CURRENT AND **FUTURE WAYS** TO CLOSED **LIFE** SUPPORT SYSTEM

MELiSSA CONFERENCE

FULLY VIRTUAL
3-5 NOVEMBER 2020

Organised in collaboration with Ghent University

IN COOPERATION WITH

esa
European Space Agency

MELiSSA
MICRO-ECOLOGICAL LIFE SUPPORT SYSTEM ALTERNATIVE

CREATING A CIRCULAR **FUTURE**

ABSTRACTS AND SPEAKERS

MELiSSA activities in Switzerland

Oliver BOTTA
Swiss Space

Office Life Support Systems are a key technology for human space flight. Traditionally, supplies like oxygen, water and food either have to be brought along on space missions or are resupplied by visiting vehicles. More recently, on the International Space Station ISS, several systems have been introduced that have allowed closing the loop, specifically on CO2 removal and O2 production as well as water recycling. With a perspective for long duration missions to the Moon and Mars, more loops need to be closed, including the carbon and nitrogen loops. In order to achieve this objective, more advanced systems will have to be developed, tested and introduced as operational systems. On the very long term, fully regenerative life support systems will be required. R&D activities in towards this objective include breadboard activities on the ground, ground demonstrators, technology precursors and systems (to be validated in analogue environments and on board the ISS). In addition, research activities in the areas such as plant characterization, genetics, microbial identification and others provide fundamental insights for LSS. Finally yet importantly, such developments and the associated technologies have a high potential to be beneficial for terrestrial applications. The MELiSSA project, implemented through the E3P and other programmes at ESA, provides a framework for European actors to engage further by providing opportunities for concrete projects, but also to enter into national and international collaborations. The project provides a balanced set of activities that include space flight hardware, research and technology developments combined with perspectives for terrestrial applications. Switzerland continues to support the MELiSSA project within the frame of E3P, following the decisions taken at Space19+. The Swiss MELiSSA community is diverse and covers universities, research institutes as well as industry. A recent survey performed by UNIL, EPFL and ESTEE has provided a consolidated view of this landscape. While in the past MELiSSA projects in Switzerland were conducted more or less individually, the survey has shown that the community trends towards a critical mass, and that there is a willingness to engage further and coordinate its activities. This is in line with the objective of the Swiss Space Implementation Plan to foster robust and diverse networks and clusters, which are essential ingredients for innovation.

ECLSS Russian activities

Viacheslav K. ILYIN
IBMP, Moscow

The long-termed space expeditions conditions require creation of mostly confined life support systems for crews in spacecraft with cyclic regeneration of substances in artificial ecosystem, because food supplies will significantly exceed the mass and volume of a closed-loop nutrient system. One of the main conditions for increasing closure factor of life support systems is processing of waste in order to return the maximum proportion of the contained elements to the cycle of substances on board spacecraft. Mandatory components of such a cycle of nutrients should be modules, that perform the functions of producers, consumers and reducers. Various microorganisms fermenting organic waste with the formation of solutions of mineral compounds and biogas can act as reducers. The feasibility of using microbial fuel cells (bio-electrochemical systems) for liquid organic waste fermentation is currently widely debated. In biological life support systems of spacecraft they can also be used to purify wastewater of various origins from organic substances, nitrogen, phosphorus and sulfide. Replacing of conventional bioreactors with cells of biofuel elements, connected in consequence chains, it is possible to achieve a deeper purification of organic waste and their transformation into a mineral form. One of the most important tasks in a closed life support system is to control the flow of nitrogen and nitrogen-containing compounds. As a rule, nitrogen accumulates in the wastewater in the form of ammonium and usually the process of its removal includes sequential nitrification and denitrification. Standard biotechnological treatment methods require large amount of organic matter and energy for oxygen supply (nitrification process) by aeration, and large amount of activated sludge. In the cell of biofuel element, ammonium is removed from the wastewater and nitrified at the cathode without special aeration. Thus, nitrification stage ceases to be limiting in the fermentation of organic waste and the removal of nitrogen from wastewater.

Status and Future plan of JAXA microbial monitoring from ISS and beyond

Toru Shimazu,
Japan Space Forum

We are conducting microbial monitoring in ISS JEM module from 2009 with passive sampling tools. We have to bring back sampled tools to Japan to analyze them, usually 1-2 months after the sampling. For future gateway or further explorations, we could not recover the samples for analysis. So we have started to accommodate on board real time microbial analyzer from air and water. We will present the status and future plan of JAXA microbial monitoring from ISS and beyond ISS.

The Role of Plants as Food and Life Support for Exploration

Ralph FRITSCHE & Lucie Poulet
NASA

PACMAN project: Designing, building and testing the prototype of a Plant Characterization Unit Claudia Quadri, Lorenzo Bucchieri, EnginSoft The Micro-Ecological Life Support System Alternative (MELiSSA) is a European Space Agency (ESA) program, which aims to develop a closed-loop life support system for long-term human space missions, based on the principle of a lake ecosystem. The MELiSSA loop has been structured as an assembly of 4 compartments, created to simplify the behavior of an artificial ecosystem and to allow a deterministic engineering approach. In particular, the higher plant compartment (HPC) has the objective of growing plants for food production, while recycling water and revitalizing air with carbon dioxide absorption and oxygen production. With the objective of a better understanding of plant growth and the exchanges with the environment, a ground based pilot test plant of a Plant Characterization Unit (PCU) has been designed, engineered and manufactured. The scope of the PCU is not food production, but the measurement of most of the parameters that characterize the plant growth which ultimately yield food, produce oxygen and water while absorbing carbon dioxide. Characterization is the keyword of the PaCMAN project. The challenges of the engineering will be addressed, particularly relative to the leak rate requirements. To overcome this critical issue a pressure management with active pressure control has been designed. Minimize the leak rate allows to reach one of the main objectives of the growth tests which is to establish water and gas mass balances in order to characterize the plant behaviour during the photosynthesis and respiration phases. Particular attention was paid to homogeneous environmental conditions both in the root zone and in the chamber in order to have balanced thermal, humidity, light and root uptake conditions for each plant. Furthermore, plant growth parameters can be controlled and monitored in order to ensure an optimal growth environment: state of the art sensors have been tested on a breadboard and included in the hydroponic system to measure and manage the nutrient delivery system in order to gain further understanding of plant modelling.

P, K, Mg, Ca, Na balances in a closed system combining aeroponic lettuce cultivation supplied with grey water

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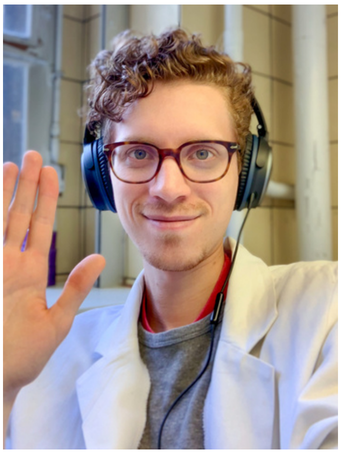
Implementation of closed loop life support system (LSS) in the future extraterrestrial colony is necessity. In most cases it means the need for elements and water recovery from waste streams. Recovered resources will almost always feed the biomass cultivation unit. The presented paper shows a scenario when aeroponic lettuce cultivation is fed with untreated grey water (water used for hygiene purposes, washing, etc.) and complete nutrient solution. Possible use of untreated grey water is worth exploring as in case of promising results it will imply that simplification of treatment process is possible. It was investigated if anionic surfactants present in nutrient solution will influence lettuce composition (in terms of elements: P, K, Mg, Ca, Na) both in edible and inedible parts. This in turn would affect the overall mass balances in the life support system, which was also calculated. Literature data was used in order to estimate the elements balances for the remaining components of the life support system (urine, feces, condensate, nitrification rate etc.). The experiment was carried out in seven aeroponic modules. Six of them were fed with grey water, while one was a control unit. Three different anionic surfactants (Sodium Laureth Sulfate (SLES), Sodium Dodecylbenzene Sulfonate (SDBS), Sodium Methyl Cocoyl Taurate (SMCT)) at two concentrations (high > 1.0 g/L and low ca 0.07 g/L) were tested. The choice of surfactant type and concentration was based on the space literature e.g. Anderson et al., 2018. High levels of surfactants were used to model minimal grey water production on space colony so resulting stream is concentrated. On the contrary, low levels were used to model terrestrial grey water production with higher water use and diluted resulting stream. The experiment lasted 42 days for five modules supplied with SDBS 0.08g/L, SMCT 1.8g/L, SLES 1.70g/L, SLES 0.07 g/L and control unit (clean nutrient solution). In the remaining two units (supplied with SDBS 1.0g/L and SMCT 1.5g/L respectively), all lettuces died on the 19th day of the experiment, thus those units were not taken into account in further consideration. In most tested cultivations mass balances of P and K were generally unimpacted (slight or small differences between reference) by surfactants, while for Ca, Mg, K and Na greater differences appeared. The results were compared to previous studies concerning lettuce cultivation on grey water, although they were conducted mostly in soil and hydroponics. It must be noted that authors provided only composition of lettuce edible parts. In some cases these studies was in compliance, but in other they differ significantly. Taking into account received results on lettuce composition elements recovery rates via urine nitrification were calculated. Generally, ca. 50% of phosphorus, 70% of potassium, 25% of magnesium, 50% of sodium (beside concentrated SLES, which had higher recovery rate 70%). can be recirculated to the aeroponic system.

Lunar Nutritional Grower (LuNG): Assessing the viability of a lunar hydroponic system

Saad RAYEES & Sebastian GARCIA
MSE TU Berlin (with Sebastian Garica)

Optimization of a controlled environment food production unit for space applications Thomas Bartzanas, Agr. University of Athens The lack of fresh food in space, as well as the high cost of transporting it from Earth, is one of the major problems that space agencies and space companies will have to solve in the next few years; this also limits long duration travels and space tourism. The future of space missions and the widespread presence in space require appropriate nutritional provision for space travelers with minimal Earth resupply. This issue is crucial to maintaining optimal nutrient quality and maintaining the mental state of travelers on long journeys, which is largely determined by weight loss, changes in blood, and the effect of radiation on their bodies. In this aspect we are working with AstroPlant, an educational citizen science project with the European Space Agency (ESA) and the research consortium MELiSSA. Our focus is the optimization of its internal micro-environment and the development of optimum fertilization strategies for different crops, in order, through the AstroPlant approach to be able to propose specific cultivation protocols and management strategies not only for space applications but also for terrestrial applications (areas facing extreme climate conditions and urban farming solutions). Initial experimental results and the numerical analysis (computational fluid dynamics) for the heat and mass flows inside AstroPlant for a variety of initial conditions (different lighting, air temperature and humidity) are presented in this paper.



Improved lettuce yield and quality by microbial treatments in vertical farming

Thijs Van Gerrewey
Ghent University

There is an increasing demand for more quality agricultural products with a limited impact on the environment. Taking advantage of positive plant-microbial interactions can be a solution to improve crop quality and robustness, but it has not yet been introduced as a concept in hydroponics. The development of peat-reduced growing media for horticulture is needed to tackle sustainability concerns related to the peat production process. However, little is known about the impact of alternative growing media on rhizosphere microbial communities. An experiment was set up to better understand the relationship between growing media composition, rhizosphere microbial communities, and crop yield and quality. Ten growing media mixtures were composed of 4 raw material groups containing 2 different raw materials each: 60 % v/v Peat (black peat or white peat), 20 % v/v other organics (coir pith or wood fiber), 10 % v/v composted materials (composted bark or green waste compost) and 10 % v/v inorganic materials (perlite or sand). Lettuce (Lactuca sativa) seeds were sown in the growing media mixtures. Plant roots were inoculated with lettuce rhizosphere community extracts collected at 5 different locations in Flanders, Belgium (L1-5). Different yield and quality parameters were determined after harvest. Using principal component analysis (PC) the influence of the growing media mixtures and rhizosphere extracts on the yield and quality parameters was investigated. Results showed significant interaction between growing media and rhizosphere extracts for all parameters tested. Growing media mixtures containing green waste compost significantly increased yield compared to other growing media mixtures. Rhizosphere extract L3 significantly increased yield and LAI compared to control. Rhizosphere extracts L1 and L4 significantly increased total phenolic content compared to control. PC analysis showed that, overall, the tested yield parameters contributed most to the variation in plant yield and quality data. The rhizosphere extract L3 and the growing media mixture containing black peat, wood fiber, green waste compost, and perlite were the biggest drivers for yield. DNA amplicon sequencing of the rhizosphere microbial communities is needed to explain the effects of the rhizosphere extracts on lettuce.

The Role of Plants as Food and Life Support for Exploration

Lucie POULET & Ralph Fritsch
NASA

An in-depth understanding of plant growth and development processes in reduced gravity is required in order to efficiently and effectively grow plants in space during long-duration missions. They have the ability to ensure functions such as food production, air revitalization, and water purification. Here, plant gas exchange modeling is addressed on short-term physical response, mid-term biological response, and long-term growth response, based on a coupled mass and energy balance. An indication of main hypothesis on which the work is based, of the followed methodology and of the used sources The lack of buoyancy-driven convection changes the gas exchange at the leaf surface, which decreases photosynthesis and transpiration rates, and ultimately can translate into reduced biomass production in the long run. The model presented here follows a chemical engineering approach, using the single round leaf assumption, including gravity as an entry parameter. A mass balance with stoichiometric limitations enables the computation of mass exchange fluxes and an energy balance relates them to heat transfer fluxes. Leaf surface temperature and biomass production are subsequently computed. A third-party independent set of parabolic flight data and gas exchange measurements at the leaf and canopy level using an infra-red gas analyzer (Li-6800) in 1g lab conditions are used to validate the model. A presentation of the main results of the paper and of their added value with respect to the substantive knowledge of the addressed matter and / or of literature on it There are threshold values for gravity, ventilation, light, and stomatal conductance, which dictate the magnitude of changes in leaf surface temperature and photosynthesis rate. Predictions on several days of growth in partial g levels using the model show impaired growth response. Plant gas exchange has been extensively modelled in 1g conditions, but it is the first time it is modelled in reduced gravity, to our knowledge.

Can microgreens serve as fresh food in space or are space conditions too harsh?

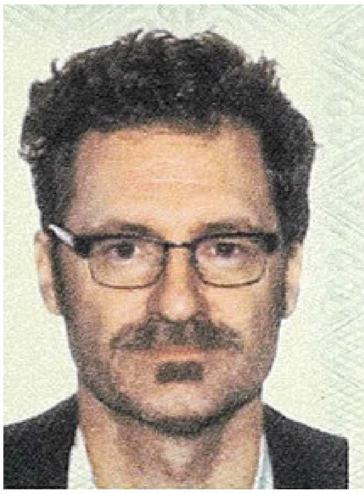
Nele HOREMANS
SCK-CEN

Plant growth and physiology in space have been of increasing interest and concern as the possibilities for long-term manned space flights have increased. In addition to delivering oxygen and water plants can be an essential source of carbohydrates, amino acids, lipids and essential vitamins. Space conditions impose stress on plants on several fronts. Plants are growing in microgravity, in concealed containers, in an atmosphere often enriched in carbon dioxide and the plant hormone ethylene but low in oxygen and they are exposed to enhanced levels of radiation. In general, space conditions alter plant growth and development by disturbing basic metabolic processes including gravity perception , photosynthesis and reproduction. The basic mechanisms leading to these disorders are still not fully elucidated. Within this project the use of microgreens as food for astronauts will be studied. Microgreens are defined as edible seedlings of different plants (vegetables, herbs, or grains, including wild species) and have been shown to contain considerably higher levels of essential vitamins and antioxidants compared to their mature counterparts . Additionally they are small in size, intense in flavour and crisp in texture hence they could form a welcoming change in an astronaut's diet. In this project the hypothesis was tested that space conditions of increased radiation and decreased gravity would adversely affect the growth and nutritional value of microgreen plants. To this extent three different plant species red arrow radish (Raphanus sativus L.), red cabbage (Brassica oleracea L. var. capitate), and opal basil (Ocimum basilicum L.), were selected based on their high nutrient value and grown as microgreens. These microgreens were grown for 7-14 in a hydroponic system during which they were exposed to gamma radiation (15.1 mGy/h) and/or microgravity (~0.1 g) or control conditions. After harvest, proteins, total antioxidative capacity, carbohydrates, lignin and pigment quantity were determined. The results of this study so far showed that the nutritional value of selected microgreens was differently affected by radiation, microgravity or the combination of these factors. The strongest response was found in overall starch levels. Additionally, except for the fresh weight and the proteins, most of the tested parameters showed a plant specific response. In general, it can be stated that the fresh weigh of the plants decreases when they are exposed to gamma radiation and to the combination of gamma radiation and microgravity. The radiation treatment also induced in increase in proteins which is possibly caused by the expression of proteins that function in the stress defence.

Microbial fuel cells with peroxide production for blackwater treatment

Sudeep POPAT
Clemson University

Microbial fuel cells (MFCs) represent a potential technology for producing electricity and/or chemicals from organic waste streams. In MFCs, anode-respiring bacteria (ARB) oxidize simple fatty acids such as acetate, produced from the activity of anaerobic microbiomes, and respire to an electrode. The electrons thus respired move through an external circuit to the cathode, where complete reduction of oxygen leads to power production in the system. The cathode reaction can be tailored to produce hydrogen peroxide by catalyzing only partial reduction of oxygen. MFCs with peroxide could potentially be attractive technology of treating high-strength wastes at remote locations to produce peroxide to use for applications such a greywater treatment, disinfection, or cleaning. One possible application of such MFCs is for space missions, to couple blackwater treatment with peroxide production. In this presentation, I will present work that we have done in improving the efficiency of both the anode and cathode reactions. First, at the anode, we have compared feeding fecal sludge (without addition of urine) and blackwater (with addition of urine) in terms of resulting current density and coulombic efficiency. We have shown that current densities up to 2 A/m2 are possible when feeding complex waste such as primary sludge modified to result in concentrations of chemical oxygen demand (COD) and ammonia that are similar to the two waste streams of interest. Notably, for fecal sludge simulant primary sludge, a significant fraction of COD was diverted to methane (>20%), while for blackwater simulant primary sludge, COD conversion to methane was limited to <5%, suggesting that higher ammonia concentrations in the later likely inhibit methanogenesis. Gene expression data for the mcrA gene in methanogens confirmed the inhibition. In the absence of methanogenesis, diversion of H2 produced from fermentation was likely diverted to acetate and then electrical current through syntrophic partnership between homoacetogens and ARB. Gene expression data for the fthts gene in homoacetogens confirmed significant homoacetogenic activity. Second, at the cathode, we have optimized peroxide production through optimizing electrode structure and electrolyte conditions. We have shown that higher loading of carbon electrocatalyst on cathodes leads to lower peroxide production, likely due to slowed peroxide diffusion within the electrocatalyst layer when carbon loadings are high. We used X-ray computed tomography to image the structure of the electrocatalyst layers and confirmed that there is a significant reduction in porosity when electrocatalyst loading is increased beyond 1.5 mg/cm2. We have also shown that electrolyte pH has a significant impact on the efficiency of peroxide production, with a non-linear relationship between pH and efficiency. Overall, our results lay a platform for further development of MFCs with peroxide production for treatment of organic waste streams with concurrent peroxide production, with one attractive application being blackwater stream during space missions.



Microbes in Hydroponic Crop Cultivation in Space

Danny GEELEN
Ghent University

Recycling technology is essential for long term human manned space exploitation and it is becoming a must for creating a sustainable agriculture here on earth. Our contribution to closing the Melissa loop is to address the cultivation of crops as a food source. Crop cultivation in space meets many challenges. Crops consume light, water, carbon dioxide and a range of minerals. These elements need to be provided at an apropriate balance, adjusted in accordance of the life cycle of the crop. In addition, crops need to be protected from pests that in space most likely originate from bacterial, fungal or viral disease. The main goal of our research is to create microbiological diversity contributing to plant health and its resistance to diseaseuse and contributes to the efficient use of organic fertiliser. Several research projects have been completed to address our objective. A closed gully was build with a closed hydroponic nutrient solution system to allow the investigation of microbial growth at the root level and to perform root shoot mass balance analyses. Our current project focusses on the use of urine derived fertilisers and associated microbiomes of the rhizosphere. A largely untapped source of potential plant nutrients is urine, which contains phosphorous and nitrogen-based compounds that are common plant fertilizers. Urine derivative nutrient preparations were added to rain water and used to grow hydroponic greenhouse lettuce. The rhizo-communities of the plants grown with these nutrient preparations were analyzed for differences in community composition, differential abundance compared to the control samples, differential abundance explained by horticultural metrics and leaf compound concentrations, indicator species, network properties and network hub-taxa.

Seed orientation affects seedling development in hardware for experiments in space

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ADry seeds are used as initial material for most of the experiments aimed to investigate effects of space factors on plant growth and crop production. During pre-flight setup of the experiments, seeds are placed dry in the hardware, and the processes of plant growth in space usually starts by adding water to initiate seed germination. During seed germination in microgravity conditions, initial direction of the root tip development is critical to achieve root establishment into the compartment designed for root growth. The correct direction of root growth ultimately affects final morphology and positioning of the whole seedling. WAPS (Water Across the Plant Systems) is an experiment funded by ESA to be performed in the BIOLAB payload on ISS. The project aims to disentangle direct from indirect effects of microgravity on plant growth. Dry seeds of Vigna radiata (azuki bean) will be placed in a growth chamber before flight, launched to ISS and inserted into BIOLAB. At experiment activation water will be injected to initiate seed germination. At this stage, for the success of the experiment, it is critical that the radicle develops into the root compartment and the hypocotyl and the stem into the shoot compartment. To achieve this goal, WAPS science teams have performed several specific experiments aimed to deepen the knowledge on dynamic processes of seedling morphology at the early stage of development. The scientific hypothesis was that, during the early stage of seed germination, radicle development is oriented according to the embryo axis and that root tropisms occur at a later stage. Research activities started with an accurate analysis of the seed morphology and embryo shape within the seed. Subsequently, an experiment was performed to compare the orientation of the radicle protruding from seeds placed to germinate with the embryo in different positions. Data showed that seed orientation affected early root growth and direction. At the stage of radicle protrusion and elongation, root direction resulted to be the same as that of the embryo axis; the phenomenon was independent from seed orientation in relation to the gravity vector. Therefore, data showed that radicle protrusion occurs with no gravitropic response. At a later stage the root tip orientation changed according to a clear positive gravitropism. Considering that the scientific hypothesis was confirmed, results were used to identify two seed positions as best to facilitate root growth into the WAPS Root Compartment.

In-Situ resources bio-utilisation for Life Support Systems (REBUS)

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The theme of bioregenerative life support systems (BLSSs) and space exploration is one of the key elements of the European Commission's Horizon 2020 Agenda; the Global Exploration Roadmap defined by the International Space Exploration Coordination Group, as well as the exploration roadmaps of the Italian and European space agencies. The major national space agencies agree on defining the Moon and Mars as important objectives of human space exploration for the next two decades. Almost 60 years after Yuri Gagarin's historic flight, we know that humans can survive in space, but the challenge to face nowadays is to guarantee him a long-term stay. As mission objectives move away from Low Earth Orbit, BLSSs development becomes essential: these systems must be able to regenerate the necessary resources for the crew during space missions, offering adequate living conditions and minimizing the need for supply from Earth. The theme of bioregenerative environmental control and LSSs is strongly connected to traditional sectors such as agriculture and civil engineering, for which it has a high potential to transfer the knowledge and technologies developed for Space, with significant impacts regarding environmental sustainability, resource optimization and energy efficiency. Today, these traditional themes are accompanied by that of circular economy, which is an economic model designed to be self-regenerated: biological materials must be returned to the production cycle and those of technical origin must be designed to deliver the highest value before disposal. The ReBUS project, intends to launch a national research line for the creation of a BLSS in Space, based on the integration of different organisms (higher plants, fungi, bacteria, cyanobacteria, insects). The research aims at minimizing the use of exogenous resources while maximizing both the use of the in situ available resources (i.e. Lunar and Martian soils, water, and gas present in the atmosphere) and the recycling of the organic matter produced in the system itself (e.g. crop residues, crew physiological waste). ENEA, CNR, Istituto Superiore di Sanità (ISS), Thales Alenia Space, Kayser Italia, Telespazio, University of Tor Vergata, University of Pavia and the University of Naples Federico II participate in the activities of the project which is funded and coordinated by the Italian Space Agency (ASI). Stefania De Pascale from the Department of Agricultural Sciences of the University of Naples Federico II is the project scientific coordinator of the project.

Hydroponic nutrient solution monitoring for crop characterization

Øyvind MEJDELL JAKOBSEN
CIRIS, NTNU Social Research

Aiming towards crop-based, regenerative life support systems for human space exploration, there is a need to characterize, understand and model plant growth and metabolism. Scientific studies call for closed-loop research facilities offering a high degree of monitoring and control. In this context, the European Space Agency initiated the development of a Plant Characterisation Unit (PCU), a hydroponic research facility with closed atmospheric and liquid compartments. The PCU aims to characterize crops such as lettuce, wheat, soybean and potato with respect to O2 and CO2 production and consumption, nutrient consumption and biomass production, based on complete life cycle experiments. Additionally, activities to upgrade the crop-cultivation chamber of the MELiSSA Pilot Plant (MPP) were recently initiated, aiming at improving its capabilities and performance by capitalizing on the PCU development. A hydroponic sub-system requires real-time monitoring and subsequent control. This presentation summarizes identified monitoring requirements and the attempted fulfillment of these, given boundary conditions such as hardware budgets and facility objectives. The work presented includes selection and testing of different analytical technologies and strategies, covering a wide range of parameters including electrical conductivity, pH, temperature, dissolved O2, dissolved CO2 and multiple macro- and micronutrients. Experiments included assessment of sensor stability, selectivity and accuracy, in addition to long-term monitoring of a recirculating hydroponic facility under realistic conditions. Results allowed identification of sensor sets within limited budgets for precise monitoring of the nutrient solution pH and conductivity, in addition to dissolved oxygen and carbon dioxide by optical sensors. Additionally, evaluation of different technologies real-time measurements of macronutrient and some micronutrient concentrations resulted in implementation of selected hardware for nutrient monitoring, including an optical NO3- sensor with promising accuracy, stability and selectivity. Ion specific electrodes demonstrated potentials of reasonably priced sensor systems for real-time monitoring of nutrients, especially when accompanied by automatic recalibration. However, results also illustrate challenges for long-term use, such as drift, selectivity and stability, with biofouling adding complications upon continuous long-term use. The presentation will summarize the analytical hardware implemented in the hydroponic loops of the PCU and the crop-cultivation chamber of MPP, in addition to results and experiences from sensor testing. Although the presented work includes a limited number of technologies, sensors and repetitions, the experiences illustrate pros and cons of different analytical possibilities, typical challenges and observed potentials, as contributions towards improved monitoring and control of tomorrow's hydroponic systems for both Space and Earth.

EBIOS: an approach to build bioregenerative life suppost sytem for terrestrial and planetary application

Barbara BELVISI

Abstract 1

Life Support Systems (LSS) have been designed historically for short duration missions, but longer stays will require to maximize the degree of self-sufficiency of the life support system while maintaining an Earth-like living environment. In the context of Interstellar Lab concept EBIOS (Experimental BIOregenerative Station), the first space-inspired village on Earth, a Bioregenerative Life Support System (BLSS) has been designed to sustain life in an enclosed environment. A downscale prototype has been envisioned to test and validate the system before the construction and the set-up of the first village in California. The BLSS consists of four compartments: habitat, food production unit, biological water recycling unit and a waste treatment system. It has been designed to reach high closure for air, water and food loops. The objective of this work is to find the equilibrium of the material flows between the compartments by using a model based on a system of stoichiometric equations (Mass balances for a biological life support system simulation model. T. Volk and J.D. Rummel). These mass balances describe the humans and plants metabolisms as well as waste recycling, which has been modified to match the process of the selected waste technologies. The result is the estimation of the consumables flow rates in order to design and size the system. However, the aim of the model is not to allow for active control of the system, and it will be upgraded with a new dynamic one fed with experimental data. Concerning the food production, a crop selection has been performed to fulfill the energy and nutrients daily requirements along with the minimization of the cultivated surface and the maximization of crop diversity. The result of this selection, in terms of food composition, affects the balance of the system. Furthermore, the food production and storage are not anymore constant when the crop-growth is included into the model. By using the steady-state model as a base, a daily estimation of the imbalances of the main streams is computed according to the stage of development of the plants. The first results from the model show that the oxygen and carbon dioxide levels for humans and plants are similar to values in literature. A resupply of oxygen is expected during the initial phase until part of the plants reaches maturity. During stationary operating conditions, the levels of consumables are foreseen to oscillate around the equilibrium. In order to minimize the amplitude of these oscillations, physical-chemical (P/C) processors will be necessary.

Abstract 2

Closed ecological life support systems (CELSS) are gaining more and more attention thanks to recent developments in the domain of space exploration as well as terrestrial applications in the light of fast climatic changes due to anthropogenic activity. Although, CELSS have proven to be feasible both theoretically and experimentally, there is a large room for improvement. Many decades of investigations conducted by different teams around the world showed that ecological life support systems based on biological elements, such as humans and higher plants, are highly promising to maintain self-sustained existence (e.g., Biosphere-2; BIOS-3; etc.). This project is devoted to the development of a CELSS with fully controlled environment for construction of bioregenerative villages on Earth, as well as for future manned space missions, in particular Martian and Lunar, that involve extraterrestrial settlements. EBIOS-1 (Experimental BIOregenerative Station), is planned to be constructed on the territory of the Mojave desert: a hostile environment with extremely small annual rainfall and highest temperatures on the North American continent. Four major system elements of EBIOS-1 are: food production, waste management, water recycling and air revitalization systems. Before construction of a full-scale village, installation of a test prototype is required in order to experimentally refine and stabilize the system. The main goal of this work is to develop a food production technique based on higher plants as a major element of the closed system. Three main objectives were targeted to reach this goal. First, to ensure the delivery of sufficient amounts of biologically significant elements to all crew members. Second, ensure the ample diversity of food based on psychological aspects. Finally, to optimize the surface allocated for crops growth taking into account both mass limits and costs of potential missions. In order to select crops that fulfill these objectives, we developed an algorithm based on certain parameters. The latter include averaged daily requirements for all biologically significant elements intake, recommended serving sizes and frequencies for each crop as well as for amount of daily food consumption per person (FAO/WHO/USDA/EFSA databases). Furthermore, the algorithm aims to increase the diversity of selected crops for maximum diverse menu choices, while minimizing allocated surface for each crop. Our approach is to combine two currently widely used agricultural techniques: traditional soil- based crop cultivation and vertical farming based on modern aeroponic techniques. The latter is designed to maximize the efficiency of every crop by increasing its yield per allocated area, while decreasing the duration of growth phases. The system setup is based on biophysical properties of selected plants (such as ambient temperature, nutrient solution composition, photoperiod, light spectra, CO_2 level, etc.), accompanied by monitoring and control units, enabling real-time system adjustment. Greenhouse for soil cultures is designed to grow fruit trees and those crops, whose efficiency is proved to be reduced when using soilless techniques. The controlled environment inside the greenhouse aims to maintain tropical forest conditions. At the same time, it plays an important role of a natural garden.

Development status of the nutrient delivery system of PFPU, Precursor of a microgravity Food Production Unit

Giorgio BOSCHERI
Thales Alenia Space

Development, implementation and operations of food production system for microgravity applications present multiple technical challenges that include the food safety, microbial population management, humidity management, nutrient delivery management, extracting and/or injecting gases from/to liquids. Thus, considering the number of issues and their respective criticality, the cost-conscious development of a food complement production unit for space application requires a step-by step approach based on a modular technological demonstrator. PFPU is a study of a modular food complement production unit demonstrator, aiming at a statistically representative production of edible tuberous plants in micro-gravity. The study is performed within the MELiSSA framework under contract with the European Space Agency, carried on by an Italian consortium led by Thales Alenia Space Italia. The PFPU key subsystems have been designed, built and are being tested. We herein present the nutrient delivery system technologies under development as well as selected COTS, reporting the key findings of the test campaign.

Microgravity mimetics on the development of multifunctional bioreactors systems for efficient cell growth

Ayşe KÖSE
Ege University Bioengineering Department

Living cells are highly sensitive and responsive to the rapid changes in their living environments. The rapid stimuli make them the produce tremendous responses for their survival and viability. With the advances on understanding behavioral models of organisms, it is getting clearer to develop sustainable utilities with the aid of microorganisms. Recently It is understood that cells response to low shear stress and microgravity like environment is changing with respect to classical bioreactor cultivations. Lowering the fluid dynamics regulates the microalgal physiology, gene expression and even the percentage of valuable molecules produced intra- and extracellular level. In this study we designed a novel bioreactor (LSB-R) with dual horizontal blades to create a low shear rate environment mimicked by the idea of cells response to microgravity environment. The major obstacles studies are the lack of control and the constant production of bioreactors, which are currently commercially produced and have similar characteristics, due to their low working volume and rotating body. LSB-R is thought to be able to overcome the problems of bioreactors that provide low shear stress. In this way, it is aimed to obtain a product which can reach a wide commercial range and to create a new field for academic studies. The fluid flow regime and hydrodynamic stress inside LSB-R was extremely mild. The average shear stress went up to 0.8 Pa for LSB-R however almost 100-fold increase compared to conventional bioreactor systems. The counter wise rotating blades created microgravity like zone in the center of the bioreactor. The velocity vectors revealed that there were almost no fluid movement was observed in the bioreactor center. The cells were hanging in the center with the effect of velocity vectors however, cells could reach the gas and nutrients efficiently due to the fluid flow inside the whole bioreactor. The effects of the LSB-R have been shown on cell wall and motility mutants of microalgae Chlamydomonas reinhardtii with preliminary pilot study. Cell survival rates of microalgae protoplasts increased 2-3-fold compared to classical cultivation. The results indicate that; LSB-R design has great advantage on lowering shear stress, eliminating excessive hydrodynamic forces, providing mild operation conditions with control apparatus and ease on scale up. Another study has been done to see the potential of cells to develop spheroids from HepG2 hepatocarcinoma cell line for spheroid formation and in vitro drug testing applications. The results show that LSB-R can be utilized for microgravity experiments for animal cell cultures, too. The size of the spheroids was 100±5 µm and the extended cell growth period was also observed. The novel design, LSB-R is capable and flexible for the means of growth and development of microorganisms and animal cell culture. The ease in the scale up and control is advantageous, and LSB-R could be implemented for outer space microorganism cultivation experiments for pharmaceuticals, drug testing, cell growth and behavior, and edible biomass production.

PRIAM: A compact intensified artificial light photobioreactor adapted to life support for human space exploration

Charlene Thobie
Brochier Technologies

For a life support for human space exploration, photobioreactors can answer the problem of the atmosphere regeneration because it produces oxygen and fixes CO2. But it also be used to the food production, if the micro-organism cultivated is edible and to the liquid waste treatment. One of the main constraints to cultivate microorganisms in space is that it is necessary to produce sufficient food in a restricted place. In recent years, photosynthetic microorganisms have tended to become a high-potential "biotechnological offer" in many sectors of activity. Their production, however, requires the development of specific technologies culture providing the conditions necessary for the photosynthetic growth [1]. It is in this context that PRIAM has been developed to achieve breakthrough performance compared to existing systems. PRIAM integrates Lightex® technology - Brochier technologies, based on woven side emitting optical fibers, which allows to design a plane multilayer photobioreactor with internal volumetric illumination. It was designed by integrating the latest scientific developments in photobioreactor engineering, while meeting the specific expectations of a biotechnological development of microalgae, with a constant productivity, and satisfying high constraints of control and robustness. The concept of PRIAM technology which aims at the controlled production of photosynthetic microorganisms, while having a high productivity (3.8kg/m3/day of dry biomass) by strongly decreasing the volume of culture per unit of illuminated surface (as = 500 m-1) is particularly suitable for the industrial production of molecules with high added value or thanks to its compactness and high volume productivity can respond to one of the issues of the life support for human space exploration. So, PRIAM technology could be adapted to edible biomass production in space. This technology by optimizing light spectra can also be used to induce physiological forcing for the production of metabolites of interest to produce a biomass quality. Algolight, a start-up, will manage this technology to meet the needs of the market to produce microalgae in a controlled and intensified way. This presentation highlights the applications using Lightex® technology and the potential of the PRIAM photobioreactor for the production of algae in a controlled environment but also the influence of low culture thickness and high biomass concentration on hydrodynamics, gas-liquid mass transfer and biofilm development. It has indeed taken many studies to achieve better control of these different points to obtain a very high-volume productivity in PRIAM.

Design of a module for cultivation of tuberous plants in space: the PROJECT “PRECURSOR OF FOOD PRODUCTION UNIT” (PFPU)

Roberta PARADISO
University of Naples Federico II

The tuberous species potato (Solanum tuberosum L.) is a candidate crop for space cultivation in Bioregenerative Life-Support Systems (BLSSs), based on technical and dietary criteria, including environmental requirements, yield potential and nutritional value. Plant growth, yield and quality are very dependent on physical and chemical characteristics of the root environment, as a consequence providing favourable conditions to the root system is essential to support a successful cultivation. However, unique growing procedures are needed to effectively cultivate plants in Space, where reduced gravity alters the liquid and gas behaviour, making critical the distribution of water, oxygen and nutrients and the control of moisture in the plant root zone, as well as the water flow within the plant and between the plant and the surrounding environment. The root growth of tuberous plants in the most common hydroponic systems (e.g. nutrient film technique) can be limited because of the water logging and the poor root aeration. However, for microgravity application, separating root and tuber zone is difficult to realize, due to difficulties of water containment and lack of density-driven separation of the liquid and gas phases. As a consequence, cultivation of tuberous plants in microgravity will require alternative systems based on the selection of suitable inert substrates, the development of a no-mixed phase system and the design of specific nutrient delivery systems. The ESA MELiSSA project “Precursor of Food Production Unit” (PFPU) aims to design a modular system for cultivation of edible tuberous crops, for food complement production in microgravity. Specifically, the objective is to realize a demonstrator to be preliminary tested in ground conditions, in the view of successive Space application in microgravity, on board of the International Space Station (ISS). The PFPU demonstrator consists of three key modules: the Root Module, the Nutrient Module and the Microbial Contamination Control Module. Among these, the Root Module (RM) is the module physically hosting the plant, accommodating roots and tubers, assuring the separation between the root zone and the aerial zone, and providing the capability to measure the environmental conditions in the rhyzosphere (e.g. temperature, moisture). The aim of the presentation is to describe the step-by-step procedure adopted to realize the RM, including the design, the building and the ground testing of its prototype. Following a step-by-step approach, a sequence of tests was performed as follow: - Phase 1 (Laboratory): Substrates hydrological characterization, aimed to investigate the hydrological behaviour of the proposed materials; - Phase 2 (Laboratory): Sensors calibration and Water distribution system set-up, aimed respectively to calibrate moisture sensors and to test the designed porous tubes system for distribution of water and nutrient solution in the selected substrate; - Phase 3 (Growth chamber): Tuber seeds germination and plant growth tests, aimed to identify the best substrate for potato plants during the early phases of development (tuber sprouting and seedling establishment), and to verify the plant ability to complete a tuber-to-tuber cycle in the proposed system layout.

The SEMiLLA Platform as a means to create environments to develop the Circular Economy concept

Clara PLATA RIOS
SEMiLLA IPStar

Circular Economy (CE) is a new paradigm where the traditional 'take, make, waste' production and consumption model turns into another where three principles drive the action: design out waste and pollution, keep products and materials in use, and regenerate natural systems. This new model means reaching new ways of thinking, the development of new technologies and the involvement of the whole society to have success. MELiSSA space research has developed program's driving elements are recovering of food, water and oxygen from organic waste carbon dioxide and minerals, using light as source of energy to promote biological photosynthesis. It is an assembly of processes aiming at a full total conversionrecovery of the organic wastes and CO2 to produce oxygen, water and food. That means the development of many technologiesmany technologies have been developed that have a direct application in the framework of the CE principles. SEMiLLA IPStar was established in 2005 as the technology transfer partner and member of the MELiSSA space research program. SEMiLLA is mandated to identify opportunities for MELiSSA circular technologies on Spaceship Earth whichEarth, which is in direct need of circular solutions to preventfor optimal resource exploitationmanagementwhere perhaps the need is even more urgent than in space. With this aim, the SEMiLLA platform was created to pursue the following objectives:
- Research:
o To Develop joint R&D projects to bring space tech toadvance terrestrial systems by space tech transferapplications.
o To Find new ways of using existing technologies and develop new novel technologies that can help fostering CE. - Innovation & Terrestrial implementation: o Develop and spread new circular business models.
o Raise understanding howthat organizations could benefit from becoming a node in a loop system.
o Help organizations adopt CE principles.
- Talent: o Identify skills needed in the CE framework.
o Prepare young professionals for the new jobs that will be created in the CE framework.
o Push young researchers, students and entrepreneurs to work in the CE frameworkield.

Mission to Mars inspires food project in the Democratic Republic of the Congo

Felice MASTROLEO
SCK-CEN

In the frame of long-haul manned space exploration mission, the European Space Agency together with academic and industrial partners are developing a high tech recycling system that will enable the production of fresh food, water and oxygen from organic and inorganic wastes produced during the mission. The system is based on the joint work of higher plants and microoragnisms inhabiting bioreactors. Among the latter, the cyanobacterium Limnospira indica a.k.a. ‘Spirulina’ will be specifically used for oxygen and super food production. Other important aspects of the MELiSSA project are the benefits for Earth applications and the education of young people. In that purpose, SCK•CEN, one of the founding members of the MELiSSA consortium, set up with a local NGO in D.R. Congo the INSPIRATION project. With INSPIRATION, it is aimed to combat malnutrition in Congo by promoting and supporting local production of Spirulina. This type of cyanobacterium is rich in proteins, vitamins, essential fatty acids and minerals and therefore ideal as a food supplement. Moreover, Spirulina is fairly easy to grow in open ponds. This project includes a joined educational program aiming to train local university researchers, and to experiment alternative resources utilization for the production of Spirulina. As an example, the use of struvite, a cheap by-product of waste water treatment, is being tested as alternative nitrogen and phosphorus source. Altogether, the INSPIRATION project aimed to promote and support a circular economy approach meaning creating a framework for an economy that is restorative and regenerative by design. During this presentation, we will share our experience in transforming the high tech lab experience into low tech field applications and education that can be useful in Equatorial Africa.

Investigating volatile fatty acids conversion to CO_2 by the MELiSSA bacterium Rhodospirillum rubrum in various culture conditions

Felice MASTROLEO
SCK-CEN

The second compartment (C2) of the ESA's Micro-Ecological Life Support System Alternative project was designed to mainly process the volatile fatty acids (VFAs) coming from the first compartment but also to produce CO_2 from the related catabolic process. Removing VFAs from the water stream is crucial to ensure a good rate of nitrification in the third compartment while CO_2 supply is needed for the fourth compartment where higher plants and cyanobacteria are grown. C2 is inhabited by Rhodospirillum rubrum, a purple proteobacterium that shows highly versatile metabolic capabilities but so far, the net production of CO_2 in MELiSSA related conditions, meaning acetate, propionate and butyrate degradation in anaerobic conditions with light as energy source, was not reported. In this work, we set up a simple and robust protocol for batch cultivation of R. rubrum in anaerobic conditions with 80 µmol/m2*s halogen illumination, and allowing CO_2 production follow-up using a standardized colorimetric method. Our preliminary results put forward a net production of CO_2 in light anaerobic conditions when acetate was used a sole carbon source. Surprisingly a similar net production of CO_2 was also measured when using a mixture (9:1) of acetate and propionate (124mM net C concentration) while photo-assimilation of propionate was previously reported to be dependent on CO_2 fixation. Ongoing work includes the introduction of butyrate as extra carbon source and follow-up of VFAs consumption using ion exchange chromatography. In addition, possible improvement of net CO_2 production using microaerophilic conditions is also envisaged. These new results will be summarized during this presentation.

IGLUNA 2020 - A Space Habitat - MELiSSA POMP Team - Cyanobacteria and higher plant production on recycled urine

Grace MARGARET CRAIN
ETH Zurich Group of Plant Nutrition

"IGLUNA 2020 is a project coordinated by the Swiss Space Center which aims to stimulate student education and exchange through an international and multidisciplinary project. The purpose is to demonstrate technologies to sustain life in an extreme environment for a space habitat. The project started in September 2019 and shall be completed with a Field Campaign and exhibition at the VERKEHRSHAUS – Swiss Museum of Transport and on the Pilatus Mountain in Lucerne, from the 10th to the 19th July 2020. MELiSSA (Micro-Ecological Life Support System Alternative) is the European programme for the regenerative life support systems. It was established to gain knowledge on regenerative systems, aiming at the highest degree of efficiency, reliability, and autonomy and consequently to produce food, water and oxygen from the metabolic waste streams and crop/kitchen produced by the crew. The MELiSSA Foundation is a non-profit organisation created by the MELiSSA partners to harmonise the research done by the MELiSSA PhDs and postdocs. Although the PhDs and postdocs are focusing their work on different parts of the MELiSSA Loop, the IGLUNA 2020 project brought six of them together in joining forces and demonstrating the potential of the MELiSSA technologies for sustaining life in extreme habitats. The MELiSSA-IGLUNA team mission is based on the creation of a complete recycling system for long-term space missions focused on the biological conversion of human urine for food and bio-based oxygen production. The MELiSSA inspired closed-loop presented in IGLUNA consists of four compartments: i) a Microbial Fuel Cell (MFC): oxidation and conversion of organic compounds into electrical current, and CO2, ii) a Membrane Aerated Nitrification Reactor (MANR): oxidation of ammonia into nitrate, which is less toxic and volatile compared to ammonia, by biological nitrification, in a microgravity compatible bioreactor, iii) a) a Photobioreactor (PBR): production of cyanobacteria (Limnospira) and O2 from the nitrate-based solution produced by the MANR, and in parallel b) a Hydroponic Plant Production Unit: production of higher plants and O2 from the nitrate-based solution produced by the MANR. The project is supported by sponsors contributing hardware, supervision, logistics or funds. The visitors of the IGLUNA2020 exhibition will become familiar with regenerative life support systems and their potential for Terrestrial Applications. This joined initiative will aid in demonstrating the viability of linked visibility for the MELiSSA technologies for life support systems, but also for terrestrial applications. Keywords: Life Support Systems, Education, Citizen science, IGLUNA2020."

The MELiSSA Foundation and the future of ESA sponsored LSS research via the Pool ofMELiSSA PhD (POMP project)

Max MERGEAY
MELiSSA Foundation

Since 2015, the MELiSSA Foundation sponsors doctorates (Ph.D.) in science and engineering to prepare the next generations of MELiSSA scientists and to promote mobility and cross-fertilization between the MELiSSA community. The POMP project is mainly supported by the countries directly involved in the MELiSSA project (including Belgium, Italy, Spain and Switzerland), but also by some other countries interested in the project such as Romania and the United Kingdom. The doctorates have a duration of 4 years with typically 1 year to be spent in one of the MELiSSA partner institutes or organizations. This is a major and quite specific feature of the POMP project. In the same spirit of integration in the MELiSSA community, postdoc fellows have to spend 3 to 6 months with another MELiSSA partner. In this respect, the MELiSSA pilot plant in Barcelona has a central place. Cofounding of the PhD projects did also occur via the host universities. By acting so, the MELiSSA POMP program facilitates the interactions between universities, research centers, industrial partners and technology implementers associated with the MELiSSA community with a permanent focus on the preparation of space flight experiments and on terrestrial applications strongly focusing on the global trend and transition to a circular economy. At the moment, the MELiSSA Foundation supports (or has supported) 3 students and 1 postdoc in the POMP 1 project and 5 students and 1 Postdoc in the POMP 2 project (since 2018). Negotiations about the preparation of a POMP 3 project to start in 2020/21 are in progress. The POMP program is a stabilizing factor to the MELiSSA R&D efforts, by easing out the gap caused by the 3 years ministerial cycles and the average 12 months period required for actual contract implementation. Integration of young scientists is also facilitated by the organization of MELiSSA Summer universities (MSU) every 2 years, where other Ph.D. students, not directly supported by the MELiSSA Foundation, are also invited and are actively interacting with the POMP fellows. The MELiSSA Foundation gives also much attention to the scientific production of the whole MELiSSA community and regularly updates the list of peer-reviewed publications, book chapters, that are produced by the MELiSSA community. Young scientists emerging from the POMP programs begin to bring their valuable contribution to this publication list. Besides, by coordinating the communication efforts upon the instructions of the MELiSSA consortium, the Foundation hopes to bring its contribution to the interactions between the numerous MELiSSA partners as well as with external partners and stakeholders. The Foundation stimulates reactivity and flexibility, and is poised to participate, in the future, to the ESA sponsored space life support efforts that are of importance to both space and terrestrial applications (circular economy). Keywords: MELiSSA Foundation, Life support, Ph.D. and post-doctoral fellow-ships, space and terrestrial applications, communication, dissemination.

SECOND DAY

MELiSSA compartments integration: Continuous operation of interconnected liquid and gas phases of a packed-bed nitrifying bioreactor, an air-lift photobioreactor and a rats isolator

Enrique PEIRO
UAB

MELiSSA (Micro Ecological Life Support System Alternative) is an international effort developing technology for regenerative life support to enable long-term human exploration missions in Space. It is conceived as a loop of interconnected bioreactors providing the basic functions of life support and has established a Pilot Plant to demonstrate and integrate the technology developments to achieve such a target, operating the interconnected compartments in a continuous mode for long-term duration experiments under the supervision of an ad-hoc control system. The integration process of the MELiSSA Pilot Plant is performed sequentially, based on the status of advancement of the different compartments. The work presented here focuses on the integration of the liquid and gas phase of a nitrifying packed-bed bioreactor, an air-lift photobioreactor and a rats isolator as a mock crew. The liquid effluent of a nitrifying packed-bed bioreactor colonized with a co-culture of Nitrosomonas europaea and Nitrobacter winogradskyi was connected to a photosynthetic air-lift photobioreactor colonized with the cyanobacteria Limnospira indica. Additionally, the gas phase of the air-lift photobioreactor and the rats isolator was connected in a closed gas loop. The main goal of this integration step is focused on fulfilling the oxygen requirements of the animal compartment hosting three rats, at different biomass concentration in the air-lift bioreactor, that in turn is fed by the effluent from the nitrifying bioreactor. Light provided to the air-lift photobioreactor (according to its light control law) was the key variable to adjust the global oxygen and carbon dioxide balance between the photobioreactor and the animal compartment gas phase. Several hydraulic residence times were tested including different influent flows (20-30-40 L/d). Oxygen set-point of the animal compartment was sequentially changed to test the system dynamics once the steady state in the liquid phase was achieved. Results showed an accurate adjustment of the operation of the integrated system and a rapid capacity of the control system to drive and maintain oxygen level at the selected set-point in the animal compartment whereas the photobioreactor was fed by the effluent of the nitrifying bioreactor at a different hydraulic residence times. A step further in the integration of the different compartment phases is achieved by coupling the gas phase of the nitrifying bioreactor to the already integrated gas loop containing the air-lift photobioreactor and the animal compartment gas phases in a closed gas loop system. Therefore, in this configuration, the air-lift photobioreactor provided simultaneously the oxygen required for nitrification and respiration of the animals. Functional tests demonstrated the correct functionality of the hardware installed and the three compartments have been connected successfully with no impact on the controlled variables in each system. The tests include several hydraulic residence times and oxygen set-points in the animal compartment. Results from the three-compartments connected will be presented.

The connection of physical-chemical and biological processes for future closed life support systems for space applications

Alexander Tikhomirov
IBP SB RAS

The creation of highly closed life support systems (LSS) for long-term space missions is associated with the use of both physical- chemical and biological methods of processing human waste for their subsequent inclusion in the circular process. Such "hybrid" LSS are potentially the most promising for rapid and environmentally friendly mineralization of organic waste, followed by the inclusion of products of such mineralization in the intra-system mass exchange. One of the key problems in creating such hybrid LSS is the integration of technologies based on waste processing by biological and physical-chemical methods. On the example of the current experimental model of a closed ecosystem, physical and chemical technologies for processing human waste and including solid, liquid and gaseous products of their processing in circular processes are considered. On the other hand, biological processes of oxidation of a number of organic wastes in the biological substrate for growing plants of the phototrophic unit, oxidizing processes of organic matter, production of oxygen and water for humans and utilization of pollutants, obtaining nutrient solutions for plants due to physical-chemical and biological mineralization of organic waste are considered. We consider the close relationship between the processes of physical-chemical and biological oxidation and synthesis of substances for the stable maintenance of human habitat in a closed ecosystem. The closure principles of circular processes based on the coupling of physical, chemical and biological processes in the current physical model of a closed ecosystem are analyzed.

Report on LunAres Research Station new hygiene module using grey-water treatment during analog missions

Leszek Orzechowski
Space is More

LunAres Research Station is an analog habitat established in 2017 in Pila, Poland. Facility was created to conduct medical and psychological studies on isolated analog astronaut teams. Infrastructure of the habitat consists of isolated 250 square meter Extra-Vehicular Activity Area and 176 square meter habitat with eight designated modules containing office, biological laboratory, mechanical workshop, gym, kitchen and storage, sleeping pods, common area, and a hygiene module. In 2019 new hygiene module was designed and build, that allowed for grey-water collection, it re-use and treatment. The module is located in 30m2 mobile sea container equipped in basic hygiene installations and devices accessible for disabled. Additionally there is an experimental space included, which can be separated from hygiene rooms to maintain specific conditions for potential hydroponic (or other) laboratory compatible with water system in the module. All installations in the container are accompanied by separates flow meters for detailed monitoring of water usage. The grey-water recycling system is designed for treatment of around 250l of water per day and collects grey water from three different sources: 2 sinks and a shower. The filters and membranes included in the system allow for re-using grey water for flushing toilet and watering plants. The module also includes a dry toilet that produces compost as well as separated compartment destined for a greenhouse. The toilet is primed with a substrate for initiating composting – coconut husk fibres. This allows for extended collection pre usage in hydroponic system. With the start of the mission season in April of 2020 we plan on testing new facilities and conduct studies during analog campaigns. With the new system, the concept of closed loops in the habitat can be tested and developed, as well as its impact on the psychological and physical performance of crew members during missions. Studies will now also incorporate new hygiene module. In the near future greenhouse compartment will be equipped with hydro, aero and aquaponics. The first stage of a new project of LunAres is already completed. We have built a new sanitary module, capable of storing, recycling and purifying gray water. The potential of the new installation will be now examined. The results of the planned experiments, coming from the first analog missions, will be presented at the conference.

An innovative, preventive acting "bioinspired" antimicrobial surface based on peptides for space and Earth

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Antimicrobial surfaces are a well suited technology to prevent and reduce microbial loads in sensitive areas, where high humidity and temperature levels are causing increased microbial loads. These can endanger human health, health of organisms e.g. in bioregenerative life-support systems as well as technical equipment. Antimicrobial surfaces are preventively beneficial • in spaceflight – e.g. in confined environments in LEO and during exploration activities, to support breeding activities of e.g. algae in bioreactors and for biological experiments, and furthermore to meet the COSPAR planetary protection policy • as well as also on Earth - in hygiene areas during medical activities and food handling, in swimming baths, bathrooms, public transportation, submarines, greenhouses etc. For its dedicated use in space as well as on Earth, antimicrobial surfaces must be free of any toxic substance, otherwise higher non-target organisms would be affected. That means, that synthetic chemicals, silver, copper etc., as used until now, are not a suited solution - which in addition might lead to resistances of the bacteria to these toxic substances and are acting rather unspecific. A suited alternative to overcome these problems are bioinspired technologies as using antimicrobial peptides from nature (e.g. from frog skin etc.), immobilized on surfaces. High flexibility concerning the microbial target, acting specifically, low toxicity and an absence of resistances are the main advantages. As a logical step, the goal of the ESA-funded project BALS (Bioinspired antimicrobial lacquer for space) was the development of a new innovative antimicrobial acting lacquer based on peptides. Project partners were OHB System, Fraunhofer Institute for Manufacturing Technology and Advanced Materials (IFAM) (both Bremen, Germany) as well as the German Aerospace Center, Institute of Aerospace Medicine (Cologne, Germany). The developed antimicrobial lacquer with immobilized peptides showed an antimicrobial activity against S. cohnii and E. coli, compared to a reference lacquer without peptides. Its adhesion strength on space relevant substrates was demonstrated in a ECSS-Q-70-13A-test series, measuring the peel and pull-off strength using pressure-sensitive tapes. In addition, the absence of effects on higher organisms and the environment was shown in a laboratory aquatic biological multispecies test system (AquaHab®). With the successful demonstration of feasibility and use (TRL 4) of such a bioinspired antimicrobial lacquer and including these promising test results, all preconditions are now given for the further development and qualification until a full commercial exploitation, ready to be used in application fields in space and on Earth.

The use of synthetic ecology for the sustainable production of vitamin B12 enriched algal biomass on long space missions

Ellen Lucy Harrison
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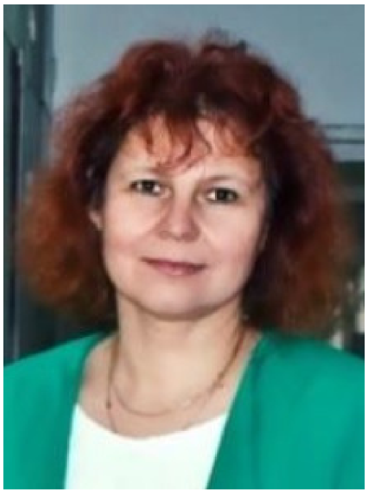
Vitamin B12 is a complex tetrapyrrole that is essential for human health. It is synthesised only by certain prokaryotes, and the process requires over 20 enzymatic reactions. In the MELiSSA loop the nutritional needs of astronauts are met by the organisms grown in compartment IV which is split into the higher plants (IVB) and the photoautotrophic bacteria (IVA). Higher plants have no B12 requirement and therefore do not make or store the compound. The photoautotrophic bacteria, Limnospira indica (formally Arthrospira platensis) as a cyanobacterium makes a form of B12 that is less bioavailable to humans and so astronauts on long space missions using the MELiSSA loop would be at risk of developing a B12 deficiency. This can lead to symptoms including impaired neurological functioning, which would be detrimental to the mission's success. Like humans many species of eukaryotic microalgae accumulate B12 when it is provided, and have been shown to obtain it from various B12-synthesising bacterial partners when grown in co-culture. The aim of this project is to investigate methods for producing a B12 enriched algal biomass that is safe for human consumption, using the edible algae Chlorella vulgaris and Haematococcus pluvialis. Rhodospirillum rubrum, an α-proteobacterium, currently being investigated by the MELiSSA project for compartment II, is thought to be safe for human consumption. It has been shown to synthesise B12 and grows under various environmental regimes. As a proof-of-concept approach R. rubrum has been grown with various algal partners to investigate the dynamics of the co-culture and its stability over time. In addition, the ability of L. indica to synthesise the less bioavailable form of B12 has been investigated so that in the future L. indica could be grown with a B12 synthesising bacterium or confirm that it will not contaminate the system with the less desirable form of B12. Together this information can be used to generate an edible algal biomass enriched with bioavailable B12 for astronauts. Not only is this useful for long-distance space travel but also on Earth with the rise of vegetarian and vegan diets increasing the proportion of the population at risk of B12 deficiency.



Anaerobic biodegradation processes for organic waste utilization followed by algal biomass accumulation

Lyudmila Vladimirova Kabaivanova
Bulgarian Academy of Sciences

Research related to Life Support Systems for long-term space flights is deepening in recent years. Some approaches were developed for finding a way to involve microorganisms in processing and recycling of unwanted waste in order to enable production of oxygen, water and food autonomously when staying long in space. For the purpose of MELiSSA the highest degree of biomass degradation and liquefaction must be achieved for more complete nutrient recovery in the following compartments. Waste material is generated daily by the crew. Anaerobic digestion is a waste-to-energy technology employing a consortia of anaerobic microorganisms and resulting in continuous biogas production, together with intermittent release of effluent digestate, rich in undigested solids, organic and inorganic compounds and metal salts. In this study we reveal the possibility for utilization of the digestate obtained after anaerobic digestion of organic waste as nutrients source to maintain and enhance microalgal growth. The ability of microalgae to photosynthetically fix carbon dioxide with oxygen release, producing biologically active substances, their short growth cycle and easy biomass accummulation were involved. As they can colonize different environments this group of organisms represents one of the most promising sources for different applications. Good growth and development was observed for the green microalga Scenedesmus acutus in digestate from anaerobic digestion processes of cellulosic wastes as a medium. Corn stalks were subjected to anaerobic biodegradation by a microbial consortium. All experiments started using culture fluid from an anaerobic methanogenic bioreactor as inoculum. A laboratory anaerobic bioreactor with working volume of 2 cm3 at 37°C worked at semi-continuous mode for 30 days. The temperature was 37 ± 0.5 ºC and pH was in the normal range 6.5 - 8.5. Algal growth was estimated following the increase in its weight. The obtained liquid fraction of the digestate appeared to be an appropriate medium for algae cultivation. Results showed good microalgal growth rate and biomass production (0.53mg/mL). C/N ratio in the digestate was determined to be in the optimal range from 15.6:1 to 19.7:1. Diminishing of nitrogen content from 0.013g/L to 0.003g/L was established during growth and development of green microalgae. Another important feature of microalgae to be used for phytoremediation to reduce the nutrient content in different waste waters due to their ability to assimilate nutrients into the cells was proved. In this way the resultant water could be used. Based on the laboratory scale study we can conclude that the anaerobic biodegradation (72%) was reached and Scenedesmus acutus has the potential to utilize nutrient content of not diluted digestate from a mesophilic process with substrate corn stalks for its mass growth. The enormous challenge of reducing the volume of wastes to generate liquid and gaseous fractions which could be used in the production of food, water and oxygen in space was faced.

Elimination of microbial hazards for the crew in low gravity conditions using the micro- and nanocapsules filled with 8HQ for the sustained antimicrobial surface decontamination

Oksana Travkova
University of Potsdam

The disinfection of surfaces in weightlessness is very difficult due to the need to use liquid disinfectants that do not spread in zero gravity conditions, but tend to collect in drops. For the sustained antimicrobial surface decontamination, we suggest to use in the top coat the micro- and nanosized containers filled with the broad spectrum biocide 8-hydroxyquinolin (8HQ) which can be released either slowly (by diffusion) or rapidly by the capsules damaging (e. g. via surface abrasion). 8HQ is a heterocyclic compound with diverse bioactivities which are exerted through its chelating ability and include antineurogenerative, anticancer, anti-inflammatory, antidiabetic, antioxidant and (which is most important) antimicrobial activity against Micobacterium tuberculosis, Escherichia coli, Streptococcus mutans, Candida albicans, Staphylococcus aureus (including MRSA and other drug-resistant pathogens), HIV etc. The median MIC of 8HQ is 12.5 ppm indicating the extremely active nature of this compound [Short B.R. et al., 2006, J Antimicrob Chemother.]. Development and characterization of the soft-shell micro- and nanocontainers as well as the investigation of the active component release is presented in this report. Synthesis of the polyurea particles filled with 8HQ was performed by interfacial polyaddition procedure. Physico-chemical characterization (size and surface charge) was done using Zetasizer Nano-ZS, visualization and morphology study were carried out with the aid of scanning electron microscope. The active agent release was investigated using fluorescence microscopy and UV-Vis spectroscopy. The laboratory results show that the stable filled particles of low polydispersity grade can be obtained in sizes which can be varied on demand between 0,5 and 3 µm. The surface of particles was successfully modified in terms of hydrophobization and hydrophilization, which allows to use the capsules in different top coating systems ranging from conventional solvent-based mixtures to novel environment friendly water-based top coating formulations. Gradual release of content from intact particles and rapid release from damaged particles has been shown as well. However, further multidisciplinary and multitechnological approach is needed to arrive at a specific application.

Potential for water independence from the grid on a household level by combined rainwater and greywater reuse: assessment through simulation

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Water management in most of the developed world is currently practiced in a highly centralized manner, relying on extensive piping networks for potable water supply and for separated wastewater evacuation through sewers. Besides leading to major infrastructure and energy costs to transport water the centralized approach stifles innovation. In the context of increased urbanization, water scarcity and necessity for water reuse, decentralization of wastewater management is an option worth considering. Greywater, originating from most household origins except the toilet (blackwater), lends itself excellently towards reuse as it is much less contaminated than blackwater. Rainwater can be treated to drinking water level. Combined decentralized reuse of greywater with rainwater caught on the roof surface could imply the possibility of complete independence of any sewer system or potable water grid. Simulations were performed, ranging from detailed mass-balances, e.g. analysing the water saving potential, to implementation in appropriate simulation software. Accumulating contaminants form a considerable concern for small closed-loop systems, as removal of contaminants is not complete. Therefore, analysing which contaminants are of most concern may alter system operation, specifically regarding the purge of contaminants out of the system. Salts and trace contaminants, in particular, are susceptible to accumulation as they are not removed through biological treatment and only to a small extent through most membrane filtration practices. A purge of circulating water out of the system must be included in any case, as rainwater causes a net influx of water. Simulations determined whether this purge sufficed to achieve an adequate water quality. The aim here was to define which contaminants are most likely to accumulate and how this purge will be implemented in the system. Particular attention was paid on the membrane bioreactor (MBR) as a key treatment technology for either greywater or rainwater.

Nutrient recovery from urine using bio-mineral producing bacteria

Ana Soares
Cranfield

Bio-mineral formation is a widespread phenomenon in nature. It refers to a series of processes involving selective extraction, uptake and incorporation of elements from the local environment into functional structures under strict biological control. Organisms can exert their influence on construction and synthesis of minerals through changes in local chemistry with different shaping strategies. Compared with inorganic minerals, bio-minerals are often characterised with more intricacy and morphological diversity, and generally associated with biological functions such as structural support, mechanical strength, protection, storage, etc. Although the mechanisms of struvite through bio-mineral production in municipal wastewater have been recently documented (Leng & Soares, 2018) current studies are focused in nutrient recovery from urine. Urine is rich in phosphate, ammonia, calcium etc. and it is practically sterile. Initial experiments have successfully completed in urine with known bio-mineral producing bacteria, demonstrated that the bacteria produced urease and were able to grow at high pHs (>8-10). Urease hydrolysed the urea to ammonia and carbon leading to an increase in pH. The urea hydrolysis and the resulting high pH constitute serious limitations for resource recovery technologies in urine streams that can be potentially be overcome by bio-mineral formation bacteria. In urine cultures inoculated with B. antiquum and M. xanthus, the phosphorus was content was reduced and recovered as struvite and calcium phosphate with yields of approximately 1 g/L urine. These initial findings are promising incentives for potentially developing sustainable resource recovery from source-separated urine. Future studies will look to understand the evolution of the microbial system and impacts bio-mineral yields to distinguish key factors for their efficiency.

Helical and linear morphotypes of Arthrospira sp. PCC 8005 display genomic differences and respond differently to acute 60Co gamma irradiation

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SCK-CEN

The multicellular cyanobacterium Arthrospira has been studied for many years because of its excellent nutritive value as a food- and feedstock and its many applications in biomedical sciences. We study Arthrospira sp. PCC 8005 as a principal organism and edible endproduct of the MELiSSA bioreactor, a life support system being developed by the ESA. Variations in Arthrospira trichome geometry and morphology have been observed under various environmental conditions including nutrient availability, temperature, UV spectrum and salinity [1]. The variable irreversibility of this morphological switch has led researchers to suggest the occurrence of mutations either arising spontaneously or brought about by environmental factors. Our previous research has showed that Arthrospira sp. PCC 8005 can withstand very high cumulative doses of 60Co gamma irradiation of up to 5000 Gy [2]. In this study we investigated the differences between the helical form (designated P6) and the new linear form (designated P2) in terms of post-irradiation growth, antioxidant activities, glutathione content, pigment production, trehalose concentration, and TEM ultrastructural analysis as well as the response of these two strains to 60Co gamma irradiation (i.e., cumulative doses of 900, 2100, and 5000 Gy using the RITA facility of SCK•CEN with a dose rate of 600 Gy.h-1). In addition, we obtained the full genome sequences for the P2 and P6 strains (both of vintage 2018) and performed a comparative sequence analysis between these sequences and the genome sequence of Arthrospira sp. PCC 8005 strain previously determined by us (3) (updated version v5 of 2014, available at NCBI under Genbank assembly accession number GCA_000973065.1). Both strains recovered equally well from cumulative 60Co gamma doses of up to 2100 Gy (but P2 grew better in respect to P6 and even in respect to non-irradiated control cultures). However, whereas P2 fully recovered from 5000 Gy, the helical strain P6 did not. Also, although helical Arthrospira typically repositions itself in the water column through the action of gas vacuoles and hence floats either on or just below the surface [1], the helical P6 trichomes sedimented and the linear form P2 floated. Assays for total antioxidant activity, glutathione content, and pigments showed statistically significant (but minor) dose-specific differences between P2 and P6, while the distinct difference in trehalose content indicated that the P2 strain metabolizes trehalose much faster than the P6 strain, presumably as a dedicated response to cellular radiation damage. For both strains, TEM analysis showed ultrastructural damage at 5000 Gy (to what degree is hard to say owing to the multicellularity of Arthrospira) but not for the lower doses. Comparison of the P2 and P6 genomes revealed a difference of 168 SNPs, 48 indels, and four large insertions affecting 41 coding regions across both genomes. As only nine of these regions encoded proteins with a known function, no conclusive genotype-phenotype associations could be made at this time. We recently extended our analyses with RNAseq transcriptomic analysis to check whether any non-coding RNA's are at play and to address the genotype-phenotype associations for strains P2 and P6 in more detail. Please note that some words need to be in italis (e.g. species names) or superscript e.g. 60Co, affiliation number, etc.

Overview of experiment results from the first research campaign of the EDEN ISS greenhouse facility in Antarctica in 2018

Paul Zabel
DLR

EDEN ISS is a European project focused on advancing bio-regenerative life support systems, in particular plant cultivation in space. A mobile test facility was designed and built between March 2015 and October 2017. The facility incorporates a Service Section which houses several subsystems necessary for plant cultivation and the Future Exploration Greenhouse. The latter is built similar to a future space greenhouse and provides a fully controlled environment for plant cultivation. The facility was setup in Antarctica in close vicinity to the German Neumayer Station III in January 2018 and successfully operated between February and November of the same year. During that nine month period around 270 kg of food was produced by the crops cultivated in the greenhouse. Besides the mere production of food for the overwintering crew (10 people) of the Neumayer Station III a large number of experiments were conducted. These experiments delivered valuable data for engineering of space greenhouses, horticultural sciences, microbiology, food quality and safety, psychology and operation of a food production facility in a remote environment. Component and subsystem validation was conducted to better understand engineering issues when building a space greenhouse. Fresh edible and inedible biomass was measured upon every harvest, dry weight ratios were determined and crop life cycle data was collected. More than 400 plant and microbiological samples were taken for the microbiology, and food quality and safety scientists working on the project. Some samples were composed of freeze dried plant tissue, but most samples were frozen at -40°C and shipped to Europe for analysis in specialized laboratories. A survey with the overwintering crew was executed to get information about the impact of the greenhouse on the crew during the nine month long winter season. Operation procedures for horticultural tasks, but also for system maintenance were developed and tested. The required crewtime, energy and resources demands were measured. This presentation shows for the first time a complete overview of the research results of the 2018 EDEN research campaign in Antarctica close to the Neumayer Station III.

From waste to resource; closing the loops in the urban water, energy and food nexus - Amsterdam case study

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Circular Economy is an increasingly popular approach adopted by companies and governments in mitigating challenges regarding food security, waste management or resource depletion. The knowledge and technology developed in the MELiSSA project during the past 30 years can be translated into terrestrial applications in helping cities to transition to a circular economy by transforming the waste streams and residues into resources. Amsterdam is a city with the big ambition to unlock the potential of the circular economy, and the sustainable food case being one of the models for this transition. Accordingly, a study was done to assess the technical feasibility of MELiSSA technologies in developing circular food systems. Three characterised domestic waste streams from households were used as inputs in the models: i) all streams mixed: black (= brown + yellow) and grey water, ii) black water separated from greywater, and iii) fully separated brown, yellow and grey water streams. The outputs described as energy (kWh), nutrients (N, P, K) and water fit for irrigation, per person per day, were used in a mass balance with the requirements of four candidate crops (tomato, cucumber, pepper, lettuce) cultivated in a controlled environment. Three MELiSSA technologies were used in modelling the input/output balances assessing the circularity of the food systems. The MELiSSA C1 liquefying compartment was based on a continuous stirred tank reactor performing anaerobic digestion for biogas production. Energy consumption in controlled environment agriculture is influenced by a diverse set of factors such as light, temperature control or other operational systems, but an estimate for the lettuce cultivation was made for the purpose of the study. Digestion of the black water stream yielded an output of energy enabling to cultivate 1.7 kg of crop per person per day. The energy recovered could also be used to offset the operational costs of the other reactors. The C2 compartment of the MELISSA loop is the photoheterotrophic production of purple non-sulfur bacteria (PNSB). These microbes convert volatile fatty acids mainly to biomass, which can be used as microbial fertiliser and biostimulant. The yield determined the potential of N, P and K recovery, which resulted in average vegetable production of 2 kg per person per day. The effluent of the purple bacteria can be treated by the next MELiSSA technology, which comprises a bioreactor and multiple membranes for urine and greywater treatment. It was calculated that 76% of the water could be recovered to ESA hygienic level, and between 80 and 90% water can be recovered to be used as irrigation for food production. Therefore, the irrigation water recovery would result in an average of 2 kg of vegetables that can be produced per person per day. This preliminary feasibility study concluded that the MELiSSA technologies can recover nutrients and water from the municipal wastewater and hence offsets the requirements for an average of 2 kg of vegetable crops, per person per day. The study highlights the potential of the life support systems for terrestrial applications and the transition to a circular economy. Follow-up proof-of-concepts will validate the study and highlight the challenges in implementing innovative space-based water and nutrient recovery systems. Keywords: MELiSSA, Earth Applications, Amsterdam, Circular Cities, Waste to Resource.

Application of the energy cascade model (MEC) on Lettuce crop grown in controlled environment at two different scales: A small growth chamber and a vertical farm

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Future human Space colonization will require the permanent presence of astronauts on Planet outposts, at a great distance from Earth. In this scenario, resupplying all the food to support human activities from Earth is not sustainable and must be integrated with the production of fresh food directly in situ, in Advanced Life Support (ALS) systems. The development of these closed systems goes along with technological innovation in agriculture, in the direction of sustainability and automation, for the remote management of environmental control and plant growth. Environmental factors and plant responses are strictly interconnected since any change in the environment provokes modifications at the plant morpho-physiological level, affecting plant behaviour in terms of water and gas fluxes, which in turn re-modify the environment itself. In this context, the application of explanatory models which, by using environmental factors as input, can predict crop biomass, photosynthesis and energy balance, become fundamental. The implementation of these tools allows to simulate plant responses due to environmental disturbances during the production, thus helps to understand the direction and magnitude of changes in plant behaviour. The aim of this study was to evaluate whether the Energy Cascade Model (MEC) can be equally and reliably applied on lettuce cultivation trials conducted in facilities at different scales. Therefore, the outputs of the MEC model were evaluated against data collected during experiments conducted in two different controlled environment facilities: a small growth chamber at the University of Naples Federico II and a multi-layer vertical farm (UAg Farm) at the University of Arizona Controlled Environment Agriculture Center (UA-CEAC). Both experiments were conducted on green and red-leaf ‘salanova’ lettuce under nominal and off-nominal growing conditions. In the first experiments conducted in the growth chamber, the study mainly focused on the effect of different air VPDs obtained fixing the temperature and changing the relative humidity during the cultivation trials. Results showed that these environmental parameters had significant effect on evapotranspiration rate and stomatal conductance and impacted the predicted output the most with significant differences between differently coloured leaves. Whereas, in the experiment conducted in the vertical farm we added different light intensities and short-term exposure to high levels of electrical conductivity simulating a water stress condition and incremental CO_2 enrichments. Preliminary results confirm that MEC model can be a suitable solution for monitoring and controlling edible plant growth in Advanced Life Support (ALS) systems.

Modelling long-term continuous operation of the nitrifying Compartment in the MELISSA Pilot Plant

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MELiSSA (Micro Ecological Life Support System Alternative) is a regenerative life support system conceived as a loop of interconnected bioreactors providing the basic functions of life support. A very relevant aspect in the development of this system is the understanding of each one of its compartments and the development of reliable mathematical models enabling their design, operation and control. The MELiSSA Pilot Plant has been established to demonstrate and integrate the technology developments to achieve this target. One of the main steps in this process is nitrification, consisting in the oxidation of the ammonium present in the organic wastes from the crew (i.e., urine). The nitrification compartment in the MELiSSA pilot plant is using a culture medium with ammonium as Nitrogen source, as a first approximation. It is a packed-bed up-flow bioreactor with an axenic co-culture of nitrifying bacteria: Nitrosomonas europaea and Nitrobacter winogradsky immobilized on polystyrene beads (Biostyrene®). The results of the continuous operation of this reactor during a three year period will be presented. A model of this bioreactor has been developed, based on the knowledge generated on the biological mechanisms behind its operation and the different operating conditions used, such as increasing ammonium loads. The proposed model implies a step forward in the understanding of such a system and has been addressed especially to require lower computational effort compared to current 1-D models available, a relevant aspect for its future use in control of the bioreactor operation. This is achieved through a novel approach in biofilm modelling in which the quasi-static substrates diffusion equations are solved in a microscopic setting, coupled with a newly proposed macroscopic model capable of describing the global biofilm growth and consolidation phenomena. The use of the model enables an accurate description of the overall reactor operation and sets the basis for the development of control laws reinforcing a robust and reliable operation for this bioreactor as part of a complete system with very strong requirements in this direction.

Global Control Loop of MELiSSA Life Support System

Baptiste Boyer
Sherpa Engineering

We develop a control system that ensures the survival of the crew while minimizing the external resources needed. We look in particular into the quantity balance between the production of matter and the consumptions. To do so, we use a dynamic model of the compartments to calculate the flow exchanges of the MELiSSA Life Support System. The flows considered are the possible critical flows for the operation of the loop: O2, CO2, water, food, etc. 2. Hypothesis and methodology: MELiSSA loop is a complex system with several separate compartments which are controlled locally. We believe that its global control requires a general vision of the system and the abstraction of some details. In that way, we use simplified models based on the knowledge models of the different compartments. They have been developed and adjusted by the laboratories that designed them. We perform simulations using a complete model of the loop developed under Simulink. We build our model from a modular and functional approach. Each sub-system is independent and internally controlled, and a general controller orchestrates the whole system. Data used are mainly taken from the MELiSSA literature. 3. Results: We present a complete model of the MELiSSA loop, composed of the different compartments, the chemical reactions between the different materials, and the materials flowing between the compartments. In its previous work, Laurent Poughon has developed a static model of the entire loop. Based on his work, we develop and validate our model by simplifying the compartments model and adding a dynamic behavior and a global control. The complete model with its optimal control shows that the natural production of the loop does not cover the consumptions, due to biomass losses in the compartments. This means that it is necessary to add in input external resources, and control them to improve the loop efficiency. We choose to add nitric acid, ammonia and carbon dioxide as they are the natural input of the nitrification compartment, the photobioreactor and the higher plants chamber. Adding a small amount of these resources can significantly increase the global loop coverage. We demonstrate the feasibility of the loop control, and present a dynamic and modular control system that ensures the crew survival in different malfunctioning scenarios. Our model with its control system can be used as dimensioning model to determine the quantity of external resources needed for a fixed-term mission with a defined crew. It can also be used as environment and contextualization model for the development and testing of the different compartment. It can be a common reference frame for the laboratories working on them.

Exploring the impact of irregular metabolic efficiencies and the space environment on the survivability of a regenerative life support system through agent-based modeling

Angelo Vermeulen
TUDelft

The survivability of a regenerative life support system during long-duration missions in interstellar space was simulated using an agent-based model (ABM) of MELiSSA. In this ABM, humans, plant plots and bioreactors are modeled as virtual agents that interact with each other. The ABM approach enables the introduction of heterogeneity at the level of agent attributes, and thus allows for the study of complex interactions that cannot be observed in more traditional equation-based modeling techniques. Properties, behavior, life cycle (e.g. growth curves and susceptibility to nourishment deficits), and rules of interaction are specifically modelled for each type of agent. The behaviors are modeled according to the major chemical reactions and productivities of each MELiSSA compartment, as documented in publicly available sources. A first series of simulation experiments investigates the survivability of the system under stress conditions of two types: stochastic metabolic efficiencies and environmental stressors. The following environmental stressors were conceptually simulated: cosmic radiation reducing metabolic functionality, and particle impact causing loss of life support system elements. A second series of simulation experiments explores the impact of different policies for nutrient distribution, both under normal and aforementioned stress conditions. The research on which this paper reports is part of the E|A|S (Evolving Asteroid Starships) project by the DSTART team at Delft University of Technology. The project entails conceptual research on interstellar travel, including onboard regenerative ecosystems.

Membrane microgravity humidity separator

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CNR-ITM

The separation of water vapour from gaseous streams by membranes is a recent operation, which is attracting significant attention in these last years for many applications (plume of cooling tower, flue gas dehydration etc.). Very recently, Thales Alenia Space and CNR-ITM started a collaboration in the framework of the ESA MELiSSA PFPU (Precursor of Food Production Unit) project aiming at using membrane humidity separators for space applications within a plant growth facility for microgravity applications. In the extensive research activity at CNR-ITM on this membrane operation, hydrophobic porous membranes are utilized for condensing water vapour contained in gaseous streams, allowing the permanent gas permeation through the membrane, while keeping on the membrane surface (feed side) the drops of the liquid phase, even the small ones. The hydrophobic nature of the polymeric membranes not only avoids water droplets dragging, but also promotes vapours condensation exploiting the principle of dropwise condensation where, when condensation takes place on a surface that is not wet by the condensate, water beads up into droplets and rolls off the surface. The typical pore dimension of the membranes used is of 0.1-0.2 micrometers, well above the kinetic diameter of any gas present in the feed; therefore, the membrane cannot have any selective separating function among the various gases as well as it will not be able to retain the water vapor that has not been condensed. In this collaboration, a devoted device was designed and built for operating in steady state in microgravity. In a typical application, the membrane unit is vertical oriented and the condensed water is usually collected on the bottom of the condenser since the drops fall down. Specific solutions are being investigated to promote water harvesting for different orientation 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. Here, we will present some results measured with the designed separation unit as function of the main variable affecting the separation process.

Modelling and testing of a root module irrigation unit

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In the framework of the ESA PFPU (Precursor Food Production Unit) 2 project, this work concerns the modelling and testing of a Root Module Irrigation Unit (RMIU) to be used in the Plant Growing Unit (PGU). The novelty of the study is based mostly on the use of new substrates for cropping plants - represented by cellulosic sponge and PVA sponge - and of CFD simulation tools. The activities described concern three successive and preparatory phases: i) media characterization, ii) simulation scenarios and iii) experimental testing. Prior to the simulation and experimental operations, some constraint variables that condition the proper functioning of the system have been calculated, as - for example - maximum volumetric capacity of the substrates, irrigation volumes to be applied, threshold water content for optimal root water uptake, and so on. This preliminary phase aims also to determine the functional performances of the cellulosic and PVA sponge by measuring the hydrological characteristics of the media (water retention curve and hydraulic conductivity curve). Once defined the initial and boundary condition for the RMIU, modelling with Hydrus1D and Hydrus2D models has permitted to optimize the geometry of the system, i.e. the distance between emitters/tubes, and diameter of the emitters on the tubes, and hence the flow rate. The two models have been used in zero gravity condition and/or in terrestrial gravity condition and the results have been evaluated in terms of homogeneity of water distribution at the end of irrigation, and in terms of water availability for root water uptake process. In the last phase, the findings obtained with the simulations have been implemented and tested. The main objective of the test phase has been that of verify and optimize the irrigation unit in terms of ALISSE criteria. The success of the test has been verified with the identification of irrigation scheduling that assure an optimal water supply in the upper part of the substrate. During the irrigation experiments, sensors of volumetric water content and matric potential have given the opportunity to compare experimental data with simulated output. The test phase has also confirmed that given a emitters density and flow rate, is ever possible to obtain the expected performance by varying the time schedule of delivery, also introducing pulsating irrigation for example or by varying the geometry of the system.

Synthetic urine treatment by a defined bacterial consortium for urea hydrolysis, nitrification and COD removal in an up-flow packed bed reactor

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UAB

Human urine treatment will be needed in future space life support systems to provide stable nitrogen (N) substrate for phototrophic food production. Human urine contains 85% of the crew's N uptake, mainly present as urea. To enable food production, it is desirable to convert urea to nitrate, which can biologically be achieved through combining ureolysis and nitrification, based on a consortium of three groups of bacteria: i) urea hydrolyzing bacteria (heterotrophs and/or AOB), ii) ammonia oxidizing bacteria (AOB) and iii) nitrite-oxidizing bacteria (NOB). Additionally, human urine treatment should also degrade the present organic compound into carbon dioxide, which afterwards can be used by plants and cyanobacteria for the photosynthesis. Organics degradation can be performed by the heterotrophic strains. As, human urine organic matrix is very complex, the degradation is a challenge for a synthetic bacterial community. Up-flow packed bed reactor was operated in a continuous mode to test 10% synthetic urine treatment by a defined bacterial community of nitrifiers and heterotrophs at the MELiSSA Pilot Plant. 10% synthetic urine medium composition was developed for the experiment, based on the real human urine composition. Nevertheless, the complexity of human urine makes really challenging to mimic its composition. The main goal of the conducted research was to evaluate urea conversion rates into nitrates and organic removal rates. Nitrosomonas europaea and Nitrobacter winogradskyi have been previously selected as AOB and NOB representatives respectively. Heterotrophic strains were selected based on its urea hydrolysis and organic removal rates in 10% real human urine. Preliminary screening tests were conducted in 10% real human urine to ensure good performance of selected strains in a real case scenario in the future space life support system application. Selected heterotrophs needed to meet specific requirements: nonpathogenic, non-spore forming, ureolytic, active in a consortium with nitrifiers. Tested strains included Pseudomonas fluorescens, Comamonas testosteroni, Acidovorax delafieldii, Delftia acidovorans, Cupriavidus necator, Pseudomonas putida. Based on highest urea hydrolysis rates P. fluorescens (41.30±0.83 mg N/L*d), C. testosteroni (35.80±9.52 mg N/L*d), C. necator (22.20±7.02 mg N/L*d) and A. delafieldii (17.20±4.05 mg N/L*d) were selected to be tested in the consortium with the two nitrifying strains. Organics degradation efficiencies for selected heterotrophic strains were similar: P. fluorescens (28.8±3.64 %), C. testosteroni (22.41±4.28 %), C. necator (26.23±4.07 %) and A. delafieldii (29.76±1.11 %). Finally, the bacterial consortium composed of the two nitrifying and the four heterotrophic strains was cultivated in 10% synthetic urine in a up-flow packed bed reactor. Results from the reactor operation will be presented.

Cyanobacterial biomass production on Mars and Moon regolith and its utilization as a feedstock for other microorganisms and higher plants

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ZARM - Center of Applied Space Technology and Microgravity

Authors: Tiago Ramalho, Nikolas Alansson and Cyprien Verseux The feasibility of long-term space missions on the Moon and Mars depends on their level of self-sufficiency: a full supply of consumables from Earth is unrealistic given high cargo launch costs and impediments associated with long-distance resupply flights (1). Closed-loop systems address this issue; however, they suffer from unavoidable material losses (2). In-situ resource utilization could complement them by providing a continuous and reliable input of supplies without increasing payload mass in proportion to mission duration. Some species of cyanobacteria were suggested to be suited for this task, as they could be fed from resources available in the Martian regolith and atmosphere (3–5). In this study, selected strains of diazotrophic cyanobacteria were screened for their ability to grow on Martian and Moon regolith simulants in pure water. All strains effectively grew in the provided conditions, although with contrastingly different rates. Soluble compounds were then extracted from cyanobacterial biomass and successfully used as only nutrients for a model heterotrophic microbe (Escherichia coli) and higher plant (Lemna sp.). These preliminary results are part of a larger project aimed at using cyanobacteria to feed regenerative life-support systems (e.g., MELiSSA) from resources available on site, both to increase their sustainability and to offset imbalances.

MELiSSA in the space: safety and reliability issues

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For more than 30 years, the MELiSSA team has been working from the initial concept to the current situation, where the MELiSSA Pilot Plan compartments are functioning and the cycle closing is approaching. During the 2000s, some contracts sponsored by ESA were awarded to study the adaptation for Space of the MELiSSA concept. Different aspects were review, mainly consumption, mass, volume and crew time. However, aspect as safety or reliability were not faced. This paper review the conclusions of the performed studies and take a look to critical aspects as safety and reliability.

Impact of a closed life support system on human microbiome and health

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INRAE

From early life onward, environment dominates over host genetics in shaping the human microbiome, which in return interacts with the immune system, playing a fundamental role in modulating risks of chronic diseases. Within confined environments, such as a closed life support systems, human are exposed to drastic changes compared to non-confined environments. With limited exchanges with out-side, increased contact with indoor materials, antimicrobial and cleaning products, a confined environment will imply exposure to a limited and selected diversity of microorganisms and antigens and increased exposure to chemicals. Additionally, specific space-flight conditions such as radiation and microgravity will further modify airborne, equipment and human microbiomes. In such conditions, bioprocesses involved for food production, product recycling, wastes processing and refinery, surrounding inhabitants, might increase emergence of new microbial contaminants, such as viruses and other pathogens. Finally, long-term consumption of pre-processed food might deeply influence the gut microbiome. These specific changes and their impact on microbiome and immune homeostasis will have to be monitored, in flight or ground experiments, to address the role of the microbiota in maintaining immune function and health of the crew in such confined environments. Given the association between indoor microorganisms, diet, human microbiome and health, specific bioactives, microorganism or synthetic microbial communities present indoor or in the diet could serve preventive objectives. Closed life support systems, with the possibility to control the environmental input, give a unique opportunity to precisely measure the impact of surrounding bioactives on human microbiome, immune system and health. Air spraying of particular mix of beneficial bacteria or nutritional supplements might mitigate or prevent development of chronic diseases due to the alteration immune function during confinement. With the aim of enabling discoveries on the role of gut microbiome in human health and disease, MetaGenoPolis research projects lead to the translation of recent discoveries into applications in nutrition, prevention and therapeutics towards an improved human health through innovative strategies to restore host-microbe symbiosis. Our unit already collaborates with University Hospital Tübingen on the ICELAND research project with the objectives to measure the effects of prolonged stay in an environment with limited antigen diversity (Concordia station, Antarctica) on human adaptive immunity and the gut microbiota during two consecutive seasons, including a nutritional supplementation to counteract or prevent potential health consequences. Linked to the International Human Microbiome Consortium (IHMC), MetaGenoPolis has been pioneer in the development of standardized faecal sample collection and biobanking as well as gut microbiome characterization and association with human diet, environment and health, using quantitative metagenomics approaches, based on shotgun next generation sequencing of whole microbiome. More recently, MetaGenoPolis deploys a similar expertise for the characterization of human saliva and skin microbiomes and their association with human diet, environment and health.

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

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Microbial produced oxygen and microbial edible biomass are very interesting sustainable resources for future space travelers. The Arthrospira-B flight experiment was a pioneering experiment to transplant the cyanobacterial oxygenic photosynthetic bio-process to space. A space compatible photobioreactor was built, allowing online measurements of both bio-oxygen production rate and microbial growth rate in space. Four of such bioreactors were integrated in the ISS Biolab incubator and were operated in batch mode for a duration of 1 month. They contained the cyanobacterium Limnospira indica PCC800, previously know as Arthrospira sp. PCC8005, or under the product name Spirulina. The experiment was performed in parallel on ISS and on ground. The bio process was modeled, using a novel and dedicated model for the growth of Limnospira in membrane photobioreactors, and the space grown biomass was analyzed in detail post-flight. The Arthrospira-B flight experiment showed it was possible: (1) to build, qualify, and operate a photobioreactor in space, (2) to restart the cyanobacterial cultures in the bioreactor in ISS after a period of storage and upload, (3) to maintain an axenic photosynthetic active culture in the bioreactor, under space conditions, (5) to monitor online the oxygen and biomass production of the culture, and (4) to implement successfully ground commands to adapt bioreactor conditions and allow several crew interventions. A good fitting was achieved between the predictive simulation and the experimental results obtained for oxygen and biomass production in the space bioreactor, when a good mixing of the liquid phase was maintained. The pigment and proteomic profiles of the biomass confirmed full activation of the photosynthetic cellular processes, in space as on ground. These data show it is feasible to start-up and operate a microbial bioreactor in ISS. Space conditions had no significant negative effect on the microbial photosynthetic bio-process. Keywords : MELiSSA, photobiobioreactor, flight experiment, Limnospira, Arthrospira, model.

Genetic responses of metabolically active Arthrospira sp. PCC 8005 to chronic high-dose gamma irradiation as determined by RNAseq transcriptome analysis

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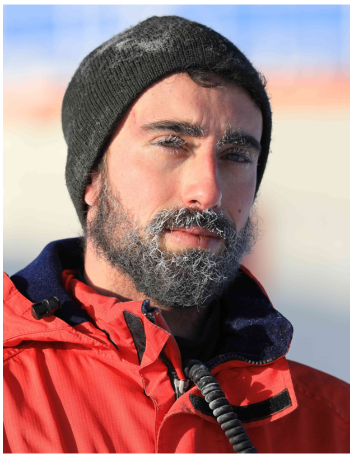
SCK-CEN

Two morphotypes of Arthrospira sp. strain PCC 8005, denoted as P2 and P6, were subjected to chronic gamma radiation at a dose rate of ca. 80 Gy.h-1 for a period of 3 days under continuous light. Samples were taken for post-irradiation growth recovery and transcriptional analysis at time intervals 16, 40, and 68 hrs (corresponding to cumulative doses of 1300, 3200, and 5400 Gy, respectively). Arthrospira sp. PCC 8005 being highly resistant to ionizing radiation (IR) [1], both morphotypes recovered equally well from 1300 and 3200 Gy. However, while the P6 helical type took about 13 days to recover and regain normal growth, the P2 straight type recovered much faster i.e. within 6 days, indicating differences in their radiation stress response. To investigate these differences, P6 and P2 cells exposed to the intermediate dose of gamma radiation (3200 Gy) were analyzed for differential gene expression by RNAseq analysis. From these analyses (at >=two-fold change and FDR=< 0.05) it emerges that upon exposure to IR the P2 strain (straight trichomes, higher IR resistance) pursues cell stability via the enhanced expression of certain genes. For instance, sseA encodes a 3-mercaptopyruvate sulfurtransferase generating sulfane sulfur species that may help to generally protect cells against oxidative stress, while isiA encodes a Chl-binding protein with an important role in protecting the photosynthetic machinery. Other P2 genes uniquely induced by IR encode proteins or enzymes involved in chlorophyll biosynthesis (chlB), carbohydrate biosynthesis (pflB), transcription (sigG), DNA damage & repair (mutT), heavy metal resistance (czcD), vitamine B12 biosynthesis (cbiN), and membrane lipid homeostasis (plsC). Likewise, a substantial number of genes were uniquely down-regulated in P2 upon exposure to IR, including genes involved in circadian regulation (kaiA, kaiC), photosystem (petJ, ndhD, hliA, rfpX) and cell wall (ddpX, plsX) organization, transport (secE, corA, cysP, narM), fimbrial assembly (pilC), transcription (nrdR, hisR, sigD, sigE), and translation (valS, rnc1, trmH, rpsO). IR-induced genes unique to the P6 strain (helical trichomes, slower post-irradiation recovery) included those involved in protein stability (htpG, groL, clpB), PSII assembly (psbI) and amino acid biosynthesis (cysR, huyA, proA), while IR-repressed P6 genes were involved in cell physiology (murC, mreB), photosystem formation (psaX, psbN, psb27), nitrogen metabolism & assimilation (aapJ, amt1, glnA, glnB, ntcB), carbon fixation (gap1), antioxidant synthesis (trxA3, aat2), lipid synthesis (fabZ), DNA damage & repair (helD), phosphate transport (pstB2), and transcription (arsR). Although the two Arthrospira sp. PCC 8005 morphotypes P2 and P6 have 401 differentially expressed genes (DEGs) in common (out of a total of 1051 and 773 DEGs for P2 and P6, respectively), pointing to an overall response to gamma irradiation, each substrain seems to follow slightly different routes. We previously reported marked differences in growth and antioxidant capacity between both types, and whole-genome comparison also showed that P2 and P6 differ in 168 SNPs, 48 indels and 4 large insertions [2]. An in-depth association between previous and current data is needed to further elucidate IR-resistance in Arthrospira sp. PCC 8005. Please note that some words need to be in italics (e.g. species names) or superscript e.g. 60Co, affiliation number, etc.



An experimental device for studies on cyanobacteria at low pressure, in the frame of BLSS

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Data is scarce on how microorganisms cope with low atmospheric pressure, and with low partial pressures of specific gases. Such knowledge would benefit the development of biological life-support systems (BLSS), of which microorganisms will be critical components [1]–[4]. First, if enclosed within crewed compartments, microorganisms may be exposed to the lower-than Earth atmospheric pressure considered for future space vehicles and habitats (see for instance [5]). Second, more extreme atmospheric conditions may be faced if BLSS are deployed outside to spread over larger surfaces, to rely on local resources, or both. Among microorganisms of interest are cyanobacteria: aside from the role of Arthrospira sp. in the MELiSSA loop, diazotrophic-litothotrophic species are being considered for processing Martian resources into substrates for BLSS [6], [7]. Fed with materials available there—atmospheric gases, water mined on site, and mineral nutrients from the regolith [6], [8]—, they could be used for direct production (e.g., of oxygen and protein-rich dietary supplements) but also to support the growth of other organisms which, themselves, could perform LSS processes (e.g., part of MELiSSA) and bring further biotechnological capabilities [6], [9]. Efficiency will largely depend on the behavior of cyanobacteria under non-Earth atmospheres: while getting close to Mars's atmospheric conditions (low total pressure, high pCO2, low pN2) would simplify the system, minimize the mass of structural materials and consumables, and lower the risk of organic matter leakage [6], [7], [10], changes in gas composition and pressure affect cyanobacterial behavior. A good compromise remains to be determined. To help address the need for knowledge on microbial metabolism at low pressures, we are developing an atmosphere-controlled, automated, photobioreactor-like device. Its nine one-liter culture vessels can be programmed independently for commonly-adjustable growth parameters (e.g., light intensity, stirring speed, and temperature), as well as for atmospheric pressure and composition. O2 production can be measured in real time, and sampling of gas and liquid can be performed without interrupting experiments. Our primary use will be for investigations on cyanobacterial behavior under low-pressure, high-CO2, low-pN2 atmospheres, as part of a longer-term project of reducing the Earth dependency of Martian BLSS. This talk will outline the rationale behind cyanobacterium-based BLSS, describe the device we are developing, and present first experimental results. [1] F. Godia, J. Albiol, J. Montesinos and J. Pérez, J. Biotechnol., 99, 319–30 (2002) [2] L. M. Steinberg, R. E. Kronyak and C. H. House, Life Sci. Sp. Res., 15, 32–42 (2017) [3] L. Hendrickx and M. Mergeay, Curr. Opin. Microbiol., 10, 231–7 (2007) [4] C. Verseux, I. Paulino-Lima, M. Baqué, D. Billi and L. Rothschild, in Ambivalences of Creating Life. Societal and Philosophical Dimensions of Synthetic Biology, K. Hagen, M. Engelhard, and G. Toepfer (Eds), Springer-Verlag, 73–100 (2016) [5] J. Norcross et al., NASA Johnson Space Center, Houston, technical report JSC-CN-34806 (2013) [6] C. Verseux, M. Baqué, K. Lehto, J.-P. P. de Vera, L. J. Rothschild and D. Billi, Int. J. Astrobiol. 15, 65–92 (2016) [7] C. Verseux, PhD thesis, University of Rome "Tor Vergata"", Rome, Italy (2018) [8] K. Olsson-Francis and C. S. Cockell, Planet. Space Sci. 58, 1279–85 (2010) [9] L. J. Rothschild, Biochem. Soc. Trans. 44, 1158–64 (2016) [10] K. M. Lehto, H. J. Lehto and E. A. Kanervo, Res. Microbiol. 157 69–76 (2006)

Production of high-quality edible biomass with high levels of antioxidants by genetic engineering of the photosynthetic microalga Chlamydomonas reinhardtii

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Carotenoids constitute a widely distributed group of lipid soluble pigments that are synthesized by plants and microorganisms: these pigments are essential in human diet being involved in eye desease prevention and being precoursors for vitamins production. Among the carotenoids, the secondary ketocarotenoid astaxanthin (3,3′-dihydroxy-β,β-carotene-4,4′-dione) shows superior activity against reactive oxygen species (ROS) and is one of the most powerful natural antioxidants. Astaxanthin has multiple purported health benefits on biological systems due to its action against ROS: astaxanthin has potential uses as an antitumor agent, the prevention of cardiovascular as well as neurological diseases, and diabetes. Moreover, astaxanthin can be used as human dietary supplement and in aquaculture to improve fish color. Other ketocarotenoids like canthaxanthin, an intermediate of astaxanthin synthesis, has properties similar to astaxanthin, with high potential for use in human health applications. With few exceptions, higher-plants and cyanobacteria do not synthetize astaxanthin, which is currently produced industrially from unicellular photosynthetic microalgae such as Haematococcus lacustris or, to a lesser-extent, Chromochloris zofingiensis. Here, we used synthetic re-design of the key gene for astaxanthin biosynthesis to enable its constitutive overexpression from the nuclear genome of Chlamydomonas reinhardtii, a green alga approved for human consumption by both FDA and EFSA. We found that up to 50% of native carotenoids could be converted into astaxanthin and more than 70% into other ketocarotenoids in the engineered strains. Modification of the carotenoid metabolism did not impair growth or biomass productivity of C. reinhardtii, even at high light intensities. Under different growth conditions, the best performing strain was found to reach ketocarotenoid productivities up to 4.3 mg L-1 day-1. Astaxanthin productivity in engineered C. reinhardtii shown here might be competitive with that reported for Haematococcus lacustris (formerly pluvialis) which is currently the main organism cultivated for industrial astaxanthin production. In addition, the extractability and bio-accessibility of these pigments was much higher in cell wall deficient C. reinhardtii than the resting cysts of H. lacustris. Engineered C. reinhardtii strains could thus be a promising strategy to produce edible strong antioxidants improving human health in space environment where the risk of oxidative stress due to space radiations is high.

Impact of carbon source and light intensity on the production of PHA by Rs.rubrum

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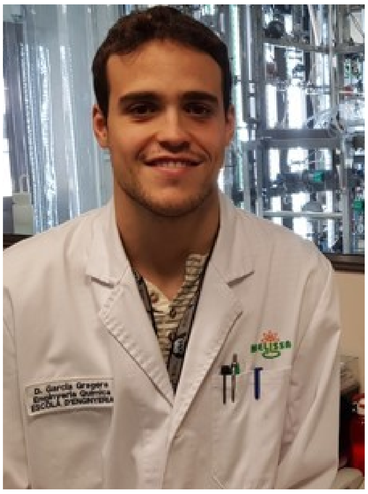
U Mons

The wide metabolic versatility of purple bacteria makes them an intensively studied group for biotechnological applications. Purple bacteria are able of both heterotrophic and autotrophic growth and have the capacity to assimilate a very large range of carbon sources. These faculties have led the European Space Agency to consider Rhodospirillum rubrum, a model purple bacterium, as a member of the MELiSSA loop. In that frame, the photoheterotrophic metabolism of Rs. rubrum could be used to assimilate, in the second compartment, the volatile fatty acids (VFAs) formed during the anaerobic fermentation in first compartment of the loop. Analysis of VFA photoheterotrophic metabolism by Rs. rubrum highlighted that polyhydroxyalkanoates (PHA) granules were accumulated in response to specific conditions. Recently, it has become of industrial interest to evaluate these polyesters as potential biodegradable plastics for a wide range of applications ranging from packaging to biomedical devices. On an industrial scale, the use of photosynthetic bacteria such as Rs. rubrum, that can harness sunlight as an energy source and use VFAs, a low-cost substrate, as carbon source could help to reduce the production cost of those materials. Physiologically, PHAs are used as internal carbon storage in bacteria when a nutrient (N,S,P) is lacking. However, in our case, PHA production is observed under nutrient enriched conditions. Our hypothesis is that PHA production could be used as an electron sink because of the consumption of NADPH during the process. In order to optimise the production of PHAs by Rs. Rubrum, it would be helpful to have a better understanding of the parameters driving the metabolism towards the PHA production. In this study we used different growth conditions, known to impact the redox state of the cell (i.e. the presence of bicarbonate in the medium, the adaptation of the strain to a substrate, a change of light intensity and the use of a mix of VFA as carbon sources). The assimilation of VFAs is known to require bicarbonate (HCO_3^-) supplementation. HCO_3^- could be used as electron sink and is thus competing with PHA production. PHA quantitation revealed higher PHA content when HCO_3^- are added progressively or when a low level of bicarbonate is supplemented to the medium. During continuous culture, an adaptation from the strain to the substrate can occur. A test using a strain adapted to acetate showed that the PHA production of an adapted strain was lower than for the wild type strain. Another important parameter that can influence the redox state of the cell is light. Our study showed than when growing on acetate an increase of light intensity from 50 to 150 µmol photons/m²s is linked to an increase in PHA content in the wild type strain. Finally, in the prospect of using waste as carbon sources, the probability of the cell to grow using only one carbon sources is low, thus the impact of the presence of simultaneous VFAs as carbon source was tested and resulted in a reduced need of HCO_3^- and a lower production of polymer.



Characterisation of an air-lift photobioreactor with a LED based illumination system

David Garcia Gragera
Melissa Pilot Plant

MELiSSA (Micro Ecological Life Support System Alternative) is an international effort developing technology for regenerative life support to enable long-term human exploration missions in Space. It is conceived as a loop of interconnected bioreactors providing the basic functions of life support and has established a Pilot Plant to demonstrate and integrate the technology developments to achieve such a target. One of these compartments is an 83L photosynthetic external loop air-lift photobioreactor where Limnospira indica is cultivated in a continuous operation mode. Its mission is to provide O_2 from photosynthesis, capture CO_2 and produce an edible material. The work presented focuses on the operational and biological characterisation of the photosynthetic bioreactor after the design and installation of a LED-based illumination system, with the aim to define the best operational conditions for O_2 production as the main target.. Illumination system provides a a maximum PFD (Photon Flux Density) of 1700 µmol·m-2·s-1, which is two times the output from the previous halogen system. A DoE (Design of Experiments) methodology approach was used in order to obtain a surface response. Two manipulated variables were studied: dilution rate (D) and PFD ranging from 0.01 to 0.04 h-1 and from 163 to 1700 µmol·m-2·s-1 respectively. The bioreactor was operated in continuous mode and main parameters such as O_2 production, biomass concentration, NO_3^- consumption and biomass composition were followed. Each condition was tested until 3 HRT (Hydraulic Residence Time) with stable results were reached (steady-state). Once it was accomplished, a new operational condition was tested. Initial results from this methodology showed a maximum rO_2 higher than 0.04 g·L-1·h-1 was obtained with a D of 0.025 h-1 and PFD of 930 µmol·m-2·s-1 with a biomass concentration of 1.05±0.07 g·L-1, as best result. However potential photoinhibition occurred when a 1 g·L-1 cell density culture working at 0.025 h-1 was exposed to a PFD of 1700 µmol·m-2·s-1. Considering these results, a full series of experiments at different values of these two main variables has been performed and the obtained results will be presented. In parallel to the bioreactor characterisation, these experiments are valid for obtaining the radiative properties of L.indica. Several advances have been made in the recent years regarding the methodological approach for reaching such properties and an update in our case of study is mandatory. Input parameters such as morphological and structural characteristics of L.indica as well as the photosynthetic pigments content were obtained. Samples from previous experiments were analysed by confocal microscopy techniques to obtain the size distribution and morphology of the cells. Phycobiliproteins and chlorophyll content are obtained by extraction and spectroscopy measurement. All these input parameters enable to finally reach the scattering cross-section, absorption cross-section and phase function of the strain used.
Keywords: MELiSSA, Life support, Photobioreactor, Limnospira indica, Radiative properties, Photon Flux Density, Design of Experiments.

The effect of phosphate starvation on nutrient uptake and cellular content of the microalgae Desmodesmus communis & Chlorella protothecoides

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Effluents from wastewater treatment plants contribute to surface water eutrophication. Conventional wastewater post-treatment is efficient but expensive and it generates excess waste. Microalgae is often proposed as an alternative because of their ability for complete nutrient removal from wastewater, but this approach is rather slow. Altering the external phosphorus availability can enhance the algal phosphate uptake. Also, nutrient availability regulates the cellular chemical content. Thus, manipulation with nutrient availability can add value to algal role in circular systems. In this study we aim to demonstrate that the biomass phosphorus-starvation enhances phosphate uptake and improves algal cell chemical content. MATERIALS AND METHODS Microalgae D.communis and C.protothecoides were used for the experiment. Algal biomass was exposed to P-deficit conditions for various time periods. The experiment was run in a batch regime using an effluent from small municipal WWTP. The change in concentrations of algal biomass, DIN, DIP and biomass polyphosphate concentrations were measured to assess the algal strain and its starvation period efficiency. RESULTS AND DISCUSSION D.communis showed the highest specific growth rate (0.72 d-1), obtained after 7-day P-starvation period. For C.protothecoides the phosphorus deficit resulted in lower growth rate. P starvation for 7-days promoted DIP concentration decrease - D.communis reduced DIP content by ~89% within 24 hours. Total nitrogen removal for both strains did not exceed 50%. Both strains showed higher Poly-P storage in biomass after 7 days of P-starvation. C.protothecoides accumulated more Poly-P (245.3 μg mg-1) than D.communis (160.8 μg mg-1). These results and similar studies (Schmidt et al., 2016; Wu et al., 2012) show that biomass exposure to P-deficit conditions can enhance the phosphate uptake from wastewater. It could potentially reduce the HRT and reactor size in a pilot-scale system. Higher cellular Poly-P content makes the biomass suitable for nutrient supplementation for higher plant growth. Increased accumulation of cellular Poly-P content, observed for both strains after phosphorus starvation indicates to higher lipid production potential (Chu et al., 2015). Higher Poly-P accumulation was observed in wastewater with higher N:P ratio, indicating to nitrogen-limited process. Thus, a consistent wastewater composition is important to maintain high Poly-P accumulation.

Microalgae: from oxygen and food production in Space to groundwater processing on Earth

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European Space Agency

Future human space flight missions in the coming decades should bring us back to the Moon for longer stays and for the first time on the way to Mars. The long duration of those missions and the increased distance from Earth will require us to be as independent as possible from Earth resources. That applies among others to the Life Support System (LSS). Physico-chemical systems are capable of regaining O2 from the exhaled CO2 and recycle water. However, to produce food, biological technologies will be required. Biological components might complement the physico-chemical technologies already in use to create a Hybrid LSS. A potential candidate to be used for biological technologies are microalgae, which compared to higher plants offer a higher harvest index, higher biomass productivity and require less water. A promising microalgae species for spaces applications is Chlorella vulgaris, a spherical single cell organism, with a mean diameter of 6 µm. Chlorella is ideal for long term cultivation as part of a LSS as it can grow in a wide range of pH and temperature levels and CO2 concentrations and it shows a high resistance to cross contamination and to mechanical shear stress. At the Institute of Space Systems, research on Chlorella vulgaris for space applications has been carried out for over a decade. The research has been focused at a system level, including the design of the required infrastructure and the cultivation techniques for the long-term cultivation of microalgae in space. That includes the experiment PBR@LSR (a DLR, IRS and Airbus project) which was send to the International Space Station in 2019 and worked for two weeks. The experiment could not run for the expected amount of time due to a technical problem. Thus, further research in this area is still required, to demonstrate in space the functionality and feasibility of the hybrid LSS approach, the long-term stability of an algae system and the photosynthetic conversion of concentrated CO2 into O2 and biomass. Microalgae-based photobioreactors can be used to reduce the required resupply mass for space missions. But, the systems being developed for space applications can also help to solve problems on Earth. Requirements for space applications are quite restrictive in terms of available resources and safety. Thus, the knowledge gained for space applications can help designing more efficient and controllable microalgae-based-systems to be used for Earth applications. A potential usage is the reduction of nitrate and phosphate levels in wastewater from agricultural applications. At IRS, as a technology transfer project funded by DLR Space Administration, a scalable test-stand of a microalgae-based system, the PBR@Earth, is being developed to investigate intake rates and quantify and assess the produced biomass. Two important factors in the design are the scalability and automation of the system. PBR@Earth development is complemented by a Boysen-foundation financed project which focuses on the design of an adjustable lighting unit and a biomass sensor. This presentation will show the link between Space and Earth applications, and provide an overview of the current status of PBR@LSR and PBR@Earth.

Solid-Liquid Separation Technology for Biomass Harvesting in Bioreactors

Marie Vandermies
QinetiQ Space

Long-distance crewed space missions for exploration of new habitats in outer space might be reality within a few decades. Advanced regenerative Life Support Systems will be required to process resources and waste for the production of water, oxygen and food. Biochemical conversions driven by microorganisms is a mandatory key technology. Biomass is produced during these processes has to be harvested for further processing and use. A review of candidate technologies compatible with zero and reduced gravity conditions is presented. Separation technology for the cyanobacteria will be highlighted as a case study. Candidate technologies available to separate solids from liquid phase include, among others, filtration and (forced) gravitation. Additionally liquid removal can be achieved by heat- or vacuum-driven evaporation. Centrifugation being far more complex, and less efficient in the case of variable cell densities, this review focuses on separation by filtration. More specifically, filtration processes are determined by flow configuration, pore size, membrane geometry and membrane material. The feed flow can be tangential or normal to the membrane surface, resulting in tangential or dead-end filtration. The former is more complex, expensive, and requires a higher setup volume. The latter is prone to membrane fouling and clogging. Pore size sets the type of particles that will be retained by the membrane, and therefore defines the type of filtration process (i.e., macrofiltration, microfiltration, ultrafiltration, nanofiltration, reverse or osmosis). The smaller the pore size, the higher the filtration driving force. Membrane geometry is selected based on specific area, while membrane material is selected based on its compatibility with process conditions. Furthermore, the driving force type (i.e., gradient of pressure, concentration, electric potential, or temperature) and intensity influence the liquid flux across the membrane. All these features and parameters have to be weighted in regard to the process requirements in order to reach an adequate trade-off. Within MELiSSA cycle, the cyanobacterium Limnospira aims to provide the consumer compartment with oxygen by photosynthesis. During this process, carbondioxide is metabolized in new cells. To maintain the biomass concentration inside the photobioreactor at desired level, supernumerary cells are harvested by filtration and the recovered culture medium is reinjected in the photobioreactor. A compact dead-end filtration unit with cellulose depth filter sheets was validated. Culture medium containing 1 g/l could be concentrated in a filtration cake with a dry mass of 7% at a maximum operating gauge pressure of 1.5 bar. The filtration unit capacity is estimated to 500 g of dry cell weight per m^2. The obtained results allowed the design of an automated filtration unit.

Characterization of oxygen production from photo-bioreactor for ISS cabin technology demonstrator

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RUAG Slip Rings SA

Oxygen regeneration from ambient carbon dioxide is a fundamental technology building block for future life support systems for space applications. BIORAT Phase B2 project consists in the development of an on board technology demonstrator preliminary design for the ISS cabin to be hosted in EDR2 facility. The design of the flight hardware demonstrator will be supported by tests results obtained in a representative bread board model (BBM). This project phase has started on December 2018 and will be running for 30 months. In this paper, we present the result of life tests performed on the liquid loop subsystem BBM developed so far. This BBM allows to recycle supplied CO2 into oxygen and biomass and consists, among other functionalities, in a dedicated photo-bioreactor for the continuous cultivation of algae (Limnospira indica PCC 8005), which are responsible for the consumption of CO2 and for the production of oxygen and in a membrane module for the exchange of oxygen & carbon dioxide between the liquid phase in the liquid loop to the gaseous phase of the ambient air. These life test results allowed to gain experiences on long duration algae cultivation (27 days) and to perform correlations of existing mathematical model of the cultivated algae growth in term of biomass and oxygen production to the actual measured values of the liquid loop BBM. The life tests results, this characterization and future development are presented and discussed in this paper.

Coupling urine treatment and water recycling with Limnospira indica cultivation

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Mons University

Long-duration human space missions require considerable amounts of water, oxygen (O2) and nutritious biomass to support the crew. Carrying sufficient supplies for 5-6 persons, for a return trip to Martian or Lunar bases is not feasible with present launch technology. Additionally, the spaceflights must be well-equipped to deal with metabolic waste streams. It is therefore important to look for practical alternatives to support life on such missions. One solution is to aim at self-sufficiency in terms of food, water and O2 production and waste management by employing regenerative life support systems (RLSS) like ESA's MELiSSA (Micro-Ecological Life Support System Alternative). The cyanobacterium Limnospira indica (formerly Arthrospira sp. PCC 8005) has been reported to assimilate both organic and inorganic nitrogen (N) sources. The possibility to use nitrified urine, as recycled source of nitrogen, water and other elements, and gaseous carbon dioxide, as recycled carbon source, for Limnospira indica cultivation could facilitate oxygen and food production. However, the high concentrations of various salts in urine have been reported to be inhibitory for Limnospira indica growth. Furthermore, source separated urine is highly unstable and thus needs to be stabilized to avoid the loss of nutrients, downstream usage problems and unsafe emissions. Partial nitrification of urine generates different N forms, mainly nitrate but in case of process instability also nitrite. It is therefore important to investigate the tolerance of Limnospira indica to high concentrations of different salts and N-sources (as mixture), in order to valorize urine as Limnospira indica medium. The present study evaluated the salt tolerance and the effect of mixed N-sources on the growth, the biochemical composition and the general metabolism of Limnospira indica. The preliminary experiments indicated that Limnospira indica can grow at high concentrations of salt which are normally present in human urine. In fact Limnospira indica cells exhibited comparable growth rates in Zarrouk Medium and Zarrouk medium supplemented with a 'urine-like' salt concentration. The effect of high salt concentrations on the metabolic and physiological characteristics of Limnospira indica has been analyzed. The study results clearly indicated that it is possible to valorize nitrified urine and N-source mixture for Limnospira indica cultivation. These results have opened new avenues towards solving the issues of wastewater remediation using photosynthetic cells. Such a possibility would further bring us step closer towards the (1) creation of photosynthetic biorefineries for production of high-value co-products using cheaper nutrients and (2) creation of waste-to-value based circular economy by embedding cultivation of photosynthetic microorganisms with wastewater remediation.

Design and control of a bioanode for CO2 recovery in regenerative life support systems

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Establishing a manned base independent of resupply from Earth presents a unique waste treatment challenges with a critical need for near complete recycling of essential resources from organic wastes produced on site. New technologies for primary waste degradation are being investigated in the context of the MELiSSA loop, the regenerative life support system of the European Space Agency (ESA). This system must retain nutrients and produce CO2, as inputs for downstream food production compartments. Optimized fermentation can only achieve 40-50 % efficiency in degradation of the standard MELiSSA waste, and drives carbon primarily to volatile fatty acids while only ~15 % of influent carbon is converted to CO2. In this study we demonstrate the utility of a microbial electrochemical cell (MEC) to drive carbon recovery towards CO2. Bio-anodic oxidation of fermentation effluent through an MEC has the potential to favour CO2 production over methane, and provide in situ pH control by electro-migration of hydroxyl ions produced at the cathode across an anion exchange membrane. In this study we present result of a scale up from a 0.008 to 0.08 m2 bio-electrochemical cell with customized control and automation features implemented to improve process stability, and gas recovery. Overall, total COD removal efficiencies were achieved above 80 %, and VFA removal efficiencies of 95 to 100 % could be achieved in both permeate and defined medium tests. Coulombic efficiencies between 75 and 90 % were achieved across different tests indicating a strong selection for the bioanodic (CO2 producing) process. Under continuous operation with fermentation effluent this system has reached VFA removal efficiencies above 95 %, total organics removal efficiencies of 88 %, with 60 to 70 % of fed organic carbon recovered as CO2. This work makes an important contribution towards the technical development of microbial electrochemical systems and demonstrates their potential for use in regenerative life support systems.

Microbial analysis of the MELiSSA waste degradation compartment 1 (C1) and isolation and identification of C1 dominant bacteria

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Micro-Ecological Life Support System Alternative (MELiSSA) is a concept developed by the European Space Agency (ESA), that evolved out of the need for a regenerative life support system for long-term space missions. Inspired on a lake ecosystem, MELiSSA consists of four biological compartments (C1 and C4) conceived as a closed-loop system that combines activities of different organisms to recycle organic waste to water, oxygen, and foods for the space crew. In the C1 compartment, a thermophilic anaerobic microbial consortium liquefies the solid waste to produce CO2 for the C4 compartment and produce ammonium, volatile fatty acids (VFA's), and minerals for the C2 compartment. To date, little is known about the composition of the MELiSSA C1 microbial community in terms of taxonomy and functioning. This research aims to acquire knowledge of the C1 microbial community in order to be able to design a mechanistic control model for the C1 compartment. The long term (up to almost 4 years) dynamics of the C1 microbial community structure in three different C1 reactors was studied by 16S ribosomal RNA (rRNA) gene/transcript amplicon denaturing gradient gel electrophoresis (DGGE) and high-throughput Illumina sequencing. In addition, dominant and potentially keystone species of the C1 community were isolated and identified in order to further characterize their roles and interactions in waste conversion. The data demonstrated a high stable community composition in C1 reactors up to day 1454 after inoculation. Over 2,500 different operational taxonomic units (OTUs) were identified. The C1 core microbiome, OTUs shared between all 3 reactors was composed of 500 OTUs which represented 93% of the total bacterial community. Seven of these shared OTUs, related to Caprociproducens, Thermoanaerobacterium, Unclassified Ruminoccocaceae, Lactobacillus, Fonticella, Leuconostoc, and Moorella, accounted for 75% of the total C1 bacterial community, with Caprociproducens and Thermoanarobacterium being the most dominant. Nine different strains were isolated of which one of them appeared to be the dominant C1 bacterium Thermoanaerobacterium. The organism was most related to the obligate anaerobic T. thermosaccharolyticum. Other isolates related to the facultative anaerobic Lactobacillus and Bacillus, and to the obligate anaerobic Clostridium baratii.

Improving ammonification to nitrate production in bioconversion of organic fertilizers to liquid products

Yankai Xie
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Organic fertilizers contain recovered nutrients, yet require microbial mineralization to produce ammonium and/or nitrate as suitable nitrogen species for fertigation and hydroponics. This study aimed at improving mineralization in a treatment step, testing the effects of inoculum type and concentration, temperature and dissolved oxygen level on a plant- and a microbe-based fertilizer. With anaerobic digestion sludge as inoculum for anaerobic treatment of the plant-based product, its concentration at 0.1 or 0.4 g VSS/L did not show obvious differences in both mineralization rate and efficiency. However, the adapted ‘second-generation’ inoculum showed improvements at 20°C, with the ammonification rate constants reaching 0.077 d-1 and the mineralization efficiency 61%. Furthermore, mesophilic conditions (35°C) increased the rate constant to 0.249 d -1. When using nitrifying activated sludge as inoculum for aerobic treatment at 35°C, the rate constant was even higher 0.349 d-1. The microbe-based product showed to be faster and more efficient in mineralization, facilitating its use as sustainable fertilizer.

Plants in Space

Hristina Tsenova Kostadinova
High Language School "Ivan Vazov"

The more space science develops, the clearer it is that future generations of our civilization will control, inhabit and travel in space around the Earth. It is hard to imagine that astronauts' long missions in future will be realized in an only technological environment. Building a life-sustaining system for astronauts on a mission to Mars is our main goal. For the development of an effective life-sustaining unit for use on long space and crewed flights, we need fresh food. The plants, we selected, are radishes (Raphanus sativus) but it is also necessary to work on the supply of proteins and fats. By calculating the specific nutritional needs of astronauts, we can keep them healthy on long duration space explorations. Water recycling is absolutely necessary. It reduces the load on board the spacecraft. The effect of the physical activity on the oxygen consumption has to be taken into account on spacecraft. For the successful growth of radishes, the photosynthetic active radiation supply has to be at least 2μmol/m2/s. In the 2 μmol/m2/s treatment, the most effective influence on both mass and length of cotyledons exerted the red and the blue light. On the other hand, the accumulation of the photosynthetic pigments was influenced by the blue and green spectrum of light. In the 0,5 μmol/m2/s treatment, the mass and the length of cotyledons were influenced significantly by the yellow and the blue spectrum of light. While the white and the red light exerted an influence on the accumulation of photosynthetic pigments. I've been working on this project for five years. At the moment our experiments are being concentrated on Spirulina and how it develops when influenced by red, yellow,green and blue LED light. My students are ages 15-16 years old. We are working at our school and we have the help of University of Plovdiv "Paisii Hilendarski".

Soybean hydroponic crop production with human urine derived waste products

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The use of processed human waste products, specifically human urine, has regained popularity as a potential fertilizer for crop production. This comes with the increased interest in alternative cropping developments for Earth and other planetary operations. For example, the process of urine nitrification (the biological oxidation of ammonia/ammonium to nitrite followed by the transformation of nitrite to nitrate) and hydroponic (soilless) crop production is explored as a linked process for life support systems in space exploration and Lunar/Martian bases. However, using urine in solution for hydroponic crop production comes with some challenges. Nitrified urine contains mostly nitrogen, phosphorus, and potassium with traces of other micronutrients (B, Cu, Zn, Mn, Fe, and Co); however, these nutrients are not all readily available or in the correct balances for optimal plant uptake. In particular, phosphorus, which precipitates before the nitrification process to form e.g., struvite or hydroxyapatite. In hydroponics, all nutrients must be in solution and available for plant uptake. Struvite and hydroxyapatite have shown promise as fertilizers in field trials, but their use in hydroponics is unknown due to their low solubility. We are testing the use of a human urine derived fertilizers (including nitrified urine and phosphorus precipitates) on hydroponic soybean production for space missions. We are looking closely at how the nutritional composition of the plant and nutrient solution changes overtime. We hypothesize that the nitrified urine alone is an adequate source of N, but we expect the plants to experience P, and possibly micronutrient, deficiency in the hydroponic system. The P precipitants (urine derived struvite and hydroxyapatite) may provide the system with additional P and micronutrients, yet the solid state of the precipitants is not ideal for hydroponics. Thus, we hypothesize that if added to the nutrient solution, the P precipitants may work as a slow release P fertilizer, but may need the help of additional chemical or biological additives.

Producing ink from Organic Waste (OW-ink) for additive manufacturing in space

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Blue Horizon S.a.r.l.

Expanding human presence beyond low Earth orbit relies on the readiness of new capabilities and technologies. Different technologies are required for the realisation of stable and sustainable human outposts on the Moon and Mars, maximising self-sustainability through closed loop life support. Launch mass requirements are the single most significant limitation of our current space mission capability. The limits of what we can economically launch and safely land severely restrict current missions and disqualify many others from consideration. Materials need to be highly customizable, allow variation (in sizes and shapes) and produced quickly on site. For this, additive manufacturing is considered one of the key enabling technologies for a large number of applications. Recent advances have enabled additive manufacturing of not only inorganic materials such as metal, polymers and ceramics but also organic material such as cells, bacteria, etc. arranged in functional organic tissues and materials. AM methods and capabilities vary according to the materials used (so called “inks”), cross-linking mechanisms after AM deposition and extrusion techniques of the AM. Each technique supports a particular range of control over matrix architecture, mechanical properties, degradation, and biological components. Exploitation of these features used in conjunction with the application-specific “ink” formulations creates a platform for fabricating customized materials and devices. Within this work in progress, we focus on the generation of “inks” from solid organic waste (hereafter called OW-inks) which is in line with different top-level open questions of ESA´s roadmap #7 “Support life in hostile environments” concerning recycling of waste, biocompatibility of new materials and the generation of new bio-inspired functionalities for materials. OW-Inks for additive manufacturing can address the issues mentioned above as they can offer functionalities such as mechanical permeability and chemical resistance, which are superior to that of their traditional inorganic counterparts. The different applications, after AM, in which these OW-inks can be used, and their overall feasibility and viability will be determined within this study. Furthermore, their functionalities will also depend on the additive manufacturing processes itself and the capabilities for using these inks have advanced in order to manufacture i.e. bio-active surfaces, building blocks, etc which can be interesting for space exploration. In a first step in this concept study the different steps of the process for producing ink from solid organic waste are analyzed (highlighted by light blue rectangle in Figure 1). The overall feasibility and viability of the process for space exploration and for a pre-selected range of commercial applications on Earth shall be assessed. Figure 1: Overview of processes to produce OW-inks for additive manufacturing from solid organic waste for different space applications. Circled in light blue the part of the overall process which are the main focus of this work in progress. In the dark blue boxes, materials are indicated which serve as feedstock for the different processes, shown in green arrows.

Two-phase system for anaerobic digestion of corn extract for biohydrogen and biomethane production

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The Stephan Angeloff Institute of Microbiology

The biological treatment of organic wastes is very important issue concerning either the Earth based solutions for overcoming of pollution problems, useful products production or concerning the life support systems during piloted space missions or extraterrestrial bases. The waste product corn extract was selected in the present work as a model substrate rich in proteins and tested for subsequent production of the energy carriers biohydrogen and biomethane. Two phase system based on two connected anaerobic continuous stirred tank reactors with working volumes of 3 dm3 and 10 dm3 was applied for biological treatment of corn extract. First bioreactor operated at 37 ℃ and the second one worked at 55 ℃. During the first phase of the process the other liquid products are volatile fatty acids with high butyrate concentration followed by acetate concentration ranging from 1:1 to 8:1. These organic acids are substrates for the methanogenic microbial community in the second stage. Usually, the anaerobic digestion processes are conveyed by consortia of microorganisms. The metagenomics analysis of the anaerobic microbial community was performed for estimation of the major groups of microorganisms involved in the microbial degradation process. Genus composition in the sample of first anaerobic digester revealed that main representatives belong to Clostridium. The simple model was proposed for mathematical modeling of the process. The possibilities for application of digestate for recycling in the two phase system was also discussed.

Investigating volatile fatty acids conversion to CO2 by the MELiSSA bacterium Rhodospirillum rubrum in various culture conditions

Felice Mastroleo
SCK-CEN

The second compartment (C2) of the ESA’s Micro-Ecological Life Support System Alternative project was designed to mainly process the volatile fatty acids (VFAs) coming from the first compartment but also to produce CO2 from the related catabolic process. Removing VFAs from the water stream is crucial to ensure a good rate of nitrification in the third compartment while CO2 supply is needed for the fourth compartment where higher plants and cyanobacteria are grown. C2 is inhabited by Rhodospirillum rubrum, a purple proteobacterium that shows highly versatile metabolic capabilities but so far, the net production of CO2 in MELiSSA related conditions, meaning acetate, propionate and butyrate degradation in anaerobic conditions with light as energy source, was not reported. In this work, we set up a simple and robust protocol for batch cultivation of R. rubrum in anaerobic conditions with 80 µmol/m2*s halogen illumination, and allowing CO2 production follow-up using a standardized colorimetric method. Our preliminary results put forward a net production of CO2 in light anaerobic conditions when acetate was used a sole carbon source. Surprisingly a similar net production of CO2 was also measured when using a mixture (9:1) of acetate and propionate (124mM net C concentration) while photo-assimilation of propionate was previously reported to be dependent on CO2 fixation. Ongoing work includes the introduction of butyrate as extra carbon source and follow-up of VFAs consumption using ion exchange chromatography. In addition, possible improvement of net CO2 production using microaerophilic conditions is also envisaged. These new results will be summarized during this presentation.

SpaceBakery – a closed ecological plant cultivation system and bakery for extended stays on Planet Mars and their applications for Planet Earth

Lucie Beckers
Puratos

A Moonshot statement was made by Puratos in 2018: Learn to bake bread on Mars. Why? The people that will set foot on Mars need to have access to sufficient food supply. Puratos decided to team up with Urban Crop Solutions, Magics, Flanders'FOOD, SCK.CEN, Gent University and Hasselt University and started together the joined SpaceBakery project, with an official start in January 2020. The project idea is to develop a Closed Ecological Plant Cultivation System and Bakery, suitable for a Mars mission concept, but designed and engineered for direct equivalent applications for Planet Earth: a closed and self-sustainable modular system which is independent from agricultural land or climate and optimal use of resources. The specific objective of the project consortium has direct parallels with the challenges of developing closed ecological systems and bioregenerative life support technologies for space applications. The research combine a series of emerging and disruptive technologies as a speaking plant approach for crop modeling, agrobots, AI, automated indoor farming, closed loop farming, microbiology and natural food fermentation. Key objectives are the design of a smart and energy efficient crop production system, the modeling of wheat growth within the specific conditions of the closed environment, the valorization of plant fibers as source of nutrients within the closed cycle, the identification of beneficial microorganisms to increase the nutrient availability for plants, the characterization of microbial communities present in each step of the production cycle and their influence on the natural fermentation processes in bread and the selection of a diverse range of crops to create nutritional and tasty bread products. Within a period of two and a half years, this consortium aims to achieve the development of a closed ecological plant cultivation system and bakery that can provide the first Martians with bread and also generates insights for sustainable and nutritional food production here on earth.

Space Architecture for a Moon Village - Life Support Elements

Daniel Inocente & Brigitte Lamaze
SOM, ESA

Owings & Merrill LLP (SOM), in partnership with the European Space Agency (ESA) and faculty of the department of Aeronautics and Astronautics at the Massachusetts Institute of Technology (MIT), have proposed space architecture and master planning strategies for the first full-time human settlement on the Moon. One aspect of ESA's space exploration efforts in Low Earth Orbit, the Moon and Mars is to aim at "developing new concepts for international exploration activities, encompassing novel cooperation opportunities open to all nations and industrial actors". The partnership envisions future missions to the lunar surface that will be driven by cooperation and sustainable planning strategies. The "Moon Village" idea presented by the ESA Director General is a vision for an open architecture based on global cooperation among multiple nations and multiple partners combining their various expertise for the common objective of enabling long-term exploration of the lunar surface. Advancement of new and emerging capabilities supported by commercial expertise, transferring proven technologies toward addressing challenges in space will result in the construction of an early outpost for safe, flexible and efficient human exploration. Achieving this initial goal would produce operational experience for the planning and extensive development of an eventual sustainable and permanent lunar ecosystem that will support a variety of human activities for scientific exploration, industrial development and commercial initiatives. This paper will present a holistic approach to considerations for achieving self-sufficiency in space habitation architecture by leveraging advancements in life-support and environmental control technologies. Located on the edge of Shackleton crater near the lunar south pole, the study aims to maximize the use of closed loop, regenerative life support systems and In-Situ Resource Utilization. Results from past activities, including a recent concurrent design facility study will be presented.

Plant prebiotics in BLSS for human nutrition in space

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Higher plants have a central role on food production and resource recycling in BLSS. Plant contains a vast array of molecules some of which are not digested by humans but are metabolized by the gut microbiota thereby influencing the microbiota and the host functioning. Such molecules are named prebiotics. The role of prebiotic in determining wellbeing and health is of increasing evidence even with reference to their potential role for mitigating problems related to long term space missions. Notwithstanding the emerging role of prebiotics in human nutrition, there is still a limited knowledge on the regulation of their synthesis and accumulation in plants in particular in relation to the role that the environment exerts on controlling their synthesis, accumulation and molecular characteristics. Our first working hypothesis is that with adequate analytical methods prebiotic can be identified, quantified and characterize even in plants proposed for BLSS. In fact, analytical characterization of plant material produced in projects on BLSS was rarely sufficiently detailed and suitable to highlight the presence of prebiotics. A second working hypothesis is that more research should be performed on the role of the environment in controlling the synthesis and accumulation of prebiotics in plant grown in BLSS. This could allow increasing our basic knowledge on prebiotic related plant metabolism, synthesis and accumulation. Furthermore, this would allow including prebiotic production as one of the relevant functions of BLSS. Research on prebiotic mitigation of space induced health and stress problems for humans can rapidly become a hot topic in the space research community. We analyzed the presence of prebiotic in plant material produced under fully controlled plant growth experiments and on species that are among those selected for use in BLSS by different projects. Different type of prebiotics were found in the analyzed species and tissues, including inulin, fructo-oligosaccharides, raffinose family oligosaccharides, polyols, cell wall components with variable monomer composition and polyphenols that also appear to have prebiotic properties. Large variability was recorded with respect to prebiotic presence, amount and type among the analyzed species and even between different tissues of the same species. Our results suggest that with proper analytical methods we can quantify relevant amounts of prebiotics in vegetable and therefore modify the quality evaluation of such type of food when considering it for BLSS. Our results suggest that the species selection and the control of the growing conditions in BLSS should take into account the production of prebiotics as a relevant output of the systems. New knowledge on the possibility to modulate the abundance and type of prebiotics on vegetables would be of general interest and benefit for Earth based food systems.

THIRD DAY

Design and simulation of circular water systems: the UWOT model

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The existing limitations of centralized water management strategies, as well as the challenges the corresponding, conventional water systems face [1–3], have led to the emergence of circular water systems as a promising alternative [4,5]. Borrowing concepts from circular economy [6], circular water systems prioritise water reduction, reuse, recycling and replenishing over the traditional “take-produce-dispose” model of linear water management [7]. This is achieved by combining multiple distributed technologies in an integrated fashion - and as a supplement to centralized systems – in order to design hybrid systems at different spatial scales (houses, neighborhoods, regions). The technologies that are combined differ per project and may include decentralized options at the tap level, such as water-aware appliances or greywater recycling, as well as more upscaled, regional measures such as rainwater harvesting, sustainable urban drainage, aquifer storage and recovery and sewer mining. The effects of the combined usage of these technologies are evident across urban water cycle domains, as these systems achieve drinking water (DW) reduction, control of wastewater (WW) streams, as well as stormwater (SW) peak and volume retention. Moreover, the introduction of these technologies within urban clusters achieves broader beneficial effects to the city level and contributes to climate-proof, adaptive and resilient societies [8]. Despite the promising outlook of circular water systems, the methods and tools to assess the performance of these distributed solutions and provide management support for integrated projects are either context-specific or untested in real, combined cases across spatial scales [9]. This study presents a generic, simulation-based model, the Urban Water Optioneering Tool (UWOT), and provides an overview of its applications for systems across different spatial scales and diverse circular water contexts. Starting from an in-household scale and the corresponding appliances, UWOT is able to upscale and simulate demand signals – and the way they are covered by different sources – at a neighborhood, region or entire city level [10]. UWOT simulates both standard, linear urban water streams, including DW, WW and runoff, as well as integrated interventions in any of these domains, following a signal-based logic of demand generation, aggregation and transformation from tap to source [11]. This enables the exploration of cases that transition from the business-as-usual, linear case of water management to a progressively more circular water model. Encapsulating multi-year research experience across multiple European (EU) projects and use cases, UWOT has been applied in circular water settings that span from the household and neighborhood scale [12–14] to whole city modeling [11,15,16].

This study focuses on recent advances that include: (a) a model application for the (re-)design of a Dutch circular water neighborhood in Limburg, which combines DW, WW and runoff retention measures; (b) an application in Athens quantifying the effects of a novel sewer mining option; (c) a broader demonstration for the regional case of Westland, where rural circular water technologies are combined with urban interventions to close the regional water cycle. The two latter cases are part of the NextGen EU project, aiming at demonstrating circular water solutions across ten European demo cases. References 1. Muller, M. Adapting to climate change: water management for urban resilience. Environ. Urban. 2007, 19, 99–113. 2. Baird, G.M. A game plan for aging water infrastructure. Journal-American Water Work. Assoc. 2010, 102, 74–82. 3. Moe, C.L.; Rheingans, R.D. Global challenges in water, sanitation and health. J. Water Health 2006, 4, 41–57. 4. Gleick, P.H. Water management: Soft water paths. Nature 2002, 418, 373. 5. Newman, P. Sustainable urban water systems in rich and poor cities - steps towards a new approach. Water Sci. Technol. 2001, 43, 93–99. 6. EU Commission Closing the loop - An EU action plan for the Circular Economy; 2015; 7. Tahir, S.; Steichen, T.; Shouler, M. Water and Circular economy: A white paper; 2019; 8. Makropoulos, C.; Nikolopoulos, D.; Palmen, L.; Kools, S.; Segrave, A.; Vries, D.; Koop, S.; van Alphen, H.J.; Vonk, E.; van Thienen, P.; et al. A resilience assessment method for urban water systems. Urban Water J. 2018, 15, 316–328. 9. Peña-Guzmán, C.A.; Melgarejo, J.; Prats, D.; Torres, A.; Martínez, S. Urban water cycle simulation/management models: A review. Water (Switzerland) 2017, 9, 1–29. 10. Makropoulos, C. Thinking platforms for smarter urban water systems: fusing technical and socio-economic models and tools. Geol. Soc. London, Spec. Publ. 2017, 408, 201–219. 11. Rozos, E.; Makropoulos, C. Source to tap urban water cycle modelling. Environ. Model. Softw. 2013, 41, 139–150. 12. Rozos, E.; Makropoulos, C. Assessing the combined benefits of water recycling technologies by modelling the total urban water cycle. Urban Water J. 2012, 37–41. 13. Makropoulos, C.K.; Natsis, K.; Liu, S.; Mittas, K.; Butler, D. Decision support for sustainable option selection in integrated urban water management. Environ. Model. Softw. 2008, 23, 1448–1460. 14. Bouziotas, D.; van Duuren, D.; van Alphen, H.-J.; Frijns, J.; Nikolopoulos, D.; Makropoulos, C. Towards Circular Water Neighborhoods: Simulation-Based Decision Support for Integrated Decentralized Urban Water Systems. Water 2019, 11, 1227. 15. Nikolopoulos, D.; van Alphen, H.-J.; Vries, D.; Palmen, L.; Koop, S.; van Thienen, P.; Medema, G.; Makropoulos, C. Tackling the “New Normal”: A Resilience Assessment Method Applied to Real-World Urban Water Systems. Water 2019, 11, 330. 16. Bouziotas, D.; Rozos, V.; Makropoulos, C. Water and the City: Exploring links between urban growth and water demand management. J. Hydroinformatics 2014.

Grey water recycling from space to earth

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FGWRS

One of the challenges of space life support developments is the very limited number of applications, namely only the iSS despite a very long term R&D period. Taking into account this constraint, the space agencies have always the tendency to give priorities to recurrent developments. Fortunately, for life support developments, terrestrial environment issues present major synergies with the space objectives. Based on the long term effort of ESA, and especially MELiSSA project, to developp water recycling system, the company FGWRS which develops the know-how of FIRMUS France, has considered the commercial applications. With Sherpa Engineering (member of MELISSA) we have created a simulator.and we are able to review, size and predict the functioning of a grey water recycling process paired with energy recovery in collective use buildings (hotels). FGWRS® has a demonstrator that runs continuously on 4 hotel rooms in Monaco and all the parameters are collected and analysed (water quality, temperature, treatment rate, ...). We will also showcase this ESA technology on the Monaco Pavilion for the Dubai 2020 expo and in the training locker rooms of the Roland Garros 2020 tennis tournament. There is no denying that there is no point in using drinking water for toilet flush, laundry, floor cleaning and even showers; all the more, as it becomes more and more urgent to save water. The time has come to transfer this know-how, acquired from ESA technology, to more practical applications on earth.

Consolidating the Swiss activities and rationale for ALSS and MELiSSA development

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In 2019, over twenty Swiss stakeholders active in the field of Advanced Life Support Systems (ALSS), including those directly involved in the R&D and transfer of related space- and Earth-based technologies, expressed their strong interest in the ongoing MELiSSA project of ESA by endorsing a Position Paper. Elaborated in the context of the preparation for the recently held ESA Ministerial Council Space19+, the overall objectives of this Swiss Position Paper were: (a) to develop an ALSS roadmap, encompassing the three main pillars of the Swiss Space Policy and (b) to demonstrate that developments in the field of ALSS would be enhanced by a consolidated and concerted effort from the Swiss stakeholders, especially within the framework of the MELiSSA project. The presentation details the results of a survey on Swiss activities, interests and strengths in ALSS and shows that: • the Swiss MELiSSA and ALSS stakeholders encompass a broad range of public and private organisations exhibiting diverse and complementary scientific and technological skills and know-how; • the emerging and dynamic Swiss ALSS community has reached a critical size and momentum; • the Swiss ALSS community has gained a clear perception of the assets and uniqueness of MELiSSA and is demonstrating a precise understanding of potentials for collaboration; • the active participation of the community can be demonstrated by more than 30 R&D and technology transfer projects, covering most of the dimensions and topics of investigations on ALSS. The presentation describes how the Swiss ALSS community is fostering the continuation of the current political support and looking towards long-term vision and planning in order to: • investigate topics relevant both for human space exploration and for their associated Earth-based applications; • attract and increase engagement of the Swiss non-space ALSS organisations in space exploration; • enhance synergies between Swiss players and the MELiSSA community; • further consider developing a dedicated testbed in Switzerland to experiment with ALSS concepts. In addition, the presentation highlights the conclusions of the Position Paper, showing that the following actions are highly timely: • consolidating the Swiss activities and rationale for ALSS into an active and productive cluster during the next European Exploration Envelope Programmes (E3P); • positioning Switzerland as a key player in space and terrestrial ALSS with a strong potential for contributions to the developments and collaborations within the MELiSSA project; • promoting the international visibility of Switzerland in the fields of manned space exploration and circular economy. One of the key outcomes of the Paper is the need for a resolute and continued financial support for MELiSSA activities remaining within ESA (via E3P), in order to maintain a steady flow of collaborative partnerships and the continuity of technology development within MELiSSA. The discussed approach does not only focus on fostering MELiSSA projects within Switzerland, but also on facilitating the emulation of such Position Paper on ALSS in other European countries.

From wastewater treatment to space-inspired resource recovery with the Biomakery concept at the La Trappe brewery

Ralph Lindeboom
SEMiLLA IPstar

The La Trappe brewery is situated on the premises of the OLV van Koningshoeve Abbey in Berkel-Enschot, the Netherlands. The beer is brewed according to the Trappist principles with an active community of 22 monks involved. The site also has a restaurant where Trappist products are being served, which is hosting an increasing number of visitors, ~200.000 visitors annually. As a consequence 320 m3/day of brewery and cheese production process water and an estimated 18 m3/day of municipal water, from the monastery and the restaurant, need to be treated. In the vision of the monastery it is important to make efficient and sustainable use of all resources and therefore a novel ‘Biomakery’ was envisioned for upcycling process streams in the H2020 NEXTGen-project. Central in the Biomakery is the Metabolic Network Reactor (MNR), which works as a linear-cascade reactor, consisting of 15 reactor cells, each working as an aerobic biofilm reactor using both living natural aquatic plant roots and artificial root structures as habitats of attached growth ecosystems. It is then followed by a dissolved air flotation unit and a drum microfilter to remove particles from the brewery effluent. The smaller municipal treatment line is intended to use 2 reactor cells, an anoxic and aerobic biofilm reactor, followed by smaller yet similar particle separation technology. The MNR on the brewery line demonstrated the ability to reduce most important water quality parameters below discharge limits (125 COD mg/L, 7-10 mg TN/L and 1-2 mg TP/L under stable influent conditions). After concentration by the DAF and drum microfilter, the produced sludge is separated by belt press and will be tested for application as soil enhancer. Efficient upcycling of resources is also the core interest in developing regenerative life support systems as the micro-ecological life support system alternative (MELiSSA) concept from the European Space Agency. Two complementary MELiSSA technologies are being investigated, using both an experimental and modelling approach for further optimizing the reuse of all process streams on site. Inspired on the 2nd MELiSSA compartment (C2), it was hypothesized that an open pond system based on Purple Non-Sulphur Bacteria (PNSB) could convert the COD and N load in the brewery process water into a value-added protein rich- biomass containing pigments. Preliminary experiments in such raceway reactor at the University of Antwerp demonstrated significant growth of purple bacteria, while removing N and COD, and further optimization experiments at the brewery are ongoing. A 2nd MELiSSA technology is focussing on water reuse. Our modelling approach using Biowin and WAVE showed that the effluent of the MNR needs limited additional steps for successful direct filtration through reverse osmosis. Preliminary filtration tests performed at FIRMUS support the modelling outcome and demonstrated the potential to convert the raw brewery process water into potable water using MELiSSA membrane technology. This study has shown that two MELiSSA technologies developed for human life support carry the potential to optimize the circularity of a Trappist brewery. We expect that many more terrestrial situations could benefit from MELiSSA technology to improve circularity.

Microalgae-based biofacade as a solution to support sustainable access to food, energy, and water in urban centers

Flora Girard
ETU UNIV NANTES

Photosynthetic microorganisms are used in the MELiSSA loop because of their ability to produce edible biomass while enabling to treat gas (i.e. CO2) and liquid (i.e. nitrogen, phosphorus…) effluents (photoautotrophic compartment IV). This concept is also relevant when considering the need to develop sustainable access to food, energy, and water in urban centers. By developing adequate photobioreactor (PBR) technology, microalgae can be grown on building façade, enabling to create exchanges loop with the building to reduce its environmental impact while creating an “active” façade producing edible biomass. Thus, microalgae-based biofaçade appears as a solution of interest to introduce benefits of photosynthetic growth for the reduction of environmental impact of human activity in urban centers while using large solar illuminated surfaces from building facades. The aim of the presentation will be to summarize our last results and the foregoing work to prepare a successful future implantation of microalgae-based biofacade in urban areas. Hypothesis As a first example of mutual benefit for both systems, the “thermo-active façade concept” can be developed : PBRs can be seen as a shell and with appropriate interactions between both PBR and building this could potentially decrease the building's thermal energy needs, while also reducing the energy need for culture temperature regulation (thermal symbiosis). Then, based on microalgae metabolism the use of wastewaster and CO2 produce by the building would offer great advantages related to GHC emission reductions, energy saving and nutrient recovery (chemical symbiosis). Main results The optimization of the “thermo-active façade” concept, through modeling of thermal exchanges and pilot-scale studies in outdoor actual conditions, highlights positive results. Indeed, with appropriate interactions between both PBR and building, the culture medium is maintained at an average temperature of 15-35°C, and this will reduce the energy need for culture temperature regulation by about 5 times, when compared to constant culture grown at 23°C, without any impact on biomass productivity. Moreover, the model to simulate the thermal exchanges was validated with a 5% deviation on temperature prediction on average and was then used for systematic optimization of the PBR-building. Our work is currently extended to the optimisation of this “symbiosis” between the building and the PBR through a system modelling approach led at the building scale, with the aim to implement the use of water, and available chemical nutrients in gas (i.e. CO2) and liquid wastes from the building for microalgal photosynthesis. This is conducted in the framework of the DISCUS project, an international collaborative project which brings together teams based in Nantes (France) and in Los Angeles (University of California Los Angeles). This international collaboration will give the opportunity to compare challenges and opportunities within those two countries for the successful implantation of these innovative sustainable solutions in cities presenting different urban scales, regulation frameworks, as well as economic and social acceptance.

BIOFACADE : a symbiosis between micro-algae production and buildings

Anouk Legendre
XTU architects

Invented by XTU Architects (patented in 2009) and developed by the SymBIO2 consortium, SymBIO2 biofacades consist of a microalgae culture system built in a high-performance facade. The biofacades are made of a juxtaposition a planar, vertical, ultra-thin (just a few centimeters) and highly productive «curtain wall photobioreactors». Working in symbiosis with the building, these photobioreactors maximize the use of solar flux for both microalgae cultures and temperature regulation, reduce water consumption for algae culture by nearly 90% compared to classic open-pond cultures, and have a volumetric productivity 30 times higher than the latter. Moreover, they are made as light as possible thanks to geometric and material optimizations, while remaining in compliance with building safety standards. BIOFACADE: AN INNOVATIVE DEVICE FOR BUILDING SUSTAINABILITY Microalgae allow us to live on a planet where the air is breathable due to the photosynthesis. To this day, microalgae remain one of the most significant carbon sinks of the planet, thus contributing to climate change mitigation. The symbiosis between the building and the microalgae farming activity results in a reduction of production cost by 30% for the algae farmer and energy consumption by 50% for the building's heating and cooling system («RT 2012» reference, French Thermal Regulations), but also waste water up cycling and the production of oxygen as well as valuable biomass for various applications. The vertical microalgae farming technique provides the advantage of using very little land, which is interesting in very dense cities. THE GREAT VALUE OF MICROALGAE Given the countless benefits and richness of these tiny unicellular organisms, starting with their much higher productivity compared to terrestrial plants and their content of protein, lipid, omega 3 and 6, antioxidants, and other naturally formed molecules, the microalgae farming branch is growing exponentially to respond to markets needs such as healthcare, cosmetics, food, biomaterials and fuels. NEXT STEP The In Vivo project, winner of the Réinventer.Paris competition («reinventing Paris»), will display a «biofacade» of nearly 500 m² above the Boulevards des Maréchaux in the 13th district of Paris, whose construction will start in 2021. Set in the heart of a unique concentration of life and city sciences expertise, this laboratory building will produce highly sustainable source of proteins through algae biomass, responding to an unmet consumer demand for high quality food products. The challenge is now how to transform a successful research program into a reality. Indeed, technologies progress faster than building regulation, real estate law or acceptability for third parties.

A model for the global carbon cycle

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Université de Lausanne

We present a model for the main possible flows of carbon in both the Biosphere and the Anthroposphere through various forms: as organic carbon in the terrestrial Biosphere, as CO2 in the atmosphere, etc. In particular, we look into the role played by carbon capture and storage (CCS) and carbon capture and utilization (CCU) technologies, which are a few of the possible instruments to mitigate anthropogenic climate change and to address the issue of circular economy on a global scale. 3. Main hypothesis, followed methodology, used sources: We believe that CCS and CCU processes can be part of the global solution to climate change and we CCS and CCU processes to climate change mitigation. In order to do so, we use the Simulink-based PhiEMI software to build our model and to perform simulations based either on actual data collected from the existing literature or on hypothetical scenarios in the world future. The data used are mainly taken from the IPCC, World Bank and IEA reports. 4. Main results: We present a complete model of the global carbon cycle, composed by the natural carbon cycle and the anthropogenic carbon cycle, included the most recent technological processes which can the anthropogenic carbon cycle, i.e. CCS and CCU. While the existing literature on the cycle of carbon dioxide presents a certain number of analysis of the natural carbon cycle, the anthropogenic carbon cycle, or a combination of the two, there are no publications which include CCS and especially CCU. In light of their increasing importance, it is therefore necessary to create a new model of the carbon cycle which incorporates these options. Keywords: carbon capture, carbon storage, carbon utilization, circular economy, industrial ecology, resource recovery and reuse, simulations tools.

Petit Langoustier isolated Island, global sustainability program

Marc Frilet
Petit Langoustier

The Petit Langoustier is an uninhabited island satellite of the famous Porquerolles Island located offshore Provence within a national Park.
A Fort has been built on Petit Langoustier during the XVII century and the French State has concluded a long term lease with two families having long relationships with Porquerolles for full rehabilitation under its original state without commercial use and in compliance with National Park objectives .
Such an isolated island with difficult sea access, no connection to grid and no water sources ,but with a conspicuous heritage tower and attached barracks is an ideal platform for testing products and processes which in an inclusive manner would permit to support the life of 10 individuals throughout the year in global autonomy, toward zero waste and zero carbon footprint. This objective is at the core of the project and several steps have already been made.
The association “Petit langoustier Fort en Mer » has recently been created to develop the project including a scientific council more particularly in charge of the Sustainable Island Laboratory aspect (SILAB).
The promoters and the association are keen to participate to the Melissa conference to present the current status of the SILAB project and exchange on a shared objective of creating a circular future with a view to use the SILAB platform as demonstrator of some of the Melissa and Alisse cutting edge technologies not yet ripe for terrestrial commercial development and to maximize integration with other devices and processes .
With the help of individuals and NGO representative deeply involved in sustainability issues specific to Porquerolles over populated by tourists and sailing boats in summer months some preliminary ideas have emerged on the type of the Melissa and Alisse technologies which could be demonstrated on the Fort Island and adapted to local needs to gether with a possible communication program.

Grey water re-use during music festivals using a mobile constructed wetland and a mobile drinking water system

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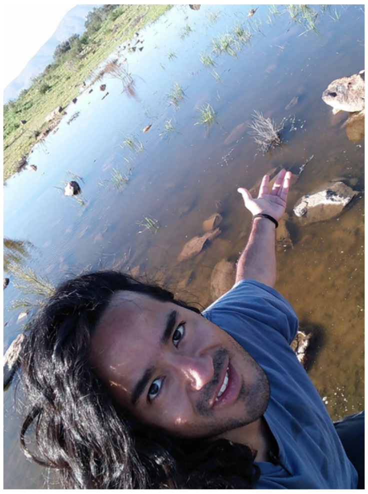
(Grey) water treatment and re-use is an essential prerequisite for a sustainable music festival, often operated on a decentralized location. In such areas these temporary events are typically not connected to a sewage network. In our study, a combination of a vertical flow based mobile constructed wetland (area of 15 m2 and a volume of 15 m3) and a membrane based drinking water system was developed (and tested for 3 years) to treat grey water (GW) as well as grey and black water (GW+BW) at different festivals in Flanders (Belgium). During the development phase, experiments were performed in order to determine which types of influent could be treated (GW and GW+BW) and challenge tests were then carried out to determine the maximum optimal loading rate. The conclusion was that GW is treated preferentially and that the system could be operated at an effective optimal loading rate of 0.9d. During the demonstration phase, the mobile constructed wetland (treating GW from 66 showers) was coupled to a drinking water treatment using activated carbon, UF and RO filtration system. Good removal of COD, BOD, TSS and TN were observed as 90%, 95.4%, 97% and 24.7% removal was obtained respectively. The drinking water system was able to further polish the GW and produce water that met the Belgian drinking water standards. As such, GW can be reused for drinking purpose at festivals, thereby increasing significantly the sustainability of water use at temporary festivals.



Bacteria and Archaea distributions in California ephemeral wetlands

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Microbial communities play multiple important ecological roles in water and soil—from acting as symbionts, parasites, and pathogens, to regulating biogeochemical cycles. Quantifying microbial community variation within and across ecosystems is therefore important to our understanding of the structure and function of ecosystems. This study addresses the microbial community composition in temporal wetlands that transition from a desiccated/terrestrial stage in to a totally aquatic -vernal pools- (Zedler, 1986). Our results evidence a marked differentiation between microbial communities for sediments in comparison with water column, regardless soils saturated with water. Archaea are constant residents in sediments across vernal pools soils. Additionally the transition from desiccation to aquatic stage doesn't have a significant turnover effect in species composition for Bacteria and Achaea soil communities. On the other hand, following a 4000km latitudinal transect across California, USA and Baja California, Mexico, there is no evidence of a transition in microbial taxa assemble dictated by such gradient. Community isolation as a function of distances between sites, is distinguishable with closest vernal pools sharing microbial taxa, in contrast of those distant vernal pools. - This study represents the first attempt to describe microbial communities in California and Baja California vernal pools. - Environmental filtering is not playing a significant role in determining microbial community composition in water-saturated soils, in comparison with dry soils. - Biogeographical patterns described in classical ecology can explain taxa assemble and microbial distributions across the landscape.

High temperatures affect pollen fertility more than altered gravity: bottlenecks in the reproductive cycle of micro-tom

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To be successful any long-term mission in space must be as much as possible independent from Earth supplies. In this framework, bioregenerative life support systems (BLSS), such as the Micro-Ecological Life-Support System Alternative (MELiSSA), are implemented with higher plants to transform organic wastes and carbon dioxide providing food, oxygen and purified water to the crew involved in long-term space missions. Many of the plant species currently studied for cultivation in space are grown to produce leafy crops starting from seeds produced on ground. However, to support long-term space missions, BLSSs require the full completion of the reproductive cycle of the space crops on board spacecraft. The choice of the candidate plant species for cultivation in BLSS is a critical issue that should firstly point on criteria of reproductive fitness, rather than growth rate, productivity and resource use efficiency. In this framework, Solanum lycopersicum L. represents one of the best candidate crops able to achieve a full seed-to-seed cycle for space missions. Among tomato cultivars, Micro-Tom represents a model crop for cultivation in space because of its dwarf habitus and short life cycle. Previous studies endorsed the use of Micro-Tom as a space crop, reporting that this plant can grow from the seed-to-seed under simulated microgravity. However, interaction with other common earth environmental factors such as RH and temperature have been generally overlooked. This interaction might represent a bottleneck to achieve the seed-to-seed-cycle in space because pollen viability and germinability are well known to strictly depend on temperature variations and because changes in temperature regimes can frequently occur in space environments. Our research is included in the ReBUS (In situ Resource Bio-Utilization for life Support system) project by ASI. Specific aim was to evaluate the effect of both simulated microgravity and temperature variations on pollen fertility of Micro-tom. In order to minimize variability within sporophytes, we firstly grew Micro-tom plants in the same growth chamber with an optimal air temperature (control treatment, CT), from sowing to the development of the first inflorescences. Thereafter we moved part of the plants in growth chambers with higher and lower temperatures (higher temperature treatment, HTT; lower temperature treatment, LTT) in order to compare the effect of different temperatures on pollen viability and germinability, during different flowering stages. The effect of simulated microgravity on pollen viability and germinability, was evaluated by uniaxial clinostat, keeping temperature at optimum. Results confirmed that microgravity under optimal temperature, does not affect pollen germinability and showed that viability of pollen strictly depended on temperature.

Indeed, pollen viability of CT remained high throughout all flowering phases, while pollen viability of HTT and LTT decreased during all flowering phases. Most importantly, HTT and LTT pollen viability resulted significantly lower than CT at anthesis and loss of viability occurred much before anther dehiscence. Moreover, HTT and LTT pollen was never able to germinate, showing that their flower cannot succeed in the production of seeded fruits. Overall, results showed that the effect of temperature on pollen functionalities determines a critical bottleneck in the seed-to-seed cycle and must be taken into account for all scientific experiments and hardware development aimed to grow Micro-Tom plants in space.

Nitrify to support life: MELiSSA's development path of an essential process

UAntwerpen

This work sketches the research and development logic, history, achievements and future of nitrification-based processes in the micro-ecological life support system alternative (MELiSSA) concept developed by the European Space Agency. A nitrification-based process is a crucial nitrogen (N) conversion step in most regenerative life support systems. A crew member consumes 9-19 g protein-N per day, and excretes the same amount, mainly under the form of urea in urine (7-16 g N per day). Nitrification is pivotal to multiple purposes in long-duration human spaceflight, ranging from stabilisation of waste streams to the production of useful compounds: water, N2 as inert gas, and/or nitrate for food production. The latter is the ultimate goal of a full MELiSSA loop, with nitrogen, after carbon, being the most abundant element to recycle. Pivotal in nitrification is the collaboration between aerobic ammonia and nitrite oxidizing bacteria (AOB and NOB), with Nitrosomonas europaea and Nitrobacter winogradskyi selected as most suitable organisms for MELiSSA. The process should additionally convert biodegradable organic matter to CO2, to avoid downstream biofouling issues and enable carbon recovery. If urine directly enters the nitrification unit, ureolytic bacteria produce ammonium (NH4+) from urea to enable nitrogen conversion. The goal is a reliable and predictable process under dynamic conditions, coupled to other MELiSSA processes. Ground characterization and demonstration activities ranged from microbial physiology in flasks at molecular level, up to reactor demonstration, including mathematical modelling and control law elaboration. Extensive testing occurred on the two important streams, namely urine and effluent from the upstream MELiSSA CI unit (anaerobic fermentation), with formulated media and real waste streams. Functional nitrification has been achieved with open, undefined consortia, as well as with synthetic, gnotobiotic communities, i.e. enabling an increased level of process predictability and safety. The process was shown to enable refinery and concentration of nutrients from urine, as well as downstream water recovery from urine, condensate and grey water. Reactor types included systems based on suspended and attached growth (biofilm on a fixed or moving bed). In terms of aeration, sparging with air and oxygen has been shown, along with gravity-independent membrane aeration. For effluent withdrawal typically a membrane is used, generating a bacterium-free stream. Besides validation of the target performance, the effect of key parameters on the microbial communities has been studied, and a predictive control law was implemented (e.g. concentrations of oxygen, nitrite,…). Long-term stability was demonstrated in the MELiSSA pilot plant in Barcelona for more than a year. Several runs showed that the process effluent was suitable to produce microalgae and crops.

Ongoing work focuses on (i) dealing with urinary organics, (ii) avoiding base addition, (iii) coupling to the food and oxygen production compartments, and on (iv) producing inert gas. Two flight experiments (BISTRO and NITRIMEL) have been successfully performed for nitrification-based processes. These demonstrated the reactivation potential of a set of key microbes and consortia, exposing many organisms for the first time to elevated levels of radiation and the impact of preparation/launch logistics. Results were promising, with good post-flight activities for nitrification (AOB and NOB), ureolysis, denitrification and anammox. Two more flights are currently under preparation, being URINIS A and B. The challenge addressed here is to obtain in-flight activity in the International Space Station (ISS), both in batch incubations as in a first continuous reactor test. Finally, the R&D work on nitrification has yielded several terrestrial valorization examples that are established. Other cases are in the development pipeline.

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

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Urine is an essential resource in regenerative life support systems (RLSS), as it presents a major flux of water and nutrients (e.g. nitrogen and phosphorus). Currently, on board of the International Space Station, only water is recovered from urine using physical-chemical processes and hazardous chemicals, while the nutrients are concentrated in a toxic brine. In this study, a novel treatment train for water and nutrient recovery from urine was investigated, combining (bio)electrochemical processes and nitrification. Since ammonia volatilization can pose a hazard to the crew, the urine (33% dilution) was first stabilized to prevent urea hydrolysis into NH3/NH4+ (total ammoniacal nitrogen, TAN) during storage. By recirculating the fresh urine over the cathodic compartment of an electrochemical cell, the pH was increased to 12 due to water reduction at the cathode (7 kC L-1 urine to reach pH 12). The high pH inhibited urea hydrolysis for over 18 months and triggered precipitation of bivalent cations (>85% Ca2+ and Mg2+ removal), reducing the downstream scaling potential. Subsequently, stabilized urine was treated in a microbial electrolysis cell (MEC) in order to remove the organics (chemical oxygen demand, COD). Up to 85% of the COD was removed, generating energy in the form of hydrogen gas at the cathode. A current production of 6-20 mA was obtained at COD loads of 200-340 mg COD d-1 and HRT between 2.5-6.7 days, corresponding to coulombic efficiencies of 25-45%. The MEC community was dominated by, amongst others, members to the genera Geobacter, Pseudomonas, Arcobacter and Comamonas, known to comprise electroactive bacteria, i.e., bacteria capable of transporting electrons to an electrode. Besides COD oxidation, the MEC hydrolysed 80-100% of the organic nitrogen, resulting in a TAN-rich COD-low stream, which was fed into a membrane aerated biofilm reactor (MABR) composed of three parallel modules with hollow fiber membranes in order to convert TAN into non-volatile and non-toxic nitrate by nitrification. Full nitrification (effluent TAN and NO2--N <10 mg N L-1) was obtained at a loading of 200-230 mg N L-1 d-1 (HRT between 5.5-8.8 days), without N loss when the MABR was operated on MEC effluent, whereas denitrification and partial nitrification occurred when the MABR was operated at the same N loading rate on raw stabilized urine without a MEC pre-treatment. In-situ electrochemical pH control was implemented in the recirculation loop of a nitrification reactor in order to enable full nitrification without base addition. Hydroxyl production at the cathode compensated for the acidification by nitrification. The treatment train yielded a stable nitrate-rich nutrient solution, suitable for microbial protein production based on microalgae, for instance belonging to the cyanobacterial genus Limnospira, previously described as Arthrospira (‘Spirulina’).

Supplementation with phosphorus and micronutrients yielded a >80% conversion of nitrate-N into edible biomass. Since the oxidation of COD and N in the MEC and MABR is not based on bubble-dependent gas/liquid mass transfer, the concept is compatible with microgravity conditions. Moreover, by integrating electrochemical cells in the treatment train, the use of chemicals and the logistics related to their storage can be minimized. As a highly N- and resource-efficient technology train, the concept can be useful for MELiSSA (micro-ecological life support system alternative), the RLSS programme from the European Space Agency (ESA), as well as for other RLSS.

Adaptation of activated sludge biomass for nitrification of concentrated urine

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Urine is a metabolic human waste rich in nitrogen and phosphorus, as well as other valuable components. After treatment, it can be used as a fertilizer for biomass cultivation. Application of the nitrification process, transforms urine into a liquid fertilizer with nitrogen in a form best absorbed by plants cultivated in soilless system. The assumption of using biological wastewater treatment and soilless cultivation (aeroponics / hydroponics) is in line with the latest trends in the design of systems in renewable life support systems in space facilities. The nitrification process itself is well recognized in terrestrial conditions, but the issues of purifying concentrated urine for soilless cultivation pose some challenges. Firstly, urine alkalinity is limited and enables only partial nitrification of urine, so it is necessary to provide additional alkalinity to reactor to obtain fully nitrified fertilizer. Constant addition of alkalinity is challenging as no easy source of alkalinity will available in extraterrestrial conditions. Secondly, very high salinity of urine will inhibit the nitrification process and development of acclimatized consortium is therefore required. Moreover salinity will be further increased through constant addition of missing alkalinity .Thirdly, the nitrification reactor should be operated at pH which is optimal for soilless cultivation so nitrified urine can be directly used as a fertilizer without pH correction or lower yields. Optimal pH for soilless nutrient solution is in range 5.5-6.5, which is far below nitrification optimum (7.0-8.0) and most of nitrifiers are not able to thrive in such acidic conditions. Nitrification in low pH and high salinity was tested before, however these conditions were tested separately. Despite these challenges, nitrification of concentrated urine is worth testing, as dilution of urine before nitrification reactor will lead to reactor's volume increase due to hydraulic constraints. Our hypothesis is that it is possible to conduct nitrification on concentrated urine with high salinity and low pH. The paper presents results of urine nitrification start-up, which lasted over 100 days. Inoculum was activated sludge taken from municipal wastewater treatment plant. During startup, after exceeding tolerance limit of salinity, sudden drop in nitrification efficiency occurred which indicated exchange in nitrifiers consortium for species better suited to high salinity. After this episode nitrification rate was restored within few days and further increase in urine loading proceeded generally undisturbed. When assumed urine loading was reached, the pH was gradually reduced to the level of 5.8. Despite the increased salinity and lower pH, the process rate did not decreased and stable nitrification was achieved.

Urine running in circles - human "waste" as a resource for horticulture & agriculture

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Until now, anthropogenic nutrient cycling is primarily based on the recycling of nutrients from livestock farming, biogas production or composting of agricultural and domestic residues while agri- & horticulture still mainly use synthetic mineral fertilisers. On the other hand, excessive proportions of nutrients are concentrated in aquifers and surface waters, originating from the application of mineral and organic fertilisers in food production or livestock farming but also from wastewater-related pathways. In order to achieve a sustainable circular economy, the high concentration of nutrients in municipal wastewater should be recovered. Therefore, human urine is a key source of “urban mining”, containing 80 % of the nutrients in urban wastewater, in a volumetric share of only 1 %. Source separation and the subsequent processing of urine allows for (i) the integrated recycling of nutrients (N, P, K, S and more), (ii) the prevention of chemical cross-contamination e.g. heavy metals or micro plastic, and (iii) the elimination of pharmaceuticals. Therefore, innovative, safe and resource-efficient technologies have been developed that effectively recover & upgrade essential plant nutrients from source-separated human urine and produce novel high-quality bio-based mineral fertilisers. There is, however, still a need to research the integration of urine-based fertilizers into horticulture with respects to fertilizer efficiency in different production systems, or the assessment of potential trade-offs, such as higher GHG emissions. For such “proof-of-concept” we conducted a set of three complementary experiments under greenhouse and open field conditions. Our focus is on two nitrified urine fertilizers (NUFs) with different NH_4^+/NO_3^- ratios, namely Aurin (1/1) and C.R.O.P. (<1/2). The conducted experiments comprise of (1) a substrate-based pot experiment with maize, (2) a hydroponic experiment with tomato using the nutrient film technique (NFT), and (3) a practice-oriented field experiment with white cabbage. Results from soil and substrate-based experiments indicate, both NUFs are viable substitutes to established synthetic mineral fertilizers (urea) and organic recycling fertilizers (Vinasse). Ammonia emissions from NUFs were significantly lower compared to urea or synthetic urine. In hydroponics we found no significant difference in total biomass production and nitrous oxide emissions between NUFs and the full synthetic mineral control. Yet, Aurin showed lower marketable fruit yield because of damage by blossom end-rot, explained by the high NH_4^+/NO_3^- in Aurin affecting the uptake of cations in NFT systems. To conclude from all three experiments, urine-based recycling fertilizers succeeded the proof-of-concept as fertilizer alternative. The different application options for NUFs in horticulture largely depend on the NH_4^+/NO_3^- ratio, respectively on the level of nitrification during urine processing.

Opening the ‘yellow box’: Main organics in urine and their fate during nitrification and microalgae cultivation

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In regenerative life support systems, like the European Space Agency’s Micro-Ecological Life Support System Alternative (MELiSSA) loop, an essential goal is to safely refine and upgrade secondary resources from waste streams to nutritious food. One of the simpler manners to produce edible biomass is based on the cultivation of the cyanobacterial microalga Limnospira. With human urine representing the major nitrogen source in long-duration spaceflights, it is an important fertilizer stream for on-board food production. After stabilization through (partial) nitrification, urine can be used in the production of Limnospira biomass and oxygen. As urine also contains organics, typically expressed as chemical oxygen demand (COD), it is important to identify and investigate their fate in the MELiSSA loop. This study focused on the respective MELiSSA compartments III (nitrification) and IVa (microalgal conversions). The objective was to identify and quantify the main organic compounds in fresh and (partially) nitrified urine, in order to further investigate their effect on Limnospira growth. Based on a NASA report (Putnam 1971) and a paper referring to the human urine metabolome databank (http://www.urinemetabolome.ca/), the COD values of the organic compounds measured in fresh urine were identified and calculated. As for the NASA paper, 16 compounds were retained accounting for 80% of the COD in urine, for the human urine metabolome this were 56 compounds. The 3 most abundant organic metabolites in fresh urine were: creatinine, hippuric acid and citric acid, they contribute to ca. 30% of the COD in fresh urine (respectively ca. 15, 10 and 5 % of the COD). The fate of these compounds was experimentally screened after complete and partial nitrification. Identification and semi-quantification of organics was done using HPLC-HRMS (high-performance liquid chromatography high resolution mass spectrometry) and comparison with reference standards and Open Access spectral databases. Influent and effluent samples were collected from two distinct set-ups treating human urine, from Eawag (Switzerland) and Ghent University (Belgium). The influent urine was stabilized chemically, electrochemically or by natural hydrolysis. The different stabilization methods led to various organic compounds in the influent samples, despite the fact that the total COD in these samples was comparable to the COD in fresh urine. More specific, the amount of Volatile Fatty Acids (VFA) in the natural hydrolyzed influent samples contributed for 65% to the COD, while they were negligible in the other influent samples. Furthermore, it can be concluded that creatinine, hippuric acid, citric acid and VFA (as well as the main part of the COD) are largely removed during (partial) nitrification. This first screening experiment highlighted the complexity of organics in real urine. It is therefore recommended that studies aiming to remove specific compounds use real urine whenever possible. Secondly, it was shown that nitrification of urine with open complex microbial communities removes most organics, including those contributing most to urine’s COD content.

Control of pH and process modelling contribute to stable alkalinity-limited urine nitrification

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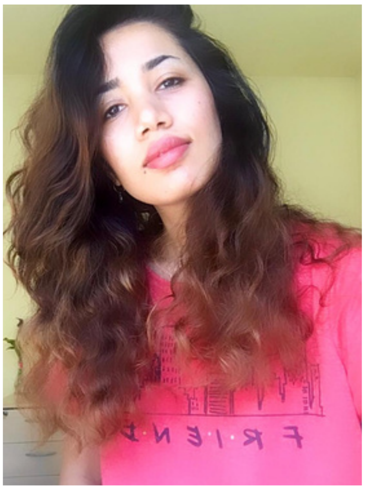
Eawag

In the MELiSSA loop, urine is the main vector of nitrogen but the urine compounds have to be stabilized prior to its utilization as fertilizer. Nitrification is an appealing biological process for urine conversion. In the nitrification process developed at Eawag, no base has to be added, if the pH is carefully controlled within a suitable operational range with fresh urine inflow. Due to the limited alkalinity present in urine, roughly 50% of the total ammoniacal-nitrogen (TAN) is converted to nitrate. Despite this partial conversion, ammonia volatilization is prevented, because nitrification causes a pH drop converting free ammonia (NH3) into non-volatile ammonium (NH4+). This so-called alkalinity-limited urine nitrification can be advantageous compared to full nitrification, because no chemicals (i.e. base) have to be dosed and less oxygen is consumed. However, it makes the process susceptible for process failures, namely: (1) Nitrite accumulation and the washout of nitrite-oxidizing bacteria (NOB), (2) complete cessation of nitrification at high pH and (3) unwanted growth of acid-tolerant ammonia-oxidizing bacteria (AOB) at low pH causing a further pH drop and the release of harmful gases (e.g. NOx). For space application, a robust, controlled and predictable operation is required. Currently, the optimal pH for stable and efficient alkalinity-limited nitrification is not known. Therefore, we investigated the impact of different pH setpoints on process stability and the potential three failure scenarios. Three 12-L continuous-flow stirred-tank reactors were run in parallel and fed with two real urine streams: a more diluted with a TAN concentration of 2000 mgN/L (first run), and a more concentrated with a TAN concentration of 4000 mgN/L (second run). To establish stable nitrification, five reactors were first operated at pH 6 and one reactor at pH 5.8. After 25 days, the pH setpoints were changed to 7 and 8.5, and in the last reactor the inflow was turned off completely. At pH 6, nitrification rates between 50 to 300 mgN/L/d were observed and no nitrite (<1 mgN/L) accumulated. After the pH change to pH 7, nitrite started to accumulate for both inflow concentrations probably due to the washout of NOB and reinforcing insufficient nitratation. For the diluted urine, the pH change to 8.5 resulted in NOB inhibition due to free ammonia. For the concentrated urine, complete cessation of nitrification was observed. After the inflow was turned off, the pH dropped first to around 5.4. Subsequently, in a second drop the pH decreased below 4.5 after 35 and 20 days for the diluted and concentrated urine, respectively. This indicated the presence of acid-tolerant AOB in the system. A mechanistic mathematical model of the system was developed. The extended activated sludge model includes heterotrophs, acid-sensitive AOB, acid-tolerant AOB and NOB. So far, the model can predict the failure scenarios qualitatively. Upon complete validation, this model can be used to identify pH ranges of stable alkalinity-limited urine nitrification and to optimize the nitrification rate. Overall, this study contributes to a robust resource-efficient nutrient recovery technology from urine, that is valuable in Space as well as on Earth. Funding: The authors would like to acknowledge the MELiSSA Foundation for funding the project via the POMP2 (Pool of MELiSSA PhD) program. Key-words: Stabilisation, nitrification, sub-system modelling Addressed challenges: Characterization and mechanistic modelling of a complex microbial community.



Urine nitrification in space: assessing the effect of space conditions on synthetic nitrifying communities

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For long- term space missions, oxygen, food and water are crucial for the astronauts' survival. This limitation has always been considered as a major hinder for deep space exploration where extra cargo from Eearth is inapplicable. Hence, technologies for in-situ production is necessary. Urine is an important waste product of the human metabolism, and contains, in addition to water, a variety of nutrients that can be utilized for the production of food. Nitrification is one of the key processes involved converting urine into a valuable nitrogen source, and this biological process relies on active nitrifying microorganisms. Nitrification is a key component of ESA's Micro-Ecological Life Support System Alternative (MELiSSA). Despite nitrification being well established in terrestrial applications (in mixed cultures for wastewater treatment), reactors proof of concept of nitrification in space awaits further demonstration, although two experiments (NITRIMEL and BiSTRO) demonstrated that the key organisms involved in the nitrogen cycle can be reactivated following flight to the Low Earth Orbit (LEO). The URINIS project (URine Nitrification in Space) builds on this and aims to demonstrate in a flight experiment to the International Space Station (ISS) that a synthetic consortium composed of an ureahydrolyser (Comamonas testosteroni), ammonia oxidizing bacteria (AOB; Nitrosomonas europaea) and nitrite oxidizing bacteria (NOB; Nitrobacter winogradskyi) is able to nitrify urea to nitrate in space. The main objectives of URINIS are to determine the optimal pre- and post- mission culture's storage and timeline, the optimization of culture conditions in fluid processing apparatus (FPA) in terms of inoculum ratios, pH, phosphate buffering and oxygen limitation, etc. URINIS will also assessing the effect of simulated microgravity and space radiation conditions during the growth of each of the strains, as a pre-flight experiment check. Simultaneously, a set of analytical procedures will be developed and fine-tuned (i.e. transcriptomics, proteomics, metabolomic, phenotypic characterization and biofilm analysis) to gain a comprehensive understanding of the changes of the phenotypic diversity, molecular mechanisms regulation and metabolic responses of the microbial cultures when exposed to space conditions. Ultimately, all these will pave the road to further develop the knowledge needed for deep space exploration within the MELiSSA loop.

Keywords: Nitrification, Urine, MELiSSA

How to gradually acclimate MELiSSA's nitrification compartment to urine mixed with VFA-rich anaerobic digestion (C1) effluent

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Within the context of targeting an overall water recovery rate higher than 90 to 95 % by appropriate integration of the various individual treatment units, the objective of the Black Water Treatment Breadboard (BWTB) project was to study and validate the performance of the Black and the Yellow Water Treatment processes based on, respectively, the MELiSSA C1 and C3 compartments, when coupled directly (without the MELiSSA C2 compartment). The following challenges and opportunities of the BTWB project were identified: (i) urine addition implies a high ammonium and salt loading to the C3 which might impair the nitrifiers, (ii) direct coupling of the C1 to the C3 implies a high organic carbon loading to the C3 which will promote the heterotrophic biomass and thus increases competition for oxygen with the nitrifiers, and (iii) sterility conditions are relaxed when the C3 is run with a non-axenic microbial culture instead of pure nitrifier strains. Full nitrification of the ureum (and removal of organic carbon) in the urine fed to the C3 reactor could be achieved at a temperature of 25 °C. A strategy to gradually increase the loading to the C3 reactor has been validated and consists of carefully monitoring the free ammonium and free nitrous acid concentrations (which depend on the pH). Furthermore, the pH itself should also be monitored closely and remain above 7 to avoid nitrifier inhibition but below 8 to prevent free ammonia volatilization. The salt loading was found to be non-inhibitory when urine is diluted with distilled water at a 1:5 ratio or higher. As for the coupling, the fixed bed configuration (with, e.g., Biostyr® beads) of the C3 needs to be transformed to a fluidized bed configuration (with, e.g., 50 % K1 AnoxKaldnes carriers) to avoid oxygen transfer limitation problems which impede a further increase of the reactor loading. By gradually replacing the urine dilution water with the C1 effluent, a total nitrogen loading to the C3 of at least 2.8 g per day and a C/N ratio of at least 4 can be reached without nitrification issues. One possible bottleneck that was identified is the C/N ratio for which an apparent limit value of 4 was observed (also supported by literature evidence), while the C/N ratio will be around 8.58 when the target loading is applied. Nevertheless, Komorowska-Kaufman and colleagues (2006) did not observe lower nitrification efficiency with C/N ratios up to 9 if temperatures remained above 15 °C. This makes us confident that a gradual increase of the organic carbon loading across this C/N ratio threshold, allowing for sufficient adjustment of the microbial community, is possible. The C3 reactor was inoculated with a nitrifying microbial community (called ABIL sludge purchased from the Belgian company AVECOM) of unknown species. While community characterization is ongoing, the robustness of this complex community in the C3 reactor was demonstrated by multiple observed fast recoveries of the nitrifying activity after removal of inhibitory stressors.

Experimental set-up to investigate the transpiration process

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The development in the field of space engineering is aimed at achieving another milestone - the creation of a colony on other celestial body (Mars or Moon). Missions of this kind will be long-term and long-distance, which results in the need to design regenerative life support systems able to produce food, water and oxygen. Physicochemical systems (like the ones in the International Space Station) are robust technologies to recover oxygen and water but not able to produce food: in order to have this capability, biological processes are required. In this case, they are called bioregenerative life support systems. The European project developing this type of technology is the MELiSSA (Micro-ecological Life Support Systems Alternative) project. MELiSSA is using microorganisms and higher plants to produce O2 and food, remove CO2 and recycle water. However, these systems are strongly influenced by environmental conditions (like gravity, temperature, O2 and CO2 level, pressure, relative humidity, etc.), and to be able to precisely control these systems, fundamental understanding and mathematical description of all processes involved is required. One of the process that needs to be better understood is transpiration: in fact, water transpired from plants is considered as a source of potable water for the astronauts. The concept of the experiment conducted to investigate this process and first data will be presented: the study is focused on effects of temperature and humidity of the air and salinity of the nutrient solution on transpiration. The main goal of this study is to maximize transpiration without harming the plants. As a model plant, spinach was selected (spinach consist mainly of leaves, has a relatively high salinity resistance, and a short growth cycle). The experiment was carried out in an environmental chamber that has a closed water circulation system and the ability to control and record temperature, humidity, duration of day/night cycle and airflow. Nutrient solution parameters (pH, conductivity, dissolved oxygen) was measured and controlled. At the end, fresh mass, dry mass, water content, leaves surface of the plants and water balance was measured. Data collected during the experiment will be used to further verify the mathematical model of the transpiration process.

Novel bioinformatics tools for microbial monitoring and clinical diagnostics

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Developing a balanced ecosystem for regenerative life support, and tight monitoring of microbial water quality in life support water circuits is essential for deep space missions, e.g. lunar or Mars missions. However, a follow-up of the microbial communities in the extreme ecosystem, the water circuits in a space habitat, and monitoring of bacteria infection within space-crew members are far from being straightforward. Recent developments in new high-throughput sequencing technologies have revolutionised molecular biology, including the study of microorganisms, an approach often referred to as metagenetics. Additionally, monitoring and track the crew health and the predicting assessment of possible outbreaks are made possible with such technologies, using an approach often referred to as microbial whole-genome sequencing (WGS). Despite rapid advances in metagenomics and WGS technologies, their integration into routine microbiological monitoring/diagnostics and infection control has been hampered by the need for downstream bioinformatics analyses that require considerable expertise. We have developed one-stop software, named OCToPUS, for 16S metagenetics analysis, thereby leading to a highly accurate assessment of microbial dynamics (Mysara, Njima, et al., 2017). OCToPUS is an assemblies several algorithms tackling the different analysis challenges, namely Chimeras, sequencing noise and analysis resolution (Mysara, Saeys, et al., 2015, Mysara et al., 2016, Mysara, Vandamme, et al., 2017). Similarly, a comprehensive bioinformatics pipeline (BacPipe) that enables direct analyses of bacterial WGS data obtained from high sequencing technologies (Xavier et al. 2020). BacPipe is an ensemble of state-of-the-art tools for the identification of the bacterial genotype (MLST, emm typing), antibiotic resistance genes, plasmids, virulence genes, and single nucleotide polymorphisms (SNPs), starting from the raw reads. The outbreak module within BacPipe can simultaneously analyse many strains to identify evolutionary relationships and transmission routes. These user-friendly bioinformatics tools have implemented successfully in studies to assess complex microbial communities in a extreme environment conditions such as deep subsurface geological clay formations (HADES, Boom clay, Belgium; and Mont Terri, Opalinus clay Swiss, Moors et al. 2013), cooling water circuits of nuclear reactors (Props et al., 2016), the human gut microbiome in several space simulation isolation experiments (Princess Elisabeth Antarctica research station, Mars Desert Research Station and Lunar simulation) as such revealing microbial composition in unseen depth. BacPipe is is integrated in EBI-SELECTA, a project-specific portal (H2020-COMPARE), and both tools are freely available: https://github.com/M-Mysara/OCToPUS and https://hub.docker.com/r/mahmed/bacpipe.

Polishing goat farm waste water in view of advanced phosphate removal

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In this study, a phosphate removal is studied as a polishing step for decentralized wastewater treatment of goat farm effluent. Economical and practical aspects are looked upon. The waste water in this case was already treated by a septic tank and constructed wetland first, and it shows good performance in COD (90%), BOD (95%), TSS (95%) and TN (60%) removal. However, the phosphate removal in this system is limited and discharge limits are not met. To solve this problem, granular filtration with different many low cost natural and commercial substrates is examined. After batch test and column test, the Iron Oxide Coated Granular sludge with Sand core (IOCG+S), a waste material from the drinking water industry, was chosen for further pilot scale studies. IOCG+S shows 1 to 4 mg PO4-P/g sorbent adsorption capacity when the influent concentration was 40 mg/l PO4-P. In pilot tests, the concentration of phosphate in the effluent of the granular filter was below 0.1 mg/L. Furthermore, FTIR, XRD, BET, SEM, SEM-EDS were used to characterize the IOCG+S, and investigate possible shifts in characteristics after adsorption. Also, lab scale column experiments are carried out to verify the factors which can affect the performance including HLR and competitive inhibition from other anions.

The effect of nitrogen availability on cold acclimation of Arabidopsis thaliana plant

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Upon transfer of plants to low non-freezing temperature an excitation pressure is built on Photosystem II (PSII) due to slowing of the Calvin-Benson cycle enzymes kinetics. This leads in a decrease of photosynthetic carbon assimilation. Exposure of fully expanded leaves of Arabidopsis thaliana plants to sustained low temperature results in gene expression, protein content and leaf metabolic alterations and an establishment of a new steady state with higher photosynthetic rate compared to that prior to cold exposure. This process is called dynamic photosynthetic acclimation and is completed in a time scale of days. The plant's ability to acclimate to lower temperature depends on the genetic background and the energy and nutrient budget of the plant. Nitrogen, as one of the essential elements for protein synthesis, affects the ability of Arabidopsis plants to acclimate to sustained cold conditions. Apart from being an essential element nitrogen is tightly linked with carbon metabolism with nitrogen assimilation acting as a sink of carbon skeletons, electrons and reductants originating from the photosynthetic apparatus. In this study Arabidopsis thaliana plants, Columbia 0 ecotype, were grown in different nitrogen concentrations and their ability for cold acclimation was assessed. Plants were grown in 20 oC day/18 oC night in sand and were watered three time per week with Hoagland solution modified to three total nitrogen concentrations: 15mM, 5mM and 2.5mM with 93% of nitrogen originating from nitrate and 7% originating from ammonium. Nitrate to ammonium ratio was kept constant since a change in that ratio could generate changes in physiological and biochemical processes. After 8 weeks, fully plants were transferred in 5oC for a week before photosynthetic acclimation was evaluated. In Arabidopsis thaliana plants maximum photosynthetic carbon assimilation is expected to increase after a week of cold exposure with the concomitant up-regulation of the Calvin-Benson cycle enzymes. Limiting nitrogen availability, however, resulted in a shortage of nitrogen that is necessary for protein synthesis, especially Rubisco that accounts for up to 30% of leaf nitrogen. Only plants grown in 15mM N showed higher maximum photosynthetic rate after cold exposure. Chlorophyll data, however, suggest that in these plants light harvesting antenna was broken down and nitrogen was re-mobilized probably towards Rubisco and other Calvin-Benson cycle enzymes. Plants grown in lower nitrogen concentrations where unable to acclimate to cold but did not exhibit signs of extreme stress with maximum photosynthetic rate remaining in the same level before and after cold exposure.

Microgreens for Human Nutrition in Spaceflight

Christina M Johnson
NASA Postdoctoral Program

For long-duration spaceflight missions, providing adequate nutritional needs of the crew is a documented risk. This can be mitigated with dietary supplementation of nutrient-dense young vegetables including microgreens. Why microgreens? Traditional crops can take months to mature, but microgreens are ready to harvest in within two weeks, and they can be grown in small volumes. This rapid turn-around time makes them practical for any spaceflight scenario where crew time is limited, or when power, mass, and volume limitations prevent growing crops to maturity. In this literature review we will introduce microgreens that have been screened by the USDA for nutritional content, and discuss their nutritional benefits in relation to the specific needs of astronauts, as defined by the NASA Human Research Program. We will specifically highlight the benefits of iron, magnesium, potassium, and carotenoids. We also discuss potential lighting and fertilizer regimens that could be used to further improve nutrient content of microgreens grown in microgravity. Developing microgreens for spaceflight will give us access to crops that are specifically designed to meet crew nutritional needs for future exploration missions.

Proposal for MELiSSA Overall Control Loop Architecture

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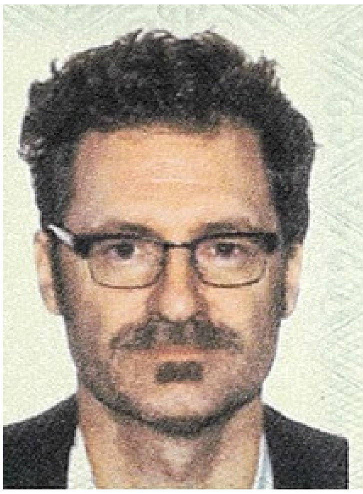
UAB - UCA

Closed regenerative life support systems represent an essential tool for the future of human space exploration. The micro-ecological approach used in MELiSSA is an interesting option to address long-term manned missions in space but requires a high degree of systems knowledge to guarantee the controllability of the mission in a safe manner. This study proposes the development of a hierarchical control architecture of the MELiSSA closed-loop for optimal resource management and control purposes following ALISSE criteria. The approach used in our research bases the prediction and control of the whole MELiSSA loop on the use of models with different complexity, from linear low-order models to full mechanistic models. The proposed control architecture is divided in three functional model-based hierarchical levels, which share information among them. From a functional point of view, Level 1 contains the predictive controllers that set input actions; Level 2 is responsible for restoring setpoints and guaranteeing stability; Level 3 assigns resource demands according to mathematical optimization tools and plant information. Broadly, setpoints, constraints and input actions are sent downwards and plant information is sent upwards through the control hierarchy. Modelling complexity, sample time and execution time are also adapted to the corresponding level in the hierarchy. While lower levels of control architecture use low-order transfer functions, higher levels in the hierarchy use non-linear mechanistic models. Computational simulations corresponding to the integration of the control and optimization layers are presented including both liquid and gas phase for nitrification, photosynthetic and crew compartment with one human. Oxygen concentration has been used as a critical variable to evaluate the proposed strategy under different operating conditions. The obtained results demonstrate that a hierarchical control architecture approach based on the use of predictive controllers and rolling-horizon optimizers is a good strategy to properly distribute resources with heterogenic consumption and production dynamics in a complex system like MELiSSA. The conception of the overall control loop in a discretized form brings in modularity in the modelling system. Hence, it is possible to update mechanistic models while they are improved; or even extend the hierarchical architecture with metabolic models.



Microbes in Hydroponic Crop Cultivation in Space

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Recycling technology is essential for long term human manned space exploitation and it is becoming a must for creating a sustainable agriculture here on earth. Our contribution to closing the Melissa loop is to address the cultivation of crops as a food source. Crop cultivation in space meets many challenges. Crops consume light, water, carbon dioxide and a range of minerals. These elements need to be provided at an apropriate balance, adjusted in accordance of the life cycle of the crop. In addition, crops need to be protected from pests that in space most likely originate from bacterial, fungal or viral disease. The main goal of our research is to create microbiological diversity contributing to plant health and its resistance to diseaseuse and contributes to the efficient use of organic fertiliser. Several research projects have been completed to address our objective. A closed gully was build with a closed hydroponic nutrient solution system to allow the investigation of microbial growth at the root level and to perform root shoot mass balance analyses. Our current project focusses on the use of urine derived fertilisers and associated microbiomes of the rhizosphere. A largely untapped source of potential plant nutrients is urine, which contains phosphorous and nitrogen-based compounds that are common plant fertilizers. Urine derivative nutrient preparations were added to rain water and used to grow hydroponic greenhouse lettuce. The rhizo-communities of the plants grown with these nutrient preparations were analyzed for differences in community composition, differential abundance compared to the control samples, differential abundance explained by horticultural metrics and leaf compound concentrations, indicator species, network properties and network hub-taxa.

MATISS-1 et 2: Microbial aerosol tethering on innovative surfaces in the international space station

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Biocontamination in manned spacecrafts and future habitats could have significant impacts on crew health and biodegradation of equipment. In particular, there is a need for decreasing the dependency of crew action for cleaning actions. Anti-microbial surfaces that inhibit or reduce the ability of microorganisms to grow on the surfaces are of high interest. In the context of future manned mission scenarios of longer duration, higher isolation, and the utilization of an increasing number of closed-loop life support systems, we hypotheses that the coating or surface modification must be effective 'in-use'. Matiss experiment (Microbial Aerosol Tethering on Innovative Surfaces in the International Space Station) is developed by ENS de Lyon, CEA Leti, Saint Gobain, and CNES. It aims to demonstrate that surfaces with hydrophobic properties already implemented in numerous terrestrial applications could be a possible technology transferable on the scale of spacecraft. By reducing the contact area of a water droplet with surfaces, the hydrophobicity allows limiting the contaminated fraction of the surface. It also limits the adhesion of microorganisms to surfaces. In this presentation, we will present the technological demonstration Matiss-1 implemented during the PROXIMA Mission by the European Astronaut Thomas Pesquet. The properties of the particles on these first returned surfaces were analyzed by optical microscopies and will be presented. Based on preliminary results obtained from Matiss-2 surfaces, we will discuss the kinetics of the contamination of the hydrophobic/control surfaces.

PARAGEN 1.0 a synthetic bacteriocin gene collection for rapid in vitro antimicrobial peptide selection for the microbial control of industrial fermentations

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Syngulon

Bacteria possess a vast reservoir of genes coding for antimicrobial peptides (bacteriocins) that are secreted in the environment. These peptides have potent killing functions that bacteria use to selectively limit the invasion of their ecological niche by bacterial competitors. These antimicrobial properties make bacteriocins very attractive to shape the bacterial flora composition used in industrial fermentations (for a review see Hols et al., 2019). To explore the potential of bacteriocins we have designed a collection (PARAGEN 1.0: Gabant and Borrero 2019) allowing us to identify relevant bacteriocin(s) for shaping the fermentation environment where the microbial composition must be controlled. These bacteriocins and bacteriocin cocktails can be applied to green chemistry, food/feed and human health. Results and challenges using bacteriocins will be presented. Cited publications: Hols et al., Mobilization of microbiota commensals and their bacteriocins for therapeutics, Trends in Microbiology, 2019. Gabant and Borrero, PARAGEN 1.0: A standardized synthetic gene library for fast cell-free bacteriocin synthesis, Frontiers in Bioengineering and Biotechnology, 2019.

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

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Bacterial adhesion, proliferation and biofilm formation is a major issue for confined manned space stations. It has been demonstrated that use of antimicrobial coatings containing heavy metals may lead to leakage of these heavy metals to the water condensate. Current metal-free solutions show several limitations including long-term toxicity, increase of pathogen resistance, limited antimicrobial activity and chemical, mechanical or adhesion failures. Herein we will present two coating methods that could potentially overcome such drawbacks and generate surfaces with broad-spectrum and long-term antimicrobial properties. In the first "dry" approach, we combined hydrophobic plasma polymer films and bio-sourced antimicrobial molecules and in the second "wet" approach, durable nanocomposite polymer films based on polyvinyl alcohol (PVA) embedding nanoparticles with bio-sourced antimicrobial agents were prepared by a wet approach (spin-coating /bar-coating). We performed the antimicrobial testing according to the ISO22196:2011/JIS Z 2801 standard to identify the most performant coatings and the cytotoxicity testing according to ISO 10993 standard to identify the less cytotoxic coatings. Both approaches generated promising antimicrobial activity and no or very low cytotoxicity. Tailoring the chemical composition, the thickness, the cross-linking ratio and the nano- and microstructure of the different coatings resulted in thin films stable in harsh conditions (water-24h, humid atmosphere at 50°C for 110 days and in salted environment for 6 days (salt spray test). Antimicrobial properties were conserved after the ageing testing. The antimicrobial coatings were active when deposited on both stainless steel and polyimide substrates. The high stability of the plasma coatings is due to the highly cross-linked microstructure embedding the antimicrobial molecules. For the nanocomposite PVA-based polymer coatings, boric acid molecules bridge alcohol bonds of the PVA together, promoting a stable coating as well. For both approaches, the antimicrobial agent is kept inside the coatings and a contact killing effect is obtained. For the plasma "dry" approach, the nature of the antimicrobial molecules as well as the hydrophobic properties are important parameters to obtain a broad-spectrum antimicrobial activity. For the "wet" nanocomposite approach, the concentration of the antimicrobial agents i.e. nanoparticles and antimicrobial molecules, are critical parameters to provide a broad-spectrum antimicrobial effect without generating cytotoxicity or biasing the high stability. Both approaches are therefore promising for future space (manned indoor) and terrestrial applications. This work is part of an on-going ESA project (contract no. 4000126324/18/NL/KML).

Towards an inventory of ecotoxicological effects metals and alloys on marine environments after over fifty years of launching activities

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Introduction Coastal Areas support increasing population worldwide, and are submitted to increasing and diversifying environmental pressure from human activities. Among others, are for long incriminated agriculture residues causing algal blooms, insufficient efficiency of continental water treatment plants, increasing concentrations of micro plastics and other residues issued by terrestrial industrials activities and even side effects on coral survival of over-use of solar cream… but marine areas are also submitted to constraints generated by increased maritime transit, petrol drills, and since the sixties, launching activities. Recently, the European DIRECTIVE 2008/56/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 17 June 2008 established a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive). It states that "The marine environment is a precious heritage that must be protected, preserved and, where practicable, restored with the ultimate aim of maintaining biodiversity and providing diverse and dynamic oceans and seas which are clean, healthy and productive. " (restauration could be conducted by 2020, i.e . now.). This requires a better knowledge of side effects, impacts and eventual toxicity of compounds, materials and activities on marine coastal areas, especially for those that remain un- or poorly characterised , among others launching activities. With this aim, our group focused at : 1-rewieving literature on existing studies with focus on fate of relevant compounds in marine environments especially in deep sea environment (such as lost container behaviours) 2-collecting general ecotoxicological data on relevant, poorly documented compounds and materials enlightening when required, insufficient knowledge, on their toxicity, biodegradation routes (organics) or general metabolism (both organics and metals) , possible colonization rate by marine organisms, and potential accumulation in marine food webs. 3- setting up experimental assessments in microcosms for a set of given materials and compounds Literature review Starting from a list of compounds commonly or specifically used by diverse industries including launching, it first appeared from our initial investigation that insufficient literature was available for numerous material and compounds for what concerns their behaviour in marine environments, their bioavailability, chemical/physical behaviour, and ecotoxicity for marine species. This appeared particularly true for deep sea environments that are characterize by lesser oxygen, lower temperature and higher hydrostatic pressure. For what concerns launching activities in general, focus was particularly made on metallic compounds, especially aluminium that enters among main propellants composition: Indeed it first appeared from foreign literature databases mining (the case of the MIR station, for instance), that the return rate of metallic part could globally reach 7.7% exceeding largely those of organic compounds.

Among metals, Aluminum should be a point of particular focus as it constitutes certainly the largest part of propellant returning to earth, all launching activities considered globally. In addition available ecotoxicological data for aluminum and its alloys demonstrate that indeed, an ecotoxicological risk exists for this compound, although analysis performed on marine species in different marine environments (especially deep sea ecosystems) remain scarce. Similarly, while for given metallic alloys (especially for the Cd and Cr based alloys), ecotoxicological effects are expected to occur, as shown from literature analysis, direct toxicological assessment of specific combination of metals used in aircrafts and launchers are mostly missing. Experimental assessment and Summary of results A feasibility study was thus performed and experimental conditions were set up to assess behaviour of selected metals and alloys in marine environment: Feasibility was demonstrated for commercial samples used in aircraft industry in surface shallow water conditions. 3 conditions were modelled (oxygenated sea water, water sediment interface, anoxic intra-sediments conditions). Electric conductivity was experimentally followed and SEM (Scanning Electronic Microscope) and a chemical analysis with ICP-MS (Mass Spectrometer) were used to evaluate corrosion rate and mechanisms in sea water of given alloys in a 3 months study. In parallel, a toxicological investigation was conducted on mussels (Mytilus edulis). Our analysis demonstrated that according to experimental conditions, corrosion and/or bio-corrosion were responsible for liberating (bioavailable) metals in sea water. However our study did not show direct short term effect of (bio)corrosion on filterers (i.e. musels). However, we suggest that further experimental assessment are to be conducted in more appropriate conditions of pressure, temperature and oxygen content aiming at modelling deep sea ecosystems and with longer incubation periods to derive final ecotoxicological conclusions.

Metallic copper rapidly inactivates the metal-resistant Cupriavidus metallidurans

Laurens Maertens
SCK-CEN & U Namur

Metallic copper rapidly inactivates the metal-resistant Cupriavidus metalliduransThe disinfecting properties of copper have been recognized for centuries, which led to its recognition as the first solid antimicrobial material by the US Environmental Protection Agency in 2008 [1]. The ESA backed BIOFILMS project aims to use metallic copper surfaces with different surface topologies to combat biofilm formation aboard the International Space Station and future manned spaceflight. We studied the effects of metallic copper on Cupriavidus metallidurans CH34, an extremely metal-resistant strain, and a species which has been isolated from different sources on-board the ISS [2]. We opted for a “wet contact” approach to mimic the drinking water reservoirs aboard the ISS, where bacterial contaminations are persistently present. C. metallidurans CH34 cultures were washed and resuspended in sterile mineral water (OD600 of 0.1), and added to conical centrifuge tubes containing either a copper plate, a stainless steel plate, or no plate. This setup was placed on an orbital shaker at 30 °C. Viable counts in the no-plate control remained stable throughout the experiment, as did the percentage of dead cells measured by flow cytometry. In the stainless steel condition, similar observations were made. In the copper plate condition, a near 5-log decrease of viable counts was seen within 3 hours. After 24 hours, viable counts increased to 106 CFU/ml by 144 hours, an observation that has not yet been made in similar experiments, and then started dropping off steadily until the end of the experiment. In the copper condition, flow cytometric events migrated to a zone suspected of containing permeabilized cells. Interestingly, there was a lag of ca. 24 hours in this data, indicating the conversion of culturable cells to viable but non culturable cells, in combination with a gradual killing of cells. Biofilms formed on the stainless steel plate contained much more biomass than biofilms formed on the copper plate, and in addition, this biomass consisted of a higher percentage of live cells, confirming the killing effect of the copper plate. In conclusion, the fate of heavy metal resistant bacteria in prolonged wet contact with metallic copper seems more complex than anticipated, in contrast to the rapid killing seen in many other strains. Follow-up research will focus on the copper-specific resistance mechanisms of C. metallidurans CH34 and their relevance in contact with metallic copper.

Decentralized sanitation & waste water treatment concepts

Peter Scheer
SEMiLLA Sanitation BV

Semilla Sanitation's goal is to treat water in a way that is more sustainable than current treatment methods, that closes the loop between wastewater supply and clean water demand, and that produces additional value over and above the value of the water that it started with. Worldwide, access to clean drinking water and hygienic sanitary facilities still is a big problem. Waste water is polluting groundwater. SEMiLLA Sanitation developed a concept based on ESA Space technology, where a circular solution is provided. Toilets, infrastructure (fixed and mobile), drinking water units, waste water units and growing systems. Building blocks to provide a closes loop system. A drinking water unit converts surface water to drink water, shower water and rinse water. Sanitation units for good sanitation. A blackwater unit converts shower water and toilet water to compost, (irrigation)water and fertilizers. A solution for festivals, humanitarian aid, disaster relief, temporary, semi-permanent, permanent and Smart Cities. The concept can help festivals, NGO,s, governments and the United Nations with her work to provide people (in need) with drinking water and hygiene, without contaminating the environment. A permanent application provides a revenue model where the stakeholders and the local population literally and figuratively reaps the benefits of the system; drinking water, hygiene, vegetable & fruit and economic growth. Creating a circular world by transforming wastewater into a resource within a closed loop system, in order to provide these basic necessities – sanitation, clean water, and nutritious food – is the mission at the core of Semilla Sanitation's work.