will lessen the requirement of costly resupply missions. One promising approach is the use of a fermentation membrane bioreactor (FMBR) for in situ resource utilization (ISRU) in space environments. Researchers at Kennedy Space Center, and in collaboration with DARPA's B-SURE program, designed and operated an FMBR that combines the principles of microbial fermentation and membrane filtration to produce value-added products from alternative feedstock resources, such as wastewater, carbon dioxide, and sunlight. FMBR operates by leveraging microorganisms that are genetically engineered to convert raw materials into target compounds. These microbes are cultured within the FMBR bioreactor chamber, where they metabolize these alternative feedstocks under controlled conditions. The culture is continuously fed to allow for greater cell densities therefore increasing product yields. An

ultrafiltration membrane separates the microbial culture from the chamber vessel thus allowing for continuous extraction of the desired product while retaining the biomass.

This setup offers several advantages including enhanced cell densities due to the continuous style of operation, solid-liquid separation especially useful for cultures that excrete the target compound, and the ability to maintain optimal microbial growth conditions via an extensive sensor suite and control system. The application of FMBRs in outer space biomanufacturing addresses critical sustainability issues such as waste recycling and in situ synthesis of pharmaceuticals, bioplastics, and other value-added products. By providing a versatile platform for producing goods and commodities on-site, the FMBR reduces the dependency on Earth-supplied resources, lowers mission costs, and enhances the self-sufficiency of space habitats. As space exploration progresses, the integration of FMBRs into life support and manufacturing systems will be crucial for the viability and sustainability of human activities beyond Earth.

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How Does Space Microgravity Change the Taste of Wine? Insights from Immersive Simulated Space-Environments

Currently, alcohol is not permitted in space due to security concerns regarding gas sensors found in the international space station (ISS) and unknown effects in microgravity conditions. However, it is important to consider its consumption for future space travel, especially as an indulgent option for commercial flights. Therefore, this study aimed to assess the sensory perception of red wine in space simulated conditions. A trained

panel consisting of 10 participants tasted six Shiraz samples from different regions in Australia in two different simulated conditions in immersive rooms with 180° screens. Environment one consisted of a space-simulated setting with view of the Earth through the ISS porthole with a simulated seating position and environment two simulating on Earth conditions dining outdoors in a vineyard. Each room had their corresponding

sound to obtain the full environment experience. The BioSensory® application was used to display the sensory questionnaire and record the videos from participants to capture their biometrics (emotions and physiological responses). Multivariate data analysis showed that samples tasted in each simulated environment were successfully separated, this was confirmed by the cluster analysis that was able to correctly group samples in both simulated conditions. Samples tasted in

the space simulated environment were positively associated with aroma, taste and mouthfeel intensity, while samples tasted in Earth conditions had a positive relationship with blood pressure, and emotional responses. Findings suggest having wine as an option may be positive, especially for space tourism; however, more research is required to explore the effects of alcohol consumption in microgravity.

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Bubbling Perceptions in Space: A Study on Carbonated Water in Simulated Immersive Space Environments

The main hydration source for astronauts in space is plain water, which may be boring for some people. Hence it would be interesting to explore other healthy sources such as carbonated water, which is often considered less boring but thirst quenching. Therefore, this study aimed to assess the carbonated water perception in space simulated immersive environments. Four commercial carbonated waters with different mineral content with two treatments (i) control, and (ii) audible sound treatment (frequencies within 20 75 Hz) were used. A total of 12 trained panelists tasted the samples in two immersive rooms (i) Earth environment simulating an outdoors dining setting and (ii) space environment displaying the view from the international space station porthole and a simulated microgravity seating position. Both rooms had the corresponding audio for the environment. Questionnaire was displayed in the BioSensory® application using the quantitative descriptive analysis (QDA®)

method and video was captured to obtain the biometrics (emotional and physiological responses). Results from the multivariate data analysis showed that samples were perceived different in space and Earth.

Descriptors such as bubble size, bubble speed, fizziness, tingly, and aftertaste were negatively associated with samples taste in simulated space conditions. Furthermore, samples tasted in space were more associated with emotion-related variables such as smile, joy and relaxed, and heart rate and blood pressure. On the other hand, the cluster analysis was able to correctly group the samples in each environment. Findings from this study suggest that including carbonated beverages in the astronauts options for hydration may be beneficial in terms of their emotional state without significantly affecting their perception.

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■ ROGER GUCCIARDI

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Evolution of the life support systems over years - the study of the AIAA database.

Although it is not the intention to be back to the early days of balloon and planes, it is fair to observe, from very short duration and short distance from the Earth to the preparation of the long-term mission beyond LEO, the focus of ECLSS R&D drastically evolved over years. Thanks to the access to the American Institute of Aeronautics and Astronautics (i.e. AIAA) database we have carefully studied the evolution of ECLSS over years and extract some key figures which can be useful for the ECLSS community either has historical information, bibliography and/or potential recommendations. In this study, firstly, we recall the international context, summarise the objectives of the key programs (e.g. Air fighter, MERCURY, GEMINI, APOLLO, EVA, ..). Then we identify and describe the key challenges (e.g.

Human physiology, atmosphere control, water treatment, toxicology, microgravity operation, nutrition, closed loop design, ..), major success and achieved milestones, the involved organisations (e.g. US Navy, USAF, NMRI, NASA, U Wright, U Florida,..). For all these steps key players have been identified, their background, position and responsibilities described. To our knowledge such analysis from 1940 to 2025, had never been done so far. Taking into account the well know interest of MELISSA project to scientific and engineering education, it could become a niche chapter of ECLSS education book, MSU lectures, ESA education program, ISU or inputs to concern exhibition and space museums.

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Market and End-User Analysis of Lunar Regolith Simulant: From Production to Application in Regolith-Based Agriculture

One crucial resource for In-situ resource utilization (ISRU) is Lunar Regolith, which could be used for construction, production of propulsion fuels, and space agriculture. Lunar Regolith Simulants mimic various physical and chemical properties, thus representing an essential tool to enable Earth-based research and testing. However, despite large advances in our understanding of Lunar Regolith Simulants and their usage possibilities, significant challenges are yet to be addressed. Competition and geopolitical conflicts are not conducive to collaboration and the dissemination of knowledge. Additionally, research is impeded by technological, financial, and logistical

gaps between Lunar Regolith Simulant producers and end-users. We present a comprehensive and detailed analysis of the current landscape of Lunar Regolith Simulant manufacturing and usage, with a particular focus on Lunar Regolith-based agricultural research. We identify trends in production capabilities and customer requirements, and show that high costs and supply chain constraints, along with challenges on Lunar Regolith Simulant fidelity, hinder research progress. Our work emphasizes strategies to align the needs of the research community with the Lunar Regolith Simulant production and serves as a resource to foster future collaboration.

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Adaptation of Plant Biotechnology to Microfluidic Systems

The adaptation of plant biotechnology to microfluidic systems represents a significant advancement in the pursuit of more precise, scalable, and resource-efficient methodologies for studying plant cells in vitro. Microfluidics enables the manipulation of microscale volumes within controlled environments, offering unparalleled opportunities for high-resolution monitoring of plant cell physiology, intercellular communication, and metabolite biosynthesis. Modular microfluidic platforms, particularly those employing semi-permeable membrane-integrated layered architectures, allow for spatial compartmentalization of cell populations while enabling molecular diffusion between them. This configuration mimics the complexity of tissue-like signaling and supports investigations into cell-to-cell communication, hormonal regulation, and proliferation control mechanisms in plant

systems. The ability to maintain distinct microenvironments and introduce dynamic flow conditions further enhances the experimental flexibility of these platforms. Moreover, microfluidic technologies facilitate the miniaturization and automation of plant biotechnology workflows, including the screening of growth regulators, stress responses, and secondary metabolite production. Compared to traditional bioreactor systems, microfluidics offers significant advantages in terms of reagent economy, temporal resolution, and integration with optical and analytical tools. Overall, the convergence of plant biotechnology and microfluidics has the potential to reshape experimental plant science by providing robust platforms for both fundamental research and applied biotechnology, including synthetic biology, phytochemical engineering, and precision agriculture.

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SOS microgravity! App: Inputs and expected returns to the Space Research Life Science community from a Space Omics and Simulated Microgravity Lab serious game

Space research keeps being a top priority for the more important economies in the world, with emerging agencies as China, even after the COVID and war in Ukraine and Gaza context. The next step in space colonization, to visit again the Moon and soon the arrival of the first human to Mars from the Gateway platform, is ready to begin. In this context, the need of space agriculture to sustain life out of Earth is demanding. Integrate the information coming from the Space Biology, Life Support Systems and Astrobiology researchers into hypothesis generation models is required. In that regard, this proposal aims to take advantage of our experience in spaceflight, partial gravity and simulated microgravity experiments in the life science domain to provide

new tools that help integrating two decades of spaceflight results to produce a real advance in our global knowledge of life support systems out of Earth. We will implement a gamification strategy applied in the creation of the SOS: Microgravity! mobile app. It will allow us to share Space Omics knowledge with the society, taken advance of public interest for Space Exploration and Mobile Apps combined. More than that, younger gamers will be more engaged to STEM careers by this, and could choose if they help us creating new in- game knowledge, that we will supervise and cross-reference, or they prefer to buy in- game research, so they will be funding us to boost the humanity desires of Space Exploration. Previously, we would like to recruit

experts in life support system and/or astrobiology research to help us in creating the tree of knowledge to be included in the game and to validate the proposals of gamers in a beta test. The final outcome of the project will be an internet game able to

generate life support systems solutions, based on scientifically sound facts, that may be potentially usable as a hypothesis generation datasets.

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Moon Farming with Microbes: Opportunities for Bio-assisted Substrate Regeneration in Space Habitats

Future space missions will necessitate sustainable food production systems that can operate under extreme and nutrient-poor conditions. One of the primary challenges is facilitating plant growth on lunar regolith, which lacks essential nutrients and contains potentially harmful compounds. Our mission is to explore the potential of nitrogen-fixing microorganisms, particularly cyanobacteria, to biologically enhance the regolith and support plant cultivation as part of a closed life support system. By integrating microbial life into

extraterrestrial substrates, we aim to promote nutrient cycling and enhance plant development without relying on external resources. Preliminary results indicate that microbial treatments positively influence plant health and growth in simulated off-Earth conditions. This approach contributes to the development of regenerative life support systems by combining biological resilience with in-situ resource utilization, offering solutions for both space-based agriculture and future applications in degraded terrestrial environments.

VENELIN HUBENOV

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Volatile fatty acids production during anaerobic co-digestion of different cellulose containing substrates

Cellulose is one of the most abundant natural polymer molecules on Earth. Because of its features, it serves as main material used for a great variety of products such as hygiene materials. It is determined not only the main waste material on our planet, but also on board of manned spacecraft missions. Waste utilization in closed loop systems involves an anaerobic biodegradation step. A key parameter is the composition and quantity of volatile fatty acids produced as intermediates. In our study pretreated mixed microbial inoculum for removing methanogens were used. Three types of materials were used as a supplement for the anaerobic biodegradation of wheat straw as a model complex lignocellulose substrate. The results for the quantity and composition of resulted volatile fatty acids production showed that volatile fatty acids synthesized during the anaerobic biodegradation of alkali pretreated wheat straw exceed about

two times the volatile fatty acids produced obtained using untreated straw at thermophilic conditions. The major component in the mixture was acetate, followed by butyrate at ratio about 4:1. Addition of more easily degradable substrate as waste algal biomass or corn steep liquor led to increase in the total volatile fatty acids produced and changes in this ratio to about 2:1, as increased hydraulic retention time leads to a reverse of this ratio in favor of butyrate to about 3:1. Volatile fatty acids are very important metabolites and their presence in the resulting liquor from the anaerobic biodegradation step has an impact to the following subsequent processing steps. Understanding the factors that influence their quantity and composition of the resulting mixture would help develop technological levels in their processing at different stages of closed-loop systems for effective resource utilization.

KEYWORDS: volatile fatty acids, anaerobic co-digestion, cellulose wastes **Acknowledgements:** This research was funded by the Bulgarian National Science Fund, Grant KP-06-IP-CHINA/3

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Effect of simulated micro and hyper-gravity on gene fitness in bacterial biofilms

Biofilms are defined as communities of bacteria that are embedded in a self-produced matrix of extracellular polymeric substances (EPS) often adhering to a surface. The formation of biofilms provides a protective shield for bacterial cells, thereby increasing the risk of the persistence of pathogenic bacteria in

industrial and medical settings and as well in the context of the space environment. For instance, the proliferation of bacterial biofilms inside the water recovery system in the International Space Station (ISS) is posing a significant risk to life-support systems and the health of astronauts. To elucidate the specific

cellular responses that are triggered during biofilm formation under extreme environments such as encountered in altered gravitational fields, we propose to use a CRISPR-silencing approach to facilitate the identification of genes that are important for this bacterial lifestyle under these conditions. By leveraging previous transcriptomic studies in the strong biofilm-forming *B. subtilis* NDmed strain, we targeted coding and non-coding genetic elements that were up-regulated during the early stages of biofilm formation, to perform CRISPRi-mediated gene knockdown and phenotyping. Our results demonstrated

that CRISPRi-mediated silencing is an effective tool for studying biofilm phenotypes in bacteria. We thus constructed a library targeting >400 genes to perform CRISPRi pooled screening in *B. subtilis* NDmed. This approach will be ultimately applied to challenge the *Bacillus* biofilm under various simulated gravity levels at ESTEC (European Space Research and Technology Center, ESA, Netherlands). This work provides proof of principle for the effectiveness of using a CRISPR-silencing approach to perform functional studies of genes involved in biofilm formation, structure, and regulatory pathways.

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Study of the bacterium *Cupriavidus necator* for a space application in life support: Waste recycling, Food and Biopolymers.

According to the International Space Exploration Coordination Group, human space exploration is stepping up a notch. The end goal is to send Humans to Mars before 2050. Due to the distances involved and the duration of the flight, replenishments will become strongly limited. The astronaut life support system must be able to maintain friendly conditions for humans by continually converting wastes into valuable compounds such as Single Cell Proteins (SCPs) and PolyHydroxyAlcanoates (PHA). Two waste streams and one microorganism were identified: urea from the crew's urine, volatile fatty acids (VFAs) coming from a first stage of anaerobic waste digestion, and the bacterium *Cupriavidus necator*. The objective of this work was to demonstrate the feasibility of producing SCPs and PHAs from VFAs and urea for a space application. The best strain of *C. necator* for this application was selected and characterized among five wild type strains. Since life support systems operate continuously as loops, continuous culture experiments were chosen and the effect of dilution rate and carbon sources on biomass composition was investigated. This macroscopic approach was combined with single cell and molecular analyses thanks to flow cytometry and proteomic to characterize microbial physiology. Finally, the nitrate anaerobic respiration of

C. necator was characterized in order to significantly reduce the oxygen requirement for SCP production. Two strain screenings were performed based on biomass composition and growth parameters: one under aerobic conditions and the other under anaerobic conditions. In both cases, the wild-type strain CECT 4623 was selected. Continuous experiments under aerobiosis led to the total transformation of the carbon and nitrogen source into biomass with a mix of SCP and PHAs. Regardless of the carbon source and under carbon limitation, the protein content increased (from 55.0 %CDW to 78 %CDW) with decreasing dilution rate while nucleic acids and PHAs decreased (from 8.8 %CDW to 4.8 %CDW and 9.8 %CDW to 0.6 %CDW respectively). In these conditions, acetic acid led to the best biomass composition while propionic acid induced more nucleic acid accumulation and growth inhibition. Flow cytometry revealed the presence of heterogeneities while proteomic showed the adaptation of *C. necator* metabolism and phenotype to the carbon source. Finally, continuous experiments under anaerobiosis (in the presence of nitrate, at pH 7.5) led to a biomass composed of a mix of protein and PHA (around 1/3 and 2/3 respectively) no matter the nutritional condition applied, which demonstrated the possibility of dispensing with oxygen.

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Identification of the anaerobic microbial consortia involved in anaerobic waste utilization processes

Anaerobic biodegradation of complex waste materials such as plant lignocellulosic wastes and cellulose containing hygiene materials can play the role of a core technology for closed loop waste recycling systems. These processes can be conducted either at mesophilic or at thermophilic conditions. The thermophilic ones can also ensure prevention to pathogenic microorganisms growth. This is an important condition, especially for hermetically closed habitats as spacecrafts or extraterrestrial bases (Lunar or Mars). The cellulolytic microorganisms on their side, are the main factor in the cellulose conversion into liquid products which can be suitable for the

microorganisms involved in the next phases of the micro-ecological recycling system analogue. In our investigations wheat straw was used as the main model substrate for anaerobic biodegradation processes. *Proteiniphilum saccharofermentans* was one of the most abundant species found through metagenomics assay in our mesophilic processes. During the thermophilic anaerobic co-digestion of wheat straw combined with waste algae biomass both *Thermocaprocibacter melissae* and *Clostridium cellulosi* are present at about 86.8% of the bacteria grown in the bioreactor operating at automatic controlled pH in the range of 5.3-5.5. In contrast, as part of

methanogenic consortia, the main cellulolytic bacteria found in low concentration (about 5%) was *Thermoanaerobacterium thermosaccharoliticum*. This bacterium can convert cellulose and sugars into butanol. A great variety of process parameters can play a significant role in shaping the anaerobic digestion consortia and hence to impact the final liquid metabolites. By modifying these parameters, the direction of development of

the microbial community can be set depending on the desired end products. Using cultivation independent methods, the main microbial species from the complex microbial community grown during the process realized can be identified. Key words: anaerobic biodegradation, microbial consortia, waste utilization
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Adaptive response of *Arabidopsis thaliana* to combined simulated microgravity and cosmic radiation: an integrated transcriptomic, morphological, and cellular analysis

As humanity prepares for long-duration space exploration, plants will be key components of Bioregenerative Life Support Systems (BLSS), contributing to oxygen production, water purification, and food supply. However, space environments combine unique and persistent stressors such as altered gravity and chronic cosmic radiation whose combined effects on plant development remain insufficiently characterized. Our study used the MarSimulator device, which combines a Random Positioning Machine (RPM, simulating microgravity at 10⁻⁶ g) and chronic gamma radiation exposure (0.33 mSv/day from thorium nitrate), chosen to mimic average environmental conditions aboard the International Space Station (ISS). We conducted a multiscale analysis of *Arabidopsis thaliana* response, integrating transcriptomic profiling (RNAseq), root morphometry, cell cycle characterization, auxin distribution, and oxidative stress evaluation. Transcriptomic data revealed a stronger transcriptional response to radiation than to microgravity, with roots displaying higher transcriptional resilience than shoots. While each factor triggered specific gene

expression signatures, a subset of commonly regulated genes suggested shared response pathways, notably involving metabolism, cell wall dynamics, hormone signaling, and oxidative stress. At the cellular level, root growth was inhibited, and meristematic cell size was significantly reduced under combined exposure, pointing to a synergistic effect. Cells exposed to both stressors accumulated in the S phase of the cell cycle, possibly reflecting DNA repair activation. Notably, auxin accumulated abnormally in the root apex, and the expression of key auxin transporters (PIN1, PIN2, AUX1) was downregulated, indicating disruption of polar auxin transport. Together, these results support a unified model of physiological adaptation to combined microgravity and radiation, highlighting a substantial energetic cost for stress integration and developmental maintenance. This work underscores the necessity of investigating multi-factorial environmental interactions across biological scales to guide the selection of plant species best suited for future BLSS on long-term space missions.

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UTASI LORINC

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MISSION POSSIBLE - STUDY THE EFFECTS OF SPACE EXPOSURE ON 23 PLANT SPECIES SEED.

Several trials have researched the effects of spaceflight on plant seeds over decades in various countries. Although this research does not represent the earliest studies in the field, it addresses gaps in the existing literature. Many studies have explored the impact of space conditions on many plant species. Yet, humanity has to test more species and breeds of plants in Space in order to establish a successful permanent extraterrestrial habitat. Food security is also an issue in our challenging Earth environment, which represents many similar issues with extraterrestrial living. For instance, breeding new varieties, closed plant production systems, lacking resources, being in an extreme environment, etc. It is also a major challenge that the seeds have to endure induced levels of harsh environments in order to succeed in deep space missions. To answer these challenging tasks, a trial was established where we hypothesize that even short-term exposure in polar orbit will result in measurable alterations in plant development, inheritance, nutritional quality and other

economically important parameters, particularly in the lesser-studied species. 100g seeds sent from 23 plant species to deep space mimicking environment. Many of them may never sent to space for scientific purposes. After landing, plants are grown with their ground control counterparts in vertical farms and open fields. Moreover, to better understand the long-term effect of Space radiation on plant mutation, we established such a trial where some of the species planned to be on seed to seed trial for F1-F2 generations. Besides that, a SPACESEED TO SCHOOL program is also organized. These findings will contribute to our understanding of space agriculture and its potential for supporting human life on long-term missions, while also providing valuable data for future comparative studies. Besides positive effects on space agriculture this mission could be useful for Earth to breed new varieties to cope future challenges like vertical farm production, extreme weather, over population, etc.

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ELENA LUCIANI

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NutriAxiom Adaptive Nutrition for Psychophysiological Resilience in Closed Ecosystems

In long-duration missions beyond Earth, nutrition is no longer a passive component of survival it becomes an active system of physiological regulation and psychological support. NutriAxiom is a research framework under development that aims to investigate how targeted nutritional strategies can promote psychophysiological resilience in confined, resource-limited environments such as analog habitats and future space missions. The project will design and test short-duration analog missions simulating stressors typical of spaceflight conditions: isolation, sensory monotony, circadian disruption, and limited autonomy. Within these settings, NutriAxiom will explore the impact of nutrition on: Biological adaptation body composition, inflammatory/metabolic markers" Endocrine balance cortisol, melatonin, stress biomarkers" Cognitive performance and emotional stability vigilance, executive function, mood"

Metabolic responses glycemic control, energy expenditure Beyond its functional role, nutrition will be investigated as a psychological anchor: a structured, sensorial and symbolic element that can support emotional well-being, restore rhythms, and reinforce perceived control in closed systems. The project will focus on the use of Mediterranean diet, microdiets, and novel functional foods designed for use in regenerative life support systems and constrained terrestrial scenarios. NutriAxiom is conceived as a platform for future applications in space health, bioregenerative systems, and emergency nutrition. Its integration of metabolic, cognitive and emotional endpoints reflects the need for a truly interdisciplinary approach to food in extreme environments where every meal can influence not just performance, but survival and mental integrity."

TIMO MARTENS

Solar Research - Fraunhofer Institut ISE

Hexagonal modular lunar habitat concept that integrates MELISSA-inspired closed-loop life-support systems

The rapid advancement of lunar exploration necessitates the development of sustainable and modular habitat solutions capable of withstanding the harsh conditions on the lunar surface. The 'Modular Hexagonal Habitat Architecture' project proposes a hexagon-based structural design for extraterrestrial settlements, emphasizing adaptability, scalability, and resilience. By leveraging a hexagonal configuration, the habitat maximizes spatial efficiency while optimizing structural integrity under extreme thermal and radiation conditions. The design integrates advanced life support systems, radiation shielding through layered material compositions, and sustainable energy

solutions, including high-efficiency solar panels and regenerative water systems.

Furthermore, the modular nature of the habitat enables easy transportation and assembly using reusable spacecraft such as SpaceX Starship, allowing for phased construction and future expansion. This presentation will outline the technical considerations behind the hexagonal design, including material selection, structural load distribution, and thermal regulation. Additionally, it will address the logistical framework for habitat deployment and the potential for future adaptations with the MELISSA project

ORSOLYA MEIER

PhD student - University of Debrecen

Research towards a novel biomanufacturing-based blss system using giant reed (arundo donax) as a multifunctional clonal propagated biotech space-plant candidate

O. Meier¹, P. Szatmári¹, L. Utasi², E. Picoli³, Cs. Tóth¹, N. Bákonyi¹, Sz. Kovács¹, J. Koroknai¹, P. Makleit¹, N. Elhawat¹, T. Alshaal¹, Sz. Veres¹, É. Domokos-Szabolcsy¹ and M.G. Fári¹ ¹Department of Applied Plant Biology, University of Debrecen MÉK (Debrecen, Hungary); ²Egreen Farming Solution & Csillagváros Co. Ltd (Budapest, Hungary); ³Universidade Federal de Vicosa (Vicosa-MG, Brasil) The demand for sustained economy generates challenge for basic science and technology in the 21st century. This demanding environment stimulated the rapid development of plant biotechnology utilizing more and more cutting edge science. The data shows the upward trend in Europe toward the generation of new green industries grouped under the biorefinery concept. Developing Bioregenerative Life Support Systems (BLSS) is critical for achieving sustainable human habitation in space, too. The BLSS

is a highly promising way of addressing this limitation, even more so if it can be combined with in situ resource utilization (ISRU). The ISRU approach aims to reduce terrestrial input into a BLSS by using native regoliths and recycled organic waste as primary resources. The combination of BLSS and ISRU may allow sustainable food production on the Moon and Mars. This task poses several challenges, including the effects of partial gravity, the limited availability of oxygen and water, and the self-sustaining management of resources. Lunar and Martian BLSS will, therefore, most likely include plants, which are necessary for food production. In addition, they provide air revitalization and water purification capabilities, and could be used for other functions including, for instance, pharmaceutical production, among many others. Arundo donax is a robust, sterile, clonally propagated decaploid monocot grass species which has been

introduced around the world by humans as an ornamental and economic plant. *Arundo donax* is well known for its ability to adapt to a wide range of marginal environmental conditions from wetlands to the semi-desert, high or low pH, high salinity, halogenated organics or heavy metal contaminated soils, from continental to tropical climate. The mechanism and stability of this wide range of adaptation abilities have great biological potential, especially for the future space agriculture. Giant reed (*Arundo donax* L.) is one of the most well-studied perennial biomass crops because of its high productivity and potential to store carbon. The massive availability of biomass generated by this crop motivates the search for its possible biomanufacture use for the generation of high added-value products through implementing the biorefinery approach. In view of its importance, the University of Debrecen started the scientific foundation of the first Hungarian BLSS and Biomanufacture research program, focusing on giant reed biotechnology in 2009. As part of this initiative, a special biological research and experimental plant cultivation space, the BIODROME Debrecen,

was constructed. According to our best knowledge, *Arundo* has not been included so far in the ISS space plant programs, nor in the plans for Mars and Moon space agriculture research. Despite the fact that *Arundo* is one of the most important model plant species for the terrestrial biomass-based biomanufacture industry and green chemistry. Hence, our research team has launched the 'Terraphon' research programme to investigate giant reed as a novel candidate space plant, exploiting its exceptional multifunctionality in biomanufacturing as the following: *Arundo donax* is a robust, decaploid grass species. The genetic variation within this species is very limited due to the lack of sexual reproduction. The investigation of polymorphism of the DNA of American, Australian, and Eurasian populations revealed a high level of relatedness and pointed to founder effects. However, a wide range of well-adapted ecotypes is known to grow under very different environmental conditions, and the mechanism of these adaptations remains a very exciting scientific question

CO-AUTHOR(S) : This plant can be mass propagated by industrial-scale somatic embryogenesis called Syn-plant technology using synchronized sustained embryogenic callus culture technology, which allows the establishment of mega plantations within a few months. These pl

ALANNAH MESCHEDER

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Pyrolysis of Inedible Biomass as a Tool for Nutrient Recycling in Controlled Environment Agriculture

Long-duration human space missions require a circular approach to resource management. Resupply opportunities will be limited, so every waste stream must be considered a resource. In future food production systems, the continuous demand for plant nutrients and carbon dioxide will be matched by a steady generation of inedible biomass. This research investigates pyrolysis as a method to convert this biomass into biochar a stable, carbon-rich material with potential applications as both a nutrient source and a growing medium. Pyrolysis offers several advantages for space applications: it operates in the absence of combustion, and at temperatures exceeding 500/ °C, it effectively sterilizes the biomass, eliminating pathogens that could pose risks to crew health or crop productivity. To integrate the resulting biochar into controlled environment agriculture (CEA) systems, which predominantly rely on hydroponic or other soilless cultivation methods, this study proposes a steeping

process similar to compost tea production. This approach allows for the extraction of soluble nutrients into a liquid form compatible with CEA nutrient delivery systems. To assess the feasibility of this nutrient recovery method, the chemical composition of the resulting biochar tea will be analyzed and compared to conventional nutrient solutions used in hydroponic CEA setups. The goal is to evaluate whether pyrolysis, in combination with steeping, can contribute meaningfully to nutrient cycling in a bioregenerative life support system (BLSS). Following nutrient extraction, the residual biochar presents further potential as a growing substrate. Possible applications include its use as a soil amendment for regolith drawing on studies involving compost-regolith mixtures or as an additive to existing soilless media. These additional uses support a circular, resource-efficient strategy for organic waste management in food systems, both in space and on Earth.

VERONICA ORLANDI

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Applications of surface-wave plasmas for life-support systems in space

Surface-wave-sustained discharge is a plasma (SWP) that is excited by propagation of electromagnetic surface waves that allows the generation of uniform plasmas in volumes. They were widely used for microbial sterilization, particularly the inactivation of bacteria on surfaces within confined environments (Moisan, Eur. Phys. J. Appl. Phys., 2013). Effective sterilization is important for maintaining the integrity and safety of closed-loop life support systems during long-duration space missions (MELISSA Technical Note 2.8, 2005). Conventional sterilization techniques pose challenges in microgravity environments due to the high temperatures necessary to

achieve sterilization, the limitations and aging of mechanical methods, or the problems with byproducts coming from chemical methods. Drawing from foundational work and recent laboratory advancements, this review examines the physical principles of SWP. These include UV photon emission and reactive species generation to neutralize microorganisms to sterilize surfaces and equipment. The same principles can also be used to eliminate greenhouse gases and purify rare gases, showcasing the versatility of SWPs (Moisan, J. Appl. Phys., 2003). In this comprehensive review, we assess current experimental data on sterilization efficacy, material compatibility, and system

integration and highlight the unique advantages of SWP: low power consumption, ambient-temperature operation, chemical-free processing, and scalability for spacecraft systems. This also

includes how SWP could be integrated into the MELiSSa system, as well as the limits of the method and the future challenges for inclusion in a real-life space system.

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■ **OLAJUMOKE OWOEYE**

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Sustainable Energy Generation through Wastewater: A situation in the Urban City of Lagos, Nigeria

As Nigeria's economic capital and commercial nerve centre, the city of Lagos is undergoing speedy urbanization. With an estimated population of over seventeen million people, Lagos is one of the world's fastest growing cities. One of the prominent natural endowments that has borne the brunt of this rapid expansion is the Lagos Lagoon, a water body that has been used for sewage disposal for more than half a century. The large volume of sewage deposited in the lagoon on a daily basis has escalated due to the rapid growth in the city's population. The thrust of this paper is to explicate the repercussions of wanton sewage disposal into the Lagos Lagoon and to highlight the potential which Lagos has to generate massive energy from sewage waste in order to meet its energy challenges. From ten separate stations across the Lagos Lagoon, water samples were collected and analyzed to ascertain the existence of pathogenic

entities using the techniques of sedimentation, microscopy and culture.

These pH levels and Biochemical Oxygen Demand (BOD) of the samples were tested using the pH meter and BOD test apparatus correspondingly. This paper shows that sewage disposal into the Lagos Lagoon has made the water body ecologically unhealthy for aquatic plants and animals. It has also decreased the visual appearance of the environment. Further, this cruel practice has exposed some persons that come in contact to the lagoon's waters to pathogenic infections. Extant studies have pointed to the fact that sewage waste is a key energy source, with 1 kilogramme of dry faecal sludge having a calorific value of 17.3 millijoule. This paper strongly recommends the dynamic use of faecal sludge to save the Lagos Lagoon from sewage pollution and upscale energy supply in Lagos.

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■ **ROBERTA PARADISO**

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Fava Bean Plant Performance And Fertility Dynamics In Mars Regolith Simulant-Based Substrates For Space Farming

CO-AUTHOR(S) :

■ **MARTA PAREJA BOTO**

Student - Maastricht University

Building a Mars Habitat: Shelter and Shielding.

As many initiatives progress toward planning initial Mars missions, the development of sustainable local habitats is becoming a critical focus. Given the extended duration that astronauts will spend on Mars, these habitats must provide more than just basic shelter; they must address the unique challenges posed by the Martian environment, including harsh radiation which a Maastricht Science Program group previously

studied in January's project period. Addressing these challenges effectively requires collaborative multidisciplinary efforts. Collaboration with other Mars Habitat teams is needed to ensure cohesive integration. In addition, the project aims to generate insight and innovative techniques that could also address terrestrial housing issues.

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■ **BORIS PETROVIC**

Co-Founder - Veganaut INC

VEGANAUT: Methods and Technologies for Plant-Based Space Exploration

Among the inherent challenges of long-duration space missions, addressing astronauts dietary needs is vital. Current approaches relying on pre-packaged meals are limited in variety, shelf life,

and ability to support physiological and mental health over extended periods. This work introduces VEGANAUT's space mission concept centered on plant-based food systems,

emphasizing the utilization of plants as the primary food source for future space crews. VEGANAUT explores the application of a scalable and autonomous food production platform to test and validate plant-based life support technologies in space analog settings. This includes a modular and mobile plant growth system with hydroponic racks, LED lamps, water pump actuators, environmental sensors, and cameras, regulated by an embedded controller integrated with Mixed Reality (MR) and Artificial Intelligence (AI) tools. Moreover, the AI VEGA, VEGANAUT's conversational AI assistant, was developed to empower astronauts and reduce the levels of complexity of tasks associated with food production in space. Pilot tests demonstrate the operational efficacy of the AI-driven platform with minimal

crew interaction and easy integration into closed-loop life support systems. Ongoing work focuses on evaluating platform robustness for analog astronaut training through real-time support, monitoring, and optimization of human-plant-system interactions. This also includes the development of protocols with guidance on plant growth and development, systems operation, food production schedule, risk analysis and mitigation, and decision-making. VEGANAUT's vision is oriented toward the development of reliable food platforms to provide nutritionally complete and psychologically satisfying food sources in larger volumes and longer time scales. This indicates relevant pathways to expand self-sustaining solutions while addressing unique challenges posed by space exploration.

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■ ÁLVARO ROPERO LÓPEZ

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DRAFT: Dynamic Regolith Air Filtration Technology

One of the critical challenges in environmental control and life support systems is the effective removal of volatile organic compounds (VOCs) from a habitat's atmosphere. State of the art air filtration systems, like the Trace Contaminant Control System (TCCS) on the International Space Station, are effective in removing a limited number of potentially harmful gasses, but do not address the majority of the broad spectrum of potential VOCs produced by human activities. Moreover, this system relies on resupplies of consumables including chemical reagents and filter matrices that cannot be easily fabricated in-situ. This study explored a bioregenerative alternative technology, soil air filters,

which have previously been used to great effect for VOC removal in closed environments such as Biosphere 2. This experiment builds on that work by comparing conventional soil air filters with bioremediated lunar regolith simulant-derived soil air filters, reducing the need for resupplies and facilitating self-sustaining lunar settlements by adopting an ISRU approach. Data was collected over the two week ASCLEPIOS V analog astronaut mission in Switzerland, demonstrating the effectiveness of regolith-based soil air filters in a realistic environment.

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■ STEFANIA SABAU

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Comparative crop rhizosphere microbiome function in urban and space agriculture

Introduction Perchlorates are toxic soil and water contaminants originating from munitions, rocket propellants, and agricultural leachate. High concentrations of perchlorates ($\text{Ca}(\text{ClO}_4)_2$, $\text{Mg}(\text{ClO}_4)_2$) have also been detected on Mars. Perchlorates pose human health risks as endocrine disruptors and limit plant growth via impaired nutrient uptake and toxicity. This research explores how plant-rhizosphere interactions can be exploited to develop tolerant crop species with potential to mitigate perchlorate contamination. Methods Rhizosphere functions in tomato (carbohydrates, pathogen resistance), broad bean (protein, nitrogen fixation), and willow (biorefinery, phytoremediation) were explored. Crops were cultivated under regolith (MMS-1, MGS-1, LHS-1) and calcium perchlorate tetrahydrate stress. Root morphology, perchlorate fate, and microbial communities were assessed through 16S rRNA gene amplification and whole metagenome sequencing (WMS). Results All three crops tolerated most regolith types with minor short-term growth effects. Low calcium perchlorate levels (0.05 0.5 mg/pot) had no significant impact. Higher concentrations (5

50 500 mg/pot) and regolith-perchlorate mixes caused stunted growth, reduced fitness, and decreased root mass. Rhizosphere microbiome alpha and beta diversity and species-level differential abundance (WMS) analyses are ongoing. Discussion Insight into plant physiological response gave indication of tolerance and susceptibility to regolith and perchlorate stress. Capturing perchlorate fate via leaching or plant accumulation will inform ecosystem and health risks, and potential reduction by rhizosphere microbiota. Shifts in microbial species supporting stress tolerance or showing functional loss will guide in vitro testing of strains as inoculants for plant growth trials. Conclusion Tomato, bean, and willow are key crops with distinct stress tolerances and microbial associations. Testing and manipulating their rhizosphere activity under regolith simulants and perchlorate contamination will elucidate plant-microbe dynamics, support sustainable agriculture in extreme environments, and aid future space exploration.

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COSIMO SARTI

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Biopotential evaluation of Lunar Regolith for Future Development of Biological Life Support Systems (BLSSs)

Human permanence beyond Low Earth Orbit (LEO), as envisioned by the Artemis program, demands innovative strategies for In-Situ Resource Utilization (ISRU) and efficient Biological Life Support Systems (BLSSs). A major challenge is exploiting lunar regolith, currently inert and lacking biological fertility, for crop production. *Eisenia fetida* earthworms are terrestrial ecosystem engineers that could play a pivotal role in processing lunar regolith and organic waste produced by human activities at the Lunar Base Camp, thereby potentially transforming these materials into a fertile plant growth substrate. Individuals of *E. fetida* were subjected to controlled feeding trials involving the ingestion of hydrated LHS-1 simulant (60% w/w water content). Casts produced during the trials were collected and prepared for metagenomic sequencing (targeting 16S rRNA gene) to identify microbial communities introduced by earthworm gut transit. Earthworms physiological responses were monitored via sequential weighing (pre- and post-ingestion, full and empty gut states) to assess behavioral

adaptations or avoidance mechanisms towards LHS-1. Furthermore, histological analyses using hematoxylin-eosin staining evaluated potential cytotoxic effects on the intestine, investigating signs of tissue damage related to regolith ingestion. Preliminary results showed no significant avoidance behaviors toward regolith ingestion compared to standard soil, indicating the worms' willingness to process the lunar simulant. Histological assessments suggested potential damage to intestinal tissues, although technical artifacts could not be entirely excluded. Microbial analyses, currently ongoing, are expected to confirm the potential for biologically mediated substrate enhancement via gut-associated bacteria. This research represents a step towards sustainable lunar waste management and biological enhancement of extraterrestrial substrates, highlighting the feasibility of integrating earthworms into future BLSS modules to create closed-loop life support systems for long-term human habitation beyond Earth.

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ILANA SCHÜRMEYER

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SpiCy - Combinatory cyanobacterial biofilms produce oxygen under high UV conditions in the stratosphere

The SpiCy (Stratospheric Investigation of Combinatory Cyanobacterial Biofilms) mission explores the potential of biofilm-based cyanobacterial cultures for oxygen generation in extraterrestrial environments. Biological life support systems (BLSS) have gained in popularity for their potential as circular cost- and resource effective alternatives to conventional life support systems. In long-haul space missions, an active oxygen production could decrease cargo mass, rendering oxygen tanks or water tanks for electrolysis obsolete. Cyanobacteria are ancient, highly efficient oxygen producers, contributing approximately 25% of Earth's oxygen today. Their ability to photosynthesize under minimal resource input makes them prime candidates for BLSS. However, the low UV tolerance of most species currently limit their applicability in space. To address this, SpiCy investigates the oxygen production of *Nostoc* sp. in mono- and co-culture with *Escherichia coli* and

Pseudomonas putida during a stratospheric balloon flight, characterized by high levels of ionizing and non-ionizing radiation. Co-cultivation aims to promote biofilm formation, enhancing resilience to abiotic stress and enabling cyanobacterial function in high UV radiation environments. During cultivation and flight, temperature and pressure were kept constant. The stratospheric balloon experiment revealed a significant drop in oxygen production by *Nostoc* sp. in pure culture in the stratosphere at high UV influx, while co-cultures with *E. coli* maintained the ground levels of oxygen production activity. This indicates a potential synergistic effect of heterotrophic-cyanobacterial biofilms in mitigating environmental stress. Our findings support the feasibility of cyanobacterial combinatory biofilms as robust, self-sustaining components in bioregenerative life support systems for long-duration human spaceflight.

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Researches for the production of whole-body-edible (wbe) space peppers towards a biomanufacturing-based blss system

The Bioregenerative Life Support System (BLSS) research is considered a pioneering initiative of the NASA and the Soviet space research programs. The head of NASA's biological program, Dale W. Jenkins wrote the following about the importance and difficulty of the BLSS system in 1966: - Closed ecological life-support systems are one of the most difficult

scientific and engineering tasks in the space program. Manned space flight of long duration requires a complete life-support system able to supply all the oxygen, food, and water; to remove all excess carbon dioxide, water vapour, and human body wastes & In the spacecraft, a human being is confined in a restricted environment where it is necessary to miniaturize a

completely balanced microcosm or closed ecological system. The BIOS-3, the very first BLSS began to be built in 1965 and was completed in 1972 at Krasnoyarsk, in the USSR. In 1987 the European Space Agency (ESA) has been initiated the micro-ecological life support system alternative program (MELISSA). After six decades, the question remains the same. How can be artificially maintain in balance the ecosystem in a closed environment in such a way that it can ensure the living conditions for human existence? If humankind is ever to undertake long-term space missions and colonization, establishing an efficient space farming system would be essential for human survival in space. However, existing crops are not sufficiently cost effective and productive for use on space farms. Relying on plant biotechnology, the Whole-Body-Edible and Elite Plant (WBE) strategy for space crop improvement has to be considered as very promising field. The WBE strategy aims to develop crops with more edible parts, richer nutrient content, higher yields, and higher nutrient use efficiencies for space farms. In view of its importance, the University of Debrecen started the scientific foundation of the first Hungarian BLSS and WBE research program in 2017. As part of this initiative, a special biological research and experimental plant cultivation space, the BIODROME Debrecen was constructed. Within the Solanaceae family peppers can be considered as the most promising vegetable- model for the WBE-concept. This is because the green biomass of potatoes as well as tomatoes are limited for the human consumption due to their extremely high antinutritive content. Peppers (*Capsicum* sp.) are the third most consumed vegetable worldwide. Its importance is enhanced by the considerable biological value of the pepper fruit. This value is not only limited to the vitamin C content. It is exceptionally rich in carotene and vitamin A, and it also contains vitamin K. In the early of thirties of the last century a concentrated puree made from a specially bred sweet pepper cultivar based on special pulping process was one of the first superfoods with considerable high vitamin C and bioflavonoid content. The original version of this concentrate had been created by the Nobel Prize-winner Albert Szent-Györgyi and his colleagues in Hungary. The VITAPRIC served as special food supplement for both, sailors and soldiers as well as it was also very popular worldwide. Pepper has also been targeted by the NASA's Bioregenerative Life Support System program. In the summer of 2021 the first space pepper cultivation experiment was carried out on the ISS station with the NuMex Espanola Improved (NEI) pepper variety. Part of the fruit was consumed by the astronauts

and another part was returned to ground for testing. The green biomass of the NEI chili pepper was discarded. In our investigation we found that the green biomass fresh weight and fruit fresh weight ratio of the determinate type sweet pepper lines were inverse to that of the continuous growth type NEI chili. We also found that the volume utilization efficiency (VUE) of our determinate-type pepper lines grown under controlled conditions is encouraging, and can therefore be recommended for further breeding.

Among others, we use biotechnological methods from the embryo rescue technique to the anther-culture to produce doubled haploids (DH). Our pepper breeding experiments are also complemented by the study of the thigmomorphogenesis, LED lighting research, optimization of nutrient supply, researches on new generation plant biostimulants, as well as optimized utilization of green biomass and the development of alternative methods for sustainable plant protection. The final goal of our research is to produce determinate growing type Whole-Body-Edible Space Pepper cultivars (WBE) especially for the international space research programmes. Our main objectives, therefore are characterized by the followings traits of the pepper: reduced sensitivity to light deficiency, "high volume utilization efficiency," simultaneous flower bud formation, "outstanding self-fertilization potential, and fast fruit development," fruit with high biological value and thin cuticle, "optimal growth dynamics under LED light," suitability for hydroponic cultivation, "balanced development based on optimized nutrient supply," high fruit weight / fruit volume ratio," low anti-nutritive content of leaves," increase the level of special bioactive compounds. The specific objective of our study is to investigate the biochemical values of the fruits and green biomass of dedicated space pepper cultivars including vitamins, protein and macro-microelement content, as well as to study qualitative and quantitative changes of other phytonutrients with a view to their human consumption. The findings of our study could hold valuable insights for the advancement of sustainable food production and resource utilization in BLSS environments with potential applications of specially selected WBE-space pepper cultivars for long-duration space missions and beyond. The research is supported by the Excellence Program announced by the Ministry of Innovation and Technology under the number ED_18-1-2019-0028, within the framework of the Space Research thematic program of the University of Debrecen."

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EVANDROS THEDOSOIOU

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Thermo-Economic analysis of Martian Habitats

In an area of continuous technological advancements, the prospects of colonizing other planets, particularly Mars, has never been more tantalizing. Mars habitats are pseudoisolated systems, with strict requirements of occupancy, mass and energy. The thermoeconomic model mentioned in this abstract provides a versatile tool of comparison between different habitat designs and mission specifications. The lack of resources and the environmental constraints make the study of these types of habitats complex. Hence, the necessity for assumptions and compromises, notably regarding parameters like temperature, humidity, and pressure. This work introduces ARHS [is an Anglicization of a Greek spelling of the word Ares (=Mars)], a novel software tool for thermo-economic analysis of potential habitats at Arcadia Planitia, a location on Mars, providing output reports. The research and analysis of the habitat system are split into three main categories: life support systems (Oxygen/Carbon Dioxide levels), power generation/consumption

(electricity, heating), and human factors (physical/mental limits). The categories are then split into classes; flows, systems, and controllers which make the Object-Oriented Programming (OOP) used by the software more convenient. The aforementioned categorization and classification are vital for the analogous representation and comparison of different mission or habitat inputs. By offering distinct outputs, ARHS enables a straightforward selection of the most fit-for-purpose habitat for the mission or the most suitable mission for the habitat. To ensure the precision and reliability of the code, extensive verification has been conducted, drawing data from analogue astronaut missions and space expeditions. ARHS empowers users to compare inputs and determine the best expedition, in manner of either habitat or mission parameters, based on both cost and resources. This groundbreaking software represents a leap forward in space expeditions, providing reliability, precision, and standards. It simplifies the selection of

missions and habitats based on their features and user requirements. Furthermore, by harnessing machine learning techniques, it can be evolved from a comparison tool to an optimization tool, creating a valuable database for future space expeditions. Looking ahead, the possibilities of this software are

endless, it is intriguing to consider that once the astronauts are selected missions can be tailored to perfection for each member. Finally, the sheer potential of the OOP enables the focus not just in space but also on expeditions here on Earth, from analogue astronaut missions to deep-sea explorations.

ILONA TROADEC

Student - The Spring Institute for Forests on the Moon

Preliminary Design of a Lunar Greenhouse for Sustainable Life Support Systems on the Moon

The long-term presence of humans on the Moon requires the development of sustainable life support systems. Lunar greenhouses may be a low-energy solution to achieve self-sufficiency by producing food and regenerating oxygen. However, the extreme conditions on the Moon high temperature variations, radiation, micro- meteorites, vacuum make the design of such habitats very complex. The objective of this study is to establish a preliminary design of a lunar greenhouse sufficient for crop growth, including material selection and structural analyses, thermal management, light collection strategies, and atmosphere/pressure management. Analysis methods include finite element analysis, thermal simulations,

and simple modeling of atmosphere fluxes at a steady state. The results suggest that lunar greenhouse would be easier to manage under a blanket of 30 centimeters of lunar regolith which could mitigate up to 99% of radiation and limit thermal variations to 2°C. Furthermore, a robust thermal control system may be necessary to ensure survival during the lunar night, but power could be saved by omitting artificial light and allowing plants to hibernate, at the cost of production speed. This study highlights the challenges of using traditional greenhouse methods and natural sunlight for plant production on the lunar surface, and contributes to future development of bioregenerative life support systems.

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Wind effects on root development for space crop production applications Plants develop their architecture in varied, heterogeneous and highly

fluctuating environments such as light, temperature, gravity, wind and water. In order to maintain their development and vertical position, essential to their autotrophy and growth, various active mechanisms are involved in both roots and shoots, enabling plants to orient their growth according to environmental signals. On the International Space Station (ISS), the lack of gravity affects how plants grow and how their roots develop. For example, the stagnant boundary layer that forms around leaves in the absence of free convection slows down heat and mass transfer, depending on the acceleration of gravity and wind speed above the leaf surface. A proposed countermeasure to mitigate these effects of reduced gravity is to provide sufficient wind force to the plants, as optimal gas exchange (CO₂, O₂, water vapor) and heat transfer between the leaves and the environment are dependent on ventilation. Therefore, providing forced convection to the plants might also be necessary on the Lunar or Martian surface, to ensure optimal heat and mass transfer. The objective of this PhD research is to investigate the role of shoot zone wind in root growth and development, and the overall coupling between the aerial and the root systems. The proposed approach is twofold. On one hand, the objective is to improve the mechanistic model based

on mass and energy transfer processes currently under development. The goal is to incorporate wind effects on leaves and roots, by integrating principles of fluid dynamics, heat and mass transfer, and plant physiology. Key questions address how wind-induced forces influence root growth, development, and nutrient uptake, and how they affect shoot root interactions. Computational Fluid Dynamic (CFD) simulations will be used to characterize windflow around the plant canopy under varying velocities and gravity conditions. On the other hand, root imaging and omics analyses will be used to compare aeroponic and traditional systems, to assess morphological and molecular responses on crops such as tomatoes, lettuces, and carrots. The outcomes will inform cultivation strategies for space-based agriculture and support the design of advanced life support systems for long-duration missions. The modeling approach will build upon the expertise developed at the host laboratory, Institut Pascal (University Clermont Auvergne). Experimental validation will be performed in controlled environment chambers within the PIAF laboratory (University Clermont Auvergne), including the EDEN Luna aeroponic facility operated by the German Aerospace Center (DLR).

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