

CURRENT AND FUTURE WAYS TO CLOSED LIFE SUPPORT SYSTEMS

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CREATING A CIRCULAR FUTURE

BOOK OF
ABSTRACTS



ORAL PRESENTATION

ABSTRACTS

DIDIER SCHMITT

Strategy and coordination group leader for robotic and human exploration at European Space Agency – ESA - France – oral

TOPIC: LIFE SUPPORT ARCHITECTURE FOR FUTURE MISSIONS

About Terrae Novae, The ESA exploration roadmap

The mission of the Terrae Novae exploration programme is to lead Europe's human journey into the Solar system using robots as precursors and scouts, and to return the benefits of exploration back to society. Terrae Novae has the literal meaning of the New Worlds that encompasses the three ESA exploration destinations: Low Earth Orbit (LEO), Moon and Mars. It evokes the spirit of new discoveries, new ambitions, new science, new

inspiration, and new challenges. It symbolises the constant quest for technological, process and procurement innovations that result in new and better ways to deliver the programme. It also reflects the aspiration to actively reach out to new partners from beyond the space sector and enlarge the space ecosystem to the commercial sphere. ●

FRANK RIEMANN

ESA – Netherlands – oral

TOPICS: SOCIETAL IMPACTS AND EDUCATION

Evolution of MELiSSA MOU

Since the early days of the project, MELiSSA partners, with a leading role of ESA, set up a cooperation scheme by signing the MELiSSA Memorandum of Understanding. This MOU defines the purpose of the MELiSSA Project, main rules of collaboration including rights and obligations of the partners, including, for example, issues such as confidentiality, intellectual property, publications. Recently, due to the need to reflect the participation of five ESA directories in the project,

the growing number of commercial applications, spin-offs, collaborations and large perimeters of the project (e.g. around 50 organisations around 14 countries), an internal ESA working group was created to discuss and elaborate the new structure and rules of the MOU. This presentation provides an overview of the needs for evolution and presents selected topics from the new version of the MOU. ●

CO-AUTHORS: Franck Riemann, Aude de Clercq, Stephan Spiedel, Christophe Lasseur

BAHAR ACIKSOZ

Principal Investigator - Sabanci University Nanotechnology Research and Application Center – Turkey

TOPIC: FOOD PRODUCTION AND PREPARATION

Shortening the Breeding Cycle

On Earth, with its nearly 7.7 billion inhabitants, a figure that is expected to climb to 9.7 billion by 2050, global climate change and the slow-release rate of new crop cultivars are raising concerns regarding global food security. Long generation spans of crops are the key limiting factor in breeding. Speed breeding is a method to grow plants under LED-controlled wavelengths and intensity, an extended photoperiod and higher temperatures to accelerate the generation period from seed to seed. In wheat, achieving the desired wheat with enhanced traits grown by one generation in the field takes 8 years through marker assisted selection of four traits, in the greenhouse 4 years, finally by speed breeding around 1,5 years. In spring wheat, durum wheat, barley, chick pea, and pea, up to 6 generations per year and 4 generations for canola instead of 2 to 3 generations can be

harvested in controlled environment growth chambers by speed breeding techniques. Recently speed breeding techniques were introduced for long-day crops, however short-day plants protocol still is lacking an optimization up to date. In addition, planophile plant leaves stand perpendicular to overhead light, thus shaded inner canopy and side leaves end up less efficient in photosynthetic work. The aim of this study is to explore the questions: how can speed breeding techniques overcome the bottleneck of light saturation in photosynthesis, the impact of LED illumination recipes on early flowering, stomatal conductance and to achieve the highest photosynthesis rate, to what extent short day crops can be exposed to long day photoperiod and how the carbon metabolism affected. ●

CO-AUTHORS: Seher Bahar Aciksoz, Atilla Yazici

ANTOINETTE KAZBAR

Lecturer/researcher - Wageningen University and research – Netherlands

TOPIC: FOOD PRODUCTION AND PREPARATION

A novel multiproduct pathway towards algal food ingredients

The urgent need to shift towards more sustainable food chains is more evident than ever before. Algae, such as microalgae and seaweeds have high protein, sugar, essential oils and pigment content, with an increasing number of species receiving the Novel Foods status. However, current processing technologies are energy-intensive and rely on hazardous chemicals,

preventing them from becoming widely used. Thus, by combining a two-phase system composed of novel natural tuneable solvents, with process-intensifying acoustic fields, sustainable and efficient production of functional food ingredients is proposed through algal agriculture. ●

CO-AUTHORS: Iulian Boboescu, Michel Eppink, Rene Wijffels

CLAUDIA QUADRI

Senior Application Engineer – EnginSoft – Italy

TOPICS: AIR RECYCLING AND CO2 VALORIZATION, FOOD PRODUCTION AND PREPARATION

PaCMan Unit upgrade - New subsystems for a deeper investigation of the root zone.

In the frame of the MELISSA activity, a PCU (Plant Characterization Unit) has been engineered and manufactured under the PaCMan project. It is now based in Napoli, at the facility of the Università Federico II. The PaCMan Unit was developed with the aim to study the performance of crops to be employed for regenerative life support systems during long term space missions: transit to Mars. Plants can be controlled and monitor in all their growth cycle, characterizing thus their behavior under different environmental conditions: nutrient delivery, atmospheric environment and light for photosynthesis. Particularly the excellent air tightness achieved in the chamber for the gas phase enables the mass balance computation relative to: Oxygen production, CO2 consumption and water recycling. The unit was initially engineered and designed with a modular approach. Therefore, each unit and each component can be easily isolated and substituted. This is an important aspect from the maintenance point of view but also for further upgrades. The PaCMan 2 project has the objective to continue the development of the PCU through the upgrade the hydroponic system with the integration of two extra subsystems in order to increase the analytical capability for the root characterization. One system allows roots and solution sampling for direct measurements of the rhizosphere, the second integrates cameras for visual monitoring of the roots. From a scientific

point-of-view, the upgrade of the hydroponic loop with two new subsystems are based on the need to include the root system in future studies towards improved fundamental understanding of the biology of higher plants and the construction and validation of the mathematical models. The plant is a continuum, and scientific data acquisition needs to address the plant in its totality. The augmented root analysis capability goes in this direction. The two new root sub-systems have been designed and implemented in a new gully. Functional tests have been already performed while a life test campaign will exploit the new functionalities of the PaCMan Unit and will validate the design over four crop cultivation periods of 3 weeks each. The presentation will highlight the modularity engineering of the unit and demonstrate that this modularity has been exploited with the upgrade of the hydroponic. An engineering description of this upgrade will be illustrated, while the other partners of the Pacam2 project will:

- CIRiS illustrated the breadboard testing on the new gully subsystem for root analysis
- CIRiS illustrate the results and plant characterization of 2 life tests
- UniNA - illustrate the results and plant characterization of 2 further life tests. ●

CO-AUTHOR: Lorenzo Bucchieri

MONA SCHIEFLOE

Researcher - NTNU Social Research, Centre for Interdisciplinary Research in Space – Norway

TOPICS: GREY, YELLOW, BLACK WATER RECYCLING, FOOD PRODUCTION AND PREPARATION, LIFE SUPPORT ARCHITECTURE FOR FUTURE MISSIONS

Lettuce cultivation in a urine recycling scenario: Effects of different NH4:NO3 ratios

Off-Earth human bases require on-site food production systems that may benefit from higher plants producing nutritious food, water, and oxygen. Crop cultivation in any environment rely on

a supply of water and nutrients, and in a closed environment such as a Lunar base, these resources should and may be recovered from waste and wastewater. Human urine represents

an important waste stream in this regard, supplying the crops with nitrogen and other nutrients after required pre-processing, remineralization of urea and (partial) nitrification of ammonium to nitrate. The ESA MELISSA Plant Characterisation Unit (PCU) at the premises of University of Naples Federico II in Italy is a hydroponic research facility with closed atmospheric and liquid compartments, offering the possibility of crop studies in controlled conditions to characterize plant growth parameters in the root- and aerial zone. Results from crop cultivation tests in the PCU will serve as input to mechanistic models of plant growth for further optimization of Space plant cultivation. This presentation summarizes results of a lettuce crop test campaign performed in the PCU, with a focus on plant cultivation in a urine recycling scenario. Future urine treatment processes upstream of the crop cultivation may be dynamic, resulting in a nutrient solution with varying concentrations of ammonium, NH4+, and nitrate, NO3-, including their relative ratio. To explore these effects, two crops of lettuce (*Lactuca sativa* L. cultivar Grand Rapids) were cultivated in a nutrient solution with low and high ammonium to nitrogen ratios (NH4:N) of 0.11 and 0.50 mol/mol and an elevated concentration of Na+ and Cl- to closer

mimic a nutrient solution enriched with urine-based nutrients. A newly developed nitrate sensor-based monitoring and control system was set to maintain a constant nitrate setpoint throughout the tests, aiming for different NH4:N ratios but the same total N concentration in the two tests. A lettuce crop test with standard nutrient solution (NH4:N ratio of 0.22 mol/mol) performed by the University of Naples, was used as a baseline comparison. The results elaborate on comparisons of plant shoot- and root morphology, biomass, growth curves, O2 and CO2 production and consumption, water production, and plant nutrient content of lettuce from the three cultivation scenarios, in addition to technical aspects of the PCU and its functionalities. Considerable differences between the different NH4:N ratios were identified for several plant parameters. Additionally, results from a pre-screening-test of lettuce grown in six different NH4:N ratios spanning from 0.0 to 0.5 mol/mol are presented. In a broader perspective, the scientific and technical results may contribute to an increased life support system efficiency and improved design/control of integrated processes of wastewater recycling and crop cultivation. ●

Keywords: Higher Plant Compartment, Urine recycling, Lettuce.

CO-AUTHORS: Mona Schiefloe, +47 95891516, mona.schiefloe@ciris.no, Centre for Interdisciplinary Research in Space (CIRiS), NTNU Social Research, Norway. Øyvind Mejdell Jakobsen, +47 93233047, oyvind.m.jakobsen@ciris.no, Centre for Interdisciplinary Research in Space (CIRiS), NTNU Social Research, Norway. Antonio Pannico, +39 338 3264475, antonio.pannico@unina.it, Department of Agricultural Sciences, University of Naples Federico II, Italy. Claudia Quadri, +39 349 1614854, c.quadri@enginsoft.com, EnginSoft S.p.A., Italy Ann-Iren Kittang Jost, +47 92880298, a.i.kittang.jost@ciris.no, Centre for Interdisciplinary Research in Space (CIRiS), NTNU Social Research, Norway. Presenting author and primary affiliation: Mona Schiefloe, +47 95891516, mona.schiefloe@ciris.no, Centre for Interdisciplinary Research in Space (CIRiS), NTNU Social Research, Norway.

UDERT KAI

Senior Scientist – Eawag

TOPIC: GREY, YELLOW, BLACK WATER RECYCLING

Community shift of ammonia-oxidizing bacteria and washout of nitrite-oxidizing bacteria due to pH changes during urine nitrification

In the MELISSA loop, urine is the main vector of nitrogen but urine has to be stabilized prior to its utilization as fertilizer. Nitrification is an appealing process for urine conversion. If no alkalinity is added, about 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+). Partial nitrification conserves resources (i.e., oxygen and base) compared to complete nitrification, but is sensitive to pH changes. Therefore, the effect of pH on nitrite production and microbial community during partial nitrification was studied. Six 12-L continuous stirred-tank reactors were fed with urine at nitrogen concentrations of 1.5 or 3.5 g N L⁻¹. During 25 days, the pH was controlled at pH 6 or 5.8 with the influent using an on/off controller. Subsequently, the pH set points were changed to 7 in two reactors, one for each urine influent. In two other reactors, the pH was changed to 8.5, and in the last two reactors, the influent was completely stopped, leaving the pH uncontrolled. In addition, short-term activity assays were conducted to evaluate the effect of NH3 and nitrous acid (HNO2) on the nitrification activity of activated sludge from stable urine nitrification. Stable urine nitrification was observed at pH 6 and 5.8 with only 0.5 mg-N L⁻¹ of nitrite in the effluent. The dominant ammonia-oxidizing bacteria (AOB) was closely related *Nitrosomonas europaea*. Despite strong biological nitrite oxidation, no nitrite-oxidizing bacteria (NOB)

species were clearly identified. Nevertheless, several unknown species of the family Xanthobacteraceae were observed, which includes NOB of the genus *Nitrobacter*. Nitrite accumulated at pH 7.5, which eventually led to nitritation. A new AOB closely related to *Nitrosomonas halophila* became dominant. NOB were washed out due to HNO2 inhibition, as nitrite accumulation caused HNO2 concentrations of 0.1 to 0.25 mg-N L⁻¹, which reduced NOB activity by 40 to 65% according to the activity assays. At pH 8.5, a temporary complete cessation was observed in the reactor with the higher TAN concentration in the influent, but while no nitrite oxidation was observed at pH 8.5, ammonia oxidation recovered leading to nitritation. Again, the AOB community adapted to the new conditions, an AOB closely related to *Nitrosomonas stercoris* became dominant. According to the activity assays, the high NH3 concentration of 100 to 300 mg-N L⁻¹ at pH 8.5 caused a 60 to 80% reduction of NOB activity and therefore most likely to washout. The influent stop, led to a pH drop to about 5.5 and a long idle phase followed by a second pH decrease to pH 2.5. The AOB community shifted towards the acid-tolerant AOB *Candidatus Nitrosacidococcus urinae*. Based on the data, it is difficult to determine whether biological or chemical nitrite oxidation was predominant. To conclude, ammonia oxidation is possible over a wide range, because the AOB community always adapted to the novel conditions, while the NOB were washed out either due to NH3 or HNO2 inhibition. ●

ANNA JURGA

PhD Student - Wroclaw University of Science and Technology – Poland

TOPICS: GREY, YELLOW, BLACK WATER RECYCLING, FOOD PRODUCTION AND PREPARATION, CIRCULAR ECONOMY

Fertilizer production for soilless plant cultivation in closed life support system - lessons learned from 4 years study

This paper presents the research results collected as part of a PhD Thesis during 4 years of study on biological wastewater treatment and soilless plant cultivation for a future closed life support system. The use of nitrification process in aerobic activated sludge reactor was investigated to produce liquid fertilizer for lettuce hydroponic cultivation. Two configurations were examined: urine and a mixture of urine and greywater nitrification. The first configuration operation lasted 225 days, and the second 140 days. In general, the used reactor set-up parameters assured stable operation of the processes. The high efficiencies were achieved in both experiments. The operation of the urine nitrifying reactor was disrupted by two major failures due to prolonged exposure of biomass to extremely high concentrations of Free Ammonia. The second configuration was fewer failures and was more stable. The values of the specific nitrification rate in both experiments final, stable phases differed by an average of 50%. The feasibility of using diluted nitrified urine and a mixture of urine and greywater in soilless lettuce

cultivation realized in this study showed the possibility of plant growth, but in a limited way in terms of quantity and quality. The main yield-reducing factors were elemental deficiencies (i.e. K, Mg, micronutrients) resulting from the composition of the treated wastewater. This was confirmed by measurements of parameters such as elemental composition, stress parameters, and photosynthetic pigment concentration. When using fertilizer based on a mixture of urine and greywater, the study showed that the elemental deficiency was the main growth-limiting factor and not surfactants presence. Despite poorer yield and quality of lettuce, organoleptic tests showed no differences in consumer perception. This is particularly important about fertilizer production using circular management (both terrestrial and space), where the aspect of consumer acceptance of the final product is particularly important. Enriching the fertilizers with the missing nutrients made it possible to obtain a yield similar to the reference in terms of quantity and quality. ●

CO-AUTHOR: Kamil Janiak

ELLEN HARRISON

PhD student - University of Cambridge - United Kingdom

TOPICS: FOOD PRODUCTION AND PREPARATION

Vitamin B12, microalgae and the MELiSSA loop

Vitamin B12 is a cofactor for enzymes involved in central metabolism and is therefore essential for good human health. It is a complex molecule, based on a modified tetrapyrrole ring that chelates a cobalt ion, tethered by an upper and lower axial ligand that can vary in structure, altering its biological function. Vitamin B12 is only synthesised by certain prokaryotes and requires over 20 enzymatic reactions. On Earth, the majority of humans fulfil their vitamin B12 requirements through the consumption of meat, eggs and dairy products, products that are much more difficult to obtain on long space missions. The MELiSSA loop is one example of a biological life support system for long-distance space exploration. It contains five compartments, and through compartment IV all the nutritional needs of the astronauts must be met. Compartment IV is composed of two sub-compartments, the higher plants in IVB and the photoautotrophic cyanobacterium *Limnospira indica* in compartment IVA. Higher plants do not make, require or store vitamin B12. Furthermore, using a new microbiological bioassay for vitamin B12 that we have developed, we have shown that *L. indica* makes pseudocobalamin, a form of B12 not suitable for humans. Therefore, the astronauts would be at risk of developing

a B12 deficiency and the resulting negative health outcomes. The novel microbiological bioassay has also been used to investigate the presence of B12 in the other compartments of the MELiSSA loop. For example, *Rhodospirillum rubrum*, a bacterium historically proposed to occupy compartment II, produces cobalamin, the more bioavailable form of the vitamin. As a proof-of-concept approach we have established cocultures of *R. rubrum* and the edible eukaryotic microalgae *Chlorella vulgaris* or *Chlamydomonas reinhardtii*, which could potentially provide a biomass enriched with B12. Both microalgae have featured in space research for decades, but only in monocultures. We use these synthetic cocultures to investigate how environmental conditions found in space, such as higher ionising radiation and microgravity, could alter the dynamics of cocultures, and thereby the B12 content of the biomass. As the popularity of vegetarian/vegan diets increases, novel vegan sources of vitamin B12 are also required on Earth. Consequently, not only is this research useful for improving bioregenerative life support systems for space travel it may also improve B12 provision on Earth. ●

CO-AUTHORS: Natalie Leys, Felice Mastroleo, Matthew Davey, Payam Mehrshahi and Alison G. Smith

SUDEEP POPAT

Clemson University - United States

TOPICS: GREY, YELLOW, BLACK WATER RECYCLING, WASTE RECYCLING

Electrochemical stabilization and resource recovery from source-separated urine

Recovery of water, nitrogen (N), and phosphorus (P) from urine are essential for long-term space missions. Processing of urine currently at the International Space Station (ISS) includes dosing of concentrated sulfuric acid (H₂SO₄) and chromium trioxide to prevent microbial growth and the formation of odors from hydrolysis of urea. However, this represents a large amount of payload needed to be included for a particular mission. Instead, we propose stabilization with in-situ electrochemically synthesized H₂O₂, presenting an opportunity to reduce the costs and hazards of urine stabilization prior to filtration. Future deep space missions will require more intensive solutions to urine treatment than solely water recovery, which may include developing methods to reclaim N and P in useful forms. Our previous reactor designs for urine stabilization with H₂O₂ relied

on a Nafion membrane separating the anode and cathode chambers to prevent the anodic consumption of H₂O₂. We have now developed an electrochemical cell that relies on a sacrificial magnesium (Mg) anode to prevent anodic H₂O₂ consumption, thus allowing eliminating the need for a membrane. Mg was chosen for its low redox potential which would also avoid chlorine production, and to provide Mg ions for the precipitation of struvite (NH₄MgPO₄·6H₂O) for P recovery. This way dissolved Mg is recovered along with P in a useful form in a solution stabilized by H₂→O₂. We will present our results demonstrating this new electrochemical cell, along with the proof-of-concept of concomitant struvite precipitation and urea stabilization from source-separated urine. ●

CO-AUTHORS: Philip Arve, Prithvi Simha, Dyllon Randall

ANTONIO PANNICO

Senior researcher - Department of Agricultural Sciences, University of Naples Federico II, Portici, Italy – Italy

TOPIC: LIFE SUPPORT ARCHITECTURE FOR FUTURE MISSIONS

Characterization of three leafy vegetables in a sealed plant-growth chamber for closed life support systems

Plants are the most promising biological regenerators in life support systems as they renew air through photosynthetic assimilation of CO₂ and emission of O₂, recover purified water through transpiration, and recycle waste products through mineral nutrition, while providing fresh food for the crew's diet. The design and development of growth chambers are critical to characterize the plant performance within Biological Life Support Systems (BLSSs) for long-term manned missions in Space. To this end, a Plant Characterization Unit (PCU) was set up at the Laboratory of Crop research for Space of the University of Naples (Italy) in 2019, within the ESA-MELiSSA PaCMAN project. The PCU is conceived as a research facility for extensive scientific investigations that accommodates two separate sub-systems, an atmospheric module and a hydroponic module. This system offers the possibility to finely measure crop parameters (i.e. H₂O, O₂ and CO₂ production and consumption, nutrient consumption, biomass production), and to determine the mass balance in both the rhizosphere (hydroponic module) and the aerial zone (atmospheric module), since the two zones are separate and leak-proof. Concerning the plants cultivation, genotype is the main factor affecting crop production and resources regeneration efficiency. Therefore, lettuce (*Lactuca sativa* L.), kale (*Brassica oleracea* L. var. *acephala*) and swiss chard (*Beta vulgaris* var. *cicla*) were characterized by conducting three consecutive life tests in the PCU. Such leafy vegetables, belonging to three different botanical families (Asteraceae,

Brassicaceae and Chenopodiaceae), were suitable as candidate crops for their high harvest index, short growing cycle, and moderate canopy size as well as for their high nutraceutical value. All three life tests were carried out by adopting the same experimental set up in terms of seedling phenological stage (three true-leaves), environmental conditions (CO₂ concentration 1000 ppm, light intensity 420 μmol m⁻² s⁻¹, light/dark regime 16/8h, air temperature and relative humidity at 26/20°C and 50/70%, respectively), nutrient solution concentration and composition, and cycle duration (28 days). The results showed a higher total dry biomass accumulation in kale and swiss chard than in lettuce (+63% and +60%, respectively), similarly, the amount of water transpired per cultivated unit by kale and swiss chard was significantly higher than the water transpired by lettuce (+60% and +41%, respectively). In addition, lettuce fixed less carbon per cultivated unit area than the other two species. This finding denoted greater overall photosynthetic activity of kale and swiss chard compared with lettuce and consequently higher O₂ production per cultivated unit area (+74% and +36%, in kale and swiss chard, respectively). All three life tests were successfully completed in the PCU, providing a reference dataset for future characterization with different climatic conditions or plant species, as well as relevant data for integrating the HPC into the MELiSSA loop. ●

CO-AUTHORS: Youssef Rouphael, Luigi Giuseppe Duri, Roberta Paradiso, Stefania De Pascale

LUIGI IZZO GENNARO

Researcher - Department of Agricultural Sciences - University of Naples Federico II – Italy

TOPIC: FOOD PRODUCTION AND PREPARATION

Species selection of microgreens to be produced in space as functional food for astronaut consumption

Long-duration missions in outer space will rely on producing healthy food for astronaut consumption during spaceflight and extraterrestrial colonization. Although much effort has been devoted at developing plant-cultivation systems for space, only a few studies provide a selection of plant species to be used in space farming, and none of these refers to criteria applied to select microgreens species for space. Space farming is effort demanding since there are many constraints to be addressed to achieve viable plant growth. In this regard, there is a need to select those species that satisfy yield and food-quality requirements despite space constraints. Microgreens are functional food crops recently valued for their flavor and phytonutrient content and have been proposed as components of Bioregenerative Life Support Systems in Space by ASI with the in-progress MICROx2 Project. Candidate species of microgreens to be harvested and eaten fresh by crew members, mostly include species from the families Brassicaceae, Asteraceae, Chenopodiaceae, Lamiaceae, Apiaceae, Amarillydaceae, Amaranthaceae, and Cucurbitaceae. In this study we developed and applied an algorithm to objectively compare numerous genotypes of microgreens intending to select those with the best growth and nutritional characteristics for cultivation in space. In the mathematical model both growth and nutritional parameters were implemented with prioritization criteria to

produce a final ranking list of the microgreens genotypes. The methodology used to generate the ranking list consisted of two subsequent runs of selection. The first was based on a dataset containing parameters on growth (e.g. yield, dry weight, sowing density, 100-seed weight), nutrition (e.g. tocopherol, phyloquinone, ascorbic acid, polyphenols, lutein, carotenoids, violaxanthin), and element (e.g. nitrate, phosphorous, potassium, calcium, magnesium, sodium). These data were gathered from a thorough analysis of the scientific literature on microgreens. The second run was based on new data on phytonutrients from experiments specifically designed for this work and performed on the six top species from the elaboration of literature data. For the calculation, literature data were normalized among parameters and genotypes, whereas experimental data on phytonutrients were expressed as metabolite production per day per square meter. Based on our elaboration, *Raphanus sativus* (radish) and *Brassica oleracea* var. *capitata* f. *sabauda* (savoy cabbage) resulted with the highest score based on their growth and nutritional characteristics. Overall, our approach has proven reliable to objectively compare and confidently select the most suitable microgreens for cultivation in space. Therefore, the method can be adapted to further applications of candidate species for space. ●

CO-AUTHORS: Christophe El Nakhel, Youssef Rouphael, Simona Proietti, Gabriele Pagliarunga, Stefania De Pascale, Giovanna Aronne

NELE KIRKERUP

MELISSA PhD student - Eawag (ETHZ) - Switzerland

TOPIC: GREY, YELLOW, BLACK WATER RECYCLING

Impact of the Composition of Organics on Urine Treatment

Organic compounds are of major importance in the recovery of nitrogen, potassium and phosphate from urine. They are substrate for various biological processes and known to contribute to the malodour of untreated urine. There are various ways urine can be collected and stored. At Eawag urine is collected by NoMix toilets and stored with no further treatment in anaerobic tanks. Here, the organic compounds are converted to more easily degradable volatile fatty acids such as acetate and are a substrate for microorganisms such as sulfate reducing bacteria and methanogens. This anaerobic storage yields a urine with a different organic composition as fresh urine and is referred to as stored urine. A second form of collection and storage is immediate stabilization after excretion. In this project we focus on stabilization by adding calcium hydroxide (directly in the collection unit). The pH is increased to 12, thereby inhibiting ureolysis and other biological processes. This urine is referred to as alkalinized urine. Independent of the collection and storage method, the urine is then fed to the biological treatment, where the total ammonia is converted into nitrate by nitrifying autotrophic bacteria and organic compounds are degraded by heterotrophic bacteria. At the pilot plant at Eawag this takes place in an activated sludge reactor, however there

are other reactor setups in different research groups such as a biofilm reactor. In these collection systems and throughout the treatment, organic compounds are mainly identified by the sum parameters chemical oxygen demand (COD) or total and dissolved organic carbon (TOC or DOC). For fresh urine, the composition of organic compounds is well established by using nuclear magnetic resonance (NMR). Creatinine, hippuric acid and citric acid represent 45 to 60% of the COD in fresh urine. However, these compounds are no longer present in urine stored over few days due to anaerobic processes. Initial measurements of the pilot plants at Eawag show that acetate is responsible for 42% of the COD, but with a high variability. Measurements with the NMR method also identified compounds such as trimethylamine and dimethylamine, which contribute to malodour. As mentioned before, the organic composition and form of nitrogen differs depending on the collection and storage type. Based on the current knowledge of the degradability of organic compounds the hypothesis was established, that the biological treatment step (nitrification and organic degradation) performs better when fed with urine, where the main organic compound is acetate, hence stored urine. To test this hypothesis three aerated fed batch reactors are operated under the same

conditions and fed with three different influents: fresh urine (stored at 4°C until use), alkalinized urine and stored urine. This is an ongoing experiment. The nitrification rate and organic removal efficiency are observed. NMR measurements of the influent and effluent will show the influence of the influent

organics on the nitrification effluent and treatment performance. With this information we will be one step closer to identifying the organic compounds in different treatment steps along the treatment chain and understanding their role in urine treatment. ●

CO-AUTHORS: Prof. Dr. Kai Udert, Prof. Dr. Siegfried Vlaeminck, Prof. Dr. Ramon Ganigué

MATTHEW GILLIHAM

Director, Plants for Space - University of Adelaide – Australia

TOPIC: FOOD PRODUCTION AND PREPARATION, PROTEINS PRODUCTION, PROCESS MODELING, SIMULATION AND CONTROL, CIRCULAR ECONOMY, SOCIETAL IMPACTS AND EDUCATION, SPACE EXPERIMENT

Plants for Space - a new multidisciplinary centre focused on enabling long term-deep space habitation

Plants for Space (P4S) is an Australian based collaborative initiative linking with partners internationally focused on providing plant-based solutions for the sustainable production of nutrition and biomaterials for Space and Earth. Multidisciplinary skillsets have combined in Plant and Food Science, Systems and Process Engineering, Nutrition, Psychology, Law, and Education to explore the fundamental breakthroughs required to develop fit for purpose biomanufactured products. Our programs will use molecular-based techniques to deliver food from plants: tailored pick & eat crops to supplement dietary needs, and a suite of complete nutrition plants to solve the challenge of total caloric replacement. Plant biofactories and bioprocessing technologies developed will be vital to sustain closed environments for extra-terrestrial settlement,

and provide new advances for on-Earth manufacturing of pharmaceuticals and plant-based foods. We also aim to develop engineering solutions and new experimental platforms that have undergone rigorous lifecycle and techno-economic analyses, and explore legal and ethical frameworks to ensure our innovations are fit-for-purpose and readily translatable to meet current timelines. We will implement a bold, long-term approach to education and training to inspire more students into STEM subjects and establish a new generation of Australian Space-fluent researchers. P4S aims to provide a touchpoint for plant and food focused researchers and industry, to deliver the efficiencies and synergies needed internationally to deliver plant-based technologies that will assist in enabling sustainable long-term deep-space habitation. ●

CO-AUTHORS: Matthew Gilliam, and the Plants for Space Consortium

MATTEO BALLOTTARI

Associate Professor - University of Verona – Italy

TOPIC: PROTEINS PRODUCTION

Green algae for sustainable edible proteins production

Microalgae are unicellular photosynthetic organisms that can be grown in artificial system to capture CO₂, release oxygen, use nitrogen and phosphorus rich wastes, and produce biomass and bioproducts of interest. Here we report two different strategies for proteins production by microalgae cultivation. In the first approach, we applied a metabolic engineering strategy in the green alga *Chlamydomonas reinhardtii* to produce high value proteins for nutritional purposes. *Chlamydomonas reinhardtii* is a species approved by FDA for human consumption, and its consumption was reported to improve gastrointestinal health in both murine models and humans. Exploiting the biotechnological tool available for this green alga, we introduced in the algal genome a synthetic gene encoding for a chimeric protein, zeolin, obtained by merging ³-zein (*Z. mays*) and phaseolin (*P. vulgaris*) proteins. Zein and phaseolin are key proteins in seeds having a role of amino acid storage: the chimeric recombinant zeolin protein allow to provide an amino acid storage strategy with a balanced amino acid profile. Zeolin

protein was thus efficiently expressed in *Chlamydomonas reinhardtii* obtaining strains that accumulate this recombinant protein in the endoplasmic reticulum or secrete it in the growth medium. This biotechnological modification can be further combined with other modifications, as the overexpression of a beta-carotene ketolase inducing surprisingly high accumulation of antioxidant in *Chlamydomonas reinhardtii*, toward the production of a superfood. In a second strategy, we cultivated the fast growing species *Chlorella*, characterized by having up to 65% of proteins per dry weight, using nitrogen and phosphorus nutrients derived from anaerobic digestion of agricultural wastes. In particular, phosphate and ammonium salts were obtained by separate chemical treatments of anaerobic digestate. The results obtained demonstrate an efficient consumption of nitrogen and phosphorus compounds by microalgae cultivation with a continuous production yield of 0,17g/L of edible biomass. ●

CO-AUTHORS: Federico Perozeni, Margherita Angelini, Eliana Gasparotto, Stefano Cazzaniga, Nicola Frison, Elisa Maricchiolo, Andrea Pompa

CELIA ÁLVAREZ FERNÁNDEZ

PhD researcher - Ghent University – Belgium

TOPICS: GREY, YELLOW, BLACK WATER RECYCLING, WASTE RECYCLING, SPACE EXPERIMENT

Will be three a multitude in the nitrifying compartment? First steps towards the characterization of a novel synthetic community by using flow cytometry and atomic force microscopy.

Almost 30 years ago, the idea of Micro-Ecological Life Support System Alternative (MELISSA) loop was drafted. The creation and implementation of this support system obey the actual need to have an autonomous space ecosystem for space exploration. The proposed Bioregenerative Life Support System (BLSS), aims to produce food, O₂ and clean water on board by reusing the waste produced by the crew. One of the key processes is nitrogen recycling. Around 85% of the nitrogen intake by the crew goes to urea. This nitrogen compound can be transformed into more valuable compounds like fertilizers for the crops that will constitute the main source of dietary protein needed by the crew. Nitrogen reuse takes place in the compartment III (C3) of the MELISSA loop, where urea is transformed via ureolysis to ammonium and then via nitrification to nitrate, which will be used as fertilizer. The whole process is carried on by a synthetic community of bacteria in which urea is hydrolyzed to ammonia by *Comamonas testosteroni*, after this *Nitrosomonas europaea* and *Nitrobacter winogradskyi* oxidize ammonia first to nitrite (nitritation) and then to nitrate (nitrification). Monitoring the abundance of the different species in the synthetic community and in biofilms (the preferred mode of growth for the C3 compartment, as implemented in the MELISSA Pilot Plant) is of critical importance. This contribution aims to first develop a novel and rapid quantification method based on the coupling of flow cytometry with fluorescence in situ hybridization (Flow-FISH). Here we used conventional FISH probes CteA, Nse1472 and NIT3 for *C. testosteroni*, *N. europaea*, and *N. winogradskyi*, respectively. After incubating the synthetic

community with the synthetic urine medium, the evolution of the three different bacteria, i.e., absolute quantities and relative abundances in the community, was closely followed, along with the nitrogen species. Preliminary data showed an increase in the abundance of *C. testosteroni* during the first days, while for the nitrifiers a gradual increase was observed only towards the end, illustrating their sequential activation. Nitrifying biofilms have been reported in the C3 on the MELISSA Pilot Plant. A pipeline was developed for their visualization in batch cultures using polyethylene sheets as the carrier. In this preliminary experiment, atomic force microscopy (AFM) was selected to obtain topographic images of the possible biofilms. The incubation time was chosen to mimic the duration of the real flight experiment. After 29 days of incubation of the axenic strains and the synthetic community, biofilms were visualized on *C. testosteroni* and the synthetic community samples. In contrast, no biofilms were visualized on the substrate incubated with *N. europaea* or *N. winogradskyi*, indicating low biofilm forming potential of the axenic nitrifiers during the length of the flight experiment. Overall, the Flow-FISH enables high-throughput and rapid quantification of the three bacteria in the synthetic community and thus high time resolution understanding of the microbial community. The AFM-based visualization allows inferring the development of biofilms and their properties. These two methods provide new tools to better understand and optimize the performance of the synthetic community for N recycling. Keywords: Nitrogen recycling, Flow-FISH, Biofilms. ●

CO-AUTHORS: Mingsheng Jia, Tara Baele, Louis Van der Meeren, Siegfried E. Vlaeminck, Andre Skirtach, Nico Boon, Ramon Ganigué

ILIANA ILIEVA

Assistant professor - Space Research and Technology Institute - Bulgarian Academy of Sciences – Bulgaria

TOPICS: PROCESS MODELING, SIMULATION AND CONTROL, LIFE SUPPORT ARCHITECTURE FOR FUTURE MISSIONS

Novel approach to enhance the potential of ground preparatory activities for improved plant growing experiments in microgravity

Plants have been grown in space for more than 50 years and although great success has been achieved, post flight analyses revealed that plants respond to stressful environmental conditions occurring in space greenhouses in microgravity rather than to weightlessness itself. Hypoxia in the root-zone and absence of convection, presence of ethylene, elevated carbon dioxide and insufficient light in the leaf-zone have been identified as space greenhouse specific environmental factors causing various stressful responses in plants. Some of these stressful conditions have technogenic origin due to mass,

volume and power constraints imposed on the hardware. Others are due to alterations in the behavior of physical processes in microgravity. Since microgravity modifies the environment in space greenhouses in a way that generates stress in plants, an approach to substitute the observed complex of microgravity-specific stresses with terrestrial analogues is presented. The goal is to broaden the knowledge of plant responses to microgravity induced stresses through inexpensive experiments on Earth and thus to accelerate the development of new hardware and cultivation practices to improve plant growing in space.

PEDRO GARCIA JOAO

Research Fellow - European Space Agency – Netherlands

TOPICS: FOOD PRODUCTION AND PREPARATION, PROTEINS PRODUCTION, LIFE SUPPORT ARCHITECTURE FOR FUTURE MISSIONS, SOCIETAL IMPACTS AND EDUCATION

MEAT4SPACE - Cultured meat for human space exploration

With space agencies currently planning longer exploration spaceflights beyond low-earth orbit targeting the Moon, Mars and beyond, the stress on astronaut health and performance will be pushed to extreme levels. While nutrition alone will not fully prevent flight-associated risks, increasing evidence indicates that adequate nutrition can mitigate flight-associated risks for crew's health, such as bone and muscle loss. However, as spacecrafts and crew travel beyond low-earth orbit, delivering supplies on a regular basis becomes impractical. Therefore, in addition to the required launch mass, pre-packaged food provided at launch will likely not have enough shelf-life or stability for the complete duration of long-term missions (>2 years). To this end, the implementation of in-flight and on-site food production systems will be crucial for crew's health and performance, and ultimately contribute for the development of self-sustainable spacecrafts and extra-terrestrial habitats (i.e. Moon and Mars). Cultured meat has emerged as an alternative protein source to conventional livestock farming, and is deemed to require less land, water, and energy. Cultured meat is animal meat that is produced by directly growing animal cells and tissues through tissue engineering approaches, without harming the animal. Animal cells are isolated, proliferated and differentiated in well-defined media conditions and bioreactors, aiming at reproducing the structure, texture, and nutritional profile of conventional meat. To date, cultured meat processes have been developed for different meat types, including beef, chicken, and salmon, among others. As these technologies advance and mature on Earth, the European Space Agency (ESA) understands cultured meat has significant potential as part of the space food systems in future exploration missions. In-flight

and on-site cultured meat systems could provide crew with fresh and highly nutritive foods, improving psychological status, health, and performance of the astronauts, while minimizing flight-associated risks. Importantly, implementation and optimization of cultured meat technologies for space exploration will lead to an important technological return to society, contributing for a greener economy, and a more sustainable and secure food systems on Earth. In this context, MEAT4SPACE is assessing the viability of a multiuse cultured meat system for long-term and long-distance crewed exploration missions. To this end, this activity is divided in two parts. First, based on pre-defined mission scenarios and nutritional requirements for exploration missions, the system and implementation requirements of the cultured meat system will be established, followed by an assessment of existing technologies and their readiness and translatability to space environments. Among others, focus will be given to cell types and source species, culture media formulations, bioreactor types, and monitoring and sensing technologies. Second, this activity will evaluate how cells (i.e., muscle or fat cells) proliferate and differentiate in simulated deep space environments by exposing them to different gravity and radiation conditions. Ultimately, with the set of requirements and obtained data, a conceptual design for an in-flight cultured meat production system will be developed. This presentation will highlight the potential of cultured meat as food source in future exploration missions, summarize the mission and system requirements establish thus far, and provide an overview of the feasibility study for an in-flight multiuse cultured meat system. ●

CO-AUTHORS: Jonathan Scott (ESA), Rob Postema (ESA)

THANH HUY NGUYEN

Ph.D student - University of Mons – Belgium

TOPIC: GREY, YELLOW, BLACK WATER RECYCLING

The effects of iss-like ionizing radiation on the proteome and metabolome of ureolytic and nitrifying bacteria

Due to the characteristics of extra-terrestrial missions on space, treatments for waste are extremely important to limit the accumulation of waste and to ensure resources recovery through recycling. Urine, for instance, is a nitrogen-rich waste that can be potentially converted to liquid nitrate fertilizer for soilless crop production in space. This recovery of resource requires the nitrification of urea present in urine by using ureolytic and nitrifying microorganisms. URINIS-A project serves as a proof-of-concept experiment to demonstrate the possibility of urine nitrification onboard the ISS. In this work, the effect of ionizing radiation on a synthetic microbial community of nitrifiers is studied. The community consist of the ureolytic heterotroph *Comamonas testosteroni*, the autotrophic ammonium oxidizing *Nitrosomonas europaea* and the

autotrophic nitrite oxidizing *Nitrobacter winogradskyi*. The separate axenic strains and the entire community were studied to determine changes in the bacterial proteome and metabolome associated with exposure to ionizing radiation by mass spectrometry-based approach. Samples were exposed to an average dose rate of 5.09*10⁻¹ mGy h⁻¹ from a neutron source (Cf-252) for 72 hours, mimicking the total radiation dose of 36 ± 7.2 mGy in the ISS during a 130-day mission. Both data-dependent (DDA) and data-independent acquisition mode (DIA, SWATH (Sequential Windowed Acquisition of All Theoretical Fragment Ion Mass Spectra-processing)) was used for the proteomic analysis. This experiment will provide preliminary knowledge on the effects of space radiation on the nitrifying consortium. ●

CO-AUTHORS: Baptiste Leroy, Tom Verbeelen, Celia Alvarez Fernandez, Felice Mastroleo, Natalie Leys, Ramon Ganigué, Siegfried E. Vlaeminck, Ruddy Wattiez

PIERRE MAGNES

Development Manager – FGWRS – Monaco

TOPIC: GREY, YELLOW, BLACK WATER RECYCLING

Grey water recycling from space to earth

At a time when it is becoming more and more urgent to save water on earth, it is clear that there is no need to use drinking water for flushing toilets, washing clothes, cleaning floors or even taking showers. The research work of the ESA allowed to develop a technology which allows to solve this ineptitude. Thanks to our technical manager, we have been in touch with ESA since 1997 and been involved in several research programs and output publications. All were focused on grey water recycling for long-span spaceflights. This joint effort led to the design of a pilot to test a grey water recycling process. This ESA pilot is still in our Lab and it enabled the development of a real grey water recycling system on the French-Italian Antarctic Station Concordia. This process has been working since 2005

CO-AUTHOR: Jean-Christophe LASSERRE – FIRMUS

and has been used by more than 1,200 people without any health incident or technical issue. (No technical shutdown since it was put into service). So we thought the time was right to transfer this knowledge, through our collaboration with ESA, to several practical applications on earth. Our process shows that this technology is perfectly mastered, without any health risk. The company FGWRS (Firmus Grey Water Recycling System) was created in Monaco in 2017 to develop this expertise in grey water recycling. We will present the results of our achievements (Faimont Monte Carlo, Roland Garros, Monaco Pavilion Dubai 2020, Monaco Oceanographic Museum) and ongoing projects, even more topical this year. ●

CHIARA AMITRANO

Post-doc - University of Naples Federico II – Italy

TOPIC: SPACE EXPERIMENT

How abiotic factors change the requirements for plants cultivation in Space systems

Establishing a human settlement on Mars will require a self-sustaining colony which cannot exist without the cultivation of edible plants. The presence of plants in these planet outposts will be necessary not only to provide fresh food to the inhabitants, but also to regenerate resources. Indeed, life support systems in Space will be based on the in situ regeneration of resources (air, water, and food) that can be achieved in closed artificial ecosystems, with the help of technological innovation in agriculture. However, the main constraint to plant cultivation in closed environments in Space is the fine regulation of all the environmental factors (e.g. relative humidity, light intensity and quality, temperature). Moreover, together with these factors, the extra-terrestrial environment provides additional constraints, in particular the presence of reduced gravity, altered pressure and ionizing radiation. Among them, ionizing radiation influences plant growth and some metabolic processes in a positive or negative way depending on the type of radiation, the delivered dose and the phenological stage of the plant. Therefore, in this study we used a multidisciplinary approach to understand the effect of

the different aforementioned factors alone and in combination, in order to assess thresholds for the occurrence of phenotypic alterations. We mainly focused on microgreen species, being characterized by a small dimension, a short growth cycle, and a high nutritional content, which allow them to easily be grown in controlled environment. Then, we set-up various growth chamber experiments to test different light intensities and spectra, VPDs (vapour pressure deficits) and ionizing radiation type and dose. Data were analysed in order to evaluate to what extent the cultivation requirements would change when environmental factors deviate from the nominal conditions. The importance of possible interactions among factors was highlighted too, especially considering that specific combinations of environmental factors may be imposed to induce positive outcomes, like increments in antioxidant molecules and in biomass. In the light of the above, when designing modules for crop production in Space, all these parameters should be carefully balanced. The outcomes of this study will also be helpful to optimize crop production in controlled environment agriculture systems on Earth. ●

CO-AUTHORS: Roberta Paradiso, Carmen Arena, Rosanna Caputo, Youssef Roupheal, Stefania De Pascale, Veronica De Micco

ANA SOARES

Professor of Biotechnology Engineering - Cranfield University - United Kingdom

TOPIC: GREY, YELLOW, BLACK WATER RECYCLING

Fresh urine treatment with bio-mineral phosphorus recovery and nitrification with biocatalysts

Urine is a source of nutrients and water, but laborious to manage as it needs to be processed quickly to avoid sudden increase in pH, ammonia volatilisation, odour issues and overall deterioration, making it much harder to treat. Hence intensified and novel ways to treat urine to recover valuable nutrients and water for re-use are needed. Bio-mineral formation is a widespread phenomenon in nature that can be applied to remove and recover nutrients from urine, of special interest is struvite. Initial experiments have been successfully completed in urine with known bio-mineral producing bacteria in mixed cultures. The selected bacteria produced urease and were able to grow at high pHs (>8-10). As such, the expected urine ureolysis does not take place, but in this case under controlled conditions defined bio-mineral producing bacteria, and not in the chaotic manner observed during normal urine storage. The carbon available is also ideal to sustain the bio-mineral producing bacteria growth. As urine is rich in phosphate, ammonia, calcium etc, B. antiquum

and M. xanthus concentrated the ions and cations intra- or extra-cellularly, enabling nucleation points to form, followed by crystal growth, capturing nutrients. The phosphorus content was reduced and recovered as struvite and calcium phosphate with yields of approximately 1 g/L urine. After struvite recovery the remaining ammonia in urine was nitrified with Microvi® biocatalysts. The latter are in silico platforms that create natural habitats, that support a high density of a single microorganism that is controlled and protected in the versatile form of a composite. Due to the protected and high biomass concentrations allowed by Microvi® biocatalysts nitrification occurred in a few hours, at a rate of 23 mg/L.h and high concentrations of nitrate were obtained. These initial findings are promising to develop sustainable resource recovery from source-separated urine for both terrestrial and space exploration applications. ●

CO-AUTHORS: R.E. Colston, P. Vale, A. Nair, T. Stephenson, A. Soares

LUIS RAFAEL LÓPEZ DE LEÓN

Marie Curie Postdoctoral Researcher - LEQUIA - Universitat de Girona – Spain

TOPICS: AIR RECYCLING AND CO2 VALORIZATION, WASTE RECYCLING, CIRCULAR ECONOMY

Indoor CO2 Direct Air Capture (iCO2-DAC): CO2 As Renewable Carbon Source

Carbon dioxide direct air capture (CO2-DAC) and conversion to chemical feedstocks is a suitable solution to contribute to the replacement of fossil fuels. This is as much as important in earth as it during space missions where availability of resources is scarce and limited. In this sense CO2-DAC plays a crucial role in the regeneration of the atmosphere during human spaceflights, but also a source of carbon that can either be used to produce bioenergy (heat and electricity) or towards the production of complex commodity chemicals. CO2-DAC technologies developed so far focused on capturing atmospheric CO2 from outdoor environments. However, CO2-DAC in non-civilian indoor environments such as human spaceflights and submarines where CO2 is mildly concentrated, can help to overcome the thermodynamical limitations of CO2-DAC technologies. In addition, removing CO2 contributes to improve indoor air quality. This work aims to assess the coupling of indoor CO2-DAC and microbial electrosynthesis (MES) technologies to produce carbon-neutral commodity chemicals. Specifically, this study simulated the installation of CO2-DAC technologies in three different scenarios: (i) high school

classroom, (ii) office room and (iii) underground transport cabin. For each scenario, MES cells were designed based on the state-of-art performance parameters reported in the literature for the conversion of CO2 to biofuels (either methane or ethanol). The produced biofuels were compared as green fuels for heating the environments, assuming an external temperature of 10 °C and a target temperature of 20 °C. A preliminary economic analysis was carried out based on the current market price of electric energy to assess the feasibility of this visionary technology. Methane prevailed as the most viable solution, resulting in a substantially lower reactor footprint and power consumption than those calculated for CO2 conversion to ethanol. It was estimated that the methane produced can be used for heating the indoor at an electricity cost of 6.3-9.6 €/d per room, which is considerably lower if compared to the non-household EU average electricity cost of 125.4 €/MWh. This preliminary study based in terrestrial indoor environments can serve as a base for future research and development of technologies similar as the proposed in this work applied to indoor environments within human spaceflights. ●

CO-AUTHORS: I. Soler, K. Jiménez, J. Bermejo, I. Azzouzi, A. Cabrera-Codony, P. Dessí, B. Kraakman, I. García, L. Morán, E. Ramos, M. Dolors Balaguer, S. Puig

YOSHIAKI KITAYA

Director of Plant Factory Research Center - Osaka Metropolitan University – Japan

TOPICS: WASTE RECYCLING, FOOD PRODUCTION AND PREPARATION, PROTEINS PRODUCTION

The significance of aquaponics in Controlled Ecological Life Support Systems

Aquaponics as a biological production system that consists of hydroponics and aquaculture systems will be useful for establishing Controlled Ecological Life Support System (CELSS) in space. In aquaponics, excess dissolved ions derived from fish excretion may negatively influence fish growth, nutritionally

promote plant growth, and increase the productivity of fish and plants. I explain the significance of aquaponics in the CELSS using sweetpotato plants and propose a system for ensuring human survival in space. ●

SIEGFRIED VLAEMINCK

Professor - University of Antwerp – Belgium

TOPIC: GREY, YELLOW, BLACK WATER RECYCLING

Urine and life support: Some nitrification-based MELiSSA solutions

This presentation will sketch some of the research and development history, logic, 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 many regenerative life support system concepts. 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-based processes are pivotal to the safe management of waste streams and to the production of useful compounds: water, N₂ as inert gas, and/or nitrate for food production. The first N conversion step in biological urine treatment is the hydrolysis of urea relying on ureolytic heterotrophic bacteria producing ammonium (NH₄⁺), followed by the action of one or more aerobic ammonia oxidizing bacteria (AOB). For nitrate production, one or more nitrite oxidizing bacteria (NOB) are involved, whereas anammox bacteria (AnAOB) partner up with AOB for nitrogen gas production through partial nitrification/anammox. The process should additionally convert biodegradable organic matter, mainly to CO₂, to avoid downstream biofouling issues and enable carbon recovery, a process driven by the heterotrophs. The goal is a reliable and predictable bioprocess under dynamic conditions, coupled to upstream and downstream processes. In terms of nitrate production, 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. 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. In terms of aeration, systems are progressively moving from sparging with air and oxygen to gravity-independent membrane aeration. Besides validation of the target performance, the effect of key parameters on the microbial communities has been studied, and predictive control laws were implemented. Long-term stability was demonstrated, amongst others in the MELiSSA pilot plant in Barcelona. Electrochemical stabilization and pH control strategies were developed. Several runs showed that the process effluent was suitable to produce microalgae and crops. For nitrogen gas production, a bioreactor proof of concept has been delivered treating real urine. 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 all groups of microbes needed. Two more flights are currently under preparation, being URINIS and Biorat 2. The challenges addressed here are to obtain in-flight activity in the International Space Station (ISS), both in batch incubations as in a first reactor test. Finally, the R&D work on nitrification has yielded several terrestrial valorization examples that are established or in the development pipeline. ●

CO-AUTHORS: De Paepe, J., Van Winkel, T., Timmer, M.J., Spiller, M.

DANIEL SCHUBERT

Team Leader - DLR, Germany

TOPIC: FOOD PRODUCTION AND PREPARATION

Ground-based demonstrator for the first space-ready lunar agricultural module

The international consortium, under the lead of the German Aerospace Center (DLR) proposes the development of a ground-based demonstrator for a lunar agricultural module to support a future long-duration lunar surface base with essential consumables and life support functions (e.g., fresh food, O₂

provision & CO₂ fixation, water recycling capability, and well-being). The demonstrator will incorporate two constituents: the greenhouse module and the habitat simulator. The greenhouse module will serve as ground-based analogue realized as a collaborative undertaking between several space agencies. The

decision to pursue the development of the ground-based demonstrator will follow the completion of the coordinated study with a goal to have an operational facility no later than 2028. The design and potential subsequent development of the ground-based demonstrator will be conducted with as much fidelity to a lunar deployment as possible. Subsystem accommodation, component selections, redundancy strategies, and interface architectures will be analogue to the actual lunar

module, allowing rigorous testing of the concept and its operations on the ground for several years before transferring the lessons learned into a space-ready system design. The presentation will give a complete overview of DLR's latest development, according to its released roadmap for the development of bioregenerative life support systems (BLSS), by outlining its technological research ambitions as well as the international involvement.

CO-AUTHORS: Claudia Philpot, Dr. Volker Maiwald, Vincent Vrakking, Dr. Paul Zabel, Markus Dorn, Conrad Zeidler

AGATA SIEDLECKA

Research Assi - Wroclaw University of Science and Technology – Poland

TOPICS: GREY, YELLOW, BLACK WATER RECYCLING, WASTE RECYCLING, FOOD PRODUCTION AND PREPARATION

The alkalinity dilemma in nitrification

As in the near future human space missions are going to be prolonged and probably carried out more frequently, the current solutions for bioregenerative life support systems will need to be improved. Especially, the problem of providing food of sufficient quality for a crew should be solved. The potential solution is the cultivation of fresh food in space stations. Moreover, in the case of trials of colonisation of the extraterrestrial celestial bodies, the grow fresh food in space are going to turn out necessary. Therefore, the scientists will not escape from solving this problem. One of the considered solutions, seeming to be the most practical, is the hydroponic plant cultivation, which is described in many studies. However, a significant problem in the cultivation of plants in space is their fertilization. It is crucial to produce fertilizer on-site, by recovering nutrients from waste. One of the streams that can be used for such a purpose in space is human urine as it is a source rich in nitrogen, phosphorous and many essential nutrients suitable for plants. The production of fertilizers from human urine seemed to strike a balance. Nevertheless, it turned out that the conversion of the forms in which nitrogen occurs in urine (85% as urea and around 5% as ammonium ions and ammonia) to the forms absorbed by plants (nitrates) in the conditions of human space missions is not affordable. Such conversion could be done

by means of nitrification. However, the problem originates in the insufficient amount of alkalinity in urine, what hampers its application. The conventional way to overcome the issue is to supplement the bioreactor with chemical alkalinity sources (e.g. hydroxides) unfortunately, this is always related with cations (such as Na⁺, K⁺, Ca²⁺) accumulation in the closed loop systems, what implies the further inconveniences: these surplus cations need to be eventually removed from the system, which is generally very difficult. Instead of using human urine solely as the nitrogen and microelement source for on-site plant fertilizer production, the mixed stream of various pre-treated wastes (i.e. black water, grey water, other biological wastes like food scraps, etc.) could be regarded as the influent to nitrification bioreactor, what seems to be the only proper solution. In this easy way, both the problem of alkalinity deficiencies and/or undesired microelements accumulation in closed loops could be solved, as the proposed stream of wastes should provide sufficient alkalinity by definition. Moreover, the composition of obtained fertilizer should be more balanced and therefore more suitable for plants. In the same time, the problem of various waste streams is going to be solved. ●

CO-AUTHORS: Anna Jurga, Aleksandra Rogowska, Kamil Janiak, Daria Podstawczyk, Martyna Grzegorzek, Stanislaw Miodowski, Krzysztof Ratkiewicz

FLORENT BOUCHON

PhD student – INRAE – France

TOPICS: GREY, YELLOW, BLACK WATER RECYCLING, PROCESS MODELING, SIMULATION AND CONTROL

Electroactive biofilm development under controlled hydrodynamic in a Couette-Taylor electrochemical reactor

The activated sludge process is the most commonly used to remove organic matter from wastewater. It requires blowing air into aerated tanks to promote microbial growth thus ensuring efficient removal of pollutants. However, aeration is one of the largest energy consumers in wastewater treatment plants (70% of the total power consumption of the facility). To reduce oxygen supply, an innovative bioelectrochemical system (BES) is considered using an electrochemical snorkel. The microbial anode is one of the key components of BES since it allows empowering the system by producing electrons. However, the influence of the hydrodynamics on the electroactive biofilm

(EAB) formation and development is still not fully understood. In particular, its influence on microbial diversity, electrochemical activity and biofilm structural properties has not been studied in depth. To address those bottlenecks, the first electrochemical Couette-Taylor reactor (eCTR) was designed to study the shear stress impact on the EAB performances. The eCTR contains 20 planar graphite electrodes included in the rotating inner cylinder. Those electrodes serve as support for the biofilm growth; while, a stainless steel outer cylinder is used as abiotic cathode. Primary effluent from a sewage treatment plant was supplemented with acetate and used as electron donor in

continuous feeding. Microbial electrodes were characterized electrochemically. Physico-chemical parameters (COD, pH, conductivity, [O₂]) were measured and controlled in the reactor. The microbial diversity is characterized by sequencing 16S rDNA ribotags. Their structural properties are analysed by tomography and the expressed functions by sequencing cDNA in shotgun metatranscriptomics. Experiments were first conducted in abiotic conditions with a hexacyanoferrate (III/II) electrolyte to precisely characterize the reactor electrochemical response under different hydrodynamic regimes. Results showed a transition domain for a rotation speed of the inner cylinder of 8 RPM, which corresponded to a shear stress of 50 mPa on the surface of the electrodes. This value is correlated to the separation between the wavy vortex and turbulent vortex flow in Couette-Taylor. To quantify the impact of the hydrodynamic conditions on electrochemical reactions, the diffusive layer thickness (δ) was calculated. Its value depended on the angular

velocity ($\dot{\epsilon}$) and to the flow regime according to $\dot{\epsilon} \pm$ with $\pm = 0.4$ for the wavy vortex and $\pm = 0.6$ for the turbulent vortex flow. Bioanodes were then formed in real wastewater under constant polarization and wavy vortex flow, with performances perfectly reproducible. Maximum current density of 3.75 A/m² was reached after 10 days corresponding to a removal rate of 17 gCOD/m²/d i.e. a coulombic efficiency that ranged between 22 and 30 % for 3 months. Each bioanode was maintained for more than 6 months to study the impact of different hydrodynamic conditions resulting in shear stress from 25 mPa to 10 Pa. First results showed a short-term impact of increasing shear stress on the microbial activity whereas, the long-term dynamics of the activity appears to be correlated with several operating parameters. The discussion will show how these results can then be used to monitor EAB activity in urban wastewater treatment strategies at pilot-scale. Key words: Electroactive biofilms Bioelectrochemical Taylor-Couette reactor Shear stress».

CO-AUTHORS: Florent Bouchon (1), Ahlem Filali (1), Théodore Bouchez (1), Alain Bergel (2) and Yannick Fayolle (1)(1) Université Paris-Saclay, INRAE, PRocédés biOtechnologiques au Service de l'Environnement, 92761 Antony, France (2) Laboratoire de Génie Chimique, Université de Toulouse, CNRS, INP, UPS, Toulouse, France

OLIVIER LÉPINE

Chief Scientific and Technical Officer – Algosource – France

TOPIC: FOOD PRODUCTION AND PREPARATION

Innovative Spirulina Nutraceuticals products for health prevention in long flight and extraterrestrial habitat

The cultivation of *Arthrospira platensis* and other photosynthetic bacteria is now well established and documented within the MELISSA project. It delivers a raw biomass that can be used as superfood during long space flight and extraterrestrial habitat. *Arthrospira* s interest is that not only it contains all essential amino acids and many vitamins but also Phycocyanin a very

strong antioxidant, sulphated polysaccharides, amongst many active molecules. However, Phycocyanin, is degraded by the normal digestion process (pH 2, 37 °C, 2-4 hours). And if Phycocyanin content usually amounts between 15-20 % of *Spirulina* dry weight, other components such as polysaccharides are present in very low quantities.

KAMIL JANIĄK

Assistant Professor - Wrocław University of Science and Technology – Poland

TOPICS: GREY, YELLOW, BLACK WATER RECYCLING, FOOD PRODUCTION AND PREPARATION, CIRCULAR ECONOMY

Closing loop with biological nitrification for nutrients recovery and surfactants removal

The nitrogen can be recovered from various streams such as urine or anaerobic digester liquor by nitrification. Nitrified wastewater can be used as a fertilizer for plants cultivation. Plant cultivation is a key aspect of future long-distance space missions, and the creation of an efficient food system will not be possible without it. The production of fertilizer in space is based on the recovery of water and nutrients from wastewater, such as urine and grey water. These streams have three potential factors that could affect the growth of plants in a hydroponic system. The first is the presence of surfactants, the second is the limited presence or absence of certain nutrients, and the third is high salinity. In our study, the fertilizer production process is conducted in an aerobic, activated sludge reactor, where nitrification and the process of carbon removal take place. The effect of these factors was checked in two hydroponic configurations, with one being based on unenriched fertilizer, and the second being supplemented with lacking elements (e.g. P, K, Ca, micronutrients). Their influence on lettuce yield, quality parameters and stress response were investigated and

compared to the control cultivation. The results showed that the main cause of a decrease in the productivity of plants grown on nitrified urine and grey water is oxidative stress. This results in the disturbance of the redox balance and oxidative damage at the cellular level, which is caused by a deficiency of elements. Enrichment with elements that are lacking resulted in the restoration of proper protein synthesis and an increase in the activity of antioxidant enzymes, which was positively reflected in the qualitative and quantitative parameters of the enriched cultivation. This further proves that SMCT surfactant used during space missions after purification in a biological nitrifying reactor does not pose a threat to plants. Moreover, the use of an unenriched fertilizer based on urine and grey water can be seen to be a good sustainable alternative for terrestrial agriculture. However, with respect to space systems, it is necessary to maximize the quantity and quality of the obtained yield, and it is therefore suggested to search for additional sources of lacking elements.

CO-AUTHORS: Anna Jurga, Krzysztof Ratkiewicz, Anna Wdowikowska, MaBgorzata Reda, MaBgorzata Janicka, Piotr Chohura

CHRISTER FUGLESANG

Director KTH Space Center - KTH Royal Institute of Technology – Sweden

TOPIC: SPACE EXPERIMENT

Visits to the International Space Station and a view of future exploration

I will shortly describe how I became an astronaut and the show short videos of my two flights to ISS. Say a bit about ISS, science

and life in space. In the end talk about the plans for further human space endeavor and future visions.

EMMANUEL FROSSARD

Professor – ETH Zurich – Switzerland

TOPIC: FOOD PRODUCTION AND PREPARATION

Recycling nutrients from organic wastes for growing higher plants in the MELISSA loop during long-term manned space missions

Long-term manned space missions to Moon and Mars will soon be reality. Shipping food, water and oxygen (O₂) from Earth will be very costly because of the masses to be transported. Therefore, the crew will need a life support system allowing the recycling of wastes to produce the food, O₂, and clean water while removing carbon dioxide (CO₂) from the atmosphere of the spaceship. The European Space Agency plans to achieve this goal with the Micro Ecological Life Support System Alternative (MELISSA) loop, which includes five compartments. In principle the wastes produced by the crew will be transferred to a compartment where they will be anaerobically digested. The digestion products will be metabolized by photoheterotrophic bacteria located in the following compartment. The minerals produced therein will be forwarded to a compartment where ammonium will be nitrified. Mineral nutrients will then be transported to a higher plant compartment where food, O₂, and water will be produced for the astronauts while CO₂ will be removed from the atmosphere. Currently, most of the work done on the MELISSA loop has focussed on O₂, CO₂, carbon (C) and nitrogen (N) fluxes, while other plant nutrients have been little studied. The present assumption is that the loop provides nutrients in available forms and appropriate concentrations at the right time to higher plants. Our review

identified potential limitations, such as the formation of compounds containing nutrients in a non-available form, the accumulation of toxic elements, and N gaseous losses. Thus, new data needs to be collected, and new methods and technologies to be developed. Among the new data to be collected we need full input-output element balances, both for nutrients and toxic elements, for each step of the MELISSA loop to assess whether all elements are recycled. It will be essential to analyse the forms of nutrients and if necessary, to implement processes to deliver plant available nutrients. The impact of various plant nutritional regimes (including non-optimal regimes) on CO₂ uptake, and O₂, water and biomass production under closed conditions needs to be quantified. Among the new methods to be developed, the most urgent one would be to remove Na⁺ from urine as soon as it is produced, but without removing cations such as NH₄⁺, Ca²⁺, Mg²⁺ and K⁺ which are essential for plant growth. Finally, it will be important to develop ion sensitive electrodes that can be used on the long term for each element of interest, and a method to treat data collected by these electrodes in real-time so that plants are always supplied with the nutrients they need. These developments will not only profit long-term manned space missions but also the development of sustainable hydroponic systems on Earth.

LAURENCE LEMELLE

DR - CNRS / ENS de Lyon – France

TOPICS: MICROBIAL SAFETY, LIFE SUPPORT ARCHITECTURE FOR FUTURE MISSIONS, SPACE EXPERIMENT

Passive limitation of surface contamination by perFluoroDecylTrichloroSilane coatings in the ISS during the MATISS experiments

Future long-duration human spaceflight will require developments to limit biocontamination of surface habitats. The MATISS (Microbial Aerosol Tethering on Innovative Surfaces in the international Space Station) experiments allowed for exposing surface treatments in the ISS (International Space Station) using a sample-holder developed to this end. Three campaigns of FDTs (perFluoroDecylTrichloroSilane) surface exposures were performed over monthly durations during distinct periods. Tile scanning optical microscopy (×3 and ×30 magnifications) showed a relatively clean environment with a few particles on the surface (0.8 to 7 particles per mm²). The varied densities and shapes in the

coarse area fraction (50 - 1500µm²) indicated different sources of contamination in the long term, while the bacteriomorph shapes of the fine area fraction (0.5 - 15µm²) were consistent with microbial contamination. The surface contamination rates correlate to astronauts occupancy rates on board. Asymmetric particles density profiles formed throughout time along the air-flow. The higher density values were located near the flow entry for the coarse particles, while the opposite was the case for the fine particles, probably indicating the hydrophobic interaction of particles with the FDTs surface.

CO-AUTHORS: Laurence Lemelle, Sébastien Rouquette, Eléonore Mottin, Denis Le Tourneau, Pierre R. Marcoux, Cécile Thévenot, Alain Maillet, Guillaume Nonglaton, Christophe Place*

LUCIE POULET

Postdoctoral Researcher - Institut Pascal - Université Clermont Auvergne – France

TOPIC: FOOD PRODUCTION AND PREPARATION

Evaluating Microgreens Crop Readiness for Space Production

Microgreens are small-size, nutrient-rich, and fast-grown crops, which are considered as candidates for future space exploration missions. In particular, the ISS, the Lunar Gateway, and Mars and Lunar missions could benefit from growing microgreens to supplement astronaut diets in the near future. Research at NASA's Kennedy Space Center has focused on (1) the selection of microgreens compatible species, (2) the evaluation of microgreens food safety, (3) the use of passive wicking, on-demand watering, and hydroponics cultivation, (4) simulated microgravity growth, (5) microgreen canopy gas exchange, and (6) harvesting techniques in microgravity. This presentation summarizes this research. Microgreen species will be evaluated for their yield in relationship to the quantity of inputs water, seeds, substrate, light intensity, photoperiod, crew time required for their growth; for their organoleptic and sensory factors in order to down select species that are highly acceptable for humans; and for their microbial loads as detected in their growth environment and the food safety metrics of their edible tissue. Passive wicking, on-demand watering,

and hydroponic systems are being studied as an efficient way to deliver essential nutrients and water to microgreens, included in a microgravity environment. Growth studies in simulated microgravity (using 3-dimensional clinostats) will assess microgreens growth relative to that in 1g. Gas exchange studies on microgreens canopies in various airflows will assess their photosynthesis and transpiration. Finally, a series of parabolic flights has enabled the evaluation of different harvesting and bagging techniques in microgravity. Indeed, traditional plant harvesting methods (scissors) in microgravity could generate significant microgreen debris in the space station cabin. Two innovative techniques, coupled to a dedicated bagging method, were designed and evaluated against the control, traditional, harvesting technique. This research was supported by grants from NASA KSC's Independent Research and Technology Development Program, NASA's Flight Opportunity Program, NASA Postdoctoral Program Fellowships (L.P. & C.J.) supported by NASA's Space Biology program, and support from NASA's Human Research Program. ●

CO-AUTHORS: Christina Johnson, Lucie Poulet, LaShelle Spencer, Matthew Romeyn, Lawrence Koss, Jacob Torres, Jennifer Gooden, Mary Hummerick, Haley Boles, Oscar Monje, Gioia Massa, Raymond Wheeler

DIMITRIOS BOUZOTAS

Water Systems Researcher - KWR Water Research Institute – Netherlands

TOPICS: PROCESS MODELING, SIMULATION AND CONTROL, CIRCULAR ECONOMY

Assessing the resilience of circular water systems: a simulation-based approach using the UWOT model

Against the make-use-dispose linearity of conventional management, circular economy (CE) design principles have been proposed as a resource management alternative that reduces waste, increases robustness and promotes efficiency. These principles find use in water management as well, offering an alternative against supply systems dependent on large, centralized infrastructure that face limitations [1,2]. The application of CE principles for water has led to the emergence of circular water systems as a promising alternative to conventional water supply, where measures that promote reducing, reusing, recycling and replenishing water are prioritized [3]. This is achieved by combining multiple distributed technologies that close loops in an integrated fashion - and supplementing centralized water supply to design hybrid systems at different spatial scales (houses, neighborhoods, regions) [4]. The technologies that are combined may include decentralized options at the tap level of households and neighborhoods, 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 [5]. The effects of the combined usage of these technologies are evident across multiple 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. As a cyclic management practice, CE principles and the equivalent circular water systems have been conceptually linked to both sustainability and resilience [6-8]. However, this intuitive link is only infrequently backed by quantitative methods

that demonstrate how closing the loop contributes towards a more sustainable and resilient system, with most of the proposed frameworks reaching only the conceptual level [9]. This study provides quantitative linkages between CE concepts and resilience for water, by demonstrating a simulation-based framework that evaluates the resilience of different circular system redesigns in a regional water system. More specifically, a stress-testing framework based on a simulation model is developed, where different circular interventions are evaluated in terms of their overall resilience against future uncertainty. The evaluation of resilience is done using relevant quantitative metrics based on system reliability, on par with a method that has been previously applied to urban water [10,11]. The simulation model that is used the Urban Water Optioneering Tool (UWOT), a signal-based water cycle model fit for circular and closed-loop systems whose capabilities have been presented in a previous Melissa conference [12]. UWOT simulates both standard, linear urban water streams, including DW, WW and runoff, as well as integrated circular interventions, following a signal-based logic of demand generation, aggregation and transformation from tap (demand) to source (supply) [13]. In the context of this regional application, UWOT has been applied to quantify four different circular water strategies and evaluate overall resilience using both a deterministic and a probabilistic approach. The results demonstrate that circular water interventions lead to more resilient water systems, and that the more circular dimensions are addressed as part of the interventions (e.g. reducing, reusing and recycling water), the more robust resilience profiles become. ●

CO-AUTHORS: Dionysios Nikolopoulos, Sija Stofberg, Jos Frijns and Christos Makropoulos

ETIENNE PERRIN

Intern in ECLSS at Spaceship FR – CNES - DOA/EVH/MMS Department – France

TOPICS: GREY, YELLOW, BLACK WATER RECYCLING, WASTE RECYCLING, BIOMATERIAL, PROTEINS PRODUCTION

Assessing the integration of a bioreactor producing SCPs and PHAs from organic waste into global environmental systems

With the launch of NASA's Artemis program, human space exploration has once again become a major challenge for the world. ESA is keen to assert its position as a priority partner for this return to the Moon and for the next step, Mars, through innovative projects under its own exploration program, E3P. ESA and CNES have thus developed an initiative to study relevant concepts for these missions, the Spaceship initiative. This Spaceship project is a collaboration between three entities, the Spaceship FR (CNES, Toulouse), the Spaceship EAC (ESA, Cologne) and the Spaceship ECSAT (ESA, Harwell). The Spaceship teams also have networks of collaborators across Europe, enabling them to support and initiate R&D to reach operational concepts, with an emphasis on practical demonstrations and on an organization with a high degree of autonomy. This new impetus for human space exploration brings with it challenges such as developing reliable and optimised life support systems while seeking to give explorers options to make their exploration more comfortable and liveable. Regenerative life support becomes very interesting especially for long-term exploration. Without waste recycling processes, long-term explorations would be both more risky due to the lack of immediately available options for crews in terms of raw materials and at the same time very costly both energetically and financially due to the more massive systems for the storage of useful resources (water, oxygen, etc.). Progress on waste recycling would also have almost immediate applications on Earth in the context of

the struggle for ecology and for circular economy. The Toulouse Biotechnology Institute at INSA Toulouse, in collaboration with CNES, CNRS and INRA is currently developing a bioreactor for processing organic waste to produce food proteins (SCPs) and bioplastics (PHAs). The inputs of the reactor are the astronauts' waste, i.e. urine and faeces, the latter being previously composted and mixed with other organic. At the output of the reactor, we have PHAs, SCPs, but also functional food. The reactor output waste would be fermentation must (to be recovered) and CO2 that could be treated with the rest of the vessel/habitat CO2 emissions by CO2 removal and reduction systems, depending on which recycling loops would be the most relevant in the overall context of an ECLSS. PHAs could for example be used to produce textile yarn for repairing or even creating new clothes. They could also be used to create raw material for 3D printers, allowing for example the creation of tools adapted to situations. Finally, PHAs could be applied to the food preservation and packaging by creating plastic film. On the other hand, the production of SCPs and functional food would support the making of foods such as algae or mushrooms, but it would also allow the artificial enrichment of the nutrition provided to the crew to reduce the risk of diseases and help in their well-being state. This paper will present the integration of this reactor into an environmental system which would therefore broaden the options for long-duration explorations by affecting both survival and crew comfort. ●

CO-AUTHORS: (Grégory Navarro, mobile : +33618960037, email : gregory.navarro@cnes.fr, Organization : CNES - DOA/EVH/MMS Department) ; (Alexis Paillet, mobile : +33686491358, email : alexis.paillet@cnes.fr, Organization : CNES - DOA/EVH/MMS Department)

FRANÇOIS CLUZEL

Assistant Professor - Université Paris-Saclay, CentraleSupélec, Laboratoire Genie Industriel – France

TOPICS: LIFE SUPPORT ARCHITECTURE FOR FUTURE MISSIONS, CIRCULAR ECONOMY

Circularity indicators and digitalisation for monitoring circular space and terrestrial systems

Terrestrial and space systems may be differentiated by their finalities in a Circular Economy (CE) perspective. On the one hand, CE for space systems maximises the efficiency of materials and energy loops to allow long-term space travel and keep the crew alive and in good health. Impacts (like pollution) on the external world (space, the Moon, or Mars surface) are not an issue by themselves; economic issues are predominant during the design of space systems but are not considered during the missions themselves. On the other hand, CE for terrestrial systems also maximises the efficiency of materials and energy loops but with additional objectives. Those are to minimise the use of resources and the environmental impact while maximising the system's economic performance on its entire life cycle; the environmental impact on the external and natural world is as important as the economic performance. Moreover, more and more CE methods and tools for terrestrial systems also include a social dimension to maximise value creation for all the stakeholders, far beyond crew health issues considered for

space systems. In this sense, designing and monitoring more circular systems whether terrestrial or space systems implies the ability to measure and analyse flows and impacts at different scales and for different purposes. Beyond historical tools such as Material Flow Analysis (MFA) and Life Cycle Assessment (LCA), Circularity Indicators represent an emergent field of research for about ten years. Digitalisation is another promising field of research for CE. It studies how to better (more dynamically and close to real-time) model, simulate, and monitor industrial systems using data gathered along the value chain and digital tools embedding circularity indicators (all along with technical and economic ones). In this particular context, our recent and ongoing work in collaboration with the MELISSA project shows a clear interest in developing a simulation platform that could model and connect the different parts of a technological space or terrestrial system. Such a platform could be based on dynamic modelling of materials and energy flows sent through the different parts of the system, for example, thanks to Dynamic

Material Flow Analysis (MFA) or System Dynamics. It could also be coupled with relevant circularity indicators selected from extensive existing databases (extending, for example, the ALiSSE criteria, which are more suitable for space systems). The simulation of multiple dynamic scenarios could allow studying long-term performances and impacts. In this presentation, we propose to give an overview of these two emerging and promising fields of research circularity indicators and

CO-AUTHOR: Franck Marle

MARTIN CERFF

Life Science Engineer - Blue Horizon Sarl – Luxembourg

TOPICS: WASTE RECYCLING, BIOMATERIAL, CIRCULAR ECONOMY

From Organic Waste to Ink for 3D Printing Within the MELiSSA Loop

Streams of liquid and solid organic waste (OW) are manifold on earth and bear great potential for human exploration. Valorization of those streams on the one hand and facilitated recycling of the products on the other hand offer the chance to manufacture new materials and at the same time increase the degree of closure of natural material cycles on which human mankind relies on. Recycling becomes evident, and is without alternative, within enclosed artificial habitats when no exchange of material with the surrounding world is possible. Such artificial enclosed habitats are essential for long-distance space missions. In this light, the development of novel materials from renewable feedstock, such as bioplastic or biopolymers in a broader sense, offers new possibilities in view of a circular economy. It is the goal of the OW-Ink project to develop a 3D-printable thermoplastic (ink) material, which is mainly manufactured from Life Support System(LSS)-based organic materials and intended for space application. Initially, we investigated several potential organic waste streams contained within LSS as starting point. Among those materials, we identified the cyanobacterium

CO-AUTHORS: Vincent Berthé (Luxembourg Institute of Technology, MRT), Giacomo Gussetti (Direct3D)

LEONE ROMANO

PhD student - University of Napoli Federico II – Italy

TOPICS: AIR RECYCLING AND CO2 VALORIZATION, GREY, YELLOW, BLACK WATER RECYCLING, WASTE RECYCLING, FOOD PRODUCTION AND PREPARATION, PROTEINS PRODUCTION

SUPER FOOD FOR SPACE: from a complex biological system to a simplified plant model.

Closing the loop of a bioregenerative life support system is mandatory to sustain life during interplanetary space missions. To date, BLSS are the only suitable technology for extended-lasting mission. In these systems, plants play a key role. Scientists are currently selecting the candidate space crops working on the species that have well-supported life on Earth for thousands of years. Although these plants are the key component of today's society, they are part of a complex system that is often inefficient, especially regarding edible/waste biomass production. When looking toward a new Mars colony, it is mandatory to identify novel plant species suitable for space agriculture's extreme conditions and constraints. Scientists are

digitalisation - toward a circular economy and to expose how they can be combined to better design and monitor more circular terrestrial and space systems. We will explore existing literature and ongoing projects that may inspire MELiSSA partners and foster collaborations. Finally, we will show how we envisage bridging the gap between Systems Engineering and Circular Economy, which are often uncorrelated disciplines. ●

Spirulina as suitable biomass candidate and precursor material for the production of the ink. Spirulina combines many advantages that makes it an ideal candidate for space application. In view of future space missions, Spirulina-related processes are developed to supply the crew with oxygen and serve as food supplement. Thus, the cyanobacterium can be regarded as one of the work horses of the MELiSSA concept (Micro-Ecological Life Support System Alternative). In the course of the work we developed an end-to-end process chain in the lab which can serve as the basis for a potential add-on system to the MELiSSA cycle in the future. As a first step, a bioprocess was established including upstream and downstream operations to harvest and produce sufficient amounts of the precursor biomass. In the following steps, an ink was formulated from unfractionated precursor biomass and plasticizer, and was further processed for subsequent additive manufacturing. Finally, we could demonstrate 3D-printability of Spirulina-based thermoplastic material for the first time, without addition of other thermoplastic material species.

LUCIE POULET

Postdoctoral Researcher - Université Clermont Auvergne – France

TOPIC: PROCESS MODELING, SIMULATION AND CONTROL

Space Greenhouse Design: towards a systematic methodology

With a return of human exploration missions to the Moon in the mid-2020s and the preparation of more distant and longer-duration missions to Mars and further into the solar system, large-scale sustainable and reliable food production systems will need to be developed. Until now, only small-scale plant growth systems have been operated in space and they still pose numerous challenges. To be sustainable, greenhouse modules (GHM) for space need to use few resources, present low risk, be robust, reliable, and resilient. The objective of the SERENITY (Space & Earth Reliable greENhouse design methodology) project is to develop a methodology allowing space and terrestrial GHM design based on given constraints, using modelling, simulations, and optimization, with a chemical process engineering and system engineering approach. The first step is to review the existing mission scenarios laid down by different space agencies to define associated physical constraints (e.g., gravity, pressure, natural light, radiation level). The second step consists in identifying

under a variety of environmental constraints the thresholds for stability, reliability, and life cycle of the different GHM subsystems (e.g., lighting, watering, environmental control, plants, microbes, growing configuration). Once this contextual frame is set, each subsystem can be described with dedicated models. This will later serve the generation of competing designs and their evaluation using ALiSSE criteria. Finally, the decision can be made on a systematic approach to choose the best solution and provide recommendations for plant growth systems design in different gravity scenarios (space and Earth). In this presentation, the general frame and the step-by-step process followed to develop this methodology will be addressed. A review and definition of environmental parameters constraints, criteria pertaining to GHM stability and reliability, and subsystem modelling, will also be provided. This project has received funding from the European Union's Horizon Europe research and innovation programme under the Marie Skłodowska-Curie grant agreement N° 101067017. ●

CO-AUTHORS: François Maréchal, Claude-Gilles Dussap

GIORGIA PONTETTI

CTO - EltHub SRL – Italy

TOPICS: FOOD PRODUCTION AND PREPARATION, CIRCULAR ECONOMY

The future of food production

Futuristic hydroponic crops and a 3D food printer for nutraceuticals, vitamins and antioxidants products, starting

from fresh raw materials or from waste from agri-food processing for high nutritious personalized food. ●

SARA DE FRANCESCO

PhD student, University of Naples Federico II - Dept. Agricultural Sciences - Italy

TOPIC: LIFE SUPPORT ARCHITECTURE FOR FUTURE MISSIONS

Lactuca sativa L. plants showed different capacities to cope with ionizing radiation when exposed to increasing doses of heavy ions.

Nowadays, crop production in Space represents one of the greatest challenges for Space exploration since it will be fundamental to regenerate resources, including food, for future Space pioneers. Indeed, a balanced diet based on the integration of fresh vegetables, rich in bioactive compounds directly produced onboard, will be useful in counteracting the emergence of Space-induced diseases on humans while minimizing the food supply from Earth. However, it is essential to understand plant behavior when exposed to extraterrestrial environmental factors, to achieve this challenging goal. Among these, Space ionizing radiation represents an open issue for mammals but also for plants, being able to induce potential critical outcomes on morpho-anatomical, eco-physiological, and nutritional aspects depending on intrinsic radiation and plant properties (e.g., type of radiation, dose, plant species, cultivar, developmental stage at the time of irradiation). This study aimed to evaluate the effects of

different doses of high-LET (Linear Energy Transfer) radiation on morpho-anatomical and nutritional traits of the Salanova® green cultivar (*Lactuca sativa* L. var. capitata). Dry seeds were exposed to increasing doses (0-control, 0.3, 1, 10, 20, and 25 Gy) of iron ions (⁵⁶Fe) (between the most damaging ions in the spectrum of the galactic cosmic rays) at GSI Helmholtzzentrum für Schwerionenforschung GmbH. After the irradiation, seeds were sowed, and lettuces were cultivated in a growth chamber under controlled environmental conditions, monitoring germination, survival, and growth performances. At the harvest, morpho-biometric parameters, such as plant total area, number of leaves, and fresh and dry biomass, were measured. We also analyzed photosynthesis (gas-exchange, photochemistry), while leaf functional anatomical traits (e.g., lamina thickness, localization of phenolics, stomatal frequency) were quantified through light and epifluorescence microscopy and digital image analysis. The

phytonutrient content was evaluated by analyzing the ascorbic acid content, antioxidant capacity, total polyphenols, chlorophylls, and carotenoid content. Results showed that the responses of Salanova plants from irradiated seeds changed depending on the dose analyzed, with an evident reduction of growth performance at the 25 Gy dose. Gained information helps understand the mechanisms behind plant radioresistance to support the choice of suitable candidate species to cultivate in Space and the

CO-AUTHORS: Chiara Amitrano, Walter Tinganelli, Marco Durante, Stefania De Pascale, Carmen Arena and Veronica De Micco

CARLES CIURANS

PhD Student - MELISSA Foundation – Spain

TOPIC: AIR RECYCLING AND CO₂ VALORIZATION

Designing the MELISSA Pilot Plant Integration. Gas loop closure between higher plant chamber and crew compartment: requirements specifications, simulations and hardware

Designing a MELISSA Pilot Plant Integration Work Package (WP) is a stepwise task with several disciplines converging in a coordinated manner. In this study the sequence of the required steps is discussed in order to demonstrate how to transit from a list of requirements to the final hardware implementation and testing phase. This is done for the Integration Work package 2 consisting of the connection of the Higher Plants Chamber (HPC) and the crew compartment (an animal isolator mimicking the respiration of a human) in a gas closed loop. The Integration challenge is conceptually easy since the oxygen demand of the animal cohort needs to be satisfied with the oxygen supply from the plants, but several technical challenges should be considered. The first step in the approach is to design the list of needs, which defines the base upon which the list of requirements is built. Thus, from the needs of the gas close loop proposed, several categories of requirements are generated

CO-AUTHORS: Carolina ARNAU, Enrique PEIRO, Laurent POUGHON, Claude-Gilles DUSSAP, Olivier GERBI, Brigitte LAMAZE, Christophe LASSEUR and Francesc GÓDIA

CHRISTOPHE EL-NAKHEL

Postdoctoral researcher - University of Naples Federico II - Department of Agricultural Sciences – Italy

TOPICS: GREY, YELLOW, BLACK WATER RECYCLING, WASTE RECYCLING, FOOD PRODUCTION AND PREPARATION, CIRCULAR ECONOMY

Integration of Human Urine Derivatives in Soilless Systems Fertilization to Grow Salad Crops

Regenerative life support systems for long term Space missions aims at producing food, water and oxygen from mission wastes. However, reinforcing and optimizing sustainable food production is an urgent contemporary issue on Earth. Diminishing the depletion of natural resources is intensively studied, and efforts are exerted towards integrating non-renewable resources such as potassium and phosphorous and energy-costing elements like nitrogen with recycled nutrients when boosting agricultural production. The approach of a closed-loop fertility cycle asserts the necessity for efficient natural resource management. For this purpose, eight urine derivatives (liquid and precipitates) were integrated in the fertilization program of a soilless cultivation of lettuce (*Lactuca sativa* L.) cv. Grand Rapids, to assess the inputs of this nutrient rich waste stream. Treatments were compared in

CO-AUTHORS: Danny Geelen, Jolien De Paepe, Peter Clauwaert, Youssef Roupheal, Stefania De Pascale

definition of the shielding requirements for Space cultivation facilities. Part of the results presented here is based on the experiment Bio_08_DeMicco, which was performed at the SIS18 at the GSI Helmholtzzentrum fuer Schwerionenforschung, Darmstadt (Germany) in the frame of FAIR Phase-0. Keywords: ionizing radiation, food countermeasures, functional anatomical and eco-physiological traits. ●

including functional, operational, interface and constrictive requirements. One of the critical requirements is controlling the oxygen concentration in the animal isolator by means of adjusting either the gas flow or the light intensity in the HPC. To better understand this specific point, several simulations have been performed with models of different degree of surrogation. The simulations that will be presented include a case where only gas flow between compartments is manipulated and a simulation where gas flow and light intensities are manipulated at the same time. The number of rats in the animal cohort is also tested and the operating conditions at the higher plant chamber to satisfy their oxygen demands will be discussed. From these simulations it is possible to take decisions supported by a systems engineering approach including the hardware design, hardware implementation and the design of control strategies to guarantee successful long-term operation. ●

terms of growth, biometric parameters, SPAD index, leaf minerals and organic acids. Two solid urine derivatives (K-struvite and urine precipitate-CaO) and one liquid derivatives (ED concentrate) fostered similar growth to the commercial fertilizer. ED concentrate induced a high accumulation of potassium, calcium, malate, citrate and isocitrate while K-struvite induced high accumulation of magnesium when compared to the control. The appraisal of nitrogen- and phosphate-rich organic streams (human urine), to sustain chemical fertilization to grow lettuce in soilless systems, showed promising results as the integration of urine derivatives proved feasible. Nonetheless, future research is needed to reduce Na and Cl presence in these derivatives, due to their deleterious effects on horticultural crops grown in soilless systems. ●

RAUL HERRANZ

Spaceflight and Simulated microgravity expert - CIB Margarita Salas (CSIC) - Spain

TOPIC: SPACE EXPERIMENT

Seedling growth: results from the largest ESA/NASA Arabidopsis experiment on the ISS looking into the molecular adaptation of plants to the Moon gravity and other life support system relevant scenarios

Plants respond to their external environment through tropisms, which are growth-mediated movements. Auxin polar transport is key in gravitropism responses while phytochromes are known to play a role in the sensing and response to red light photostimulation. While responses to light (phototropism) and gravity (gravitropism) have been well characterized, little is known about the interaction between the two responses. In addition, cell growth related mutants (nucleolin gene family) are also relevant to understand meristematic competence alterations on life support systems scenarios. Those mutants were grown in the European Modular Cultivation System (EMCS) as part of the Seedling Growth series of experiments on the ISS.

On-board centrifuges were used to create gravity vectors of reduced gravity from 0.1 to 1g (on board control). In addition, plants were exposed to unidirectional blue light or red light at each magnitude of gravity. Arabidopsis samples were sequenced via RNA-seq, and subsequently analyzed for differential gene expression looking for general patterns and adaptation processes that may indicate that different genetic backgrounds or particular mutants can have a benefit to space agriculture. Thus, organisms chosen to be part of life support systems in space exploration missions should validate their fitness in reduced gravity and elevated radiation scenarios. ●

CO-AUTHORS: Joshua Vandenbrink, Louisiana Tech University, John Z. Kiss, UNC-Greensboro, F. Javier Medina, Centro de Investigaciones Biológicas Margarita Salas

MARCO GATTI

Senior Project Engineer – EnginSoft – Italy

TOPIC: PROCESS MODELING, SIMULATION AND CONTROL

A roadmap for future system studies VARSITY legacy

In the framework of the MELISSA projects, the system studies are crucial at all levels of development: from process modelling up to control laws and necessary for ground systems as well as space ECLSS. When dealing with modelling, it is essential to realize that a model of a compartment or of a process should always be developed by taking into account its connections with the system, even if it would be much simpler to consider it as a detached, separate unit. However, up to now, mechanistic models of MELISSA compartments are often developed and have been demonstrated mainly at unit or sub-units levels and within limited ranges of process behavior, generally the optimal ones. Even if it could appear trivial, it is also important to underline that model development is a progressive process and different models are not necessarily at the same level of maturity. The VARSITY project raises awareness about the obstacles to overcome on the way to system simulation of the complete MELISSA loop, giving an overview of the mechanistic modelling of single compartments and directions to follow for a robust modelling architecture, ready for the system simulation of the complete loop. This simulation requires trustworthy mathematical models of the individual compartments as well as the modelling of the connections defining the loop as a network. The validity of the simulation results will depend on the maturity of the compartment models but also on the integration capacity,

i.e. the maturity of the interfaces and connections of the compartments. In this sense, a first set of definitions for State Vectors (SV), Model Readiness Level (MRL) and System Readiness Level (SRL) are one of the important outcomes of the VARSITY project. The objective of the SV definition is to highlight the variables that needs to be taken into account for a realistic simulation of the MELISSA loop. Moreover, the SV will guide future model developments assuring compatibility between each compartment model. It is mandatory that future models are developed keeping in mind that they need to be interfaced with other models in a network structure. The SV with continuous updates has the ambition of becoming a reference for this scope. Another important issue to be considered for system studies is the model compatibility in terms of sizing. To build a realistic network of the complete MELISSA loop, a common unit of normalization for the different models need to be defined. With the introduction of MRL and SRL definitions, the objective is to raise awareness about the status of modelling and the fact that different models are not at all at the same level of development. In this way, the knowledge gaps on some processes are clearly identified and should be used as an indication about the best directions to follow in terms of model developments for future system studies. ●

CO-AUTHORS: Erik Mazzoleni, Lorenzo Bucchieri

CAROLINA ARNAU JIMENEZ

Technical coordinator - MELiSSA Pilot Plant – Spain

TOPICS: AIR RECYCLING AND CO2 VALORIZATION, FOOD PRODUCTION AND PREPARATION

Characterization of the performance of the Higher Plants Chamber in the MELiSSA Pilot Plant under batch and staggered mode of operation using *L. sativa*

Crops culture in future crewed missions in Space is essential to guarantee the supply of food, to recycle water and to regenerate air. In the MELiSSA Pilot Plant, several tests have been performed to gain knowledge from the culture of *L. sativa* (lettuce) in a Higher Plants Chamber (HPC). The data obtained from this set of experiments are relevant to characterize the oxygen production capacity and the physiology response of lettuces under a wide range of operating conditions and will also be used to design the future Integration steps of the MELiSSA Pilot Plant, that is, the connection of the HPC to other compartments of the MELiSSA loop. The experiments are divided into batch and staggered test. In all tests, lettuces had a germination period of 9 days prior entering the chamber. In the batch test, 100 (20 trays with 5 plants) lettuces were exposed to a photoperiod of 16 h of light and 8 h of darkness for 28 Days After Transplanting (DAT), with a light intensity during the light photoperiod of 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and the carbon dioxide concentration inside the chamber controlled at 1000 ppm with external addition of pure carbon dioxide. For the staggered tests, lettuces had a total residence time inside the chamber of 28 DAT. The total capacity of the chamber was distributed in four groups each one containing 25 plants (5 trays with 5 plants), with the first group of lettuces with an age of 7 DAT, the second group of lettuces with an age of 14 DAT, the third group with an age of 21 DAT and

the last group with an age of 28 DAT. The first condition tested (Test A) was the same as the conditions in the batch test. The second condition tested (test B) was using a light photoperiod of 24h and the same concentration of CO2 and light intensity as in the batch test and the first staggered test. The third condition tested (Test C) was using a 16h/8h light/dark photoperiod, a light intensity of 550 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and a carbon dioxide concentration of 1000 ppm and the fourth condition tested (Test D) consisted of using a photoperiod of 16h/8h, a light intensity of 550 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and a carbon dioxide concentration in the chamber of 5000 ppm. Overall, the mean oxygen production capacity of the chamber is visibly higher in staggered mode of operation than in the batch mode, if taking the whole length of the experiment into account. Regarding the different staggered experiments tested, the oxygen productivity was higher in Test B, where a 24 h light photoperiod was used, than in other tests. On the other hand, a reduced oxygen productivity was observed in Test C in relation to Test B and even a higher reduced productivity observed in Test A, but with a reduced variability. In terms of dry biomass at harvest and leaf area, no significant differences were detected between the tests. The information collected provides valuable data about the compartment, its dynamics and operational resilience and will help shaping the future MPP integration steps. ●

CO-AUTHORS: Carles CIURANS, Arnau VIZCARRA, Antonio PANNICO, Roberta PARADISO, Enrique PEIRO, Stefania DE PASCALE, Francesc GÒDIA

GIORGIO BOSCHERI

Advanced Life Support Engineer - Thales Alenia Space Italia – Italy

TOPIC: FOOD PRODUCTION AND PREPARATION

PFPU Microgravity Precursor Food Production Unit development status

A food production unit is a key element for future space exploration missions, with a first use opportunity in the future Mars Transit Habitat. So far, several technical issues related to the development, implementation and operations of food production system for space applications were identified. They include food quality prediction, food safety, integration strategy as well as microbial contamination, humidity and nutrient delivery management. 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. It is carried on by an Italian and Finnish consortium led by Thales Alenia Space Italia. Breadboards of the PFPU key subsystems have been designed, built and tested. A system breadboard have recently completed manufacturing and is now in the middle of its test campaign. This paper describes the PFPU development status and the associated roadmap. ●

CO-AUTHORS: Thomas Fili (TAS), Giovanni Marchitelli (TAS), Christel Paille (ESA)

ALEXANDRE SOBAS

Master student - Université Paris Saclay, CentraleSupélec, Laboratoire Genie Industriel – France

TOPICS: PROCESS MODELING, SIMULATION AND CONTROL, LIFE SUPPORT ARCHITECTURE FOR FUTURE MISSIONS, CIRCULAR ECONOMY

Specification process of a simulation platform for the MELiSSA project

The MELiSSA project consists of developing a closed-loop life support system (LSS), allowing astronauts to recycle crew waste, revitalize air and produce water and food. That will make remote exploration missions possible by reducing the spacecraft's mass, but those regenerative technologies are also destined for terrestrial contexts. There is an increasing need to develop a complete simulation platform for the MELiSSA loop. That implies considering a multi-criteria approach to provide the user with metrics complementary to the traditional Equivalent System Mass (ESM). State of the art was conducted on previous LSS projects to identify the challenges around the specification of such a simulation platform. Many similar projects have struggled in the past for technical, political, and human reasons, such as ELISSA and V-HAB. That can be explained by the technical complexity of the problem and the poor consideration of stakeholders' needs during the specification phase. In addition, the MELiSSA simulation platform has to consider its specificities in circularity, which can relate to similar challenges in terrestrial circular systems. An in-depth industrial audit with several MELiSSA project stakeholders enabled their simulation needs to be understood. Some tools already exist, such as ALISSE, a multi-criteria evaluation tool. However, the industrial audit revealed a complex use and variability in the reliability of the criteria due to difficulties in integrating the external simulation models. After several expert interviews, we represented their needs from 3 different viewpoints (Operational analysis, System Architecture & Use case), highlighting existing tools and conflicting priorities

(trade-off between flexibility, speed, and reliability). It showed the need to implement new methods to ensure the consistency and usefulness of the final simulation platform. In particular, we have identified a gap between the formulation of system architecture simulation requirements and simulation models. To bridge this gap, we consider here two complementary approaches extracted from the literature: The 'simulation request': to define the needs and causes of each simulation in a transparent and traceable way. It is enriched with 'Model Identity Cards' (MIC), a collaborative standard that simplifies simulation models specification sharing and interfacing.

"The 'Credible Simulation Process' approach allows a project manager to anticipate the resources and roles needed for each step of the specification process. These two approaches would be interesting to integrate into the MELiSSA project, as they would allow each stakeholder to use the same standard of requirements and architecture via a top-down approach. Simplified templates for applying them have been created. That contributes to spreading their interest among the project stakeholders with a view to standardization, connectivity, traceability, and digital continuity. Through this research, simulation architecture specification methods have been identified to address the project's needs at different levels of detail and technicality. These methodologies have been analyzed and discussed, proposing perspectives for future research within the MELiSSA project."

WIEGER WAMELINK

Senior Ecologist - Wageningen University & Research – Netherlands

TOPICS: AIR RECYCLING AND CO2 VALORIZATION, WASTE RECYCLING, FOOD PRODUCTION AND PREPARATION

Effect of the addition of human urine-based struvite on the growth of green bean on Mars and moon soil simulants

When humans are going to live on the moon or Mars food production will be essential for their survival. Of equal importance is waste management including human faeces. To be able to set up a sustainable agricultural ecosystem for food production reuse of faeces and non-eaten plant parts is a must. Growing crops is possible on several media, I opt to use as many resources from the celestial bodies as possible, the moon and Mars regolith and the present ice. Earlier experiments have shown that crop growth is possible on the simulant regoliths though there are several problems to solve. One of them is the almost absence of reactive nitrogen (NO3 and NH4) in the regoliths. This could be solved by (1) reusing the non-eaten plant parts as manure and regolith improvement and (2) the application of human faeces, in this case human urine. Human urine was not applied directly but in the form of struvite (MgNH4PO4), a salt that can be extracted from human urine. Goal of this experiment was to test if human urine-based struvite could fertilize Mars and moon regolith simulants and lead to a higher yield of green bean. To test the effect of struvite 15g per pot was added to Mars (MMS) and moon soil simulant (JSC 1A). As a control we used potting soil (750g Mars and moon

soil simulant, 250g potting soil). To all pots 10% (volume) organic matter was added. The experiment was conducted in tenfold and randomly placed in a greenhouse in Wageningen. During the experiment plant height and greenness (SPAD meter) were recorded. Beans were harvested when ripe and at the end of the experiment three and a half months after the start. There were significant differences present between the non-manured pots and the manured pots, with a higher yield for the manured pots. The fresh weight yield of the beans did not differ between the soil types, though the harvest was highest on the earth potting soil and lowest on the Mars soil simulant. Plants grown on manured potting soil and moon soil simulant reached on average the same height and were much higher than the control plants without struvite. The plants on Mars soil simulant were smaller but still taller than the control. It can be concluded that the addition of struvite had a significant positive effect on the production of green beans on potting soil but also on Mars and moon soil simulant. Therefore, the production of struvite from human urine could significantly improve soil quality and growth potential and help to set up a closed agricultural ecosystem for Mars and the moon. ●

CO-AUTHORS: Wieger Wamelink

PAOLO CARATELLI

Associate Professor - Abu Dhabi University, College of Engineering – United Arab Emirates

TOPICS: LIFE SUPPORT ARCHITECTURE FOR FUTURE MISSIONS, SPACE EXPERIMENT

Concept Study of a BLSS Module for LEO, Cislunar and Mars Transit stations.

With a resurgence of manned missions beyond earth orbit, the permanence of crews in space will depend by the capability of life support systems to regenerate the limited stored amount of vital supplies like oxygen, water, and food. Furthermore, extended crewed missions towards distant planetary bodies like Mars would face the challenge of no chances for resupply from external resources, both during deep space transfer and mission s permanence on the planetary surface. Therefore, the integration of bio-regenerative processes in the life support systems of future spacecrafts, seems a possible option to provide a vital sustain for the crew in an almost perfect circular economy. Differently by the early manned space exploration, a new paradigm is replacing the initial philosophy of waste and expendability, focusing instead on the principles of reusing and recycling. But this new approach has a cost in terms of compactness, maintenance, reliability, and labor intensity requested to technical systems and crews. The technological transfer from testing facilities at ground to restricted and environmentally challenging conditions onboard a spacecraft, is still representing a limit for the technical integration and preparedness of bio-regenerative systems to be successfully integrated. Therefore, the opportunity to test the performance of bio-regenerative systems in prolonged conditions of microgravity, and the physicochemical behavior of biological components exposed to galactic cosmic rays (GCR) and solar particle events (SPE), would also represent an important step forward in direction of an optimal integration of these systems. Furthermore, safety protocols and emergency procedures in

case of failure of biochemical reactors need to be properly tested and assessed in condition of microgravity. The study proposed in this paper is a project for a lab module to test, experiment and integrate bio-regenerative processes and technologies into the life support system of future LEO stations and the Gateway cislunar orbital station, which is under construction to support the future Artemis mission. Based on the frame of the current Cygnus modular cargo spacecraft by Northrop Grumman, the proposed Bio-regenerative Life Support System (BLSS) Module is adopting the same minimal architecture to be configured as a hybrid rigid/inflatable module, designed to test, and experiment bio-regenerative systems and processes in prolonged conditions of microgravity. The initial concept of the study has been recently presented by the authors at the ICES 2022 in Saint Paul MN, and this one represents a further development of its main components. The inflatable section, which hosts the greenhouse for photo-bioreactors and racks for higher plants, is now integrated with a low rpm centrifuge to improve the performance of the aeroponic system and water recovery. Furthermore, the rigid section hosting the hardware and processing compartments as per MELISSA concept, has been extended to maximize the dimensions of digesters for higher plants waste, and allow for a safe maintenance in case of failure. The integration of the rigid section with a toilet for the mission crew of four people, would also contribute to the necessary amount of organic matter, urine, and wastewater to be processed in the bio-regenerative cycle. ●

CO-AUTHORS: Peter Weiss (Spartan Space); Maria Alessandra Misuri (Abu Dhabi University); Rowdha Begam Mohamed Hanifa (Abu Dhabi University)

JOANNA KUZMA

Phd Student - Clermont Auvergne University, CNES

TOPIC: PROCESS MODELING, SIMULATION AND CONTROL

Modelling physical processes in higher plants using leaf replicas for space applications

Future Bioregenerative Life-Support Systems (BLSS) will include plants to produce food, oxygen, and water for human crews. A deep understanding of phenomena at the core of plant metabolism and of their interaction with the environment will be required. Indeed, plants are complex reactive systems, which are sensitive to their environment, their growth history, as well as their genetics. Therefore, studying physical processes that influence their metabolism without confounding factors due to genetic and biological variability is challenging. However, this study is crucial for the development and validation of mechanistic models, which will allow in-depth knowledge of plant behaviours in non-standard environments, like for example spaceflight conditions. As a source of data needed for further development or first validation of a mechanistic model, leaf replicas can be used. Thanks to their simplified construction it is possible to focus on physical phenomena at the leaf surface, without uncertainties coming from genetic variability between

plants, or biochemical and biological interactions, like a different size and density of stomata, their opening and closure, or the effects of plant stress on their behaviour. Depending on the purpose the replicas can be made of a single material or have a more complex structure and can simulate the stomatal apparatus or have a built-in heater. This presentation gives an overview of the existing plant replicas and shows how their different construction and materials allows the study of the governing mechanisms of heat and mass transfer in more detail. An emphasis is put on the energy balance, the transpiration rate, and the boundary layer conductance. The simplified equations associated to replicas combined with the use in parallel of replicas and real plants will help to estimate crop parameters. In depth understanding of these mechanisms will allow first to further develop mechanistic models of physical phenomena at the leaf level and later to upscale to whole canopy modelling. ●

JORGE MANDUSSI MONTIEL

Visitor Researcher/ Lecturer - University of California Merced - United States

TOPIC: FOOD PRODUCTION AND PREPARATION

Amphibious plants present a gigantic shift in root microbial community across life cycles

The relationship between plants and microbial communities living inside plants endophytes, is complex, especially for those plants inhabiting ecosystems that are subject to strong environmental shifts. Amphibious plants in ephemeral wetlands vernal pools, are defined by their ability to tolerate a wide range of hydrologic conditions, from completely saturated soils to progressively drier conditions. These plants serve as shelter for microbial communities that in turn may influence the host's environmental tolerance. This research aims to investigate for the first time the diversity and community composition of microbial endophytes associated with the California amphibious native plant *Eryngium castrense* inhabiting vernal pools. Whether microbial communities remain the same across plant life cycles, aquatic and terrestrial, and the environmental constraints when soils go from saturated to dry is unknown. On the other hand, is also of interest to investigate if the microbiome

of plants in more pristine vernal pools differs from those inhabiting pools under anthropogenic pressures. Roots and shoots were collected according to their aquatic and terrestrial morphological stage, across a transect of five kilometers spanning from urban to more pristine habitat, at the University of California Vernal Pools and Grassland Reserve, located in the Central Valley of California. High-throughput sequencing of the 16S rRNA region was employed to analyze the microbiome of the plant specimens. To our knowledge, this study is the first of its kind in exploring amphibious plants inhabiting ephemeral wetlands known as vernal pools. Preliminary results showed a gigantic shift in root microbial communities in comparison with the microbes in the shoots, suggesting that plants might choose the associated microbiome, according to the environmental conditions of the soil, water saturation, or desiccation. Keywords: vernal pools, microbial-symbionts. ●

PATRIZIA BAGNERINI

Professor - University of Genoa – Italy

TOPIC: FOOD PRODUCTION AND PREPARATION

Adaptive vertical farm for fresh food production in life support systems

Astronauts who will operate stations around the Moon or live and work on its surface will need life support systems that can provide air, water, but also food for their sustenance. Growing plant food in order to become self-sufficient in terrestrial food supplies plays a very important role. The aim of the research is the study and design of an innovative type of Vertical Farm, called Adaptive Vertical Farm (AVF) capable of producing fresh plant food in the orbital stations and future lunar settlements with a significantly higher efficiency than the current vertical farms. For much of their development, plants are in a growth phase where they take up little space. The current projects of vertical farms in space are evolutions of greenhouses on Earth, where the volume of each shelf of cultivation is fixed, with an unnecessary waste of space and energy. Plants need proper lighting and air circulation for their growth. With the proposed AVF greenhouse concept, the volume allotted to each cultivation shelf continuously adapts to the level of growth of the plantation and plants at the beginning of their development phase relinquish useful volume to those who are in a more advanced stage. By this technology, more cultivation shelves may be fitted in the greenhouse and nearly 100% of the farm volume is always used. The progressive adaptation of the farm structure to the plantation needs - called adaptive principle - allows to grow a greater number of crops per unit of volume than existing solutions. It has been demonstrated [1] that comparing a

greenhouse with fixed shelves to an adaptive greenhouse of the same height, controlled by an efficient scheduling algorithm, the production yield increases by about 108% (and can reach 172% in some cases). Adjusting the height of the shelves to the plantation growth consequently entails positioning the light sources always at the correct distance from the leaf canopies and providing plants with a well-controlled uniform-temperature airflow circulation in a reduced volume. The air conditioning system, operating in a downsized compartment, requires much less energy so that the optimization of the cultivation conditions and of the overall yield provides also a significant energy demand reduction compared to a fixed-layers vertical farm. This energy saving is estimated around 43%. The Adaptive Vertical Farm concept is a subject of further scientific investigation in relation to its performance versus specific types of cultivation and applications. The AVF technology is presently developed by Space V (V stands for Vegetables), a startup and spin-off of the University of Genoa. Space V designs innovative solutions for the cultivation of vegetables in space, and it owns industrial patents on the principle of adaptive cultivation at national and international level. [1] P. Bagnerini, M. Gaggero, M. Ghio, F. Malerba, M. A. Malerba, Adaptive Vertical Farm for Fresh Food Production in Orbital Stations and Future Lunar Settlements, Proceedings of IEEE Metrology for Aerospace Conference, Pisa 2022. ●

CO-AUTHORS: Mauro Gaggero, Marco Ghio, Franco Malerba

BENJAMIN THIRON

Control engineer - SHERPA Engineering – France

TOPIC: PROCESS MODELING, SIMULATION AND CONTROL

Design of the MELiSSA loop control strategy

Several technological solutions exist today to ensure the survival of a crew during space missions. The MELiSSA (Micro-Ecological Life Support System Alternative) Life Support System (LSS) aims to stand as a leader for long-term journeys. To satisfy such a goal, the LSS shall provide the crew what is needed to survive and to properly conduct its mission, and this in an optimal way. It is hence necessary to design an optimal control strategy for the MELiSSA loop. It means to find the suitable compartments, their physical flow connections and their operating points that ensure the delivery of resources like water, air, and food and which optimize evaluation criteria (the mass to carry, the efficiency of the loop, the overall risk, etc.). An optimal control strategy also means that the MELiSSA loop should ensure as long as possible the survival of the crew during failure scenarios. A strategy for the dynamic control of the complete MELiSSA loop is developed within the framework of the VARSITY (VARIous Intergration of system sTudY for model-based cybernetics for the control of complex systems) project. This strategy was designed based on knowledge models of the compartments which are assumed to operate in their nominal

behaviour, i.e., they are all assumed to fulfil their own objectives. Different flow architectures that represent the possible connections between these compartments are considered. For each architecture, a control algorithm is specified to drive the flow distribution and operate the MELiSSA loop. They are tested, evaluated and compared to find out the best configuration. Their evaluation is based on the ALiSSE (Advanced Life-Support System Evaluator) criteria in addition to a crew delivery criteria to make sure that the loop ensures the crew survival. The optimal control strategy (in terms of ALiSSE metrics) is composed of the most promising architectures and distribution controlled device. It will be tested and fully validated in a second phase with a more refined loop model based on a mechanistic representation of the compartments and of the connection network. The control system will also be extended and enhanced to fit the detail of the mechanistic model and allow for a realistic evaluation of the loop. Simulations will run with nominal operation and different failure scenarios. The same metrics will be used to evaluate the control strategy. ●

CO-AUTHORS: Philippe Fiani (p.fiani@sherpa-eng.com) (1), Olivier Gerbi (o.gerbi@sherpa-eng.com) (1), Claude Gilles Dussap (C-Gilles.DUSSAP@uca.fr) (2), Lucie Poulet (lucie.poulet@uca.fr) (2), Laurent Poughon (Laurent.POUGHON@uca.fr) (2), Lorenzo Bucchieri (l.bucchieri@enginsoft.com) (3), Marco Gatti (m.gatti@enginsoft.com) (3). (1) Sherpa Engineering, 92000 Nanterre, France, (2) Université Clermont Auvergne, 63000 Clermont-Ferrand, France, (3) Enginsoft, 24126 Bergamo, Italy.

LUIGI GENNARO IZZO

Researcher - Department of Agricultural Sciences - University of Naples Federico II – Italy

TOPIC: FOOD PRODUCTION AND PREPARATION

Light stimuli to guide roots of agriculturally-important plants in extra-terrestrial environments

Research on plant tropisms is crucial for future programs that foresee plant-based life support systems for space missions and extra-terrestrial colonisation. Although extensive research has been performed to understand mechanisms of tropistic responses in model species such as *Arabidopsis thaliana*, there is a need to investigate plant tropisms and their interactions in agriculturally-important species for cultivation in space. Gravity is an important stimulus (i.e., gravitropism), and its alteration in space can severely impair the interaction between tropistic responses of roots. Nevertheless, several other stimuli can mediate the directional responses of plant roots, including light, water, touch, nutrients, electromagnetic fields, and even sounds. In this regard, studies on tropism interactions are necessary to counteract the detrimental effect of altered gravity, and maximise plant growth and resource-use-efficiency in plant-based life support systems in space. Ground-based facilities at the European Space Research and Technology Centre offer many opportunities to investigate tropism interactions in a wide range of gravity levels by means of clinostats, random positioning machines, or a large-diameter centrifuge, allowing experiments under gravity conditions ranging from simulated microgravity to 20g hypergravity. The results presented here are part of a broad research project funded by the European Space Agency with the goal of characterising root tropism interactions

under altered gravity conditions using candidate species for cultivation in space. Specifically, in our experiments, we evaluated the interaction of gravitropism and phototropism in roots of *Brassica oleracea* (that includes many common cultivars, such as cabbage, broccoli, cauliflower and kale) under a wide range of gravity conditions to assess the relative influence of each tropism, and their effectiveness in guiding root growth orientation. Experiments were performed using a custom-made device with LEDs to provide different spectral (Blue, Red, White, Dark) and photon flux treatments (from ~30 to 80 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Overall, we showed that light stimuli can significantly affect root growth orientation both in simulated microgravity and hypergravity. Indeed, blue-light mediated phototropism was consistent among the different g levels causing a significant root curvature even at 20g. Differently, red light stimulated a weak bending of roots which was enhanced under simulated microgravity. Our results on early root development characterization indicate that cell proliferation in the root meristem is a light-dependent and microgravity-sensitive process. Cell proliferation increased in seedlings subjected to simulated microgravity and dark conditions, which is probably associated with cell growth dysfunction (i.e., nucleolus, ribosome biogenesis), leading to serious alterations in plant development. In contrast, this alteration was not found in

photo-stimulated seedlings, indicating that light is effective in modulating this essential process under altered gravity conditions. Further in progress experiments are focusing on characterising transcriptomics profile of phototropic responses under partial gravity (i.e., Moon-g and Mars-g), simulated microgravity and hypergravity conditions. We are currently

targeting these studies to develop cultivation strategies based on environmental stimuli to improve plant cultivation in near-future missions to the Moon and Mars. Our research with space analogues will support the ESA Terranova Programme in the missions of Europe's human journey into the Solar system. ●

CO-AUTHORS: Leone Ermes Romano, Maurizio Iovane, Aranzazu Manzano, Raúl Herranz, F. Javier Medina, John Z. Kiss, Jack J.W.A. van Loon, Giovanna Aronne

BLANDINE GORCE

Trainee - European Space Agency – Technology - Engineering and Quality (TEC-MMG) - Netherlands

TOPIC: SPACE EXPERIMENT

Lessons learned for life support system payloads

The Micro-Ecological Life Support System Alternative (MELiSSA) is the European project aiming at developing regenerative life support systems for future space missions beyond Low Earth Orbit. As the MELiSSA ground studies are progressing, the project's engineering approach has to be validated in space conditions, on-board the International Space Station, via precursor scientific experiments and then technology demonstrators. However, operating scientific and technological payloads in space is a challenging task because of the extreme environment, gravity and the limited hardware and resources. The collection of lessons learned (LLs) from past operational activities is a crucial step to improve the performance and optimize the complex timing and high costs of future payload operations. In this context, the MELiSSA team wishes to take advantage of the expertise acquired during past life support payloads operations to perfect their future technology demonstrators. The objective of this study is to go further than typical operational LLs collection exercises by providing technical details and concrete actions to take into account during the design and development of a life support payload. The gathered feedbacks and LLs will be compiled in technical ready-to-use recommendations to support the development of on-going and future MELiSSA life support

technology demonstrators (e.g. BIORAT 1, BIORAT 2 and Precursor of a Food Production Unit). To obtain the necessary data, the study started with a thorough evaluation of the already available information in the ESA and HRE LLs from past life support ISS payloads. The bibliography will be broadened with a set of interviews that will be conducted according to ESA-HRE's knowledge management methodology between June and October 2022. The goal is to identify the main critical points encountered, their roots and possible corrective or mitigation actions. The lessons will be gathered from various ESA, academic and industrial actors that were closely involved in the development and on-orbit operations of life support scientific or technological payloads. The topics presented here will focus on both ground development and on-orbit operations phases for each previous life support payload analyzed. The presentation will highlight the final product of our study: practical guidelines gathering all current expertise on life support systems operations, organized chronologically to provide a handy and visual reference spanning the entire payload life cycle for the on-going and future MELiSSA life support technology demonstrators. ● Keywords: Life Support Payloads, Lessons Learned, ISS Operations

CO-AUTHORS: PASTOR Alice (Alice.pastor@esa.int, ESA-Human and Robotic Exploration), AUDAS Chloe (Chloe.audas@esa.int, ESA-Technology Engineering and Quality), LASSEUR Christophe (Christophe.lasseur@esa.int, ESA-Technology Engineering and Quality)

GREGORY NAVARRO

SpaceShip France Engineer – CNES – France

TOPIC: LIFE SUPPORT ARCHITECTURE FOR FUTURE MISSIONS

SpaceShip.FR and MELiSSA: Harmonized Roadmaps for Regenerative Life Support Systems

The recently published ESA Terrae Novae 2030+ exploration strategy roadmap aims to enable Europe's participation in the first crewed exploration mission to Mars. Yet, missions to deep space still pose significant challenges in terms of Environmental Control and Life Support System (ECLSS) technologies. The ECLSS approach adopted in the current ISS spaceflight program relies heavily on terrestrial resupply and maintenance capabilities. This approach is not suitable for a future Mars transit mission which will impose the management of considerable amounts of resources (e.g. metabolic consumables, hygiene items, clothing, etc.) in complete autonomy from Earth. The use of Regenerative Life Support (RLS) technologies has

been proposed as a promising solution to tackle this challenge. In this perspective, multiple activities have been initiated in Europe for several years to propose RLS building blocks that meet the specific requirements of a crewed mission to deep space. Initiated more than thirty years ago, the Micro-Ecological Life Support System Alternative (MELiSSA) project, led by ESA, seeks to develop a circular life support system with the highest degree of autonomy. Thus producing oxygen, water, food, and potentially biomaterials, for the astronaut crew from the wastes generated during their mission. The SpaceShip FR project, led in France by CNES since 2018, also investigates the design and development of long-term habitats for human exploration of

the solar system. To lay the foundations of a future space base on the Moon and possibly Mars, SpaceShip FR is bringing together technologies and skills and building the required partner networks with academia and industry. In an effort to join capabilities in this endeavor, the MELISSA and SpaceShip projects have established harmonized roadmaps highlighting

CO-AUTHORS: Alexis Paillet, Romain Charles, Chloé Audas, Christophe Lasseur

GIOVANNA ARONNE

University Professor - Department of Agricultural Sciences - University of Naples Federico II – Italy

TOPIC: SPACE EXPERIMENT

Water Across the Plant Systems (WAPS): ground tests on hydration and air humidity to model plant growth for space experiments

Growing plants will be essential in future bioregenerative life-support systems like MELISSA. To achieve this, understanding their behaviour in environments like spaceflight is crucial. In particular, studying transpiration rate and heat exchange will give valuable information, as it relates to the leaf boundary layer in different ventilation and gravity regimes. Coupled to the development of mechanistic models of plant growth, this allows for a deeper understanding of plant behaviour in a spaceflight environment. Water Across the Plant Systems (WAPS) is an ESA project (ILSRA-2014-0020) that aims to fulfil these objectives on the International Space Station (ISS) by the growth of mung bean plants in the Biolab facility. The main goal of the experiment is to disentangle the direct effect of microgravity on plant function from the indirect effect due to the formation of stagnant air outside the plant organs (boundary layer). To reach the WAPS research objectives numerous ground tests were performed to define the best combination of environmental parameters both for plant growth and payload control systems. One set of experiments focused on watering, plant transpiration and humidity control in the growth chamber. During the space experiment, it is important that plants have access to a sufficient amount of water not only to sustain good seed germination and seedling growth and -development, but also to avoid biological phenomena (as stomata closure) that could interfere with the measurements and analyses of plant functional reactions in space. It is therefore important that plants are neither water

CO-AUTHORS: Mona Schiefloe; Lucie Poulet; Øyvind Mejdell Jakobsen; Luigi Gennaro Izzo; Ann-Iren Kittang Jost; Jean-Pierre Fontaine; Claude-Gilles Dussap

RAY WHEELER

Senior Scientist – NASA Kennedy Space Center – United States

TOPIC: LIFE SUPPORT ARCHITECTURE FOR FUTURE MISSIONS

NASA's History of Bioregenerative Life Support Research

Like many space agencies, NASA has a long-standing interest in the use of bioregenerative life support technologies for space exploration. Much of the early work focused on microalgae like Chlorella for O₂ and biomass production, with research shifting more toward higher plants for the CELSS Program ca. 1980. From 1980 through about 2010, numerous studies were carried out with crops in controlled environments to assess yields, gas exchange, nutrient, and water requirements. This included one of the first working examples of a vertical farm NASA's Biomass Production Chamber. Around 1990, NASA funded research patented the use of light emitting diodes (LEDs) to grow plants on the Space Shuttle. Since that time, vertical farming and LED

the existing synergies and complementarity between their respective activities. Paving the way for the upcoming collaboration, this presentation will bring forward the shared vision between the two projects, with a clear definition of the expected interfaces and Intellectual Property. ●

deprived nor stressed due to an excess of water. Challenges related to humidity control in the Biolab facility indicate that it might be desirable to use as little water as possible, as this may reduce the amount of plant transpiration and the following increase in humidity that must be handled by the facility control system. In this framework, hydration tests of mung beans grown in WAPS flight design pots were performed with four different watering regimes under two different relative humidity settings (30% and 85%) to investigate the effects on the plants' ability to grow healthy to the target stage. In parallel, a mechanistic model based on the single-leaf hypothesis and predicting heat and mass transfer at the leaf surface was developed. The model includes gravity as an entry parameter and is therefore adapted to reduced gravity environments. Outputs of the model include, among others, leaf temperature variations, water accumulation and transpiration, and biomass production. This talk presents the results of these watering tests and gives a comparison with their simulation counterparts obtained with the mechanistic model. The model is able to give an accurate order of magnitude of dry biomass and water accumulated throughout the duration of the test. The modelling approach applied to achieve specific goals of the WAPS experiment, could be used in the future to accurately predict water needs for other plants growing in Space conditions and as a valuable tool for designing experiments and crop cultivation setups in Space. This work was funded by the ESA Progress Programme. ●

lighting for controlled environment agriculture have expanded around the world. More recent NASA studies with plants have focused on salad crops that can be grown on early missions, like the ISS, where NASA's Veggie plant chamber and the Advanced Plant Habitat have been used both for research and to grow supplemental food for the astronauts. Throughout this time, the use of biological systems to process liquid and solid wastes have also been studied, including ground testing with hollow fiber membrane bioreactors for treating urine in u-gravity. Eventually, humans will travel to and live on the Moon and Mars, and bioregenerative systems will be crucial to these missions. ●

ERIC LANDEL

Expert – ELC – France

TOPIC: PROCESS MODELING, SIMULATION AND CONTROL

New stakes for numerical modeling and simulation of Cyber-Physical Systems

Performances of products and services become always higher and more sophisticated in order to meet the desires of their users. This becomes possible mainly by the growing contribution of electronics. Then, products are made of physical parts with multiphysics phenomena, controllers and communication. They become Cyber-physical systems. The counterpart is a significant increase in their complexity which makes their development and validation always more difficult and expensive. In automotive industry, a typical example of this tendency is the autonomous driving systems. Classically, numerical modeling and simulation remain a strong lever to support these stakes, but with particular issues: Mainly: architectural management, model management,

model transfers, model identification, traceability, etc. On these questions, methods, tools and processes are emerging and their implementations make it possible to accelerate the availability of complex simulation results in the different contexts of the projects: in phase of design for optimizing products and in validation phase for reducing the number of physical tests. At IRT SystemX, a research project AMC (Agility and Margin of Design) has recently delivered a demonstrator of a complete chain of tools to support the process of modeling a complex system. For facilitating the exchange of models between teams, a Model Identity Card (MIC) is proposed as a metamodel to expose important information without ambiguity. ●

FELICE MASTROLEO

Senior Scientist - SCK CEN – Belgium

TOPIC: SPACE EXPERIMENT

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

Microbially produced oxygen and microbial edible biomass are very interesting sustainable resources for future space travelers. Arthrospira-C (ArtC) is a space biotechnology flight experiment, to transplant cyanobacterial oxygenic photosynthetic bioprocess to space. ArtC is the follow-up and step-up of the pioneering ArtB flight experiment which flew in Dec. 2017 to ISS, this time allowing continuous culturing and variable light settings. A space compatible photobioreactor was built, allowing online measurements of both bio-oxygen production rate and microbial growth rate in space. They contain the cyanobacterium *Limnospira indica* PCC8005 (aka *Arthrospira* sp. PCC8005, or under the product name *Spirulina*). Four of such bioreactors are to be integrated in the ISS Biolab incubator in 2023 and will be operated in turbidostat mode by continuous feeding for a duration of 2 months, to test production kinetics at 4 different light settings. The experiment will be performed in parallel on ISS and on ground. The bioprocess will be monitored and steered, using a novel and dedicated model for the growth of *Limnospira* in membrane photobioreactors, and the space grown biomass will be analysed for its nutritive value in detail postflight. In this presentation we will update you on the current development of the ArtC flight experiment and we will address

following challenges: (1) to build, qualify, and operate a turbidostat photobioreactor in space, with special attention to liquid mixing and CFD modelling of reactor designs and operation states; (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 the fitting to the predictive simulation, and (4) to implement successfully ground commands to adapt bioreactor conditions and allow several crew interventions. In addition, some reference data obtained pre-flight on ground will be presented and discussed. 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 the reactor conditions on ground. These data show it is feasible to design and operate a space-compatible continuous microbial photobioreactor, which is ready to be tested in ISS. Keywords: MELISSA, photobioreactor, flight experiment, *Limnospira*, *Arthrospira*, model. ●

CO-AUTHORS: Thomas Roersma (1,2), Hugo Moors(1), Surya Gupta(1), Natalie Leys(1), Felice Mastroleo(1)

JÉRÉMI DAUCHET

Associate Professor - Institut Pascal (UCA) - France

TOPICS: AIR RECYCLING AND CO2 VALORIZATION, PROTEINS PRODUCTION, PROCESS MODELING, SIMULATION AND CONTROL

Knowledge models of photobioreactors and their paths integral formulation.

It is now widely recognized that only knowledge models can allow simulation, design, scale-up, optimization and control of photoreactive processes such as photobioreactors (PBR) [Dauchet et al., 2013; 2015; 2016; Pruvost et al., 2016]. PBR can be used for many terrestrial applications such as the production of 3G biofuels or molecules of interest, the production of biomass, depollution..., but also for space applications such as the recycling of the atmosphere: production of O₂ and CO₂ mitigation. Our laboratory has developed such knowledge models for nearly 30 years, which are used both to simulate results obtained in various European laboratories of the MELISSA consortium and to design PBR with high kinetic or energy efficiencies, compatible with the constraints of space or terrestrial applications. These reified models are nevertheless highly complex since they integrate different bodies of physics at different scales of space and time in order to reduce their parametric space. Indeed, only input data such as the shape of the microalgae, their size distribution and their pigment content are necessary to construct in a predictive way the optical properties of the microalgae, to deduce their radiative properties (cross sections and phase function) via the resolution of Maxwell's equations, the irradiance field via the resolution of the photon transport equation (Boltzmann), and then formulate, from the linear energy converters theory, the thermokinetic coupling leading to the calculation of the biomass growth rate [Dauchet et al., 2016, Rochatte, 2016]. After reviewing the different bricks that constitute a reified predictive model of PBR, we will show in this work how it is possible to express the main observables of the process (mean growth rate, energy efficiency) as a single paths integral formulation (in the sense of Feynman-Kac). This kind of formulation makes it possible to envisage a statistical sampling of the paths space by the Monte Carlo method. We then benefit from the latest advances in this method via the image synthesis and EDStar communities [Delatorre et al., 2014, Dauchet et al., 2018]. In particular, we will show that the calculation is insensitive to the geometric complexity at all scales (meshless) and to the space and time dimensions of the problem (the number of calculated integrals), while evaluating, at the same time, the standard deviation on

CO-AUTHORS: Vourc h T., Gros F., Cornet J.-F.

ALBERTE REGUEIRA

Postdoctoral researcher - Ghent University - Belgium

TOPICS: WASTE RECYCLING, PROCESS MODELING, SIMULATION AND CONTROL

Bioenergetic modelling for predicting and steering VFA production in carbohydrates anaerobic fermentation

Anaerobic mixed-culture fermentations (MCF) yield a mixture of volatile fatty acids, which are valuable as chemicals and can also be used in subsequent bioprocesses to yield high added-value products (e.g. bioplastics). In this way, MCF are postulated as valid processes to valorise organic residues. While most of MCF processes are mesophilic, thermophilic processes present a number of benefits such as a faster hydrolysis and a more

efficient pathogen removal. MCF behaviour is still not well controlled, which makes their design a challenging task. One of the main limitations is the product spectrum variability with operational conditions (e.g. pH) and substrate composition, which makes process optimization only possible at the expense of intense experimentation. In this sense, bioenergetic models, a kind of metabolic modelling, proved to successfully predict the result and parametric or geometric sensitivities. We will illustrate all these characteristics on the calculation of the average production rate in biomass (or oxygen) of an optimized solar PBR technology with light dilution over its entire lifetime (30 years), taking into account the dynamics of incident solar flux density q_0 [Gattepaille, 2021]. The approaches that we present in this work still require further improvements but are today more and more widespread for the short execution time and the new interpretive meaning that they provide in many engineering fields, in relation to the energy transition, climate change and space exploration [Delatorre et al., 2014; Dauchet et al., 2018; Villefranque et al., 2022]. Dauchet J., Blanco S., Cornet J.-F., El Hafi M., Eymet V., Fournier R. 2013. The practice of recent radiative transfer Monte Carlo advances and its contribution to the field of microorganisms cultivation in photobioreactors. *J. Quant. Spectrosc. Radiat. Transfer*, 128: 52-59. Dauchet J., Blanco S., Cornet J.-F., Fournier R. 2015. Calculation of the radiative properties of photosynthetic microorganisms. *J. Quant. Spectrosc. Radiat. Transfer*, 161: 60-84. Dauchet J., Cornet J.-F., Gros F., Roudet M., Dussap C.-G. 2016. Photobioreactor modeling and radiative transfer analysis for engineering purposes. In: *Adv. Chem. Eng., Photobioreaction Engineering*, 48, 1-106. Dauchet J. et al. 2018. Addressing nonlinearities in Monte Carlo. *Scientific Reports*, 8: 3302. Delatorre J. et al. 2014. Monte Carlo advances and concentrated solar applications. *Solar Energy*, 103: 653-681. Gattepaille V. 2021. Modèles multi-échelles de photobioréacteurs solaires et méthode de Monte Carlo. PhD Thesis, Université Clermont Auvergne, France, 2021UCFAC014 [<https://hal.archives-ouvertes.fr/tel-03600307>]. Pruvost J., Le Borgne F., Artu A., Cornet J.-F., Legrand J. 2016. Industrial photobioreactors and scale-up concepts. In: *Adv. Chem. Eng., Photobioreaction Engineering*, 48, 257-310. Rochatte V. 2016. Développement et modélisation d'un photobioréacteur solaire à dilution interne du rayonnement. PhD Thesis, Université Clermont Auvergne, France, 2016CLF22705 [<https://hal.archives-ouvertes.fr/tel-01511196>]. Villefranque N. et al. 2022. The teapot in a city : a paradigm shift in urban climate modeling. *Sci Adv.*, 8: eabp8934. ●

the product spectrum of mesophilic MCF. So far, there are models available for carbohydrates, proteins and their cofermentation. This modelling approach assumes that microorganisms harvest the maximum energy from the substrate and that the expected product spectrum is governed by pathways associated with the highest ATP yield. However, there are no models for thermophilic MCF. Therefore, the aim of this work was to develop the first bioenergetic model for carbohydrates (glucose) at thermophilic conditions. The model considers acetate, propionate, butyrate, lactate, ethanol and butanol as possible end products. In this work, glucose fermentation (10 g/L) in a continuous reactor at 55°C and a retention time of 2 days was simulated. The effect of pH was explored with simulations at pH values of 4.5, 5.5 and 6.5. Validation experiments were done mimicking the simulations and using as inoculum effluent from the C1 compartment pilot plant at UGent. In a first round of simulations and experimental validation, it was observed that the model predicted significant yields of propionate, which was not observed in the experiments. This and the fact that thermophilic propionate producers from carbohydrates are not known to exist led to discard this pathway

CO-AUTHORS: A. Regueira, L. Ma, A. Ashraf, N. Kamot, R. Ganigué

DOMINIQUE CHAPUIS

System engineer - Beyond Gravity Sliprings SA - Switzerland

TOPICS: AIR RECYCLING AND CO2 VALORIZATION, SPACE EXPERIMENT

Design & operation of a bread board model of spirulina photobioreactor equipped with a harvesting system to support ISS On Board Demonstrator development

Oxygen regeneration from ambient carbon dioxide is a fundamental technology building block for future life support systems for space applications. The BIORAT-1 (BRT-1) project consists in the development of an On Board technology Demonstrator (OBD) for the ISS cabin to be hosted in European Drawer Rack 2 (EDR2) facility. The aim of the BRT-1 OBD is to recycle the carbon dioxide from the crew into dioxygen and biomass by the mean of PhotoBioReactor (PBR) & spirulina (*Limnospira indica* PCC 8005), this corresponds to the Photoautotrophic Bacteria compartments IVA of the Melissa loop. The design of the flight OBD is supported by life tests results performed on Bread Board Models (BBM). This project phase has started on December 2018 and will be concluded by a Conceptual Design. In this paper, we present the new functionalities added to the BBM making it more representative to the BRT-1 OBD. In the previous test performed in 2019, the BBM was operated in a chemostat configuration where the biomass concentration into the PBR & the Liquid Loop (LL) is controlled via the liquid outflow flow rate. Because the inflow flowrate is equal to the outflow one (feed), it results in a large feed medium volume consumption. The major BBM hardware upgrade is to add a Solid Loop (SL) to the existing PBR & LL, its primary function is to harvest the biomass produced by the PBR to keep the biomass concentration in an optimal range in the PBR. The SL operates by filtering and storing the biomass in a

CO-AUTHORS: Dominique Chapuis; Paolo Dainesi; Céline LAROCHE; David Duchez; Baptiste Jarry; Gilles Dussap; Dries Demey; Marie Vandermies; Olivier Gerbi; Chloe Audas; Christel Paille

in the model for a second set of simulations. The enhanced model predicted butyrate, acetate and ethanol as main products in glucose thermophilic MCF, showing comparable yield values between 0.43 and 0.50 mol/mol glucose. These values do not show any particular trend with the pH, suggesting that these options are energetically alike. The validation experiments confirmed these observations as no particular trends were observed. For all three pH values the root-mean-square deviation between model and experimental data showed values below 0.2 mol/mol glucose, which indicates an acceptable degree of deviation. The gas production model yields show a close-to-equimolar CO₂ and H₂ production with values around 1.69 and 1.96 mol/mol glucose, showing a slightly decreasing trend with increasing pH values. This was successfully validated with data from the experiments with a very good agreement. In this work, we developed the first bioenergetic modelling for thermophilic glucose MCF, which was successfully validated with ad hoc experiments. This opens an avenue to predict the most likely product spectrum of a particular feeding and operational conditions and design and steer thermophilic MCF fermentation processes. ●

Replaceable Unit RU. A secondary function of the SL in particular the RU is to store the feed medium needed for the growth of the spirulina. The integration of the SL has a major impact in the BBM operation as it will allow to limit dramatically the volume of feed medium needed by the PBR from typically 1.8L/day to 0.4L/day and therefore the SL limits the uploaded mass & extends the maintenance operation periods as needed for the future OBD operation. The second major functionality added to the BBM is the addition of a multi-parameter Process Controller to the existing CSE software allowing to operate autonomously the BBM after the inoculation and between the operation mode. Among the numerous controlled process parameters, the most critical were the nitrate concentration & biomass concentration in the PBR & LL as they rely on state observers (estimators) rather than measured values. Life test was performed with the objective to operate the BBM with the same experiment duration & maintenance operation periods representative to the future BRT-1 OBD mission. Despite a shortened life test due to an unexpected change in morphology of the Spirulina, the correlation and validation of the nitrate and biomass estimators could be performed with actual sample values & available on-line biomass measurements. Lessons learned of this life test campaign have been considered to improve the robustness of the BRT-1 OBD conceptual design. ●

BLANDINE GORCE

Student - Trainee - ISAE-Supaero - ESA - TEC - Netherlands

TOPIC: LIFE SUPPORT ARCHITECTURE FOR FUTURE MISSIONS

Conceptual design of an Environment Control and Life Support System for a Mars Transit Mission

The Life Support System (LSS) currently operated on-board the International Space Station (ISS) rely on resources supplied regularly from Earth and on waste disposal vehicles burning up upon re-entry. This cargo logistics will not be suitable anymore for missions to deep space. To prepare for missions towards Mars, life support systems need to be designed to reach higher recycling levels. In the present study, we aimed for the conceptual design of an Environmental Control and Life Support System (ECLSS) for a Mars Transit Mission. This project was realized by ISAE-Supaero students led by the European Space Agency (ESA) in the frame of the Micro-Ecological Life Support System Alternative (MELISSA) project. The mission scenario considered in this study was a 1000-day-mission with a crew of 4 astronauts, with the challenge to reach a recycling rate of 98% on the water and 75% on the oxygen. To start, the needs and requirements of such a mission were assessed to enable the design of an optimized system fulfilling these constraints. A study of the state-of-the-art was later performed to list existing LSS technologies demonstrated or operating on-board ISS. Some of these technologies were selected according to the Advanced Life Support System Evaluator (ALISSE) criteria in order to design an optimized system fulfilling the mission constraints. However, due to the demanding requirements and

the high level of recyclability expected, our research highlighted the necessity for alternative regenerative technological building blocks such as the ones developed under the umbrella of the MELISSA project. Our final conceptual design enables the recycling of oxygen, water and food to limit the initial upload mass. Thanks to the regenerative technologies used, our simulation results showed that the system could be fully autonomous in oxygen and that the initial stocks of food boarded on the spacecraft would be reduced in half compared to a system solely based on ISS LSS technologies for the same scenario without increasing significantly the need of water. Several iterations were performed to optimize our conceptual design. During the iteration process, the priority was given to the efficiency criteria. Yet, to reach this theoretical result, efforts still have to be made to increase the Technology Readiness Level (TRL) of some of the selected technologies. Our final results illustrate the current trade-off to find between low TRL and system efficiency. Our study also provides a strong foundation for the design of future Mars Transit ECLSS and offers an opportunity to highlight the critical technical gaps requiring more development. ●

Keywords: Life Support Systems, ALISSE, Conceptual Design.

CO-AUTHORS: GARNIER Lucien (lucien.garnier@student.isae-supaero.fr, ISAE-Supaero), SSI YAN KAI Hugo (hugo.ssi-yan-kai@student.isae-supaero.fr, ISAE-Supaero), SCHINI Pierre-Yves (pierre-yves.schini@student.isae-supaero.fr, ISAE-Supaero), LEFEVRE Alix (alix.lefevre@student.isae-supaero.fr, ISAE-Supaero), AFUERA Elliott (elliott.afuera@student.isae-supaero.fr, ISAE-Supaero), AUDAS Chloe (chloe.audas@esa.int, ESA-TEC).

FABIO LORENZINI

Project Manager - SRL Kayser Italia - Italy

TOPICS: WASTE RECYCLING, BIOMATERIAL

BioPack: a technology for waste inhibition and compaction for Life Support Systems

Solid waste handling and management aboard the ISS has been relatively simple since many years: it consists in hand-compacting the waste material (not sorted), wrapping it into small bundles, loading up a docked resupply vehicle, and then burning it up during atmospheric re-entry. Even if the storage time of the collected waste is limited, the crew is exposed to (bio)safety risks due to the growth of microbes on the food/drinking packaging and food leftovers. This strategy for ISS waste disposal has been efficiently implemented because of the minimal needs for resource recovery due to the frequent re-supply (and trash disposal) but cannot be adopted for long-term exploratory human space missions. For this reason, space agencies are increasing emphasis on the three R's of trash management: reduce, reuse, recycle. In the past, several activities were performed in this direction (i.e. NASA heat melt compactor), developing compaction/inhibition technologies of non-sorted wastes. The results on the volume reduction performance were promising, but the physical characteristics of the product obtained and its nature allowed for limited transformation into re-usable resources (i.e. radiation-shielding tiles, reference: Heat Melt Compactor Development Progress, J.M. Lee et al, ICES-

2017-267). Under the aegis of the European Space Agency, Kayser Italia, along with Ghent University and KU Leuven, is working to improve the selected non-body waste handling and management approach in manned exploration missions, and to develop novel biodegradable packaging. This objective is to develop an innovative system to compact and inhibit selected waste by studying technological solutions to reduce human safety risks by inhibiting microbes, compact and re-process selected wastes, which also make use of the biodegradable packaging. The compacted and inhibited waste will be safely stored and later repurposed (e.g. in the Life Support System, in the MELISSA CI compartment, or in the Materials Processing Systems e.g. additive manufacturing). Kayser Italia, starting from the expected waste fluxes, is developing a system capable of treating the sorted wastes considering a four crew member mission scenario. This ground technological demonstrator can process both the padding material and food/beverage packaging. A typical cycle of the system with food/beverage packaging involves the recovery of water (using heat and vacuum), the compaction (using a pneumatic ram) and the inhibition (by dry heat). Padding waste can be treated using the same approach. The final product is a tile, biologically

inert and mechanically stable that can be further processed by life support system or additive manufacturing facilities, hence allowing a further use of the recovered resources. The system, designed to be very flexible in terms of operating parameters (e.g. duration of each

step, temperature, compaction force, vacuum level), can be used to test several waste (both for the space sector and other possible applications, e.g. maritime industry) and to define the requirements of a future flight hardware. ●

CO-AUTHORS: M. Pinna, M. Balsamo, M. Tavanti, R. Ganigué, C. Paille, and S. Ortega Ugalde.

OURANIA OIKONOMIDOU

Postdoc researcher - Aristotle University of Thessaloniki

TOPIC: GREY, YELLOW, BLACK WATER RECYCLING

Spreading and sliding of condensed air humidity droplets over metallic substrates under non-isothermal conditions

Air humidity in the cabin of a spacecraft increases continuously during a space mission due to breathing and metabolic moisture of crew members. Intense humidity is imminent to trigger bacteria growth, damage the electronics on-board and affect the overall thermal comfort. Therefore it is important to control humidity rates in the space cabin. Another aspect that determines the viability of a space mission is the adequacy of water for human consumption, cleaning and experimentation, emerging the need for water recycling. Both humidity control and water recycling issues can be treated by the dehumidification of air in spacecrafts using Condensing Heat Exchangers (CHEs). Condensation efficiency relies on the wettability of condensing surfaces. Thus, currently the metallic fins of CHEs are covered with coatings of varying wetting properties that suffer from various defects such as low mechanical durability, low adsorption or absorption rates of liquid molecules, low droplet repellency, low thermal conductivity, toxicity and environmental hazardness [1]. The present work is part of an ESA OSIP research project on the synthesis of novel fluorine free, superhydrophobic, water repellent and highly durable coatings that will enhance air dehumidification in spacecrafts. Synthesis attempts involve the polymerization of different free monomers/oligomers (silanes, siloxanes, acrylics and/or epoxy resins) and the addition of nanoparticles (i.e. Ca(OH)₂). Thin coatings are applied on metallic rectangular substrates (i.e. aluminum, copper, stainless steel) of varying roughness and thermal conductivity that act as the condenser surface of a heat exchanger. As a primary stage of this

study, preliminary wetting experiments are conducted to examine the parameters that may affect dynamic spreading/sliding performance of a condensed water droplet on an uncoated copper substrate, under the effect of complex body forces. For this purpose, Kerberos, a special custom centrifugal device, is used to change the normal and tangential components of forces applied on air/droplet/substrate contact line, due to the combined rotation and tilting of the substrate at different speeds and tilting angles. Three Wi-Fi cameras view the X, Y, X, Z, Y-Z areas of the droplet and record its 3-D motion [2]. To simulate vapor condensation, non-isothermal conditions are applied between the water droplet (30°C and 82°C) and the copper substrate (14°C and 30°C, respectively). Results show that dynamic contact angles are not affected by the initial droplet temperature, however they are influenced by the initial substrate temperature. This is due to the instantaneous temperature equalization of the droplet contact area and the solid substrate. Further experiments show that cooling down the solid substrate by 16°C, triggers both droplet spreading and sliding at lower rotation speeds and tilting angles. In future, the outcome of this preliminary research will be compared to data on the wettability of novel coated surfaces.

1. D. Westheimer & G. Tuan, Proceedings of the 43rd AIAA Aerospace Sciences Meeting and Exhibit, American Institute of Aeronautics and Astronautics, Reno, Nevada, US, 2005.

2. S. Evgenidis et al., Colloids and Surfaces A: Physicochemical and Engineering special issue (S.I. Victor Starov), 521, 38-48, 2017. ●

LAURENT POUGHON

Professor - Université Clermont Auvergne - France

TOPIC: PROCESS MODELING, SIMULATION AND CONTROL

Model structuration and review for MELISSA knowledge and control.

The MELISSA (Micro-Ecological Life-Support System Alternative) loop is the European research project on regenerative life-support system (LSS). It is composed of multiple compartments and can be represented as an integrated sum of interconnected unit operations into a circular system. In order to reach a robust performance and control of the loop, these operations and interrelations need to be deeply understood. This entails to study in the same perspective, with the same degree of accuracy and with the same knowledge level and concepts, organic waste degradation, water recycling, atmosphere revitalisation, and food production sub-systems. A structured view of the regenerative LSS functioning is a step-by-step integration of unit operation models and thus a mechanistic approach is followed to model each compartment of the loop. These mathematical models are

based on physical, chemical, and biological description of the underlying phenomena, and lead to process simulation. An effort is currently on-going to harmonize and improve mechanistic models of the MELISSA loop. This presentation gives an overview of the state of the art of the models within the MELISSA loop, for each compartment, detailing current modelling status and developments that are needed for a robust modelling architecture. Ultimately these models will need to be organised into several hierarchical levels with a decision system and interfacing with a human environment. This study is part of the VARSITY (VARiUS Integration of system sTUDY for model-based cybernetics for the control of complex systems) and OSCAR (Optimal System-in-System Control and Architecture) projects, which are funded by the European Space Agency. ●

CO-AUTHORS: Laurent Poughon, Lucie Poulet, Philippe Fiani, Olivier Gerbi, Benjamin Thirion, Marco Gatti, Erik Mazzoleni, Lorenzo Bucchieri, Claude-Gilles Dussap

TINH VAN NGUYEN

PhD student - KU Leuven – Belgium

TOPICS: WASTE RECYCLING, CIRCULAR ECONOMY

Characterization of a promising thermophilic chain elongating bacterium isolated from a MELiSSA waste compartment reactor, *Thermocaproicibacter melissae* gen. nov., sp. nov. for n-caproate production utilizing polymeric carbohydrates

Medium chain carboxylic acids (MCCAs) such as n-caproate find their many important applications as antimicrobials, perfumes, pharmaceuticals, food additives, and especially precursors of biofuels. The biological conversion of organic waste to MCCAs by chain elongating bacteria in anaerobic digestion (AD) brings important economic and environmental benefits. Production of MCCAs from organic waste in thermophilic AD has more advantages as compared to mesophilic AD, but the involved organisms are unknown. This study isolated and characterized the first thermophilic chain elongating bacterium. Strain MDTJ8 was isolated directly by plating from a thermophilic acidogenic reactor (a MELiSSA waste compartment reactor) producing n-caproate from human waste. Its genome sequence

demonstrated that MDTJ8 represents a novel genus within the Oscillospiraceae family and it was designated as *Thermocaproicibacter melissae* MDTJ8T. The genome size is remarkably smaller compared to mesophilic Oscillospiraceae chain elongators but contains conserved genes for chain elongation and energy conservation. MDTJ8T grows optimally at 50-55°C and pH 6.5. It ferments monomeric, dimeric but also polymeric carbohydrates like starch and hemicellulose to produce n-caproate and n-butyrate via chain elongation and acetate, formate, and lactate via mixed acid fermentation. MDTJ8T is of interest for elucidating the biology, biochemistry, and ecology of thermophilic chain elongation in a circular bio-economy context. ●

CO-AUTHORS: Jonah Mortier, Bin Liu, Ilse Smets, Kristel Bernaerts, Karoline Faust, Laurent Poughon, Claude-Gilles Dussap, Rob Lavigne, Tomeu Viver, Springael Dirk.

TOM VERBEELEN

PhD student - Belgian Nuclear Research Center – Belgium

TOPICS: GREY, YELLOW, BLACK WATER RECYCLING, WASTE RECYCLING, LIFE SUPPORT ARCHITECTURE FOR FUTURE MISSIONS

The Effect of ISS-like Ionizing Radiation and Microgravity on the Transcriptome of N-cycle Bacteria

In this paper, we focus on the third compartment (CIII) of the MELiSSA loop. This section is currently populated by a co-culture of *Nitrosomonas europaea* and *Nitrobacter winogradskyi* converting NH_4^+ into NO_3^- in a two-step oxidation process. The effluent from CIII serves as a N-source for biomass production in downstream compartments. In the perspective of direct treatment of urine in CIII, an additional ureolytic strain that hydrolyses urea to NH_4^+ needs to be included in the co-culture of the nitrifiers. Preparatory terrestrial experiments for a spaceflight proof-of-concept experiment for nitrification in space with a tripartite community with *C. testosteroni* as the ureolytic partner were conducted. Using RNA-sequencing (RNA-Seq), we were able to determine effects of ISS-like ionizing radiation (IR) and microgravity on their whole transcriptome profile. Bacteria were irradiated with a neutron source (Cf-252) over the course of a 3-day experiment at a dose rate of 0.5 mGy/h, approaching the total radiation dose the samples would be exposed to during a 130-day mission in the ISS (36 ± 7.2 mGy). Neither ureolytic genes in *C. testosteroni* nor nitrification genes in *N. europaea*, *N. winogradskyi* nor both gene types in the tripartite culture were affected when exposed to low-dose IR. However, RNA-Seq results did put forward increased expression of oxidative stress response genes and inhibition of genes associated to the production of reactive oxygen species. Hence, ISS-like IR appears not to be a limiting factor to the ureolytic and nitrification activities of the strains while it

triggered oxidative stress to some extent. *N. europaea* and *N. winogradskyi* were also grown under low-shear modelled microgravity conditions in a Rotating Wall Vessel (RWV) and a Random Positioning Machine (RPM). Results clearly showed that RPM simulated microgravity has a greater (yet overlapping) effect compared to the RWV on the transcriptomic profile. In *N. europaea* RPM microgravity, downregulation of *amoA2/amoB2*, coding for ammonia mono-oxygenase, was observed. This protein partly catalyzes the oxidation of NH_4^+ . Inhibition of RuBisCO activators *cbbO/cbbQ* was observed in both RPM and RWV microgravity conditions. Ammonium oxidation could be affected by localized NH_4^+ starvation. In *N. winogradskyi*, RWV and RPM microgravity prompted underexpression of the RuBisCO operon and translation genes. Cell division, replication, transcription and translation genes were also inhibited. Moreover, a shift towards nitrate reduction instead of nitrite oxidation possibly occurred. Indeed, upregulation of *nxrB2*, responsible for both nitrite oxidation and nitrate reduction, and increased production of pyruvate, the electron donor in nitrate reduction, were observed. This could be a consequence of localized nitrite deprivation, causing the cell to assimilate alternative nutrients for its energy production. In conclusion, simulated microgravity affected nitrification related gene expression in both *N. europaea* and *N. winogradskyi*. The impact of these results on future implementation of CIII in the MELiSSA loop will be discussed. ●

CO-AUTHORS: Tom Verbeelen, Celia Alvarez Fernandez, Thanh Huy Nguyen, Siegfried E. Vlaeminck, Nico Boon, Baptiste Leroy, Ruddy Wattiez, Natalie Leys, Ramon Ganigué, Felice Mastroleo

QUENTIN ROYER

Student - ISAE-SUPAERO – France

TOPICS: GREY, YELLOW, BLACK WATER RECYCLING, WASTE RECYCLING, LIFE SUPPORT ARCHITECTURE FOR FUTURE MISSIONS, SOCIETAL IMPACTS AND EDUCATION

Analog mission to test life support systems for future manned missions

In February 2023, we will take part in an Analog Mission in the Mars Desert Research Station (MDRS) which will last for 4 weeks. The MDRS is a research facility located in Utah (USA) where analog missions take place to prepare for future manned missions on Mars or the Moon. The MDRS can host a crew of seven analog astronauts. Each one has a specific role: a botanist, a biologist, a geologist, an engineer, an astronomer, a health and safety officer, and a commander. There are several facilities in the base: an observatory, a greenhouse, a workshop, and a laboratory. The crew lives in a closed environment, almost completely isolated from the outside. Each year and for more than 5 years, ISAE SUPAERO students have led missions lasting between two and four weeks. During our mission, we follow rigorous rules to make the simulation as realistic as possible: we live with a limited amount of water, of resources, and are almost completely isolated from the outside. Moreover, the food that we eat is freeze-dried. The crew lives as real astronauts on Mars: it is compulsory to perform an EVA wearing a spacesuit, and emergency situations are often simulated in the station (depressurization, injured astronaut in an EVA). The mission aims to perform scientific experiments in a closed environment, test new technologies that will have a spatial application (LIBS technology), and also live in a closed loop environment for 3 to 4

weeks, with restricted resources that may be recycled. Previous crews have led experiments on waste recycling. They used the AquaPad technology during the mission to check whether is water was drinkable. Another experiment studied the possibility to grow spirulina with the help of urine as a biofertilizer. During the next mission (Crew 275), which will happen in February 2023, we want to focus on closed-loop experiments, on two main axes: micro and macro environment. On a macro-environment scale, we will strongly monitor our use of resources (water, food, and dioxygen) and set up experiments (along with researchers) to measure the amount of waste that can be recycled and reintegrated into the loop. We may test a prototype to regenerate dioxygen from carbon dioxide. We also may measure the amount of water that we get from condensation in the air, and test a prototype for this usage in real conditions during the mission. Finally, waste recycling could allow us to produce fresh edible food to eat during the mission. On a micro-environment scale, we will set up a closed loop containing plants, bacteria, and induced chemical reactions. This will allow us to measure the efficiency of each step of the closed loop during the four weeks of the mission. ●

Keywords : Analog Mission, Closed-loop environment, Life Support System simulation

CO-AUTHORS: Alexandre VINAS, Alice CHAPIRON, Marie DELAROCHE, Corentin SENAUX, Adrien TISON

PIERRE FONTANILLE

Director - BIO-VALO – France

TOPIC: WASTE RECYCLING

Lift off biogas industry : BIO-VALO, the pilot test platform for your projects

BIO-VALO is a French innovation platform for industrials and research centers allowing the realization of studies, pilots and / or demonstrator development, dedicated to the transformation and valorisation of organic matter. Thanks to its fully equipped laboratory with 36 bioreactors of laboratory capacity (2L) and its 250L and 1m3 pilots, BIO-VALO works alongside its partners for innovation, new technology development and research, to improve the transformation and valorisation of organic matter into Biogas and biomolecules. BIO-VALO platform is positioning between 5 and 8 of TRL, allowing to test and certified the performance of green tech innovations at a semi-industrial

scale. Drawing on the know-how of its team and its network of French and European experts from industry or research, BIO-VALO will be at your side to find innovative, effective and robust solutions to improve your project. As green deep tech company, BIO-VALO developed innovative technologies related to the challenges of the future: green processes, respectful of the environment and with a positive impact on greenhouse gas emissions. BIO-VALO works in close collaboration with the Pascal Institute of the Clermont Auvergne University, which has been working for more than 20 years on the Melissa project with the European Space Agency.

CO-AUTHORS: Pierre FONTANILLE, B. CHEZEAU, CG DUSSAP

LUIGI GIUSSEPPE DURÌ

Unina - Italy

TOPIC: LIFE SUPPORT ARCHITECTURE FOR FUTURE MISSIONS

Advancement of the PFPU Root Module for the production of tuberous species in microgravity

Tuberous crops such as potato (*Solanum tuberosum* L.) and sweet potato (*Ipomoea batatas* L.) are among the leading food crops around the world. Based on technical and dietary criteria, including environmental requirements, yield potential, and nutritional value these crops are candidate crops for Bioregenerative Life-Support Systems (BLSSs) in Space. The design of a Root Module (RM) for growing tuberous plants in microgravity was one of the goals of the ESA-MELiSSA 'Precursor of Food Production Unit' (PFPU) project. The RM is a closed unit that consists of a growing media (Root Zone - RZ) with an upper headspace for tuber growth (Tuber Zone - TZ). Results obtained in the preliminary stages of the project showed the potential use of sponges (by comparative tests of different growing media) as coherent substrate to grow tuberous plants in microgravity conditions. Management of RZ water content and the TZ air relative humidity is one of the main critical issues to be considered, as well as containing contamination by fungi and bacteria of the substrate. In this regard, polyvinyl acetate (PVA) sponge has proved to be particularly effective both in terms of water holding capacity (by absorbing 25 times its dry weight) and its ability to inhibit microbial proliferation (being properly engineered for medical applications). However, preliminary results showed that high air relative humidity in the TZ caused necrosis of shoots apex and of the stolons. For this purpose, new RM configurations, designed to reduce humidity levels in the TZ, were tested. The experiment consisted of growing potato plants

(cultivar Colomba) using PVA sponge as substrate in four different RM setups: a control (C) where the RM is without the lid (fully controlled air humidity of the TZ, but definitely not applicable due to greening of tubers exposed to light), a RM with a polyethylene film (PE) lid (light- and gas-proof), a RM with a polyester fiber (PF) lid (light-opaque but gas-permeable), and a RM as PE but with an active ventilation system (AVS) that allowed constant air-exchange in the TZ. All lids had an incision in the middle that allowed the emergence of shoots. The test was carried out under controlled conditions (light intensity and quality, photoperiod, air relative humidity, and temperature), while fertigation was delivered every two days by injecting the nutrient solution into the microperforated tubes embedded in the sponge. During the experiment, the volumetric water content of the substrate and the air relative humidity and temperature of the TZ were monitored. The main results revealed higher water consumption in PF and AVS than in PE, but lower than in the C design. Moreover, the air relative humidity of the TZ in PF and AVS was significantly lower than that in PE. The best performance was obtained in the AVS design, where the plants were totally comparable to those grown in C (TZ relative humidity equal to the room environment). The test findings achieved, and the data collected, generated relevant inputs for the future development of the ultimate flight design of the RM.

CO-AUTHORS: Antonio Pannico, Luigi Giuseppe Durì, Youssef Roupheal, Stefania De Pascale

ENRIC GARCIA TORRENTS

MD-PhD student-researcher - Medical Anthropology Research Center - Spain

TOPIC: SOCIETAL IMPACTS AND EDUCATION

Open-source cellular agriculture and other one health citizen lead projects

The presentation will focus on Shojinmean, a cell-based meat nonprofit project, as a prime example of how technologies used in space exploration can and hopefully will be used to help cover basic needs on Earth. The presentation will tap on that and other experiences by citizen science collectives, open

software and open hardware projects, biohacking spaces, and activist-cientist communities devoted to the sustainable solution of one health and development problems, such as Scientist Rebellion.

ROSA SANTOMARTINO

Research fellow - University of Edinburgh - School of Physics and Astronomy - United Kingdom - oral

TOPICS: WASTE RECYCLING, CIRCULAR ECONOMY

Plastic recycling in space using microorganisms: a potential tool to close the loop

Plastics are indispensable in our everyday life, as well as in the construction, food and pharmaceutical industries, owing to their durability and adaptability. However, plastic accumulation in soils, marine environments and rocks poses a serious threat to

ecosystems. Beyond Earth, plastics play a key role too, due to their resistance and capacity to withstand harsh space conditions. Raw materials to produce new plastic consumables, e.g., fossil fuels, are not available in space, and bioplastic

production would probably suffer limitations, as the biomass required would be necessary for multiple aims (e.g., food). From this perspective, plastic recycling in space will be pivotal to upcycle carbon, produce new consumables, obtain feedstock and reduce waste production. Recycling and reuse of materials by using a closed-loop system is key for a self-sustainable human presence in space, not only to minimize the resupply of resources from Earth, but also for ethical considerations associated with space waste generated by the human presence [1] [4]. The biggest limitation is the lack of technologies enabling in-situ resource utilisation (ISRU) and indefinite recycling of materials [2]. A promising approach includes the use of microbiologically supported processes, to provide viable and efficient methods not only for human waste recycling, but also for upcycling of consumables, including plastics. Microorganisms have been shown to break down microplastics into organic nutrients to support growth, a process called biodegradation that offers exciting paths towards a circular bioeconomy [5]. Synthetic biology approaches have also been used to engineer microorganisms to produce useful molecules starting from plastic waste [6], [7]. Plastic-biodegrading microorganisms could therefore become promising components of extraterrestrial outposts. However, microorganisms respond unpredictably to space conditions [8] and can have deleterious effects on materials and crew [9]. Therefore, their potential use in human space exploration for novel applications, such as

plastic biodegradation, needs to be investigated. The Leverhulme Trust funded research project presented here aims to study the cellular and molecular mechanisms of microbe-mediated plastic biodegradation under space conditions (e.g., simulated microgravity), which will be pivotal to establish space biotechnologies. Although not providing products that are traditionally included in regenerative life support systems (e.g., food, oxygen, water), such space biotechnologies would produce primary and secondary products that could sustain biological compartments in life-support systems, and vice versa [10]. Moreover, it has the potential to be transferred and pose benefits for terrestrial applications, too, addressing crucial environmental issues. The addition of a plastic-recycling compartment could therefore be a beneficial addition to further close the loop of life support systems, and contribute to a circular economy on Earth and in space.

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GIORGIA PONTETTI

CEO - G & A Engineering srl - Italy

TOPIC: FOOD PRODUCTION AND PREPARATION

The SOLE project: a hydroponic greenhouse demonstrator for fresh food production in space

Future human long-term space exploration demands for fresh food production during missions, independently on periodic supplies from Earth. The possibility to grow plants in space positively impacts both astronaut diet and psychological wellbeing. It has been estimated that a facility of a few cubic meters is sufficient to supply the diet of 4 to 6 crew members with key vitamins and fresh bioactive substances. Soilless cultivation is the best suited for production of high quality food in space, where resources are limiting factors. In this context, the project SOLE aimed to realize a hydroponic greenhouse

based on LED lighting systems for growing plants, specifically microgreens, for space applications. The best performing light recipe, in terms of duration, intensity and spectral quality, was identified, based on plant response analyzed by nondestructive morphometric and hyperspectral systems. Data on plant growth and bioactive metabolites accumulation were monitored, in real time and even remotely, and elaborated. The dimension of the SOLE demonstrator can be easily adapted for space applications as growing plant onboard the International Space Station or in mini-/micro-satellites.

CO-AUTHORS: E. Bennici¹, E. Benvenuto¹, M. Crisconio², A. Desiderio¹, E. Di Mascio³, Francesca Ferranti², M. Garegnani^{1,4}, L. Giuliani¹, L. Nardi¹, C. Pacelli², G. Pontetti², M. E. Villani¹

THIEME HENNIS

Mission Commander - AstroPlant - Netherlands

TOPICS: FOOD PRODUCTION AND PREPARATION, CIRCULAR ECONOMY, SOCIETAL IMPACTS AND EDUCATION

AstroPlant - an educational citizen science architecture for plant characterisation

For future human space exploration, food delivery will be an issue. Current space food products have a shorter shelf-life than the anticipated Mars mission duration and limited alternatives to increase this shelf-life. However, current technology development for plant growth during spaceflight is on its way, with a high potential to link food production and regenerative life support systems for overall higher sustainability. A challenge

of plant production in space is the lack of data on plant growth (for food or oxygen in regenerative life support systems like MELiSSA) due to the low number of ground facilities available to perform experiments. This results in limited predictability of plant growth and performance. MELiSSA addresses this challenge through the development of structured mathematical models and the validation of the mechanistic part of the model

in state-of-the-art infrastructure. However, while state-of-the-art infrastructure is a powerful tool, its use for the validation of the statistical part of the model is inefficient in terms of time and cost. Data generation on plant growth behaviour under varying environmental conditions can be accelerated by developing a simplified plant growth facility version and deploying a high number of them (i.e. a statistical approach). AstroPlant is a novel IT infrastructure and network of plant growth chambers that complements the existing state-of-the-art infrastructure, and proposes to address the validation of the statistical part of the mathematical model at lower cost and potentially faster and to support rapid testing and experimentation before moving an experiment to a highly controlled facility such as PACMAN. Additionally we will look back into the past of AstroPlant, which has a predominantly educational focus. We will highlight several educational experiences and projects, and explain how AstroPlant is used as an educational tool from elementary to university level

CO-AUTHORS: Daniel Steginga, Cecile Renaud, Dafni Agoustaki, Antonio Pannico, Christel Paillé

MARION DUGUÉ

Student researcher - TU Delft – Netherlands

TOPICS: WASTE RECYCLING, LIFE SUPPORT ARCHITECTURE FOR FUTURE MISSIONS, SPACE EXPERIMENT

Evaluating the use of menstrual blood-derived cell therapy to support astronauts in long-term space missions

Stem cell transplant is currently in its early age but offers great potential to prevent or treat damages such as muscle atrophy or tissue damage due to exposure to radiation. This is specifically interesting for space flights where, due to micro-gravity, astronauts experience up to a 20 percent loss of muscle mass on spaceflights lasting five to 11 days, as well as the increased exposure to ionized radiation. Menstrual blood derived stem cells have shown a lot of potential for cell therapy because of their multilineage differentiation, self-renewal, and secretion of growth factors. Their plasticity and safety for cell therapy is specifically interesting in the context of long-term space missions and multi-planetary civilisation. Currently, menstruating astronauts decide to pause their periods during their missions as the current apparatus in spacecrafts are not adapted to female hygiene, specifically menstruation. Whilst

education. We propose to organise a session that connects past, present and future of AstroPlant and to discuss AstroPlant as a scientific approach enabling MELISSA (and other researchers) to do quick experimentation and gather data for plant modelling. This work is currently being done by 5 universities directly or indirectly participating in the MELISSA consortium. We will also look back and introduce practical applications of AstroPlant in educational contexts. The 60-90min session will be organised as follows: 1. Introduction on AstroPlant's past, present, and future; 2. Round of brief presentations where AstroPlant (and MELISSA) partners explain their plans and activities concerning AstroPlant, followed by questions from the audience. The participating partners include University of Mons, Agricultural University of Athens, University of Napoli Federico II. 3. A 15-20 minutes panel conversation with the audience about the potential, prospects of AstroPlant, and about the individual experiments. ●

urine and faecal matter are collected in separate liquid and solid compartments to be re-used and transformed, it is essential to take the advantage of a natural and regular process of stem cell production. This literature review will explore the pipeline from menstrual blood collection, cultivation in micro-gravity and the transplant of the isolated stem cells, all in the context of space flights. The outcome of this work is to assess the medical and engineering feasibility of menstrual blood-derived cell therapy, using osteopenia as a case study. Special focus will be put on the gaps in knowledge and unmet challenges to build a research agenda for every step of the pipeline to develop an end-to-end apparatus for menstrual-blood derived stem cell therapy for spacecraft utilization. This is foundational research for combining regenerative medicine and regenerative life support systems to support long-term space exploration. ●

LOUISE FLEISCHER

Executive Director – Spring - France

TOPICS: LIFE SUPPORT ARCHITECTURE FOR FUTURE MISSIONS, SOCIETAL IMPACTS AND EDUCATION, SPACE EXPERIMENT

Autonomous complex biospheres in space : moral grounds, historical perspectives and a way forwards

Human space exploration is living through a renewed interest in the general public and state fundings, and some multi billion dollars international companies claim a multiplanetary human species as a company vision. With a never-seen before investment from private investors and the massive appearance of start-ups in the sector, the story telling of human exploration is being steered towards an American narrative of entrepreneurs in lust for grandeur. The current dominating vision of human expansion, driven by American billionaires, is littered with an industrial and productivity-bound views of exploration, merely carrying the environmental errors of which we are only starting to see the consequences here on Earth. There is a definite need

for a more life-centered, less techno- and anthropocentric approach to space exploration that would include biodiversity in every steps. On one hand, going away purely utilitarian view of space expansion is the only way to envision a space sector appealing to new climate-crisis-sensitive young professionals. On the other hand, the space expansion is only worthwhile if life itself is carried away, in a sense that is way broader than humanity alone. Developing complex multi-species biospheres with high levels of autonomy and very well controlled interfaces is still a research domain in progress, let alone sending them to space. Nonetheless, promising experimentations have been carried on Earth and in space in the past decades, and a way

forward can also be set by leveraging the current interest in space tourism. A historical review of autonomous biospheres experiments on Earth or in space is presented here, and some lessons learnt are drawn. It is for example emphasized that major experiences - such as biosphere 2 - could not yet join scientific rigor to systematic approach and large ambitions, despite enhancing the importance of cross-species interactions; that most space experiments target mono-species with non-zero interactions with an outside environment (notwithstanding the recent Plantsat mission); that Earth-based experiments do show promising results that could be easily replicated in space to assess their viability, should enough funds be gathered. The recent trends in space miniaturization and standardization (the so-called CubeSat revolution) and in space tourism let us foresee

CO-AUTHORS: Dr Florian Marmuse; Mr Anatole Trepos

GAËTAN GRECO

Euro Space Center – Belgium

TOPIC: SOCIETAL IMPACTS AND EDUCATION

Mars Camp - How to raise awareness of STEM through the topic of space

With more than 1.5 million visitors welcomed and 500,000 training days already completed by individuals from 30 nationalities over the last 30 years, the Euro Space Center (ESC) has become a key player on an international scale when it comes to raising awareness and educating people about space and related sciences. The ESC is ideally located in the heart of the GALAXIA business park, which is dedicated to space industry and connected to the ESA space hub in nearby REDU. GALAXIA-REDU is the result of a partnership with the ESA and benefits from the presence of leading actors in the field of space and related activities (e.g. SES, TELESPAZIO, RHEA). The ESC has developed a diverse programme of courses for youngsters from 6 to 18 of age, designed to raise their awareness of STEM through the topic of space. Keeping up with current events, the ESC's latest project is the creation of a Mars Camp. The idea is to explore the various technologies needed to survive on Mars from the perspective of how they might find a use on Earth, particularly with respect to our imprint on ecosystems. At this stage, the project is being subsidised by a grant from the Walloon region, worth ~ 120,000. The facility for the Mars camp will cover approximately 250m² to accommodate 40 to 50 people simultaneously, split in activity groups. It will be based on a plot of land next to the ESC and modelled on the Martian landscape, taking advantage of the red clay naturally present in the area. The buildings will aim to be net zero carbon and self-sufficient, energy-wise. The activities proposed have been analysed by an educational team that has studied martian

a path forward for complex biospheres in space, featuring biospheres in CubeSats, in private space stations and on Moon and Mars bases. A first concept of autonomous biosphere in a CubeSat is presented, focusing on cost-reduction and performance monitoring. Several possible Earth applications, bridging terrestrial and spatial applications, are discussed. In order to promote an alternative vision for space exploration, away from the American techno- and anthropocentrism, we recommend to include elements of biodiversity in all space exploration programs: the recent announcements of several private or public projects of crewed stations in LEO or lunar orbit in a new opportunity for concrete projects, such as the one sustaining the Spring endeavour. ●

mission analogs, particularly those taking place in the Mars Society's Mars Desert Research Station. At this stage, workshops are expected to be held in four modules organised around one central module used for gatherings and briefings:

1. Laboratory module: identification of rock samples and understanding biological adaptations to conditions on Mars, microscopic observations;
2. Technical workshop module: producing electricity using photovoltaic cells, electrolysis, storage and use via fuel cells;
3. Greenhouse module: Providing a biological basis for Life Support Systems, including production of food through hydroponics or aquaponics, testing and measuring the photosynthetic yield of green plants and algae;
4. Airlock module: simulating extravehicular activities in a recreation of a martian landscape. Trainees will carry out scientific or technical experiments, taking samples and searching for water or ice underground. They will also discover the possibilities of technological tools for exploration like drones and mobile machines. They will work while keeping contact with small teams indoors, who will have a roadmap of operations and archive the measurements taken. In the end, three training programmes were devised, ranging from a single day-long introductory visit to a full immersive session stretching on 5 days.

Presenting this project to the MELISSA community is an opportunity to meet industry stakeholders and demonstrate the quality of the scientific work we seek to carry out. ●

DANIEL YEH

Professor of Environmental Engineering - University of South Florida - United States

TOPICS: AIR RECYCLING AND CO2 VALORIZATION, GREY, YELLOW, BLACK WATER RECYCLING, WASTE RECYCLING, FOOD PRODUCTION AND PREPARATION, LIFE SUPPORT ARCHITECTURE FOR FUTURE MISSIONS, CIRCULAR ECONOMY

The membrane bioreactor (MBR): A hybrid technology for bioregenerative wastewater treatment and resource recovery in space

Extraterrestrial surface habitat life support systems (LSS) on the Moon and Mars, as well as long-duration space travel, will require novel capabilities to withstand anticipated unique, harsh conditions. In order to provide safe, habitable environments for the crew, water purification and waste

processing systems will be required to treat all sources of water (condensate, Sabatier, urine, hygiene, fecal, food waste) in order to achieve the necessary levels of recovery needed to sustain life over the long-duration missions. The ability to recycle organic wastes creates an opportunity to recover critical elements (e.g.,

C, H, O, N, P) for subsequent food production, water purification, and atmospheric regeneration. Bioregenerative systems mimic functions of nature in engineered systems, or bioreactors, utilizing combination of prokaryotes, eukaryotes and archaea. While these systems are commonly used on Earth for wastewater treatment, bioreactors for space travel face additional challenges. Terrestrial bioreactors often rely on gravitational settling of dense flocs and granules for cell retention. For micro- or partial-gravity environments, density differential alone will not be adequate for cell retention; a gravity-independent means for cell retention is crucial. The membrane bioreactor represents the state of the art in wastewater treatment. This hybrid system combines biological processes with membrane filtration to achieve performance beyond what each can accomplish individually. The complete cell retention in an MBR allows for the decoupling of hydraulic retention time (HRT) and solids retention time (SRT), which result in a high-throughput, compact, treatment system. The Bioregenerative Water Technology Team at NASA Kennedy Space Center and the University of South Florida has developed a bioregenerative platform based on the hybrid MBR technology. The overall architecture is compact, modular, flexible, and adaptable to

CO-AUTHORS: Luke Roberson, Talon Bullard, Alexandra Smith, Daniella Saetta, Jason Fischer, Melanie Pickett

KATO CLAEYS

Architect Engineer - Catholic University of Leuven (KULeuven) - Belgium

TOPIC: LIFE SUPPORT ARCHITECTURE FOR FUTURE MISSIONS

Analog astronaut habitats and space simulation systems

As mankind reaches further beyond Earth, the duration of space missions are getting longer. The renewed interest in going to the Moon is strongly related to the desire of Mars exploration by human beings. Moon habitations are not yet common business. In history there was only one kind of Moon habitation, which was the Apollo Lunar Module, in which astronauts could stay for a maximum of a few days. These pioneering activities date back from more than 50 years ago. Since the Apollo program no humans have returned to the Moon. Analogue space missions are used to simulate space environments with the purpose to understand the psychological effect between crew members that arise during long-duration missions. The space available and the layout can have a major influence on their interactions. Therefore, it is necessary to understand the way crew members work together and the spaces they require to work productively and efficiently, to feel comfortable and motivated and to relax. An extraterrestrial habitat must respond to several harsh environmental space conditions. This has a direct influence on the crewmembers and their mental health. A habitat underground leads to different environment perception than a habitat above the ground. There are different space analogues who are dedicated to diverse environmental conditions. The way of living in space is different because the spaces are smaller and the way of moving around is different because of the lower gravity. Most of the space analogue habitats are mainly focused on a specific environmental condition. The habitats that are

CO-AUTHORS: Kato Claeys¹, Lars De Laet², Sarah Baatout^{3,1}

mission evolutions. The main subsystems of the bioregenerative architecture include: 1) Anaerobic membrane bioreactor (AnMBR): Also termed the Organic processor assembly (OPA), the function of the AnMBR is to treat organic wastes such as fecal and food wastes. These wastes are characterized by a concentration of suspended solids comprised of carbohydrates, proteins and lipids. The assigned function of the AnMBR is to break down and convert suspended solids to biogas (methane, hydrogen and carbon dioxide), reduce effluent chemical oxygen demand (COD), liberate organically-bound nutrients, and remove pathogenic organisms. 2) Phototrophic membrane bioreactor (PMBR): The PMBR is comprised of a co-culture of microalgae and bacteria. The assigned function of the PMBR is to polish the permeate of the AnMBR to further remove dissolved organic carbon, manage nutrients (nitrogen transformation, load dampening), and perform air revitalization. 3) Food processor assembly (FPA): The FPA is a food production platform (prokaryotic or eukaryotic), fueled by outputs from the AnMBR, or PMBR. For the presentation, we will describe each step of the bioregenerative architecture, and present performance data from extended trials treating analog and real metabolic wastes. ●

dedicated to Mars simulation are Mars500, FMARS (Flashline Mars Arctic Research Station) and AMASE (Arctic Mars Analog Svalbard Expedition). The NEEMO missions in the Aquarius habitat focusses on EVA in the water. CHILL-ICE is focused on living in lava tubes, where the Chinese research facility Yuegong is focused on growing vegetation. There are also space analogues in Antarctica such as the Concordia Station or the Belgian Princess Elisabeth Station where the living conditions are extreme. Another example of an analog space habitat is the EMMPOL habitat in Poland provided by the Analog Astronaut Training Centre (AATC, Krakow, Poland) and by the EuroMoonMars Society. During those space simulation missions, crews are isolated from the outside and are performing a multitude of experiments. The crew size goes up to six analogue astronauts. The habitation environment was analysed in the EMMPOL habitat via questionnaires, filled in by the crew members from EMMPOL10 and EMMPOL11 crews. A thorough analysis of the distance between crew members was measured through the proximity experiment. During this presentation, possible lunar habitats, their habitability and the results of the EMMPOL proximity experiment will be reviewed. Acknowledgements : K. Claeys is supported by KULeuven, SCK CEN and EuroMonMars and holds a MSc in architectural engineering (ULB, Belgium) and an advanced MSc in Space Studies (KULeuven, Belgium). ●

MARIA-GABRIELLA SARAH

Partnership Analysis and Development Officer – ESA – Netherlands

TOPIC: SOCIETAL IMPACTS AND EDUCATION

The MELiSSA Project in the ESA_Lab@ Initiative: A Brainstorming Platform Promoting European STEM talents

The ESA_Lab@ initiative, a cooperation platform initiated in 2017, aims to establish solid institutional links between the European Space Agency, ESA Member States academia, and national research institutions. This partnership aims to attract and educate young and talented people, encouraging them to get involved in space activities while benefiting ESA's research topics. Since its creation more than thirty years ago, the Micro-Ecological Life Support System Alternative (a.k.a. MELiSSA) project has relied on networking with multidisciplinary academic, scientific, and engineering partners from multiple European countries. The project considers it essential to inform and educate about the challenges and difficulties, approach, and results. Due to its long-term perspective, the project naturally seeks collaboration with prestigious European universities and academic institutions to progress in specific key research areas.

CO-AUTHORS: Chloé Audas, Franck Marle, François Cluzel, Stéphanie Lizy-Destrez.

MELiSSA has progressively grown into a brainstorming platform welcoming and promoting young European STEM talents, from Master's level to Postdoc positions. In the frame of the ESA_Lab@ initiative, MELiSSA has established strong ties with the French Engineering Schools CentraleSupélec (Paris-Saclay) and ISAE-SUPAERO (Toulouse). Leveraging its multi-disciplinary approach and dual space and terrestrial applications, MELiSSA strives, in the long-term, to foster cooperation with other ESA_Lab@ entities sharing the same research interests and will thus further support the growth of the whole ESA_Lab@ network. This presentation will include an introduction of the ESA_Lab@ initiative and an overview of the ongoing outreach and education activities proposed under the umbrella of the MELiSSA project at ESA_Lab@CentraleSupélec and ESA_Lab@ISAE-SUPAERO, opening the door for future educational collaborations. ●

OLIVIER GONÇALVES

Professeur - Nantes Université - Laboratoire GEPEA UMR CNRS 6144 – France

TOPICS: AIR RECYCLING AND CO2 VALORIZATION, GREY, YELLOW, BLACK WATER RECYCLING, WASTE RECYCLING, BIOMATERIAL, FOOD PRODUCTION AND PREPARATION, PROTEINS PRODUCTION, MICROBIAL SAFETY, PROCESS MODELING, SIMULATION AND CONTROL, LIFE SUPPORT ARCHITECTURE FOR FUTURE MISSIONS, CIRCULAR ECONOMY, SOCIETAL IMPACTS AND EDUCATION, SPACE EXPERIMENT

The assessment of microalgae biochemistry through NMR non-invasive approach offers new perspectives for their monitoring in photobioreactors.

The sustainable production of lipids by microalgae is widely developed among the bioprocess community targeting various applications such as feed, food, health or bioenergy. The cultivation of microalgae needs dedicated systems with the optimal enlightenment geometry. Performing non-invasive online analyses on these bioprocesses is limited to few analytical techniques, often based on optical properties and can however rarely be related to intracellular products. The real-time knowledge of the lipids accumulation in microalgae is in this case not possible. In the present communication, the proof-of-concept that the recent benchtop NMR spectroscopy device can be used for the non-invasive and selective detection of lipids inside microalgae cells is carried out following the experimental set up illustrated in figure 1

Figure 1: coupling between a photobioreactor (PBR) (Parachlorella kesslerii culture) on the left and a benchtop spectrometer (grey) on the right using a peristaltic pump (highlighted in blue). The loop connects the PBR with capillaries (accentuated in full green line) to a smooth quartz tube (accentuated in dashed green line). The sensitive volume of NMR is represented in red. More precisely, we will demonstrate in the present communication how a compact NMR spectrometer connected to a PBR can measure, in real-time and in a non-invasive manner, the total lipid concentration, and that directly on the entire cells grown in their culture medium. For the selective removing of the water signal, the W5 version of the WATERGATE pulse sequence will be employed. The experimental results indicated that the NMR signal nicely correlates ($R > 0.99$) with the offline FAME (Fatty Acid Methyl Ester) total lipid analysis

as performed by GC-FID (Gas Chromatography coupled to Flame Ionization Detector) within limits of detection and quantification of respectively of 9 and 30 mg.L⁻¹ (figure 2). The lipid specific signal appears also quite robust regarding the dissolved dioxygen, making the benchtop NMR spectroscopy an appropriate universal device for the online monitoring of lipids produced in bioprocesses.

Figure 2: 3D stacked plot of half the 310 1H NMR spectra for the sake of readability, after phase and baseline corrections. The residual water peak at 4.7 ppm and the significant growth of the main lipid peak at 1.2 ppm over the course of the cultivation are highlighted. Conclusion The present work demonstrates that compact NMR spectroscopy is able to provide a real-time signal which is representative of the total lipid concentration. The relative evolution of this signal allows the in-vivo non-invasive monitoring of the total lipids on microalgae grown under bioprocesses real conditions. Furthermore, the comparison of the NMR data with a quantitative total lipid analysis makes the NMR analysis quantitative, so lipid concentration and productivity values are calculated in real-time. In this study conditions, the limit of detection was measured at 9 mg.L⁻¹ and this limit can be tuned in the case of different bioprocesses. Benchtop NMR present also all the monitoring potential to microalgae cultivation systems. ●

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CO-AUTHORS: Dylan Bouillaud, Delphine Drouin, Jonathan Farjon, Patrick Giraudeau, Olivier Gonçalves

IULIAN BOBOESCU

Assistant Professor - Wageningen University – Netherlands

TOPIC: AIR RECYCLING AND CO2 VALORIZATION

Extracellular conversion of CO2 into sugars and other functional food ingredients: SweetAir

Current food systems are causing a quarter of total carbon emissions and represent the primary driver of biodiversity loss. These approaches need to be completely reconsidered to save what is left of the global biodiversity and reverse carbon emissions. Alternative food ingredients have been successfully produced using bacteria, yeasts and microalgae. However, some of these microorganisms require sugars, organic acids or proteins, which are produced using conventional approaches, further contributing to the problem. Moreover, these microbial cells are limited by a number of factors such as availability of enzymatic substrates and competition over metabolic fluxes. Thus, this work aims to develop an elegant way of bypassing these limitations while decarbonizing our society by directly converting carbon dioxide from the atmosphere into food ingredients using cell-free systems. More specifically, this will be achieved using zeolite carbon scrubbers which will provide microalgal enzymatic extracts rich in enzymes (e.g. RuBisCO)

and substrates (e.g. ribulose-1,5-bisphosphate) with concentrated CO2. The hypothesis is that these extracts, which allow plants to convert CO2 into glucose during photosynthesis, will be several times more active having more substrate to their disposal and being freed from cellular regulation mechanisms. In order to achieve this, novel enzyme extraction and purification approaches based on deep eutectic solvents and external fields such as acoustic and electric will be developed to preserve the structural integrity of these enzymes as well as to provide an environment where their activity is promoted and maximized. Furthermore, the other cellular components such as lipids, pigments, starch and proteins will be recovered through a multiproduct biorefinery approach. The novel insights provided by this project could provide a scalable and more sustainable source of novel food ingredients which could potentially replace current practices and help restore the climate and ecological balance on Earth. ●

CO-AUTHORS: I.Z. Boboescu, A. Kazbar, S. D Adamo, M. Barbosa, M.H.M. Eppink, R.H. Wijffels

MARTA DEL BIANCO

Researcher – Italian Space Agency – Italy

TOPICS: WASTE RECYCLING, FOOD PRODUCTION AND PREPARATION, LIFE SUPPORT ARCHITECTURE FOR FUTURE MISSIONS

The REBUS project in the context of the Italian Life Science Roadmap for human space exploration.

Human space exploration is one of the most effective drivers for scientific research and technological innovation. In view of the steadily growing international relevance of human space exploration beyond Low Earth Orbit, the Italian Space Agency (ASI) is continuously reinforcing the network of the national scientific community and industrial stakeholders for the success of future Moon and Mars exploration missions. ASI has activated four working groups with the participation of national experts from the scientific community on the macro-areas of space life sciences: integrated physiology, microbiology, biological systems for life support, and radiations. The groups have analyzed the current scenario of space life science research and have recently concluded their activities, producing a national roadmap for life sciences in the context of human space exploration. The roadmap has highlighted the need to tackle unsolved issues related to the effects of long-duration space flights and develop the required, enabling technologies. In particular, a central role is played by

those technologies based on biological systems and essential for the creation of a closed, self-sufficient ecosystem for the support of human life. In this contribution, we will present and discuss the challenges and goals identified by the Italian Life Science Roadmap. We will detail how the ReBUS (In-situ REsource Bio-Utilization for life support in Space) project aims at answering many of the open questions regarding the creation of an integrated BLSS (Bioregenerative Life Support Systems). The principles of the ReBUS project are: minimizing the use of exogenous resources; maximizing the recycling of the organic matter produced in the system itself (e.g. crop residues, crew physiological waste); exploiting the use of the in situ resources (i.e. Lunar and Martian soils, water, and gas present in the atmosphere). This is being achieved through an integrated network of organisms: higher plants, fungi, bacteria, cyanobacteria, insects. ●

CO-AUTHORS: Silvia Mari, Francesca Ferranti, Claudia Pacelli, Valerio Vagelli, Sara Piccirillo, Vittorio Cotronei

CYPRIEN VERSEUX

Researcher - ZARM, University of Bremen – Germany

TOPIC: LIFE SUPPORT ARCHITECTURE FOR FUTURE MISSIONS

Assessing the efficiency of cyanobacterium-based BLSS on Mars

A long-term presence on Mars will require being as independent of Earth as possible in terms of material resources. This independence may be reached with the help of biology, which could help perform a wide range of functions with a low impact on the surroundings. However, biological systems would best rely on resources available on Mars as recycling alone would mean that the amounts of available resources decrease over time and most organisms cannot utilize raw Martian resources directly. A solution has been proposed which lies in selected species of cyanobacteria. Their physiology is such that they could, it seems, be fed with materials available on site: water mined from the ground or atmosphere; carbon and nitrogen sourced from the atmosphere; and the local regolith, from which it has been argued that they could extract the other necessary nutrients. The cultured cyanobacteria could then produce various consumables directly, such as dioxygen and dietary supplements but also support the growth of secondary producers (plants or microorganisms) which could, in turn, generate a wide range of critical consumables. Various proofs-of-concept have

been reported in the literature and evidence accumulates that some cyanobacteria could, indeed, be fed from Martian resources and provide feedstock for other organisms of biotechnological interest. But whether a system works at all is not sufficient to decide whether it should be integrated into mission plans: its cost-efficiency must be determined and compared to potential alternatives. Among the factors that will determine this cost-efficiency is the fitness of cyanobacteria under (i) hypobaria (low pressures), and as a result low partial pressures of dinitrogen; and (ii) a dependence on regolith for all nutrients not provided as gases, which would also lead to high concentrations of highly oxidizing compounds (chiefly, perchlorates) in the extracellular medium. Another key element is the design of specific cultivation hardware. In this presentation, we will present the wet-lab and in-silico work performed at the ZARM s Laboratory of Applied Space Microbiology to study those factors and thereby assess, and improve, the efficiency of cyanobacterium-based BLSS on Mars. ●

CO-AUTHORS: Cyprien Verseux and Tiago P. Ramalho

MAURIZIO IOVANE

Post doc - Department of Agricultural Sciences, University of Naples Federico II – Italy

TOPICS: FOOD PRODUCTION AND PREPARATION, SPACE EXPERIMENT

Simulated microgravity affects pollen tube development: a crucial stage in the seed-to-seed cycle of space candidate crops

Long-term space missions will necessarily require the full completion of plant reproductive cycle to produce viable seeds to be used for plant cultivation over time independently from Earth supply. Therefore, the identification of mechanisms by which space factors can affect plant reproduction is becoming increasingly critical for plant cultivation in future human settlements in space. Within the plant reproductive cycle, pollen development represents a crucial stage to ensure seed and fruit production over subsequent seed-to-seed cycles in space candidate crops. In this regard, investigations on how altered gravity can affect pollen tube germination and pollen tube path towards the ovary might provide useful insights on space-related issues in plant reproduction. 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 effects of clinorotation as microgravity analog treatment on pollen germination and pollen tube development as potential bottlenecks in the regular seed and fruit development. The effect of clinorotation on pollen germination and pollen tube path was assessed in *Solanum lycopersicum* L. cv. Micro-Tom and *Brassica rapa* L. var. silvestris, both eligible for cultivation in space. In the control treatment pollen germinated on agar medium inside Petri dishes vertically positioned according to the 1g gravity vector, whereas in the microgravity analog treatment pollen germinated in Petri dishes rotating on uniaxial clinostat (2 revolutions per minute). The effect of clinorotation on pollen tube path was assessed comparing tube length and

tortuosity between control (1g) and clinostat (cl) treatments. Tube length and tortuosity were assessed to describe possible alterations on speed and curviness of pollen tube development under altered gravity. Both crops tested in this study showed an increased pollen tube length and tortuosity under clinorotation. However, gravity conditions prompted a differential effect on germination percent of pollen in the two species. Indeed, pollen germinability of *B. rapa* and Micro-Tom were respectively higher and lower under clinorotation compared to 1g control. Further, results showed that clinorotation can promote pollen tube elongation in germinated pollen grains by increasing curviness of pollen tube path. This phenomenon might prevent the capability of pollen in reaching the ovary inside flowers and would justify the reduced fruit production previously reported in seed-to-seed cycles performed under real microgravity. Overall, results highlighted that clinorotation can exert differential effect on pollen reproductive traits according to the species. If confirmed by an experiment in real microgravity, our findings suggest that different species can differently react to altered gravity. Therefore, a species-specific approach is needed to select the crops that can better ensure seed and fruit production over time under space conditions. In the current long-term exploration scenario, plant reproduction should be considered as crucial for future human settlements on Moon and Mars and research on candidate space crops should increasingly focus on reproductive fitness, in addition to growth rate, productivity, and resource use efficiency. ●

CO-AUTHORS: Luigi Gennaro Izzo, Giovanna Aronne

■ AIGARS LAVRINOVIS

Researcher, PhD student - Riga Technical University – Latvia

TOPICS: GREY, YELLOW, BLACK WATER RECYCLING, WASTE RECYCLING, PROTEINS PRODUCTION

Optimizing phosphorus removal for municipal wastewater post-treatment with *Chlorella vulgaris*

The excessive worldwide use of phosphorus facilitates eutrophication of aquatic ecosystems and deterioration of their ecological status. Effluents from municipal wastewater treatment plants (WWTPs) contribute to a significant amount of phosphorus loading into natural waters. Technologies for phosphorus removal in large WWTPs are well established and include chemical precipitation and enhanced bacterial uptake. However, the traditionally used treatment technologies often generate excess waste or require high capital and operational costs, especially at small WWTPs. Microalgae-based wastewater treatment is often viewed as a nature-friendly and cost-effective alternative to conventional wastewater treatment methods. Although microalgae-based technology poses environmental and economic advantages over conventional wastewater post-treatment, the phosphorus removal rate must be maintained at high levels to meet the discharge standards for good ecosystem status in the receiving waters. Considering this necessity, the microalgal phosphorus famine or starvation technique is gaining attention as an approach to enhance both phosphorus removal and high value molecule production. Still, there are drawbacks of microalgae manipulation with phosphorus availability such as reduced biomass growth rate which further lowers the total yields of high-value molecules. Also, the margin for optimum P-starvation remains rather obscure. This study aimed to find the optimum initial conditions that would result in a trade-off between the highest possible phosphate removal and accumulation rates as well as biomass growth rate at the shortest biomass P-starvation period. Additionally, protein productivity induced by P-starvation was assessed as a possible improvement in biochemical quality of *Chlorella vulgaris*. The microalgal species *C. vulgaris* was cultivated in batch conditions

to study the effect of phosphorus deficiency caused stress. The microalgal biomass was exposed to phosphorus deficiency conditions for periods varying between 1 and 10 days and inoculated at different initial biomass and phosphate concentrations. A 10-day period of phosphate deficiency, supported by low initial biomass concentration (~0.25 mg DW L⁻¹), increased the specific phosphate removal rate by 62.175% when compared to the reference conditions, and reached 7.8 mg PO₄ g DW h⁻¹. A 10-day period of biomass P-deficiency also boosted the polyphosphate accumulation and protein productivity, increasing them up to 40 and 46.8 times, respectively, if compared to reference conditions. Further, an optimization algorithm xT SAAM (<https://www.x-t.ai/>) was used to identify the most optimum initial settings for efficient wastewater post-treatment and resource recovery. The obtained optimization algorithm model results suggested one-day biomass P-starvation with low (0.25 g DW L⁻¹) initial biomass concentration as the optimum combination to achieve the highest performance. The initial phosphate concentration seemed to have less impact. The initial conditions suggested by the optimization model were validated in a sequencing batch photobioreactor running for 60 days. Results showed 101.7 and 138.0% higher phosphate removal and polyphosphate accumulation, respectively, compared to the reference conditions. Biomass protein productivity decreased by 38.7% after P-starvation. The obtained results present microalgae exposure to phosphorus stress as a supplementary tool for wastewater post-treatment to significantly increase the phosphate removal rate without notable biomass productivity loss and maintain high yields of valuable molecules synthesized by the algal cells. ●

■ ANTONIO GIANDONATO CAPORALE

UNINA

TOPIC: FOOD PRODUCTION AND PREPARATION

The Potential of Lunar and Martian regolith simulants as plant growth media

The combination of Bioregenerative life support systems (BLSS) and in situ resource utilization (ISRU) may allow sustainable food production on Moon and Mars. The ISRU approach aims to reduce terrestrial input into a BLSS by using native regoliths as soil for plant growth. Nevertheless, extra-terrestrial soil is very different from vital and fertile terrestrial soil. So it must be modified to be used as plant growth medium. Since Lunar and Martian regolith are not available on Earth, space research studies are commonly carried out on regolith simulants, which tend to replicate the geo-mineralogical properties of extra-terrestrial regoliths, assessed during the past manned missions to Moon or by rover and robotic spacecrafts landed on Mars. This work provides a brief overview of the physicochemical properties and mineralogical composition of Lunar and Martian

regolith simulants, currently produced and available on the market. Then, it describes potential strategies and sustainable practices for creating regolith simulants akin to terrestrial soil. These strategies include the amendment of simulants with composted organic wastes, which can turn nutrient-poor and alkaline crushed rocks into efficient life-sustaining substrates equipped with enhanced physical, hydraulic, and chemical properties. In this regard, we present main results from our recent scientific works focusing on exploitation of regolith simulant-based substrates as plant growth media and carried out within the Italian Space Agency project In-situ Resource Bio-Utilization for life Support system (ReBUS). Discussion will identify the main critical aspects and future challenges related to the in situ agricultural use of Lunar and Martian soil. ●

CO-AUTHORS: Antonio G. Caporale, Youssef Roupael, Mario Palladino, Stefania De Pascale

■ CHARLÈNE THOBIE

Process Project leader – Algolight – France

TOPIC: FOOD PRODUCTION AND PREPARATION

ALGOLIGHT to produce high value products in a compact controlled and intensified photobioreactor adaptable to the life support for human space exploration

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. It is in the context that Algolight, a start-up, has been developed an intensified photobioreactor named PRIAM and will manage this technology to meet the needs of the market to produce microalgae in a controlled and intensified way. The PRIAM photobioreactor has been developed to achieve breakthrough performance compared to existing systems. Its intensified technology which aims at the controlled production of photosynthetic microorganisms, while having a high productivity (3.8 kg/m³/day of dry biomass) by strongly decreasing the volume of culture per unit of illuminated surface (as = 500 m⁻¹). For it, the PRIAM photobioreactor uses 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. A dozen strains have been tested and cultivated there successfully. This photobioreactor can operate continuously with reduced maintenance. Indeed, advanced

hydrodynamic studies have made it possible to control the biofilm aspect in the PBR and the gas-liquid transfer. 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 but also helps to reduce energy expenditure. This technology 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 which is to produce sufficient food in a restricted place. In fact, for a PRIAM photobioreactor of only 100 L allowing to produce more than 0.12 t/year, a footprint of less than 1m² is necessary. So, PRIAM technology could be adapted to edible biomass production in space. Indeed, the concept of this technology could be transposed to the space application (atmosphere regeneration, edible biomass production and liquid waste treatment in a restricted place) where the main problem would be taking into account the absence of gravity and therefore the hydrodynamics and the gas/liquid transfer. This presentation highlights Algolight activities, the development and testing of its intensified photobioreactor using Lightex® technology and the potential of the PRIAM photobioreactor for the production of algae in a controlled environment. ●

Keywords: intensification, lighting system, controlled production

CO-AUTHORS: Charlene THOBIE*1, Laure PERUCHON1, Jérémy PRUVOST2, Cédric BROCHIER11 ALGOLIGHT, CRTT, 37 Boulevard de l'Université, 44 600 Saint-Nazaire, France. Tel: +33 4 37 56 85 60. Mail: charlene.thobie@algolight.com2 GEPEA UMR - CNRS 6144, University of Nantes, 37 Boulevard de l'Université, 44 600 Saint-Nazaire, France). Tel: +33 2 40 17 26 69. Mail: jeremy.pruvost@univ-nantes.fr

■ MAX MERGEAY

Director - MELiSSA Foundation – Belgium

TOPIC: SOCIETAL IMPACTS AND EDUCATION

The MELiSSA foundation and the selection of young scientists involved in research on life support in space: eight years of experience in the POMP project.

The POMP project (Pool of MELiSSA Ph.D) aims at reinforcing the scientific capacity of European Space Research, focussing on life support in space conditions, including food production and recycling of waste and used waters, and more generally, applies to the rehabilitation of polluted or damaged ecosystems on Earth. POMP student benefit from a 4 years fully financed PhD scholarship, 3 years to be spent in one European university and 1 year to be spent in the laboratory of a MELiSSA partner in a different EU country, within the MELiSSA project. This unique feature is the main originality of the POMP project, increasing the integration of European Space Research. In addition, the POMP project provides the opportunity to integrate the MELiSSA PhD pool, to attend the MELiSSA annual conference, to participate to the MELiSSA summer University and to benefit from ESA support to create a spin-off company. During the POMP1 phase, which started in 2014, and POMP2, which started in 2018, the Italian delegation, which focused on edible plants

research, preferred to independently select Ph.D. candidates. Other Melissa partners mostly though not exclusively focussed on microbial and technical aspects of life support and waste recycling in space conditions. More interactions occurred during the POMP3 exercise, as Italian fellows will spend more time with different MELiSSA partners. The MELiSSA foundation also financed some post-doctoral scientists for a 2 years term to be spent in a European university deeply involved in the MELiSSA project or space research and in a country different from that where the Ph.D. diploma was acquired. POMP1 started in 2014 and involved partners from Ireland, the Netherlands, Belgium, and Switzerland. One Ph.D and two post-doctorates (from Romania and Turkey) were completed, producing 9 publications. In Italy, 3 Ph.D. were completed and produced 15 publications. POMP2 started in 2018. Besides one post-doc from Switzerland who wrote two books, it involved Ph.D. from Belgium, Spain,

Switzerland, UK and will finish at the end of 2022/beginning of 2023. Partners who hosted them during one year are from France (UCA) and Belgium.

POMP3 started in 2021, with 2 Ph.D. in Switzerland, one in France, one in Spain and one year host MELiSSA partners in Italy, Belgium, Spain and Switzerland. In Italy, 3 Ph.D. will visit a variety of MELiSSA partners (e.g. the Belgian Nuclear Center, to study the effects on plants of radiations, a matter relevant for space research). MELiSSA summer schools were organized every two years, first in Catalonia, then in Auvergne and most recently in Sofia, Bulgaria. These summer schools became a major integration tool for the MELiSSA community and for promoting the scientific continuity and fecundity of the MELiSSA project. The POMP project (Pool of MELiSSA Ph.D) aims at reinforcing the scientific capacity of European Space Research, focussing on life support in space conditions, including food production and recycling of waste and used waters, and more generally, applies to the rehabilitation of polluted or damaged ecosystems on Earth. POMP student benefit from a 4 years fully financed PhD scholarship, 3 years to be spent in one European university and 1 year to be spent in the laboratory of a MELiSSA partner in a different EU country, within the MELiSSA project. This unique feature is the main originality of the POMP project, increasing the integration of European Space Research. In addition, the POMP project provides the opportunity to integrate the MELiSSA PhD pool, to attend the MELiSSA annual conference, to participate to the MELiSSA summer University and to benefit from ESA support to create a spin-off company. During the POMP1 phase, which started in 2014, and POMP2, which started in 2018, the Italian delegation, which focused on edible plants

CO-AUTHORS: Berangère FARGES, Max MERGEAY and Rob SUTERS

SOLVEIG TOSI

Professor - Department of Earth and Environmental Sciences, University of Pavia Italy – Italy

TOPIC: WASTE RECYCLING

The REBUS fungal collection for the space organic waste exploitation

This work is a part of the ASI project In Situ Resource Bioutilization for Life Support Systems (REBUS) and its main purpose is the selection of fungi that can grow on the organic waste produced by the crew of the International Space Station (ISS), and bio-transform it. On our planet, fungi have the crucial role of organic substances degraders. For this reason, they represent strong candidates for many processes concerning bio-regeneration for space life support, needful for extraterrestrial long time life maintenance. Moreover, fungi, during the degradation process, re-enter in the system nutrients that can be used by plants; in this process fungi transform a waste to a fertilizer, or can transform waste in new edible matter with their fruiting bodies. In the present work we present the results of two different experiments: in the first one, the fungi were used as degraders of waste, in the second one vegetable waste was utilized for growing edible mushrooms. The first experiment showed the ability of some fungi, properly isolated and selected, to reduce in terms of weight a simulant of solid organic waste. These new isolates, together with a set of Basidiomycota already belonging to the collection of mycology laboratories of DSTA, have been tested for their ability to increase biomass upon SOW that is a simulant of ISS waste formulated by ENEA - Centro Ricerche Casaccia SSPT-BIOAG-BIOTEC, Italy. This simulant is composed by food waste of astronauts diet with other materials, such as cellulose paper, coming from the human activity on board of ISS. The most active fungi in the production of biomass on the

CO-AUTHORS: Ester Rosa, Vezzola Michele, Mirca Zotti, Elena Savino

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SOW were 13 strains belonging to the following genera: Chaetomium, Dichotomopilus, Byssochlamys, Humicola, Bjerkandera, Ganoderma, Pleurotus, Schizophyllum, Monascus, Torulaspora, Penicillium and Alternaria. Only those strains that did not have airborne spores and were not pathogenic for plants and animals were selected. After the selection we artificially combined different fungal strains to test different consortia. The best consortium were composed by only 3 strains, able to reduce of about 30% of SOW dry weight in one month at 25°C. In the second experiment two vegetable wastes, potatoes and carrots, were used and compared for production of edible mushrooms. A total of 30 culture substratum bags were prepared containing 200 g of straw and vegetable residues in different percentage (25%, 50%, 75% and 100%) and inoculated with a Pleurotus ostreatus strain. The mycelium bags were incubated in a greenhouse at 15 and 20 ° C and a relative humidity between 70 and 80% for three weeks in order to obtain fruiting bodies. Mycelium development and production of mushrooms in terms of number and weight, were significantly different depending on if carrots or potatoes were used. The best results were obtained with carrots at 25%, and this condition was better than in the control where straw was at 100%. On the contrary the presence of potatoes, whether they were in small quantities or in large quantities, inhibited for the P. ostreatus production. ●

NICO BETTERLE

Assistant professor - University of Verona – Italy

TOPICS: WASTE RECYCLING, CIRCULAR ECONOMY

Development of innovative processes for the industrial cultivation of high added-value plants in a vertical farming pilot system

Hydroponic farming is an innovative cultivation system of plants of agricultural and/or pharmacological interest that can be exploited to valorize disused industrial environments through vertical cultivation. Growing hydroponically means cultivating plants without soil, using a liquid solution containing mineral salts as source of nutrients. Hydroponic farms are generally closed environments, where plants can grow in strictly controlled conditions. Thus, growers can provide the desired nutrient solution to the plants, tuning each physical parameter inside the growing module as humidity, temperature and gas composition. Despite the widespread prominence of these technologies in recent years, the sustainability of this industrial cultivation process is limited by the high demand for energy for indoor lighting and for the air conditioning of the large spaces used. These two latter factors are necessary to ensure adequate conditions for plant growth, resulting in a high associated carbon footprint. ONO Exponential Farming srl (ONO EF, www.onoexponentialfarming.com) is an Italian startup company that developed an innovative vertical farming pilot plant. The implementation of advanced technologies as machine learning and artificial intelligence for better managing cultivation parameters resulted in the reduction of management costs without any negative effects on the quantitative and qualitative yields of the plant product. Moreover, management costs are further reduced by the implementation of an automation system, by the possibility to significantly increase the availability of CO2 resulting in an increase of photosynthetic efficiency, by the reduction of possible contaminants given by the absence of

direct contact among operators and plants, and by a dynamic positioning of the cultivated plants with respect to the lights available according to plant growth phase. A multi-year collaboration between the SOLE-LAB research group at the Department of Biotechnology, University of Verona, and the company ONO EXPONENTIAL FARMING srl (ONO EF) has led to the development of innovative processes for industrial cultivation of several horticultural plants or for the synthesis of high value-added molecules in unconventional plant systems through vertical farming. The latter approach can be important for paying out the investment made for the vertical farm, which has still high operational costs, recently even higher because of several global contingencies. In the effort to develop a sustainable technology, testing was done to limit accumulation of wastes during the plant growth cycle. As an approach of circular economy, exhausted plant nutrient solutions from the pilot plant were collected and used as media for the cultivation of robust microalgae as Chlorella or Spirulina. Upon harvesting, the microalgae are treated and then used as biostimulants to improve the growth performance of plants inside the vertical farm. Moreover, the possibility of increasing the availability of CO2 inside the ONO EF-vertical farm paves the way to another important element of circular economy that is the reduction of carbon footprint of an industrial production plant. In fact, the CO2 present in industrial enriched gases can be sequestered by plants and used to enhance photosynthetic conversion of CO2 to plant biomass. As a summary, less CO2 in the atmosphere, more CO2 used by plants. ●

CO-AUTHORS: Kristina Ljumovic, Lorenzo Girelli, Simone Zecchinato, Thomas Ambrosi, Matteo Ballottari

SILVIA TABACCHIONI

Senior scientist – ENEA – Italy

TOPIC: WASTE RECYCLING

Space organic waste degradation: a new approach to microgreens cultivation

Organic waste accumulated during human space missions represents a valuable resource in the perspective of future exploration programs of the solar system. Bringing enough food from Earth takes up volume and increases the spacecraft mass and fuel cost. A viable solution is the production of fresh food, such as edible microgreens, in route using hydroponic systems and simultaneously recycling food and other organic space organic waste as fertilizer. This could cut costs and render missions beyond the low earth orbit possible. Bioprocesses based on aerobic and/or anaerobic digestion are commonly used on Earth for treating organic waste. It's an efficient way of getting mass treated and recycled with concomitant mass reduction. To perform organic waste degradation, microbial consortia were selected directly from the organic waste through an enrichment process under anaerobic conditions. The organic waste used for the experiments was prepared in laboratory

mixing food and non-food waste according to NASA data. Two microbial consortia, showing the highest degradative efficiency, were selected. Metagenomic analysis of their bacterial composition revealed that the two consortia contain only eubacteria and no archaea as in the non-degraded substrate. In both consortia, most bacteria belong to the phylum Firmicutes. Among the latter, Bacillus strains represent the dominant class in both microbial consortia. As far as genera are concerned, the enrichment process caused a significant decrease of the relative abundance of Lactobacillus and a corresponding increase of the Enterococcus one. The presence of bacteria belonging to this genus in the two microbial consortia is relevant because most of these bacteria are able to degrade cellulose that represents about 10% of the space organic waste substrate. Upon degradation, a significant mass reduction of 30% of substrate dry weight was observed. Significant decrease of the total

soluble compounds and cellulose was found whereas hemicellulose and lignin content did not vary. Furthermore, the liquid fraction of the digestate was collected to be tested as fertilizer for the cultivation of microgreen in hydroponic systems.

CO-AUTHORS: Luigi Chiarini1, Lorenzo Nolfi1, Angiola Desiderio1, Marco Garegnani1,2, Luca Nardi1, Maria Elena Villani1, Stefano Moscatello3, Simona Proietti3, Alberto Battistelli3, Silvia Tabacchioni1 1Department for Sustainability, ENEA, Italian National Agency for New Technologies, Energy and Sustainable Economic Development, Casaccia Research Center, 00123 Rome, Italy; 2Politecnico of Milano, DAER Department of Aerospace Science and Technology 3National Research Council, CNR - IRET - Research Institute on Terrestrial Ecosystems, V.le G. Marconi, 2, 05010 Porano (TR), Italy.

Its effect on the germinability of microgreen seeds was also evaluated. Keywords: microbial consortia, resources recycling, microgreens cultivation ●

gained great interest in recent years, enabling nutrient recycling from urine while facilitating wastewater management. Most source separating toilets enable water savings by using a low flush volume, but are maintenance intensive due to the natural, rapid degradation of urine (urea hydrolysis) which results in precipitation and malodor. Clogging of pipes by precipitation of salts is a common problem in low flush urinals and urine-diverting toilets and it is believed to be one of the major barriers for the widespread implementation of urine source separation. URIDIS is an electricity-driven electrochemical system that stabilizes source separated urine by alkalization. Urine is collected separately and sent to the electrochemical system, which is a closet-size box, coupled to a small urine and water buffer tank. The electrochemical cell generates alkalinity in the urine without addition of chemicals. The resulting high pH (>11) triggers precipitation of phosphate and inhibits urea hydrolysis, thereby preventing nutrient losses, odor nuisance, and clogging. The electric field drives chloride ions over the ion exchange membrane to the other compartment of the cell where it is oxidized to form hypochlorous acid, a disinfecting compound, directly in the tap/rain water which is used to flush toilets. The disinfecting flush water contains the risk for pathogen spreading,

prevents urine degradation at the toilet level thereby reducing the cleaning and maintenance requirements and flush volumes due to its higher activity. The core of the URIDIS technology was developed within the MELISSA POMP program. Urine recycling is of key interest in regenerative life support systems to recover water and nutrients, which can serve as a fertilizer for plants and microalgae. Within MELISSA, electrochemical urine stabilization was explored, as a chemical-free and safe alternative for toxic chemical stabilization using chromium trioxide and phosphoric acid, which is currently used on-board the International Space Station. At present, the URIDIS technology is further developed by HYDROHM. With support of ESA, BELSPO, Verhaert, Spraying Systems, Laufen and Stad Gent, the URIDIS technology is demonstrated on scale, by coupling installations to public toilets (The Place to Pee). Our development trajectory will be presented at the conference. Overall, this space-born URIDIS technology paves the way for efficient urine collection and treatment on Earth, contributing to a sustainable circular future, by tackling pressing challenges such as water scarcity and the disturbed nutrient cycles. The URIDIS technology furthermore improves the hygiene of toilets, which is very valuable in times of COVID, while reducing the operational costs. ●

CO-AUTHORS: Jolien De Paepe, Pieter Naert, Fabian De Wilde, Max Sabbe

CÉLINE COENE

Bioprocess engineer – QinetiQ – Belgium

TOPICS: BIOMATERIAL, FOOD PRODUCTION AND PREPARATION, LIFE SUPPORT ARCHITECTURE FOR FUTURE MISSIONS

Implementation of an automated process for a continuous *Limnospira* harvesting and the recycling of the culture medium for space applications

Future long-term space exploration missions require the implementation of circular Life Support Systems for the supply of water, oxygen and food from mission wastes. Therefore, separation systems dealing with multi-phasic streams need to be addressed. The BioHarvest (BHV) study focuses on the solid/liquid separation in space with the aim of demonstrating the continuous separation and harvesting of the cyanobacterium *Limnospira indica* (the biomass) from its culture broth under axenic conditions. The biomass is intended to be used for further food processing while its culture broth free of organic matter and resupplied with nutrients should be directly recycled into the photobioreactor (PBR). Based on a literature review followed by a trade-off analysis and feasibility tests, the technologies were selected and a breadboard model was built. The latter consists first in the Biomass Harvesting Unit (BHU) collecting the cyanobacterium culture from the PBR, separating it from its culture medium with dead-end filtration and harvesting it. Second, the Medium Filtration Unit (MFU) which is a crossflow filtration unit, further treats the culture medium to retain the dissolved organic compounds and to release water and nutrients. The breadboard is also equipped with CIP (Clean-In-Place) and the control system and electronics (CSE) which allows to operate the system automatically. In terms of productivity, the nominal daily harvesting target is 80 litres of PBR outlet per day. From this, the target is to recover at least 90 % of the culture medium and to harvest the biomass with a dry matter of 4-8%.

The performances of BHU and MHU were conclusive in batch mode and in short continuous mode: The BHU was able to retain all the biomass and the MFU retention rate of the organic matter was above 90% whereas the nutrients were not retained at all. The productivity of the MFU was also very good, with a high permeation flux allowing to treat the 80L/day. However, the continuous operation of the BHV technology could not be achieved in the long term due to biomass accumulation as a sticky cake with a high specific resistance on the BHU filter, despite backwashing cycles and intense vibrations. Yet, after optimization, the BHU performance could be improved with a greater volume treatment capacity and run duration. From this study, it appears that the most critical step is the biomass separation and harvesting at the BHU level. The future work identified here focuses therefore on this limiting factor. On the one hand, an investigation needs to be conducted on the rheological properties of the biomass as well as on the culture properties of *Limnospira*. The presence of extracellular material might indeed affect the viscosity of the concentrate and/or these components might have an affinity with the filter material. On the other hand, BHU unit needs to be modified to avoid cake formation and to improve biomass harvesting. To do so, adjustments on different parameters such as the vibration system, the ratio between filter surface and filter house volume, as well as the filter material are to be considered. ●

CO-AUTHORS: Dries Demey, Estelle Couallier, Jordan Tallec, Marie Vandermies, Céline Coene, Brigitte Lamaze

KORNEEL RABAEY

Co-founder – HYDROHM – Belgium

TOPIC: GREY, YELLOW, BLACK WATER RECYCLING

URIDIS, electricity-driven water technology for safe and sustainable toilets without chemical additives

HYDROHM, a spin-off company of Ghent University and MELISSA, is developing the URIDIS technology, an electrochemical technology that offers a solution for common problems associated with toilets today, such as hygiene, the high water consumption for flushing and the loss of valuable nutrients contained in urine. Toilets consume up to 60% of the

water in non-residential buildings. Public toilets are moreover often a source of odor nuisance, and present a hygiene risk by aerosol formation upon flushing. This may contribute to the spreading of infectious diseases. In light of the increasing water scarcity and unsustainable nutrient cycles, urine source separation (i.e., separate collection and treatment of urine) has

MAURIZIO CALVITTI

Senior scientist - ENEA - Italian National Agency for New Technologies, Energy and Sustainable Economic Development – Italy

TOPIC: WASTE RECYCLING

Entomological degradation in bio-regenerative systems for space: Study on the efficacy of *Hermetia illucens*-mediated bioconversion

Human survival in space exploration missions beyond low Earth orbit depends on the possibility of recycling, at least in part, primary resources. The production of fresh food to supplement crew diet is a fundamental requirement. On the other hand, the cultivation of plants in artificial environments requires the availability of fertilizers. Therefore, it is necessary to formulate efficient and safe circular bioeconomy systems adaptable to the extreme conditions of the extraterrestrial context. Some insect species are effective organic matter degraders. Among these, *Hermetia illucens* (also known as black soldier fly) is known for its remarkable ability to bioconvert various types of organic waste. In this work, as a part of ReBUS project, we have characterized the degradation process operated by *H. illucens*, with reference to the possibility of introducing this bioprocess into a space bio-regenerative system aimed at producing fertilizers, compost or soil conditioner for plant growth. First, a recipe for Space Organic Waste (SOW), substantially composed of food residues and paper wipes, was determined based on the composition of organic waste (NASA data) produced during the ISS missions. To these, the inedible parts of vegetables, grown as fresh food for the crew, were added. To study both the best bioconversion performance and the response of feeding in the artificial rearing conditions, in terms of insect fitness, three independent experimental conditions were evaluated, characterized by different feeding quantities (8.55, 50 and 112 mg of SOW per larva per day). To this aim, indexes of larval

development and conversion efficiency of ingested substrate were calculated to characterize and optimize the biodegradation process. Analyses of chemical components, before and after bio-regenerative processes, demonstrated in all experimental conditions a high degradation efficiency (87,2%) of the water and ethanol extractives. Even cellulose degradation, consisting mainly of the paper wipes waste, was very effective with a value of 46.1% reduction compared to the initial contents. The results obtained demonstrated the efficiency of the degradation process operated by *H. illucens* on substrates similar to mission waste. Experiments to evaluate the quality of degradation products as promoters of plant growth are currently in progress. In addition to studying the waste bioconversion action of the insect, we also included the development of insect rearing conditions in specially designed facility prototypes, which can be proposed for the space environment. Furthermore, the current use of black soldier fly larvae as human food, in some countries, opens the possibility of exploit this insect species not only for the organic waste recycling, but also as a potential supplementary source of animal proteins. These preliminary results inspire future studies and insights aimed at developing appropriate systems to exploit the bio-regenerative potential of insects in space missions. ●

Keywords: *Hermetia illucens*, resources recycling, waste bioconversion.

CO-AUTHORS: Elena Lampazzi1, Angiola Desiderio1, Giulia Lombardi1, Luca Nardi1, Maria Elena Villani1, Giulio Metelli, Marco Garegnani1,2, Stefano Moscatello3, Simona Proietti3, Alberto Battistelli3, Maurizio Calvitti1

JANA FAHRION

PhD candidate – SCKCEN – Belgium

TOPIC: AIR RECYCLING AND CO2 VALORIZATION, FOOD PRODUCTION AND PREPARATION

The impact of light, temperature and low-dose irradiation on the growth and composition of *Limnospira indica*, a component of the MELISSA life support system for space exploration

There are still many challenges to overcome for human space exploration beyond low Earth orbit (e.g., to the Moon) and for long-term missions (e.g., to Mars). One of the biggest problems is the reliable air, water and food production for the crew in space, to become more independent from Earth cargo supply. Bioregenerative life support systems aim to overcome these challenges using bioreactors for waste treatment, air and water revitalization, as well as food production. The MELISSA project was initiated by the European Space Agency (ESA) in 1988 and uses, besides higher plants, the edible cyanobacterium *Limnospira indica* (aka spirulina) for air revitalization and as an additional food source. The ultimate use of *L. indica* in space life support systems will require specific storage and culture conditions which are not commonly used on Earth. These conditions are tested in the precursor space flight experiments ArtEMISS-B and -C (ArtB and ArtC) of which the latter will fly next year to the International Space Station. In the ArtB and ArtC precursor flight experiments, *L. indica* is cultured in photobioreactors designed for microgravity conditions and controlled by light-limiting conditions, meaning that the light intensity is the parameter by which the production of oxygen and biomass is controlled. The start-up of the bioreactor in space includes a revival of the dormant inoculum (in dark and cold) and propagation in batch mode. Thereafter the photobioreactors are run in semi-continuous mode at warm temperature but low light intensities to induce slow growth allowing longer exposure to space conditions (e.g. cosmic radiation, microgravity) while demanding low amounts of resources such as energy, medium and crew time for feeding. It is known that light conditions have an impact on revival and biomass production kinetics and biomass composition. But the impact of changes in low light conditions, in combination with different temperature regimes and low-dose irradiation, is not

known sufficiently yet. Characterizing the bioprocess behavior that can be expected under these conditions is essential to detect possible changes/problems in the cultures and to know which conditions enable a controllable, reliable oxygen and biomass production, in space. Our experiments showed that low-dose gamma irradiation has a significant impact on the biomass production of batch cultures of *L. indica*. Additionally, small differences in the light intensities (i.e. 36, 45, 75 and 150 $\mu\text{E m}^{-2} \text{s}^{-1}$) have a significant impact on the maximum biomass yield and the time a culture needs to reach the stationary phase (Tstat). Lower light intensities allowed for higher maximum biomass yields, in batch conditions. For example, a low light intensity of 36 $\mu\text{E m}^{-2} \text{s}^{-1}$ resulted in a maximum biomass concentration of $3,36 \pm 0,15 \text{ g/L}$ (at 23°C), while cultures grown at 140 $\mu\text{E m}^{-2} \text{s}^{-1}$ only reached a maximum of $0,82 \pm 0,10 \text{ g/L}$ (at 25°C) (-75,8%). In continuous regimes, higher O₂ production rates were observed for the higher light intensities. In batch, small low light variations (36, 45 and 75 $\mu\text{E m}^{-2} \text{s}^{-1}$) had little impact on pigment concentrations ($0,10 \pm 0,04 \text{ mg phycocyanin per mg DW}$ (45 $\mu\text{E m}^{-2} \text{s}^{-1}$) vs. $0,12 \pm 0,03 \text{ mg phycocyanin per mg DW}$ (75 $\mu\text{E m}^{-2} \text{s}^{-1}$), only a light intensity of 140 $\mu\text{E m}^{-2} \text{s}^{-1}$ induced a significantly lower pigment concentration (i.e. $0,04 \pm 0,02 \text{ mg phycocyanin per mg DW}$). At the contrary, lower temperatures caused a significantly lower antenna pigment (phycocyanin and allophycocyanin) and chlorophyll concentration. For example, a temperature of 34°C gave a phycocyanin concentration of $0,13 \pm 0,05 \text{ mg/mg DW}$ and 21°C resulted in $0,08 \pm 0,04 \text{ mg/mg DW}$ (-38,5%). In conclusion, a low light intensity (36 - 80 $\mu\text{E m}^{-2} \text{s}^{-1}$) in combination with warm temperature (33°C) can be used to obtain a well controllable culture with a high pigment content and a high biomass production in a batch culture. ●

CO-AUTHORS: Jana Fahrion, Laia Navarro Irún, Claude-Gilles Dussap, Natalie Leys*

PIERRE VAN CAENEGEM

Head of business development & promotion department - Caux Seine développement - France

TOPICS: WASTE RECYCLING, CIRCULAR ECONOMY

The greater Caux Seine Area: the land of energy transition & circular economy

At the heart of Normandy and central to the economic regions around the cities of Le Havre, Rouen and Caen, Caux Seine is located along the Seine river. Structured around 4 strategic industrial sectors (energy, logistics, fine chemistry and aeronautics), the Greater Caux Seine Council covers a region where industry has always been present. With 36.4% of all private sector jobs in industry, the area's economy has been shaped by international businesses and a network of sub-contractors. Keen to be attentive to the evolutions in our environment the Greater Caux Seine Council has chosen to focus its strategy on energy transition and circular economy to

support structural projects such as the creation of the Air Liquide Company aiming to reduce CO₂ emissions. After the arrival in the area of the petrochemical industry in 1933, followed by chemical industries in the 1960s, the area has resolutely turned its attention to the green economy, becoming a pioneer in circular economy along the River Seine with the installation of the first industrial water plant in 1972. The area efforts in favour of the circular economy obtained national recognition when the Greater Caux Seine Council was awarded with the Circular Economy Trophy from the French Ministry for Ecological Transition in 2018, and ADEME in 2020. The Caux

Seine area is proud to be a land of numerous energy sources : petrochemicals, biomass, photovoltaic, methanisation, wind energy and the development of gas mobility. In addition to these energy sources which are already available and indeed complementary to them, the Greater Caux Seine Council is also keen to develop hydrogen projects: Air Liquide has chosen to set up in the industrial zone of Port-Jérôme. The capacity of the future project in Normandy will be more than 200 megawatts enough to meet the very significant needs of refiner customers. With this project, from 2025 Air Liquide Normand Hy will avoid 250,000 tons of annual CO₂ emissions. Another major project in the circular economy is the choice of Eastman to set-up their activities in the industrial zone of Port-Jérôme. The company plan to invest up to \$1 billion and build the world's largest material-to-material molecular recycling plant in France a facility that will recycle approximately 160,000 tonnes of hard-to-recycle polyester waste annually. Other manufacturers are

also interested to invest in Port-Jérôme. It is in this dynamic context that the economic development agency Caux Seine Développement signed a partnership in 2021 with ESA and the Ecole Centrale SUPELEC within the framework of the MELISSA project. The main idea is to benefit from the skills of the MELISSA project in terms of modelling of circular system to study, simulate and diagnose the local circularity. As a first study element, shared networks between manufacturers (steam network or a hot water network of Port Jerome industrial Zone) have been considered. There are other examples of territorial synergies: facilitating and leading networks within our industrial zones, thus encouraging inter-business exchanges (e.g. : waste recovery transformed into new materials and/or energy sources, logistical optimisation, vehicle sharing, facility sharing, HR sharing, etc.). This presentation will address the Caux Seine circularity challenges and obtained results during this first collaboration with MELISSA community. ●

ALBERTO BATTISTELLI

Research Director - National Research Council of Italy, Research Institute on Terrestrial Ecosystems, CNR-IRET – Italy

TOPIC: FOOD PRODUCTION AND PREPARATION

Chicory (*Cichorium intybus* L) for space-oriented production of prebiotic rich plant under controlled conditions for astronaut wellbeing.

Plant cultivation systems were studied for space production of prebiotic rich vegetables under controlled conditions, to sustain wellbeing of astronauts in space. Screening of various plant species and cultivars was performed prior to select *Cichorium intybus* L as the most suited for prebiotic production in space. Some chicory cultivars produce a large and edible root very rich in inulin, a confirmed prebiotic. Chicory cultivars performances were tested under various agronomical and environmental growing conditions, including nitrate availability, light intensity, length of the photoperiod, and application of biostimulants. Chicory productivity, biomass allocation in different plant tissues, non-structural carbohydrate, including inulin accumulation, and its degree of polymerisation (PD), cell wall constituents among other variables related to food quality and safety were studied. Root tissues were tested in vitro for prebiotic activity prior to be used for in vivo test in a murine, stress induced model by other members of the Rebus project. Chicory responded with a remarkable acclimation potential to growth under both fully and partial controlled environment in growth cabinet and in a low-tech greenhouse, respectively. Harvest occurred at earlier root growth stage than in normal field crops for industrial inulin extraction, when root fresh weight averages were close to 50 g. In the greenhouse start of bolting occurred at harvest while in fully controlled conditions bolting did not occur. Growth was faster in the growth cabinet than in the greenhouse. The available genetic material showed undesired variability on morphological traits. Inulin content in

young roots ranged between 45 and 55 % of the dry matter with higher contents in fully controlled grown roots. Both low and high polymerisation fructans were accumulated in the roots that showed an average DP in the range 9-10. Nitrate could be accumulated to significant levels even the root, making this aspect relevant for the implication of this ion presence for food safety in space conditions. Main relevant variables affected by tested growth parameters were biomass productivity, root/shoot ratio, content of fructans in the root, yield of roots and prebiotics. Roots also contained a relevant amount of cell wall constituents adding to the amount of prebiotic and nutritional fibre of this vegetable. Living in space causes physiological and psychological stress, that can be at least in part mitigated by prebiotic intake. Prebiotic production in bioregenerative life support systems has received very limited attention. Here we present a robust set of data showing that *Cichorium intybus* L has the potential to grow and produce prebiotic and fibre rich roots, in fully controlled environments making it as an elective specie for the use in bioregenerative life support systems in space. We also provide the first data indicating the required growing area in fully controlled conditions capable of providing the suggested daily supply of nutritional fibre, fructans and prebiotics to a crew member in space. The prebiotic potential of young root obtained under environmental control conditions were confirmed by in vitro tests and the material was used for in vivo tests on a murine model with promising results. ●

CO-AUTHORS: Simona Proietti, Stefano Moscatello, Michele Mattioni, Gabriele Pagliarlunga CNR-IRET, Filomena Nazzaro CNR-ISA, Giuseppe Colla, Mariateresa Cardarelli, DAFNE UNITUS

JACK HOENIGES

PhD student - Nantes University – GEPEA – France

TOPICS: AIR RECYCLING AND CO2 VALORIZATION, GREY, YELLOW, BLACK WATER RECYCLING, PROTEINS PRODUCTION, PROCESS MODELING, SIMULATION AND CONTROL, LIFE SUPPORT ARCHITECTURE FOR FUTURE MISSIONS

Modeling and experimental campaign of a novel, compact, thin-tube photobioreactor for high volumetric productivity

Microalgae are a prime candidate for integration into life support systems for long-term space exploration. Cultivating microalgae produces oxygen and valuable edible compounds such as proteins while upcycling waste byproducts from the crew such as CO₂ and nitrogen found in urine. However, typical microalgae cultivation systems are large and require significant quantities of water making them ill-suited to extraterrestrial applications. Thus, we propose a novel thin-tube photobioreactor which uses process intensification principles to achieve high volumetric biomass productivity. A modeling and experimental campaign is underway of an experimental pilot with a cultivation volume of approximately 100 mL consisting of two 4 mm diameter tubes connected by an upper and lower chamber to enable circulation between the two tubes. This configuration features a large specific

illuminated area and enables cultivation of microalgae at high biomass concentrations. Preliminary results for batch cultivation of *Chlorella vulgaris* have achieved biomass concentrations as high as 9 g/L and average productivities of approximately 0.8 g/L/day for with relatively low photon flux densities (i.e., 50-100 $\frac{1}{4}$ mol/m²/s). Continuous operation will be completed with targeted biomass concentrations between 15-20 g/L. A detailed model of light transfer within the system accounting for all reflection and refraction effects will be coupled with a microalgae growth kinetics model. This model will serve to guide the experimental campaign and assess the system's maximum achievable biomass productivity under different light conditions.

CO-AUTHORS: Rami Makarem, Walid Blel, Laurent Pilon, Jeremy Pruvost

FLORA GIRARD

PhD student – GEPEA – France

TOPICS: AIR RECYCLING AND CO2 VALORIZATION, GREY, YELLOW, BLACK WATER RECYCLING, PROCESS MODELING, SIMULATION AND CONTROL

Microalgae-based biofacade to develop sustainable buildings: system modeling with Modelica

Photosynthetic microorganisms are used in the MELISSA loop for their ability to produce biomass while enabling to treat gas (i.e. CO₂) and liquid (i.e. dissolved nitrogen, phosphorus &) effluents (photoautotrophic compartment IV). This concept was extended to terrestrial application with the microalgae-based biofacade. It acts as an « active » facade able to exchange matter and energy between a building and a microalgae cultivation system. The ambition of this shared usage is to create a synergy between the building and the microalgae cultivated in facade in order to develop sustainable buildings (reduce energy consumption for thermal regulation, recycling flue gas and wastewaters etc.) while creating a source of income thanks to the production of valuable biomass. The aim of the presentation is to present the system model of the biofacade developed under Modelica language. This model simulates thermal and chemical exchanges between the building and the facade photobioreactors (PBRs). The model of the thermal symbiosis is composed of two sub-models: the thermal model of a building envelop and the thermal model of the microalgae-based biofacade. An energy analysis was performed with this model, in order to compare annual cooling and heating consumption of a standard building to those of a building equipped with a biofacade. The results of this analysis show that the installation of PBRs on building facade decrease the energy consumption of the building and the PBRs. The microalgae-based biofacade is therefore a promising technology for the insulation

of buildings with the additional interest of producing biomass. The model of the chemical symbiosis evaluates the utilization of yellow wastewater as nitrogen and phosphorus source and flue gas as inorganic carbon source for the microalgae cultivation in facade. It calculates the biomass productivity according to the amount of light received in the PBR, the stoichiometric consumption of the nutrients by the microalgae and the gas-liquid mass transfer inside the reactor in order to perform a mass balance analysis on these three fundamental nutrients. With this model, a study evaluating the biomass productivity and the purification of liquid and gaseous effluents of an office building was performed. The results revealed that by acting on operating parameters (yellow wastewater dilution rate, residence time in the PBR, flow and concentration of the flue gas) the microalgae cultivated in facade could consume all the nitrogen and phosphorus of building's yellow wastewater and more than half of the CO₂ of the flue gas, without impact on biomass productivity. In conclusion, this system model is a robust decision support tool that can be used to perform energy analysis, sizing and optimization studies. The perspectives are to realize a parametric study in order to identify the optimal operating parameters depending on the location of the biofacade and to calculate the environmental benefits of using this technology to develop zero net energy buildings.

CO-AUTHORS: Cyril TOUBLANC, Yves ANDRES and Jeremy PRUVOST

FRANCESCA ZORATTO

Researcher - Istituto Superiore di Sanità (ISS), Centre for Behavioural Sciences and Mental Health – Italy

TOPIC: FOOD PRODUCTION AND PREPARATION

Chicory roots as antidote to spaceflight-induced chronic stress: a translational study in the framework of the ReBUS project Background.

During future long-term space missions, astronauts will experience prolonged confinement and isolation and will be exposed to socio-environmental stressors potentially capable of affecting individual well-being, and impairing physiological stress reactions, psychological functioning, and neuropsychological capabilities. To favour the success of these missions, it is critical to minimize the potential consequences on the well-being of crewmembers. In this respect, the use of prebiotics may be a promising approach. Prebiotics, such as fructans, are selectively fermented by the intestinal bacterial flora whereby they positively orient the composition of the gut microbiota; besides, their degradation products (short-chain fatty acids, SCFAs) are released into blood circulation, thus affecting not only the gastrointestinal tracts but also distant organs, including the brain. Here we tested whether, in a murine model of chronic stress, the daily consumption of chicory roots (*Cichorium intybus* cv Chiavari) prompts the recovery of stress-related behavioural and physiological alterations. Methods. C57/BL6 adult male mice received a diet containing 25% of chicory roots (fructans content 46.12±0.63% of the dry weight) or a control diet. In addition, half of the subjects in each group was administered a low dose of the stress hormone corticosterone (35 $\frac{1}{4}$ g/ml) through drinking water whilst the remaining half received a control solution. After 4 weeks of prebiotic (vs. control) diet and 3 weeks of corticosterone (vs. control) solution, mice underwent, during the following 5 weeks, a series of tests mapping onto cognition, attention, memory, motivation, emotionality, sociality and hormonal stress reactivity. Body weight and food intake were weekly monitored. Results. As expected, the administration of

corticosterone induced detrimental effects on multiple behavioural domains, producing an impairment of cognitive and mnemonic performances and executive functions, an increase in anxiety, a decrease in motivation and a progressive increment in body weight. The consumption of chicory roots was able to reverse the impact of chronic stress, improving cognitive abilities, reversing deficits in attentional and decisional capabilities, increasing motivation and containing the stress-induced body weight gain. In some instances, the beneficial role of the fructans-based dietary intervention was also observed in control mice that did not show the stress-related alterations. On the contrary, no beneficial effects were evidenced in the domain of anxiety and in that of recognition memory; as for spatial memory, an improvement was observed in animals not exposed to stress. Conclusions. Collectively, our findings indicate that fructans contained in chicory roots, by supporting microbial communities and/or through the release of metabolites (SCFAs), determine the recovery of numerous alterations induced by chronic stress, at both the behavioural and the physiological level. In conclusion, our data indicate that vegetables with prebiotic value can represent a valid aid to counterbalance the detrimental effects of stress and support astronauts' performances. Specifically, our findings provide a basis for future efforts to optimize the cultivation of prebiotic-rich vegetables within the bioregenerative life support systems to counteract the psychophysiological effects resulting from prolonged isolation and confinement and nurture a beneficial gut microbiome during long-duration human expeditions into outer space.

CO-AUTHORS: Claudia Soldati¹, Angela Ottomana¹, Martina Presta¹, Gabriele Paglialunga², Simone Macri¹ - ¹Centre for Behavioural Sciences and Mental Health, Istituto Superiore di Sanità, Rome, Italy; ²Research Institute on Terrestrial Ecosystems, CNR, Porano, Italy

CHRISTINE ESCOBAR

Vice President - Space Lab Technologies - United States

TOPIC: FOOD PRODUCTION AND PREPARATION

Duckweed Production for Space Life Support

Space Lab Technologies, LLC (Space Lab) is investigating duckweed plants (family Lemnaceae) as a candidate crop to provide bioregenerative life support (especially crew diet supplementation) for deep space exploration. A desirable space crop is one that minimizes use of mass, volume, crew time, and energy, and has fast growth, high harvest index, nutritious biomass, and good taste. Duckweed has enormous potential for space life support applications, recognized by NASA since the beginning of the space program. Benefits include 100% edible and nutrient dense biomass production, atmosphere regeneration (with a high capacity for CO₂ sequestration), wastewater treatment via highly efficient nutrient uptake, and

gravity-insensitive, exceptionally fast growth. In addition to rapid growth, high volumetric yield, and nutritional density, duckweed exhibits natural robustness to environmental perturbation, making it extremely attractive for space applications. However, production of aquatic floating plants in a volume constrained microgravity environment presents unique engineering challenges. μ G-LilyPond, developed by Space Lab Technologies, is a growth chamber for reliably and efficiently producing duckweed in microgravity. This presentation provides an overview of 1) benefits of duckweed as a space crop, 2) the challenges of duckweed production in a space habitat, and 3) progress in μ G-LilyPond development.

ERNESTO LOPEZ BAEZA

Honorary Professor (from A/Professor of Applied Physics - Earth Observation specialty) - University of Valencia. Faculty of Physics. Earth Physics & Thermodynamics Dept. Environmental Remote Sensing Group (Climatology from Satellites) - Spain

TOPICS: GREY, YELLOW, BLACK WATER RECYCLING, WASTE RECYCLING, PROCESS MODELING, SIMULATION AND CONTROL, SOCIETAL IMPACTS AND EDUCATION

Integrated Water Cycle Demonstration Pilot Project Using MELiSSA Space Technology

Within the recently established MELiSSA in Spain Working Group for the development of terrestrial applications of MELiSSA space technology, the University of Valencia is currently developing a Pilot Demonstrator on the Integral Water Cycle to be installed at the University's Science Campus. It will show the whole process of wastewater treatment and water reuse for the optimisation of irrigation of the campus green areas and water supply to the greenhouses, composting development and use of liquid nitrogen fertilisers, and in urban gardens to be developed. This presentation shows, on the one hand, how remote sensing (RS) Earth Observation techniques from satellites and drones support the intelligent planning and optimisation of the above applications. The large amount and variety of data used makes it necessary to apply artificial intelligence algorithms and data semantics ontologies. On the other hand, the treatment technologies implicit in MELiSSA to be applied to the joint treatment of urban waste water and organic fraction of urban solid waste on the Campus are described. Objectives: i. Separate collection of wastewater (black and yellow water) ii. Valorisation of organic matter and nutrients contained in wastewater iii. Reuse of treated water and obtaining fertiliser products for subsequent application on campus iv. Monitoring and optimisation of irrigation by RS v. Estimation of biophysical parameters by RS vi. Estimation of nitrogen and its nutrient role, also by RS and drones A selective collection of yellow (urine) and black water (faeces) will be carried out and taken to the Demonstrator (MELiSSA). There, black water will be treated by anaerobic

processes to obtain biogas (energy source) or for the production of volatile fatty acids that could be used as raw material for the production of bioplastics. Yellow water will be treated to produce nitrogen fertilisers. The treatment of black water is based on biological processes in anaerobic membrane reactors, while the treatment of yellow water is based on physico-chemical processes (URiDISTM). Both sewage treatment and urine treatment will provide water of a suitable quality for irrigation or fertigation. The organic fraction of the food waste generated on campus will be recovered together with the sludge produced in the water treatment by composting processes, which will allow obtaining an organic amendment applicable as fertiliser on the land. An estimate has been made of the flow that could be treated in the MELiSSA demonstrator. The Mathematics Faculty and the Campus Cafeteria are the project target centres, since they produce 100% domestic wastewater. The overall treatment flow is about 2.5 m³/d, that will serve as basis for setting the dimensions of the envisaged MELiSSA demonstrator. It is worth mentioning that its TRL, as a Pilot Demonstrator, could be estimated between 5 and 7 (test bed / demonstrator model in a relevant / operational environment) as the different components are in an advanced stage of development and the Pilot Demonstrator will compose them as a compact system. This is an integrating project of the Science Faculties and Engineering School and, therefore, a highly innovative academic work niche. It includes a large number of SDGs, namely 2, 4, 6, 7, 9, 11, 13, and 15. ●

VERONICA DE MICCO

Full Professor - University of Naples Federico II, Dept. Agricultural Sciences – Italy

TOPIC: LIFE SUPPORT ARCHITECTURE FOR FUTURE MISSIONS

Morpho-physiological and nutritional responses of Brassica microgreens to heavy ions: an outlook on ionizing radiation from the REBUS project

The Space exploration goals are pointing to manned missions towards Moon and Mars. Technological and scientific challenges are still to be faced including the realization of sustainable closed artificial ecosystems, defined as Bioregenerative Life Support Systems (BLSSs). In such systems, higher plants are key organisms for the regeneration of resources and for food production in case of edible species. In Space, easy-to-produce vegetal systems as microgreens would be appealing to complement crew diet with fresh, healthy, high nutritious and antioxidant-rich food, which can help counteract the diseases induced by microgravity and ionizing radiation on astronauts. However, ionizing radiation represents one of the major risks for Space exploration not only for the detrimental effects on human body, but also for the effects on mechanical, electronic, and biological components of BLSS. Among these, plants themselves can be influenced in their development by radiation, thus changing not only their nutraceutical value but also their efficiency as regenerators and consequently altering the input/output balance among the different compartments of the

artificial ecosystem. Within this scenario, the aim of this study was to analyze the effects of high-LET (Linear Energy Transfer) ionizing radiation on morpho-anatomical traits and antioxidant content of *Brassica rapa* L. subsp. *sylvestris* var. *esculenta* microgreens. Dry seeds were exposed to different doses of ¹²C and ⁵⁶Fe ions at different doses (0-control, 0.3, 1, 10, 20, and 25 Gy) at GSI Helmholtzzentrum für Schwerionenforschung GmbH. After the irradiation, seeds germinated, and microgreens were cultivated under controlled conditions. At harvest, biometrical traits, such as stem elongation, fresh and dry biomass, and total leaf area were quantified. Leaf functional anatomical traits (e.g., leaf tissue thickness and density, cell size, localization of phenolics, stomatal frequency) were quantified through light and epifluorescence microscopy and digital image analysis. The antioxidant charge of microgreens was evaluated considering antioxidant capacity and content of polyphenols, chlorophylls, and carotenoids. Results highlighted that the species shows a high radio-resistance at the dry seed stage, and even the high doses (i.e. 20 and 25 Gy) did not prevent organogenesis and did

not induce early microgreens senescence and death. However, growth responses of microgreens, from irradiated seeds, were different for the two types of radiation. A unique dose-response relation was not identified as common for all parameters analyzed. This study furnished useful insights to evaluate plant radio-resistance at early stages of development that are critical in the plant life cycle for the high sensitivity of plants. Gained information is useful to support the decision actions about the choice of suitable species to be cultivated in the BLSSs in Space and for the definition of the shielding requirements for Space

cultivation facilities. Moreover, given the different responses recorded after irradiation with the two types of ions, an hypothesis arises that the combined action of several radiation sources may have either additional or compensatory effects. Therefore, further experiments using other sources of radiation as well as galactic cosmic ray simulators are desirable. Part of the results presented here is based on the experiment Bio_08_DeMicco, which was performed at the SIS18 at the GSI Helmholtzzentrum für Schwerionenforschung, Darmstadt (Germany) in the frame of FAIR Phase-0. ●

YASH PARDASANI

MRes student - Scottish Association for Marine Sciences (SAMS-UHI) - United Kingdom

TOPICS: AIR RECYCLING AND CO₂ VALORIZATION, WASTE RECYCLING, BIOMATERIAL, FOOD PRODUCTION AND PREPARATION, LIFE SUPPORT ARCHITECTURE FOR FUTURE MISSIONS, CIRCULAR ECONOMY

Algal dormancy and revivability in space

Microalgae have the ability to provide essential life support functions on short and long terms missions. Applications include oxygen production, soil improvement, bioremediation of waste and as a source for human nutrition. However, we need species of algae that can be stored for extended periods of time and grown whenever required as re-stocking of fresh supplies or continual growth during transit may not be possible or if actively growing samples fail. Algae species such as *Haematococcus pluvialis*, *Dunaliella salina* and cryophilic snow algae may be suitable species for such long-term missions as they can produce high concentrations of antioxidants such as the red astaxanthin or the orange ²-carotene pigments. This accumulation also coincides with a period of dormancy in their encystment phase, after which the cells can be easily revived back to their vegetative forms when given favourable conditions. Another advantage of the physiology of these cells is that they may be more resistant to chronic doses of radiation when in space (due to the high antioxidant properties of the pigments). They may also be more tolerant of the effects of desiccation (as experienced in their dormant phase), and this would be a more efficient way of

storing such algae for space missions as it reduces the cost of launching and carrying algae in their liquid cultures to space. I will present the design concept, experimental design and current findings from my MRes student research project on algal dormancy and revivability in space. My experiments focus on which species can tolerate desiccation and whether this is correlated to their life cycle stage and pigment concentration. I have also exposed the algal cells to radiation doses similar to those reported on the International Space Station, at SCK Belgium. I have done the growth curves analysis, microscopic images, and lipid and pigment analysis on both radiated and non-radiated algal cultures to observe the effects of space radiation on the cells. In addition, the algal cells have been desiccated in well plates and revived after two weeks and two months to observe the effects of desiccation on the cells. Growth and metabolomic analysis will be undertaken on the revived desiccated cells. The findings from the desiccation experiments will provide insights into potential ways of storing algae for long-term space missions to the moon, Mars and beyond.

CO-AUTHOR: Dr Matthew P. Davey

DANIEL YEH

Professor of Environmental Engineering - University of South Florida - United States

TOPICS: GREY, YELLOW, BLACK WATER RECYCLING, CIRCULAR ECONOMY, SOCIETAL IMPACTS AND EDUCATION

The NEWgenerator Resource Recovery Machine for off-grid wastewater treatment: Case studies for global sanitation in India and South Africa, and implications for space colonies.

Water, sanitation and hygiene (WaSH) is at the core of the Sustainable Development Goals (SDGs). Yet, billions of people worldwide, many in marginalized communities, suffer from poor sanitation stemming from lack of wastewater infrastructure. Due to high CAPEX and OPEX, the conventional approach of centralized wastewater treatment plants served by an extensive sewer system is not an option for many communities. Accordingly, a new classification of modular and pre-fabricated non-sewered sanitation systems (NSSS) have been proposed as a micro-infrastructure alternative. Developed at the University of South Florida (USA) through the Bill and Melinda Gates Foundation's

Reinvent the Toilet Challenge (RTTC), the NEWgenerator is a solar-powered, modular, automated, wastewater treatment and recycling system capable of operating completely off-grid from energy, water and sewer. The NEWgenerator is designed to recover nutrient fertilizer, clean water, and renewable energy from wastewater and organic wastes. The core technology stages within the NEWgenerator are the anaerobic membrane bioreactor (AnMBR) (for solids, COD and pathogen removal), nutrient capture bed (for N and P removal/recovery), and electrochlorination (for pathogen removal and polishing). The NEWgenerator is capable of handling a wide range of wastewater strengths (black, yellow,

grey), intermittent flows, and prolonged shutdowns/dormancies. This presentation will follow the two-decade journey of the NEWgenerator from concept to development to commercialization, including multi-year field trials in India (school in Kerala) and South Africa (informal settlement community in KwaZulu-Natal) with the NEWgen 100, which is capable of providing service to hundreds of users per day. Focusing on the water-energy-food nexus, horticultural feasibility studies have been conducted on the direct fertigation of crops (such as Swiss

CO-AUTHORS: Robert Bair, Cynthia Castro, Hsiang-Yang (Gary) Shyu, Lindelani Xaba

DANIELA BILLI

Professor - University of Rome Tor Vergata – Italy

TOPIC: PROCESS MODELING, SIMULATION AND CONTROL

ReBUS-Cyanobacteria: The use of the desiccation-, radiation-tolerant cyanobacterium *Chroococcidiopsis* sp. CCMEE 029 for in situ resource utilization on the Moon and Mars

The POMP project (Pool of MELiSSA Ph.D) aims at reinforcing the scientific capacity of European Space Research, focussing on life support in space conditions, including food production and recycling of waste and used waters, and more generally, applies to the rehabilitation of polluted or damaged ecosystems on Earth. POMP student benefit from a 4 years fully financed PhD scholarship, 3 years to be spent in one European university and 1 year to be spent in the laboratory of a MELiSSA partner in a different EU country, within the MELiSSA project. This unique feature is the main originality of the POMP project, increasing the integration of European Space Research. In addition, the POMP project provides the opportunity to integrate the MELiSSA PhD pool, to attend the MELiSSA annual conference, to participate to the MELiSSA summer University and to benefit from ESA support to create a spin-off company. During the POMP1 phase, which started in 2014, and POMP2, which started in 2018, the Italian delegation, which focused on edible plants research, preferred to independently select Ph.D. candidates. Other Melissa partners mostly though not exclusively focussed on microbial and technical aspects of life support and waste recycling in space conditions. More interactions occurred during the POMP3 exercise, as Italian fellows will spend more time with different MELiSSA partners. The MELiSSA foundation also financed some post-doctoral scientists for a 2 years term to be spent in a European university deeply involved in the MELiSSA project or space research and in a country different from that where the Ph.D. diploma was acquired. POMP1 started in 2014 and involved partners from Ireland, the Netherlands, Belgium, and Switzerland. One Ph.D and two post-doctorates (from Romania and Turkey) were completed, producing 9 publications. In Italy, 3 Ph.D. were completed and produced 15 publications. POMP2 started in 2018. Besides one post-doc from Switzerland who wrote two books, it involved Ph.D from Belgium, Spain, Switzerland, UK and will finish at the end of 2022/beginning of 2023. Partners who hosted them during one year are from France (UCA) and Belgium. POMP3 started in 2021, with 2 Ph.D. in Switzerland, one in France, one in Spain and one year host MELiSSA partners in Italy, Belgium, Spain and Switzerland. In Italy, 3 Ph.D. will visit a variety of MELiSSA partners (e.g. the Belgian Nuclear Center, to study the effects on plants of radiations, a matter relevant for space research). MELiSSA summer schools were organized every two years, first in Catalonia, then in Auvergne and most recently in Sofia, Bulgaria. These summer schools became a major integration tool for the MELiSSA community and for promoting the scientific continuity and fecundity of the MELiSSA project. The POMP project (Pool of

CO-AUTHORS: Berangère FARGES, Max MERGEAY and Rob SUTERS

Chard) using NEWgenerator membrane permeate. The NEWgenerator is the recipient of the 2014 Cade Museum Prize for Innovation and the 2020 USPTO Patents for Humanity Award. Because the NEWgenerator can establish a closed-loop, circular-economy, micro-infrastructure in resource-constrained environments, the technology has the potential to be adapted for space colonies. The presentation will describe potential future applications in surface habitats on the Moon and Mars. ●

MELiSSA Ph.D) aims at reinforcing the scientific capacity of European Space Research, focussing on life support in space conditions, including food production and recycling of waste and used waters, and more generally, applies to the rehabilitation of polluted or damaged ecosystems on Earth. POMP student benefit from a 4 years fully financed PhD scholarship, 3 years to be spent in one European university and 1 year to be spent in the laboratory of a MELiSSA partner in a different EU country, within the MELiSSA project. This unique feature is the main originality of the POMP project, increasing the integration of European Space Research. In addition, the POMP project provides the opportunity to integrate the MELiSSA PhD pool, to attend the MELiSSA annual conference, to participate to the MELiSSA summer University and to benefit from ESA support to create a spin-off company. During the POMP1 phase, which started in 2014, and POMP2, which started in 2018, the Italian delegation, which focused on edible plants research, preferred to independently select Ph.D. candidates. Other Melissa partners mostly though not exclusively focussed on microbial and technical aspects of life support and waste recycling in space conditions. More interactions occurred during the POMP3 exercise, as Italian fellows will spend more time with different MELiSSA partners. The MELiSSA foundation also financed some post-doctoral scientists for a 2 years term to be spent in a European university deeply involved in the MELiSSA project or space research and in a country different from that where the Ph.D. diploma was acquired. POMP1 started in 2014 and involved partners from Ireland, the Netherlands, Belgium, and Switzerland. One Ph.D and two post-doctorates (from Romania and Turkey) were completed, producing 9 publications. In Italy, 3 Ph.D. were completed and produced 15 publications. POMP2 started in 2018. Besides one post-doc from Switzerland who wrote two books, it involved Ph.D from Belgium, Spain, Switzerland, UK and will finish at the end of 2022/beginning of 2023. Partners who hosted them during one year are from France (UCA) and Belgium. POMP3 started in 2021, with 2 Ph.D. in Switzerland, one in France, one in Spain and one year host MELiSSA partners in Italy, Belgium, Spain and Switzerland. In Italy, 3 Ph.D. will visit a variety of MELiSSA partners (e.g. the Belgian Nuclear Center, to study the effects on plants of radiations, a matter relevant for space research). MELiSSA summer schools were organized every two years, first in Catalonia, then in Auvergne and most recently in Sofia, Bulgaria. These summer schools became a major integration tool for the MELiSSA community and for promoting the scientific continuity and fecundity of the MELiSSA project. ●

RASTISLAV KRAMPL

BioX Technologies – Slovakia

TOPIC: BIOMATERIAL

Arthrospira - Biomass Recovery

Solid-liquid extraction in liquid cultivation systems are important part of the processes especially if they are used in food production in closed ecosystem. Our focus was testing of various methods for spirulina biomass recovery, as centrifugation, sieve filtration, microfiltration, ultrafiltration, and ultrasound separation to describe the future recovery

process suitable also for zero-g. Through the technology and various methods trade-off we have created design of the HW Demonstrator that could be used in the near future for desired isolation. The work had been done in 5 different phases with interesting results and design. Keywords: Limnospira, solid-liquid separation, cells recovery. ●

CO-AUTHOR: Adam Trcka +1 416 7078337, trcka@biox.sk, BioX Technologies

PIERRE JORIS

PhD - Toulouse Biotechnology Institute – France

TOPICS: WASTE RECYCLING, BIOMATERIAL, PROTEINS PRODUCTION

Assessing the Recycling Potential of *Cupriavidus necator* for Space Travel: Production of SCPs and PHAs from Organic Waste

Nowadays on the international space station, more than twelve replenishments per year are necessary to supply food, spare parts and scientific experiments and to take out wastes. But as it is envisaged to go longer and further in space, those replenishments will no longer be possible due to the distances to be covered. The astronaut life support system must be capable of continuously transforming wastes into valuable compounds. Two production types have been identified as critical and could be completed with a unique microorganism like *Cupriavidus necator*. On the one hand, as microgravity leads to fast muscle loss, single cell proteins (SCPs) could be used as protein rich food for the crew. On the other hand, the need for building materials for advanced housing construction necessitates obtaining them locally. The production of bacterial biodegradable plastics could be an alternative solution. Specifically, *C. necator* is well known for its ability to produce poly(3-hydroxybutyrate), a biodegradable bioplastic. By coupling the life support system to a 3D-printer, astronauts

could be provided by an unlimited amount of construction materials. Moreover, two waste streams have been considered to supply our compartment: urea coming from the crew's urine and volatile fatty acids (VFAs) coming from a first step of anaerobic waste digestion as provided in the loop 1 MELiSSA. Thus, the objective of this work, within the Spaceship.Fr project, was to demonstrate the feasibility of producing SCPs and PHAs from VFAs and urea in bioreactor. Because life support systems operate continuously as loops, continuous culture experiments were chosen and the effect of the bioreactor dilution rate on biomass composition was investigated. Total transformation of the carbon source into biomass with high SCP or PHA content was achieved in all cases. We will present the transformation performances of VFAs and urea by the bacteria in bioreactor in terms of titers, yields and productivities but also in terms of the quality of SCP and PHA produced and of the nucleic acid content. We will further discuss the envisioned integration of our process within life support systems. ●

CO-AUTHORS: Eric Lombard, Gregory Navarro, Alexis Paillet, Nathalie Gorret, Stéphane E. Guillouet

MARIJN TIMMER

PhD-candidate – Uantwerpen – Belgium

TOPIC: GREY, YELLOW, BLACK WATER RECYCLING

Nitrogen gas production and extraction from urine to compensate for gas leakage during long-term Space missions: Proof of concept for an energy-efficient microgravity-compatible bioreactor

Regenerative life support systems (RLSS) are crucial for long-term crewed deep-space missions. Maintaining an inert atmosphere in the pressurized space cabin is an important challenge, as there are unrecoverable losses from this artificial atmosphere through leaks and extravehicular activities. To mitigate these, it was estimated that for a 1000-day, 4-person

mission (e.g. to Mars), 20 kg of additional N₂ (excluding storage equipment weight) is to be hauled from Earth depending on the chosen vehicle. Local regeneration of this resource from readily available waste streams can solve this logistic problem. Urine represents the major nitrogen flux in human waste streams: 70% of all human excreta and a concentration of 7-10 g N L⁻¹. Its

stabilization is crucial for safety reasons, but its treatment offers resource recovery opportunities. Currently, only water reclamation is implemented at the International Space Station. This study, fitting in the Micro-Ecological Life Support System Alternative (MELISSA) framework, pioneered in investigating the use of astronaut urine for biological N₂ production as alternative to hauling gas from Earth. Our work explored the most resource-efficient N₂ production method, i.e. partial nitrification/anammox (PN/A), for nitrogen removal from urine. The process was implemented in a membrane-aerated biofilm reactor (MABR), to enable gravity-independent aeration. A hollow fiber contactor module was implemented to extract the produced gas. The MABR (0.27-0.36 L active volume) was inoculated with PN/A biofilm from a rotating biological contactor and operated at 26-28°C. The reactor start-up (181 d) included a phase on synthetic wastewater and synthetic urine (10%) before transition to real urine (10%). Urine was stabilized by increasing the pH above 11 to prevent spontaneous urea hydrolysis to free ammonia, as NH₃ release to enclosed spaces poses a hazard. The intermittent aeration and feeding regimes were optimized and yielded optimum N-removal rates and efficiencies in the MABR of 1000 mg N L⁻¹ d⁻¹ and 80-90%. The

produced gas was successfully extracted (p=0.1 atm), with an N₂- extraction efficiency of 99%. Carbon in the urine (Urea-C and COD-C) was converted to CO₂ (867 mg C-CO₂ L⁻¹ d⁻¹) and extracted with 95% efficiency. Inevitable for any biological N-conversion, side products NH₃ and N₂O were found with emission factor 0.1%, and 5% within expected range. N₂O-emission was strongly related to operational condition; lower vacuum strength and higher aeration time yielded N₂O emission factors less than 1.5%. The extracted gas after trace element removal consists of N₂ (35%) and CO₂ (65%), could be used in a plant compartment or, after CO₂-removal can be sent to the crew compartment to top up gas pressure. The treated urine contains less dissolved contaminants, enhancing its water recovery potential. A mission scenario analysis confirms enough N₂ production and extraction with the urine of 3 astronauts to make up for the gas-losses from an Orion-deep space habitat. The payload benefit over N₂ haulage from Earth for a deep space mission (e.g. a Mars-mission, 4 persons) will be at least 211 kg per 1000 days. This novel process opens up opportunities for modular integration within MELISSA or other RLSS and might be an important step in achieving better autonomy and resource efficiency in human spaceflight. ●

CO-AUTHORS: J. De Paepe, I. Morowa, T. Van Winckel, M. Spiller, P. Markus, R. Ganigué, C. Lasseur, K.M. Udert, S.E. Vlaeminck

MATTIA TOFFANETTI

Designer - MEG Science – Italy

TOPICS: AIR RECYCLING AND CO₂ VALORIZATION, WASTE RECYCLING, FOOD PRODUCTION AND PREPARATION, LIFE SUPPORT ARCHITECTURE FOR FUTURE MISSIONS, SOCIETAL IMPACTS AND EDUCATION

PBRSpace R&D @MEG Science

A powerful new vision for space exploration is being realised: technical, scientific and cultural revolutions are now evident in the design of systems, environments, products and interfaces for life in space. In this context, the Space Exploration Industry is rapidly rediscovering the role of the Designers and the Design Discipline as a link for promoting interdisciplinarity and human-centred technology innovation. This new Industry behaviourism laid the basis for PBRSpace, a technology-transfer driven work path characterized by a R&D process addressing the design of an advanced photobioreactor (PBR), serving as novel hybrid subsystem for the regeneration of the atmosphere and for the generation of microalgal biomass convertible into food and other subproduct integrated into state-of-the-art Life Support System (LSS) for current and future space stations artificial environments and related systems architecture. Within the research, a technical-scientific cluster examines PBR and LSS technology, starting from a general point of view and reaching up to state of the art of the integration of the former in the latter. A cultural-historical cluster instead investigates the role of the Design Discipline within the Space Exploration industry, through the deconstruction of its history into a chaptered framework. Each single chapter aims at highlighting the evolution and integration of the meta-design and design activity in the different eras identified, tracing the future of human presence in space analysing the Industry still-ongoing transformation. Everything is supported by bibliographic sources ranging from scientific papers and publications to exhibition catalogues and project papers, including NASA and ESA manuals and regulations, always referencing to state-of-the-art technology and

demonstrators. This design-driven interdisciplinary activity was developed thanks to the collaboration between two different contexts, MEG Science and Politecnico di Milano university. MEG Science is a consultancy firm, focusing on applied photobiology for non-human applications. Works includes the realisation of new systems ranging from laboratory-scale experiments, pilot machinery development, and PoC analysis, industry-scale applications and up to software applications able to decode the language of photobiological system in optimized ways. Clients range from private and state research institutions to large corporations involved in plants, animals and bacteria physiology, entomology and biochemistry. The resulting collaborative design activity concentrate numerous already tested, validated, patented, and currently used deep tech advancements that lay the basis for the technology-transfer process. Leveraging the bio-regenerative and natural photosynthetic process of microalgae for in-situ circular transformation of waste resources, PBRSpace represents a final manifestation for a novel hybrid equipment integrable within current and future space station LSS characterized by variable and controlled performance and focus on formal, interaction and feedback elements, unseen for a photobioreactor and oriented to achieve a high degree of autonomy in compliance with the constraints and limitations imposed by the environment and the current opportunities and limitations offered by the integrated technology. PBRSpace is a clear example of MEG Science reality-oriented design practice: the aim for its development process is to start with a PoC development and progress, up until to be able to do actual tests in future space stations. ●

AUDE DE CLERCQ

Head of Technology Transfer and Patent management office – ESA – Netherlands

TOPICS: CIRCULAR ECONOMY, SOCIETAL IMPACTS AND EDUCATION

Commercialisation opportunities of 30 years of Melissa's research

The ESA Agenda 2025, in particular the creation of the Commercialisation department, the Accelerator Space for a Green Future and the Inspirator on human space exploration has been welcomed by the ESA Melissa project team and the Melissa Community. With the green deal, there is a clear market fit for our waste treatment, nitrification, photobioreactors, plant characterization, food preparation, modelling and control solutions. Those specific technologies and methods developed over the last 30 years for human space flights respond to a very high degree of circularity, which is now also at the center of our earthly preoccupations. In contrast with many space

developments, MELISSA project benefit of terrestrial applications on many issues: - co-funding of R&D due to high synergies with terrestrial challenges, robustness and operation tests of the Melissa technologies on large scale demonstrators (on earth) to their adoption and deployment to support man-crewed mission, motivation of European actors to join such an inspiring project, communication and education. Therefore their transfer to earth is a top priority. We do believe that there is a window of opportunity that should be not missed, and the Melissa project needs to catch the interest from decision makers, investors and create successful business cases. ●

ROK CAPUDER

CEO - Zavod 404 – Slovenia

TOPIC: FOOD PRODUCTION AND PREPARATION

3D printing in low-gravity (3DmedLowG project): Challenges in development of hardware and food compatible printing ink

3D printing is a well established process for heated polymer extrusion and with the understanding of the benefits of the printing process the area of use is expanding into other fields. Food supplements and other active ingredients can now be deposited with similar extrusion techniques, allowing construction of precisely shaped and dosed oral forms. These techniques do not require elevated temperature, but the ingredients must be embedded in a gel or gel-like ink. The project 3DmedLowG aims to develop a printable ink capable of delivering a variety of active ingredients. During the project an experimental printing setup will be constructed. With the use of standard and coaxial printing nozzles of various sizes we will determine the recipe for hydrogel ink. A pressurized extrusion system will be used to demonstrate extrusion and adhesion properties of the ink. Hydrogels which are composed of naturally available polysaccharides provide a good starting point for the development of 3D ink for food supplements due to several reasons: (i) providing good biocompatibility, (ii) offering options for prolonged protection of the containing active ingredients, (iii) as well as the possibility to fine tune the adhesion and viscosity required for the printing matrix through e.g. biopolymer selection

and degree of cross-linking [1]. The development of the printing matrix based on 2-5 % Na/Ca-alginate network will be presented. Improved functionality will be provided from the inclusion of smaller saccharide units in the following ways: Mono/disaccharides (1-10 %) will be added to drastically shorten the time and lower the mixing intensity required to achieve sufficient hydration, as well as improve the organoleptic properties of the product. -²cyclodextrins (max. 0.5 %) will be added to enable inclusion of hydrophobic components, for additional protection of active ingredients, and to examine the change in technological properties of the printing matrix. The gelation of the matrix will be achieved by coaxial printing of Na-alginate/saccharide solution of various concentrations together with the solution of CaCl₂ (2-5%) and active ingredient. Printing performance, as well as the adequacy of input materials and the quality of the final product will be evaluated, and fail/pass criteria for their successful implementation will be determined. 1. Osojnik rnivec, IG, Poklar Ulrih, N. Nano-hydrogels of alginate for encapsulation of food ingredients. In: Biopolymer nanostructures for food encapsulation purposes. 1st ed. London, Elsevier/Academic Press, 2019. ●

CO-AUTHORS: Rok Capuder, Luka `turm, Rok Jamnik, Iza Burnik, Ilja Gasan Osojnik rnivec, Nataaa Poklar Ulrih

TIM VAN WINCKEL

Postdoctoral Researcher - The University of Antwerp – Belgium

TOPICS: GREY, YELLOW, BLACK WATER RECYCLING, PROCESS MODELING, SIMULATION AND CONTROL, LIFE SUPPORT ARCHITECTURE FOR FUTURE MISSIONS

Nitrogen gas and water recovery using the Nitrogenisor bioreactor for crewed Mars mission: A feasibility study based on stochastic mission scenarios.

The International Space Station (ISS) does a formidable job at closing resource loops. However, it is not sufficient for a crewed Mars mission. One such open loop is cabin pressure management, which relies on N₂ gas to combat cabin leakages.

Another is water management. The current vapor compression distillation (VCD) technology at the ISS recovers only 74% of the water. The Nitrogenisor (NGR) system aims at closing both the N₂ and H₂O loop for future Mars missions using electrochemical

urine stabilization in combination with biological urine denitrification with an energy-efficient membrane-aerated biofilm reactor. This study aimed to provide insights for mass and energy requirements of the NGR system and compare its feasibility to ISS technology (VCD) as well as water recovery with the brine processing apparatus (BPA) by conducting a stochastic comparative mission scenario analysis. Four crew members (CM) would partake in a short-stay Mars mission (650 days, 30 days Mars stay) as described in NASA's Mars design reference architecture using a 322 m³ pressurized deep space transit (DST) vehicle. A leakage rate of 4.54 g air/d and one contingency EVA per 30 days was assumed. Urine production was 1.68 ± 0.28 L/d with an average N content of 8.7 ± 0.6 gN/CM/d. Three configurations were compared based on mass and energy requirements: (A) NGR replacing the VCD and providing N₂ gas for cabin pressure and H₂O to the water processing apparatus (WPA). Based on experimental lab data, the NGR achieved an N removal efficiency of 77% ± 8% and N removal efficiency of 1.09 ± 0.14 g N/L/d. (B) VCD treating urine, providing distillate H₂O to WPA at 74% efficiency. N₂ gas hauled from Earth. (C) Same as (B), but H₂O recovered from VCD brine at 85% efficiency using the

CO-AUTHORS: M.J. Timmer, J. De Paep, M. Spiller, P. Markus, C. Lasseur, K.M. Udert, S.E. Vlaeminck

AGATA KOŁODZIEJCZYK

Assistant Professor - Life Sciences for Space Laboratory, Space Technology Centre, AGH UST – Poland

TOPIC: BIOMATERIAL

Kombucha-derived biomaterials for life in space

Kombucha is a microbial consortium of bacteria and yeast. It was tested 18 months on ISS and seems to be a promising biomaterial to be cultivated in space, for example during long term manned missions. In this work we would like to present our actual research related with kombucha applications in 3D printing, hydroponics and self-healing processes. We develop 3D printing method

BPA. Based on 1000 stochastic model runs, NGR (A) was able to provide 29.6 ± 5.1 kg N₂, which fulfills the mission's N requirement making the COPV unnecessary. The system was able to achieve this using lower hardware mass (161 ± 23 kg) vs 302 kg for VCD (B) and significantly lower energy requirement (NGR = 2.2 ± 0.2 kWh/kg N_{removed}; VCD = 589 ± 163 kWh/kg N_{removed}). Overall payload requirements were lower for configuration (A) (2570 ± 104 kg) vs (B) (5373 ± 306 kg), mainly driven by large amount of H₂O required to fill the VCD's efficiency gap. This could be improved with configuration (C), which lowered the mass requirements to 2152 ± 70 kg. However, BPA is power hungry (1.3 kW/kg urine), thus a trade-off needs to be made between payload and energy. Overall, the biggest limitation of configuration (A) is the increased salt loading to the WPA, which requires more multifiltration cartridges. A dedicated ion-exchange bed with acid/base rinsing or perhaps reverse-osmosis may alleviate this constraint. Overall, this study shows that replacing the VCD with the novel NGR to provide N₂ for cabin pressure management and increased water recycling is feasible and at current level of analysis preferred based on mass and energy criteria. ●

CO-AUTHORS: Waldemar Pichór, Shreyas Srivatsa, Wojtek Guzewicz, Tadeusz Uhl

BAPTISTE LEROY

Associate professor – UMONS – Belgium

TOPICS: AIR RECYCLING AND CO₂ VALORIZATION, GREY, YELLOW, BLACK WATER RECYCLING, FOOD PRODUCTION AND PREPARATION, PROTEINS PRODUCTION

Toward nitrogen recovery from unnitrified urine using *Limnospira indica*.

Urine contains most of the nitrogen excreted by human and thus represent a very interesting waste from where to recover this important resource. Human urine contains between 4 and 7g of nitrogen per litre, depending mainly of the protein intake, mainly present in the form of urea. In the MELISSA project, to recover this resource, the urine nitrogen is converted to nitrate which are then easily assimilated by cyanobacteria or plants. On earth similar strategies are also proposed to produce urine derived fertilisers. Urea conversion to nitrate is performed in three steps, urea hydrolysis in ammonium by ureolytic microorganisms followed by oxidation of ammonium to nitrite and then nitrate by nitrifying bacteria. This on-earth well mastered process is about to be tested in space in the URINIS project. Based on our previous observation, in the frame of the BIORAT2 project, we hypothesised that partially nitrified or

totally unnitrified urine could potentially be used to cultivate cyanobacteria. This hypothesis is derived from the development of culture condition in which the usually observed urea toxicity for cyanobacteria is abolished. We could demonstrate that *Limnospira indica* can be grown with urea as nitrogen source at concentration as high as 150mM with performances similar or even better than with nitrate. In these condition, very high protein content (+/- 70%) and biomass productivity (> 0,5gr/L.d) can be obtained. But urine contains much more than urea and effect of salt and organic component must also be studied before we could claim the direct recovery of urine nitrogen using cyanobacteria. Here we present results of the analysis of urine-like salinity on the growth and metabolism of *Limnospira indica* as well as of the presence of some urine relevant organic compound. Encouraging results were obtained which suggest

the possibility to use, in certain conditions, urine as a nitrogen source for the culture of cyanobacteria. Oral presentation Acknowledgments: This work was performed in the frame of the BIORAT2 project funded by Belspo and ESA. The

BIORAT2 project is part of the MELISSA program of ESA. The Bioprofiling platform used for proteomic analysis was supported by the European Regional Development Fund and the Walloon Region, Belgium. ●

CO-AUTHORS: Baptiste Leroy, Manon Gachelin, Jennifer Delfosse, Neha Sachdeva, Ruddy Wattiez

ARNAUD RUNGE

ESA – Netherlands

OPPORTUNITIES FOR MELISSA-DERIVED DOWNSTREAM SERVICES WITHIN ESA'S SPACE SOLUTIONS

ESA Space Solutions is the go-to place for promising business ideas involving space in all areas of society and economy. It has the mission to support entrepreneurs in Europe in the development of business using satellite applications and space technology. This ESA initiative aims to demonstrate and support the fact that Space is open for business and has the power to improve people's everyday lives on Earth. To do so, ESA is helping companies to integrate space data and technology into commercial services. Successful applicants receive: 1) Zero-equity funding, 2) personalised ESA consultancy support, 3) Technical & commercial guidance, 4) Access to a network of partners and 5) Credibility of the ESA brand. ESA Space Solutions are part of ESA's ARTES (Advanced Research in Telecommunications Systems) 4.0 Programme, which provide

different types of funding opportunities and support the development of sustainable services utilising space assets. ESA is particularly interested in applications of space technology or data to previously untapped consumer markets. These services typically use one or more of the following space assets: This initiative has already supported several activities in a broad and diverse spectrum of thematic areas (for example, Aviation, Health, Energy, Insurance, Tourism, Finance, Investment & Insurance) and many of these areas could benefit from the work performed by the MELISSA community (such as Infrastructure & Smart Cities, Food & Agriculture, Environment, Wildlife and Natural Resources and Maritime and Aquatic). The first demonstration project related to MELISSA is currently under preparation and aims to kick off during Q3 2022. ●

CAROLINA ARNAU JIMENEZ

Technical coordinator - MELISSA Pilot Plant – Spain

TOPIC: GREY, YELLOW, BLACK WATER RECYCLING

Hydrolysis and nitrification of synthetic urine in continuous packed-bed bench-scale bioreactors

MELISSA Pilot Plant envisions implementing in its nitrifying compartment the treatment of human urine from the crew. A biological process to perform full nitrification (i.e. Urea → Ammonium → Nitrite → Nitrate) and COD removal from urine is envisaged. The bacterial consortium able to perform this process is composed of different bacterium types: heterotrophs, which hydrolyse urea into ammonium and oxidize COD components into carbon dioxide, and nitrifiers, which transform ammonium into nitrate. When implemented in the pilot plant, 10% of nitrifying compartment influent will come directly from the crew's urine, so the process is designed to treat an inflow containing up to 10% urine concentration. As a prior step to the use of real urine, the nitrification of synthetic urine has been studied. The main objectives are to demonstrate the feasibility of urea hydrolysis, ammonium nitrification and COD removal in continuous mode and determining the optimal experimental conditions to maximize urea degradation and nitrate production rates. The research was performed at bench-scale using three packed-bed reactors operating simultaneously in continuous mode under sterile conditions and controlled pH and temperature. First, a series of tests focused on determining the optimal inoculation strategy of the bacterial consortium (i.e. simultaneous or sequential inoculation of heterotroph and nitrifiers) and the optimal start-up strategy. The operation was performed by a stepwise increase of nitrogen and carbon load

increasing synthetic urine inflow concentration from 2.5% up to 10% combined with the decrease of hydraulic residence time from 40h to 15h. The results obtained show that the inoculated consortium was able to perform full nitrification of synthetic urine, obtaining significant urea degradation (322 mg N-L-1-d-1) and no accumulation of ammonium nor nitrite, in all configurations tested up to an inlet nitrogen load of 1333 mg N-L-1-d-1. Comparing the different inoculation and start-up strategies tested, the optimal combination, in terms of maximizing urea degradation and nitrate production rates, was a simultaneous inoculation of heterotrophs and nitrifiers and using a hydraulic residence time of 20h at the initial continuous phase. Secondly, the optimization of the packed-bed support material was also studied. Different materials such as polypropylene, borosilicate glass and ceramic beads were tested. First phase optimized inoculation and continuous operation strategy was used to compare the performance of the three packed-bed supports. From the three materials tested, borosilicate glass beads offered the highest urea degradation and nitrate production rates in all the different continuous phases tested. COD degradation obtained with each of the three supports was only partial. The obtained results will serve as a basis for the scale-up of the process, as well as for the transition to the treatment of human urine. ●

CO-AUTHORS: Marcel VILAPLANA1, Joan GARRIGA1, Helen HOLZKE1, Jolien DE PAEPE2, Enrique PEIRO1, Siegfried VLAEMINCK2, Francesc GÒDIA1

CONNOR KISELCHUK

Lead - Plant Science - StarLab Oasis - United Arab Emirates

TOPICS: WASTE RECYCLING, BIOMATERIAL, FOOD PRODUCTION AND PREPARATION, LIFE SUPPORT ARCHITECTURE FOR FUTURE MISSIONS, SPACE EXPERIMENT

Advancing Opportunities for Ag-Tech in the Space Environment: Mutation Breeding Programs, Closed-Loop Developments, and Exploring Future Opportunities

The cost to access space has consistently dropped year-over-year to a point where Low-Earth Orbit (LEO) is now an economically viable arena to produce new and useful IP across a range of industries. StarLab Oasis Ltd., an agricultural research start-up based in Abu Dhabi (UAE) and owned by Voyager Space Inc., is focused on conducting in-house development (and facilitating commercial activity) of the technical products and biological organisms needed to enhance sustainable food production on Earth, while at the same time enabling closed-loop agricultural systems for further space exploration. StarLab Oasis will present our plan to utilize the space environment for new mutation breeding programs in addition to a roadmap for the development and future implementation of closed-loop technologies across several destinations in space. LEO has led to the invaluable expansion of over two hundred crop varieties with beneficial traits that are grown in our modern agricultural system, with the majority being produced by and cultivated in China. The unique environment of space, which presents a chronic dose of ionizing radiation is a relatively underutilized arena to conduct new mutation breeding programs to develop climate-resilient plants through the process of accelerating genetic gains. Chronically exposing seeds to a low dose of ionizing radiation outside of the protective magnetosphere for

several months is known to induce genetic mutations without drastically reducing seed viability, something that is difficult to replicate on Earth. Space-bred varieties have the potential to capture substantial portions of the agricultural market where the effects of climate change will become the most pronounced. StarLab Oasis looks to facilitate seed payloads for customers and conduct crop selection upon sample return at our Agricultural Research Center in Abu Dhabi (currently under development). In addition to crop improvement initiatives, there remains a selection of technical gaps within space-based Controlled Environment Agriculture that require substantial time and financial investments to overcome. StarLab Oasis is excited to explore public-private partnerships that iterate on current state-of-the-art technologies regarding, but not limited to; food safety, plant imaging, plant waste management, and nutrient solution management for use in closed-loop automated plant growth facilities on ISS, Starlab Space Station, and on the Lunar surface. In addition to presenting our technical development roadmap, we will outline a new proprietary hardware platform to help research teams from public and private institutions quickly iterate on closed-loop technical solutions and perform demonstrations aboard the ISS now, and on the Starlab Space Station in 2027. ●

CO-AUTHORS: Benjamin Greaves (Lead Engineer, StarLab Oasis Ltd., bgreaves@starlaboasis.com)

POSTER PRESENTATION

ABSTRACTS

GIOIA ARIETI

Human-Centred Designer – Danske Bank – Denmark

TOPIC: SOCIETAL IMPACTS AND EDUCATION

Measuring time inspired by the overview effect from astronauts in Space

From Space's past missions, the importance of the Cupola to support the psychological needs of astronauts emerged. Frank White created the term the overview effect : a profound cognitive shift in astronauts perception of themselves and view on life, resulting from the experience of viewing Earth from orbit or the moon. Acknowledging the loss of overview perspective on Earth, how can we design the view of life as an overview for humans on Earth? How can we design for humans reflection? This was an experimental research with the scope to prototype-to-provoke thinking about time through a more overview effect cognitive approach. The prototype-to-provoke explored physical calendars as a probe: one of the most common and accessible tools for people on Earth to overview their life and nature's cycles. In the Western world, the most adopted one is the Solar Calendar. The calendar was redesigned, creating two affordances: getting the overview, on one paper, of one year, or longer, of someone's life. Bypassing the standardized 7-day or 1-month time units; tracking time according to the perception of time passed rather than actual time passed. Among others,

experiences were identified as a variable to stimulate the perception of time passed. To further provoke, another probe was developed: the word human-centred season, defined as a quantifiable time period characterized by the coupling of human activity to distort the perception of time. The prototype-to-provoke probes were presented as a commercialized calendar to various audiences and formats: through events, talks and workshops. The traction and engagement gathered through the people introduced to the idea suggest that there is interest in the subject of reflecting on time through a more overview perspective. An emphasized link to time overview and the overview effect learned from Space psychology could amplify the communication on society and human well-being that Space missions have. Further experimentation could test the commercialization of such a concept. However, the conclusion of this design experiment leads to two open-ended, thought-provoking questions: What if we started to design more for an overview effect? What if we introduced the dimension of the human experience of time in a calendar? ●

DAFNI AVGOUSTAKI

Post Doc Researcher - AGRICULTURAL UNIVERSITY OF ATHENS – Greece

TOPICS: FOOD PRODUCTION AND PREPARATION, PROCESS MODELING, SIMULATION AND CONTROL, CIRCULAR ECONOMY

The influence of light intensity on lettuce plants using the Astroplant kit: Supporting with science-based datasets the Melissa Life Support System

Vertical farms (VFs) is a novel type of farming that gives the opportunity to farms produce big and continuous quantities of fresh vegetables in an indoor and controlled environment regardless the outdoor climate conditions. The potentials of vertical farms are very much aligned with the optimisation and development of automations and technological innovations. One of the main challenges in VFs is limited access on data and growing protocols for indoor food production. This problem occurs due to the limited number of large-scale applications in the industry but also due to the patented and restrictions that are followed by leading companies in the market contributing to limited access to full and valid data sets. MELISSA is a life supportive system that deals with this challenge by developing, validating and disseminating structured mathematical models. However, data validation is a process with high cost and time requirements. Astroplant as a pioneering IT infrastructure and network, designs and develops small-scale indoor growth chambers that can replicate experimental and growing protocols. Astroplant supports a global network between its users, validate with statistical approaches data extraction from various environmental conditions in plant growing, and finally

propose to its users growing protocols depending on their species under a much lower cost and scale. Indoor lighting in horticulture is a key component that influences plant growth and development rate. Light consists of its three main dimensions; quality (spectrum combination), quantity (intensity of photons per m² of growing area per time) and duration (photoperiod, daily light and dark provision). The goal of this research is to examine the optimal light intensity for indoor lettuce growing by using the specific LED lamp and the IoT device provided by Astroplant to its users. Specifically, this research project tests the Astroplant kit under different light intensities in order to assess and evaluate the influence of lighting configuration in plant morphology and physiology and consequently propose to the users a uniform lighting recipe that could increase their yield production under the lowest possible harvest cycle and cost. Subsequently, the upper goal of this study is to provide scientific validation and detailed specifications on the architecture arrangement of the equipment in order to improve light use efficiency for lettuce production and propose evidence-based growing protocols to farmers before moving to larger-scale applications. ●

CO-AUTHORS: Christos Vatistas, PhD Fellow, Agricultural University of Athens, Laboratory of Agricultural Constructions / Thomas Bartzanas, Assoc. Professor, Agricultural University of Athens, Head of Laboratory of Agricultural Constructions

EUGÉNIE CARNERO DIAZ

Associate Professor - Institut de Systématique, Evolution, Biodiversité : Sorbonne Université
Museum national d'Histoire naturelle – France

TOPICS: FOOD PRODUCTION AND PREPARATION, LIFE SUPPORT ARCHITECTURE FOR FUTURE MISSIONS, SPACE EXPERIMENT

Combined action of microgravity and cosmic rays on arabis thaliana early development

To reach Mars, humanity must develop life support systems in which plants will play an essential role as a source of food and oxygen, as well as in recycling waste. The current biodiversity attests to the great capacity of plants to adapt to their environment. However, a new adaptive challenge awaits plants when humans settle on the moon and then set out to conquer Mars, since plants will be immersed in a new environment with no possibility of returning to terrestrial conditions. It is important to better understand the mechanisms of plant adaptation in order to develop a strategy to design the most suitable and efficient life support systems and provide the best chance of success for long-duration human spaceflight. In space culture systems, many parameters are easily adaptable such as temperature, illumination, humidity... However, some of them are unchangeable and unavoidable such as altered gravity levels and the constant presence of radiation. In the present study, plants were cultivated on the MarSimulator device in which microgravity was simulated using a random positioning machine and radiation was provided by thorium nitrate salt. To characterize the action of radiation and microgravity on the adaptive response of plants to these factors, particular attention was paid to root growth, meristematic cell size and cell cycle. Oxidative stress and DNA damage in these cells were also assessed. Then, because of its importance in plant growth, development and gravitropism, the distribution of auxin in

roots was also studied. Moreover, a model of microgravity and radiation adaptation mechanism has been proposed based on the results obtained from a transcriptomic study (RNAseq) of whole root system. Results showed that microgravity inhibits root growth and that radiation does not seem to modify this response. However, at the cellular scale, the combined action of microgravity and radiation produce the decrease of the meristematic cell size that cannot be explained by the single action of microgravity. Thus, these two factors seem to have a synergistic effect. Irradiated cells subjected to microgravity tend to accumulate in S phase of cell cycle, while oxidative stress and DNA damage increase in these cells. The prolongation of the S-phase could result from the activation of DNA repair systems. The pattern of auxin distribution in the root apex changes with environmental factors, since this hormone tends to increase in root apices of plants exposed to microgravity and radiation. The increased presence of auxin in the meristematic zone could explain in part changes observed in the cell cycle of seedling cells exposed to radiation and microgravity. Space is a complex and new environment to which plants must adapt quickly. This study contributes to better understand the interaction between radiation and microgravity on the adaptation of plants to this new environment. This knowledge can enrich the data that will be necessary for the integration of plants of agronomic interest in life supports. ●

CO-AUTHORS: Isabel Le Disquet, Miguel Valbuena, Veronica Pereda-Loth, Eugénie Carnero Diaz

MARTIN CERFF

Life Science Engineer - Blue Horizon Sarl – Luxembourg

TOPICS: GREY, YELLOW, BLACK WATER RECYCLING, WASTE RECYCLING

BIORAT 2: Conceptual Design of the Urine Nitrification On-Board Demonstrator for MELISSA first steps

Regenerative life support technologies such as the ones investigated in the MELISSA project, which is developed by ESA and partners, are required for long-distance deep-space exploration such as Mars transit mission. It is their fundamental role to reduce the needed upload mass, generated waste and support the crew with their daily needs during the mission. Among their fundamental tasks are the recycling of carbon dioxide into oxygen and the production of fresh food, mainly by means of plants and algae. For optimal growth, plants and algae require water and nutrients which can be derived by step-wise degradation and recycling of organic waste streams. Flight precursor studies such as Arthrospira-B and URINIS have already successfully demonstrated, or are currently under preparation, that the required microorganisms survive exposure to reduced gravity and increased radiation. Besides carbon, nitrogen precursors are among the most important nutrients for Spirulina and plants within MELISSA. Currently, nitrogen contained within urine is neither recycled nor reutilized at the ISS. Source-separated urine from the crew is considered one of the most

valuable nitrogen sources for fertilizer on the space station. The BIORAT-2 project is aiming to perform the technological demonstration of urine nitrification (corresponding to compartment C3 of the MELISSA loop) on board of the ISS by means of an on-board demonstrator (OBD). Within its final function the BIORAT-2 OBD shall convert urine into a nitrate rich feed medium with the goal to enable plant and Spirulina growth and, as a consequence, help to facilitate the removal of nitrogenous compounds from the water. In order to further close the nitrogen loop, it is foreseen to couple the BIORAT-2 OBD with the BIORAT-1 OBD (corresponding to compartment C4A of the MELISSA loop) by providing the nitrate necessary to the growth of Spirulina within the photobioreactor. We have recently started with the current BIORAT-2 project phase A to assess the feasibility of the mission and to define a conceptual design. In a first step, the state of the art of relevant aspects has been investigated including nitrification processes and technology on Earth as well as tools such as mathematical models to better understand and predict process performance.

A set of key challenges has been identified to successfully transfer nitrification processes to space in the future. Among those are the sufficient supply of the involved microorganisms with oxygen to obtain a robust and axenic bioprocess which requires as little maintenance as possible. The conceptual design of processes and the bioreactor aims to achieve optimal conversion of the urine into nitrate-rich fertilizer under micro-

gravity conditions. Since, in the future, the BIORAT-2 OBD is foreseen to be operated in connection with the BIORAT-1 OBD, it is important that the OBD matches well with the needs of BIORAT-1 in terms of size and mass flow, and generates feed medium of a certain quality. Here, we intend to introduce the activity and report on our conclusion on the state of the art. ●

CO-AUTHORS: Dominique Chapuis, Paolo Dainesi, Jolien de Paepe, Siegfried Vlaeminck, Laurent Poughon, Céline Laroche, Claude-Gilles Dussap, Chloé Audas, Christel Paille, Christophe Lasseur

CARLES CIURANS

PhD Student – MELISSA Foundation – Spain

TOPIC: PROCESS MODELING, SIMULATION AND CONTROL

Control-Based Optimization of the Carbon Dioxide Use in Life Support Systems: Space Lessons for Circularity Challenges

An advanced hierarchical control approach is presented to enhance the optimal usage of resources in isolated environments like Life Support Systems. To test such an approach, the MELISSA Pilot Plant with the integration phase corresponding to Work Package 7 (WP7) is used as base for an exemplary case study. WP7 represents an interesting engineering challenge since it includes two oxygen producing compartments (a photobioreactor with cyanobacteria and a higher plant chamber (HPC) with a hydroponic culture of lettuces), two oxygen consuming compartments represented by the crew compartment (an animal isolator mimicking the crew respiration) and the nitrification compartment, and two interphase units, an Oxygen Enriching Unit (OEU) and a concentrated oxygen buffer tank (CT). Three design and control strategies are presented: (1) HPC fed with a fraction of the gas flow from the animal isolator with only control of O₂ in the animal isolator; (2) HPC fed with a fraction of the gas flow from

the animal isolator with control of O₂ in the animal isolator and control of CO₂ in HPC; (3) HPC fed with a fraction of the concentrated gas from the OEU and a fraction of the diluted gas from the OEU and with control of O₂ in the animal isolator and control of CO₂ in HPC. The control approach is based on a hierarchical control structure with three layers: A tertiary controller with resource optimization functions; a secondary controller with correction and feedback functions based on a Model-based Predictive Control (MPC); and several primary controllers based on predictive functional controllers. The simulations performed demonstrate that the use of advanced control approaches dramatically reduces the addition of external carbon dioxide in an isolated system and improves the circularity of the loop. In turn, since the internal resources are better used, the overall carbon dioxide concentration in the loop is controlled at lower levels and less accumulation is observed. ●

CO-AUTHORS: Carles Ciurans, Najmeh Bazmohammadi, Josep Maria Guerrero, Claude-Gilles Dussap, Francesc Gòdia

CÉLINE COENE

Bioprocess engineer – QinetiQ – Belgium

TOPICS: GREY, YELLOW, BLACK WATER RECYCLING, WASTE RECYCLING, FOOD PRODUCTION AND PREPARATION, PROTEINS PRODUCTION, LIFE SUPPORT ARCHITECTURE FOR FUTURE MISSIONS

Desalination of urine for biological applications under space conditions

As long-distance space exploration missions are considered, the need for advanced Life Support Systems is growing for the supply of water, oxygen and food from mission wastes. Urine valorization is a fundamental building block of these systems. In this context, the Salinity Reduction of Yellow Waters (SaRY) project main focus is to reduce the sodium content in urine to maximize water and nutrient recycling for higher plants and microalgae cultivation. Starting from a literature review followed by trade-off tests and analysis with ALISSE (Advanced Life Support System Evaluator) criteria, a preliminary concept of SaRY equipment was proposed. In this concept, urine is first collected and then stabilized electrochemically by alkalization to remove divalent cations (i.e., calcium, magnesium) and

phosphate. These nutrients can be resupplied to the final product for bacteria/higher plants cultivation. Urine is then fractioned using electro dialysis (ED) to separate the organic components from the minerals and ion exchange (IE) resins to selectively remove sodium ions. In terms of sodium removal, the first results are conclusive with more than 90% of the initial sodium content removed. Yet, to be in line with ALISSE criteria, further tests will be performed with the aim of minimizing input related to the initial ED concentrate, ED module cleaning and resin regeneration (IE). In addition, acute toxicity of the end products towards higher plants, nitrifying bacteria and the microalgae *Limnospira indica* will be evaluated. ●

CO-AUTHORS: Dries Demey, Jolien De Paepe, Nathalie Pujol, Marie Vandermies, Céline Coene, Jean-Christophe Lasserre, Fabian De Wilde, Christophe Lasseur, Sandra Ortega

NICOLAS DROUGARD

Associate Professor - ISAE-SUPAERO – France

TOPICS: FOOD PRODUCTION AND PREPARATION, PROCESS MODELING, SIMULATION AND CONTROL, LIFE SUPPORT ARCHITECTURE FOR FUTURE MISSIONS

Artificial intelligence for plant production systems: two testbeds for data collection and crop strategy generation.

With the support of the Innovspace (Fablab of ISAE-SUPAERO) the ALICE (Artificial Intelligence for Life In spaCE) project contributes to the research on Bioregenerative Life Support Systems. Indeed, this project focuses on the growth of plants and the control of their environment. More specifically, it aims at the optimization of environmental monitoring and feedback processes for plant growth, using Artificial Intelligence methods. On the one hand, plant growth systems are already being considered for long-term missions: hydroponic systems, potatoes in controlled environments, cultivation of tuberous plants, etc. These systems will need to be optimized for maximum long-term production while minimizing resource use. On the other hand, such systems, once equipped with sensors, can allow the collection of data necessary for the improvement of precision agriculture. Measurements in a controlled environment of plant growth in selected conditions, allow a better understanding of their needs and development. Such databases (some of them online, for example for phenotyping), would bring the data-driven revolution to plant growing. In the framework of the ALICE project, test beds have been set up to collect plant production system data and deriving from this data better autonomous cultivation strategies. More precisely, the goal of this research is to collect plant growth data in order to train learning algorithms and compute efficient and economical cultivation strategies, i.e. sequences of actions that maximize production over the long term and minimize resource consumption (nutrient, water, energy, etc.). A promising

direction in this field is the semantic segmentation of plants, and more generally computer vision, allowing to estimate the state of the plant from pictures. This state estimate can then be used by the system to update the current estimate and act accordingly. Long-term optimization of automation, i.e. maximizing plant growth and minimizing resources, can benefit from another hot area of artificial intelligence: Offline Learning for Planning, also known as Offline Reinforcement Learning. In these research domains, the algorithms allow to improve the past cultivation strategy, using the record of previous experiences, which is possible in a controlled plant growth system equipped with sensors. Such algorithms could allow to control these systems more efficiently, with guarantees on the improvement. The first test bed of the ALICE project is a robotic hydroponic system developed during student research projects. This robot controls the intensity and color of the light, the flow of the water and the nutrients level, measures the temperature, the pH of the water, and with its robotic arm fixed on a rail, it is able to take pictures of each plant and to move them. The last plant growing system is a Farmbot Genesis XL, an open source Cartesian coordinate robot farming machine. This robot can plant seeds, water plants, remove weeds, take pictures, and measure the soil moisture. Work on the data collected by these systems has led to algorithms for predicting plant states from sensors, and to environment-machine interaction models for extracting rational decisions based on the data. ●

CO-AUTHOR: Nicolas DROUGARD

ENRIC GARCIA TORRENTS

MD-PhD student-researcher - Medical Anthropology Research Center

TOPIC: MENTAL HEALTH DIFFICULTIES AND COUNTERMEASURES IN SPACE EXPLORATION FROM AN INTER-PERSONAL, SOCIAL GROUP PERSPECTIVE

The oral presentation will introduce key state of the art insights on how to maintain acuity in distressing environments characteristic of human space exploration, gathered via a mixed-methods research approach including an in-depth updated literature review on the topic covering published scientific literature and mission reports, a survey distributed to cosmonauts and medical ground crew, and structured interviews with a select few pilots, scholars, and specialists at the forefront

of mental health in space exploration and space analog environments. Recommendations to minimize or eliminate factors damaging crew acuity, resilience, morale, and overall cognitive and emotional health will be summarized, potential improvements to be implemented in life-support systems will be exposed, and issues requiring further attention and research will be highlighted. ●

CO-AUTHORS: Aya Hesham MD, Ain Shams U.; Gonçalo Torrinha MD, U. Minho; Dan Hawk; Yi Yuan; Tejasvi Shivakumar, PhD cand., U. Nottingham; Tomas Ducai, psy. st., U. Viena; Guillermo Dominguez, PhD cand., German Space Agency; Valagkouti Ariadni, Interstellar Labs.

ICÍAR GIMÉNEZ DE AZCÁRATE BORDÓNS

PhD student - scientific assistant - ETH Zürich - Swaziland

TOPICS: GREY, YELLOW, BLACK WATER RECYCLING, WASTE RECYCLING, FOOD PRODUCTION AND PREPARATION, CIRCULAR ECONOMY

Can human urine recycled within the MELiSSA loop provide sufficient available phosphorus to plants grown in hydroponics?

Extended space missions will not be able to depend on Earth for the supply of clean water, food, and oxygen, so they will need to be produced on board. To this end, the European Space Agency has developed the Micro-Ecological Life Support System Alternative (MELiSSA), a five-compartment life-support loop system capable of recycling crew waste to grow higher plants, that can cover all these needs. Human urine is one of the wastes produced by the crew. It contains a significant amount of phosphorus (P) and nitrogen (N), among other plant nutrients. Some of the nutrients contained in fresh human urine, including P, start precipitating almost spontaneously as they leave the human body. This can pose a problem of pipes clogging and compromise the availability of the nutrients contained in the solids to the plants. In addition, human urine can be a vector for diseases, and its handling in an enclosed space can pose a biosecurity problem. Our project aims to recover the maximum amount of P from human urine in the form of precipitates and to transform this P into plant-available forms. We also aim to provide safe products and use as few inputs as possible to

obtain them. To this end, we analysed different types of urine precipitates: I) precipitates induced by the addition of Ca(OH)₂ to fresh urine at the rate of 6 g/L; II) precipitates induced by the spontaneous growth of microorganisms in fresh urine; III) precipitates induced by the addition of urease in fresh urine; IV) precipitates induced by the addition of urease in fresh urine followed by precipitates pasteurization; and V) precipitates induced by the addition of urease in synthetic fresh urine. We are currently analysing the forms of P in the precipitates by X-ray diffraction and the total element content in the precipitates. We are also analysing the concentration of elements (including P) present in the original fresh urine and the amount of elements that could not be removed by precipitation. Next, we will extract P from precipitates with water at different pH and with cationic and anionic resins. Finally, we will carry out experiments with lettuce in deep-water culture hydroponic systems under controlled conditions, to determine the availability of the recovered P to the plants. The results will be presented at the conference. ●

CO-AUTHORS: Astrid Oberson, Geremia Pellegrini, Christa Hirschvogel, Ruben Kretzschmar, Kurt Barmettler, Kai Udert, Emmanuel Frossard.

ARMAN GRUMEL

Master Student - MELiSSA Pilot Plant – Spain

TOPICS: AIR RECYCLING AND CO₂ VALORIZATION, FOOD PRODUCTION AND PREPARATION

At-line analysis of ammonium, nitrate and potassium in a hydroponic higher plant chamber

The MELiSSA Pilot Plant has been conceived to integrate and demonstrate the MELiSSA regenerative life support system during long-term experiments. To reach this objective, a precise characterization of the nutrient flows and concentrations connecting each of the MELiSSA compartments is essential to better understand its performance and their interaction. One of the compartments is a Higher Plant Chamber (HPC) of 5 m² growing area, used to regenerate the atmosphere and produce food. It is extremely important to monitor the concentration of the main nutrients in the hydroponic solution such as ammonium, nitrate and potassium in order to characterize nutrient uptake by the plants and consequently optimize photosynthesis and food production. An ammonium, nitrate and potassium Ion Selective Electrode at-line analyser based on potentiometry (ISE 2035, Methron, Herisau, Switzerland) has been integrated in the MELiSSA Pilot Plant Higher Plant Chamber liquid loop. Analysis can be programmed and then performed automatically, requiring very little maintenance or oversight. After optimizing the main functions of the at-line analyser

reliable quantification for all three ions with errors lower than 10% has been achieved. This was assessed by performing a sequenced functional test, working with increasingly complex solutions, moving from mono-ionic standards to nutrient solution and finally analyzing fresh samples from the hydroponic loops of the HPC including the complete liquid matrix. Additionally, the 2035 ISE analyser has been used to quantify the three targeted ions every 4 hours (including an automatic calibration prior every analysis) from the hydroponic solution of a 42 days staggered test growing lettuces (*Lactuca sativa* L. cv. Grand Rapids) at a controlled pH and electroconductivity at 5.9 and 1.9, respectively. Reliable quantification for all three ions with error lower than 10% has been as well achieved. It shall be noticed, that while the electrodes can operate at a wide range of concentrations, they need to be calibrated at a narrower range to give results with optimal precision. This range differs for each electrode as it is based on the level of the corresponding ion. As a result, significant changes in an ion's concentration can cause a loss of precision of the analyser's readings. ●

CO-AUTHORS: Carolina ARNAU, Carles CIURANS, Enrique PEIRO and Francesc GÒDIA

ABDALLAH HAYDAR

PhD student - Nantes Université - France

TOPIC: PROCESS MODELING, SIMULATION AND CONTROL

Application of thin-gap bubble columns at high volumetric productivity in microalgae cultivation: study of the influence of confinement on hydrodynamics and gas-liquid mass transfer

The photobioreactors (PBRs) dedicated to the cultivation of microalgae are a promising technology for many applications such as the production of high value-added products, bioenergy or CO₂ capture... Bubble columns are often used as PBRs to cultivate microalgal biomass, as they allow both good mixing and good gas-liquid transfer, which are essential parameters for the growth of microalgae. Nevertheless, technological advances are still needed to reduce production costs and environmental impacts and increase energy efficiency. In this context, the intensification of cell production by the increase in the culture concentration for a given illuminated surface, represents a promising way to achieve optimized and eco-efficient production. In order to increase the availability of light energy to the culture, it is necessary to increase both the incident light intensity and the specific illuminated surface. For this reason, it is necessary to reduce the culture thickness. In the GEPEA laboratory, the reactors called high volume productivity PBR have been used so far to achieve this goal. However, it should be noted that important modifications occurred in such bioreactor: (i) rheology of the culture medium which is evolving with the biomass concentration (viscosity increases and rheology becomes non-Newtonian shear-thinning for high concentrations), (ii) bubble coalescence which is favored by confinement and increase of biomass concentration, (iii) biofilm development which is also increased in such configuration. These potential problems will impact the formation and the

behavior of bubbles and subsequently the hydrodynamics and the gas-liquid mass transfer in the PBR. This document presents a focus on the intensification work carried out within the GEPEA laboratory, by the use of bubble columns at 4 mm and 2 mm of thickness (e), with high confinement ratio (e/d) (d correspond to bubble diameter). Consequences of this geometrical change on the mixing hydrodynamics and gas-liquid mass transfer are studied for different solutions (Newtonian and non-Newtonian), having the same rheological properties of microalgae *Chlorella vulgaris* at high concentrations (up to 30 g.L⁻¹). Comparison is carried out with conventional bubble column. The results show that the gas-liquid mass transfer coefficient kLa is lower in a thin-gap column (e=2 mm and 4 mm) for Newtonian and non-Newtonian fluids and this in comparison with conventional columns, and it also decreases with the degree of confinement. The increase in viscosity and the non-Newtonian behavior have little influence on the gas-liquid transfer, especially at low UGsup. Furthermore, at a given superficial gas velocity (UGsup), the gas holdup is higher: (i) with a viscous or non-Newtonian fluid than in the case of water (ii) and also in a thin-gap column than in a classical one. This behavior increases when confinement ratio changes from e/d < 1 to e/d > 1. The regime transitions occur at a lower UGsup for non-Newtonian fluids compared to water and for thin-gap column compared to classical columns. All these information s allows to identify the optimal operating conditions for a good conduct of the microalgae culture process. ●

CO-AUTHORS: Walid BLEL, Jérémy PRUVOST, Charlene THOBIE, Sikandar ALMANI, Caroline GENTRIC

JOSÉE ELAN

School Director - Ecole Paule Bernard Dupont, 14230 - La Cambe, France

TOPIC: SOCIETAL IMPACT

Expore Mars

On October 18th, the Head of MELISSA project presented his activities in our school. This lecture was perfectly in line with our current education activities : The scales for our life places, initiated in the school over a year. School children, already very motivated by astronomy after the visit of La Hague Planetarium, were able to ask many questions. The time of exchange was very fruitful and constructive and often focus on astronaut daily life, environment and mission reasons.

Opportunity was taken to build a poster for MELISSA conference in Toulouse, November 2022.

The poster summarises their approach :

- Location from La Cambe to Mars, including the spaceship,
- The locations : Space, plantes, Earth, Mars,
- The space journey, represented by a red line
- School children thoughts : imagination, thinking, names in the stars, ... ●

AUTHORS: Pacôme, Timéo, Julian, Louane, Lalie, Mélyanne, Atanaël, Raphaël, Mathis, Soline, Desta, Noa, Esteban, Héloïse, Catlyne, Nathan, Gaël, Zac, Cléa, Maël, Enzo, Alice, Margot, Gabin. Kevin Pigeon.

LYUDMILA KABAIVANOVA

Assoc. Professor - The 'Stephan Angeloff' Institute of microbiology, Bulgarian Academy of sciences – Bulgaria

TOPIC: WASTE RECYCLING

Waste to energy and algal biomass production

Important representatives of marine ecosystems, algae are abundant renewable resources with high nutritional value and their bioactive substances possess different health benefits. Anaerobic digestion of lignocellulosic waste can sustainably produce renewable energy, thus coping with unwanted waste accumulation. Waste wheat straw as a renewable energy source was involved in anaerobic digestion at appropriate conditions to obtain biogas and biomethane as energy carrier. The total biogas yield reached 1116 cm³/L. The digestate after the anaerobic digestion process was used as a medium for cultivation of algae after adsorption with activated carbon for clarification. Photosynthetic microalgae used the nutrient residues from anaerobic digestion of agricultural waste and with their short growth cycle and easy accumulation of biomass could prove their economic perspectives- their low-cost cultivation has a great potential for many applications. Addition of 4 g/L of the already obtained microalgal biomass as a co-substrate back into the bioreactor led to an increase in the biogas yield compared to the sole lignocellulosic substrate. A small closed circle system, starting from anaerobic digestion of lignocellulosic waste substrates, followed by microalgae cultivation in the digestate with biomass accumulation and subsequent return of part from the microalgal biomass back in

the bioreactor as a co-substrate was realized, encouraging circular economy. The suggested scheme is a simple and low-cost technology, as the substrate used is freely available and algae as microorganisms, represent promising sources for new products and applications. They proved to grow in a waste effluent as medium. This laboratory scale study showed that the anaerobic biodegradation of wheat straw reached 72%, while the green microalga *Scenedesmus obliquus* has the potential to utilize the nutrient content of waste digestate for its mass growth and development. On the other side, algal biomass could be employed as a biofertilizer for stimulating growth of some other valuable plants for food or other purposes. Biotechnological exploitation of lignocellulosic waste is promising for sustainable and environmentally friendly energy production because of the abundant availability of these sources. The enormous challenge that remains for scientists to handle is reducing the volume of wastes available on earth and also at long long-term manned space missions, where wastes are prohibited to be disposed of in space, is to generate liquid and gaseous fractions to be used in the production of food, water and oxygen. Key words: waste to energy, anaerobic digestion, microalgae. ●

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CO-AUTHORS: 11113 Sofia, Bulgaria, 26 Acad. Georgi Bonchev str., the Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, +359 29793167; E-mail: lkabaivanova@yahoo.com; vhubenov7@gmail.com 21113 Sofia, Bulgaria, 21 Acad. Georgi Bonchev str., Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, 21 +359 29792118; E-mail: juivanova@yahoo.com Waste to energy and algal biomass production Lyudmila Kabaivanova 1 Venelin Hubenov 1, Juliana Ivanova 2

BAPTISTE LEROY

Associate professor – UMONS – Belgium

TOPICS: WASTE RECYCLING, BIOMATERIAL, PROTEINS PRODUCTION

Deciphering purple non-sulfur bacteria carbon and redox metabolism for waste upcycling and resources recovery.

Rhodospirillum rubrum, a model purple non-sulfur bacteria (PNSB), has been extensively studied in the context of the MELISSA project for its ability to assimilate volatile fatty acids (VFAs) produced during anaerobic digestion. In this context, we studied the metabolic pathways used to assimilate several VFAs when provided individually or in a blend. We observed that combination of different VFAs affect the way they are assimilated but also how the bacteria achieve redox homeostasis. Redox balancing mechanism are very important when PNSB assimilate VFAs under anaerobic conditions since these carbon sources bring more electron than biomass production requires. PNSB thus developed electron sinking strategies to get rid of this electron excess. Bicarbonate fixation, hydrogen or bioplastics production

are among the most observed electron sinking mechanism thus making the analysis of this metabolism highly promising for the development of sustainable biotechnologies. Here we summarize our recent finding regarding VFAs assimilation in *Rhodospirillum rubrum*, redox homeostasis strategies and possible biotechnological application for energy, biopolymers or even food production. Oral presentation Acknowledgement: This work was supported by the Belgian Fund for Scientific Research (Grand Equipment-F.R.S-FNRS); the Concerted Research Action ARC project [P. Cabecas, PHASYN, 2017]; the CDR -FNRS [B. Leroy, Redox homeostasis in purple bacteria]; The Bioprofiling platform used for proteomic analysis was supported by the European Regional Development Fund and the Walloon Region, Belgium. ●

CO-AUTHORS: Baptiste Leroy, Paloma Cabecas Segura, Laura Toubeau, Ruddy Wattiez

GENEVIEVE LEVEILLE

CEO/Founder - AgriLedger – United Kingdom

TOPICS: FOOD PRODUCTION AND PREPARATION, CIRCULAR ECONOMY, SOCIETAL IMPACTS AND EDUCATION

Distributed Ledger Technology in the Analysis of Earth Data and Its Use in Decision-making for Sustainable Food Systems

Idea description: Adapting to the Earth becoming warmer every year is required. The continued emissions of greenhouse gases and the effects of global warming cannot be forecasted with accuracy or perfectly mitigated [1]. As such, figuring out how localised climates and the economies linked to them are impacted is an enormous challenge. Emerging digital ledger technologies (DLT), demonstrated in projects taking place in micro economies, are now able to inform decision-making and planning as the provision of access to relevant and localised information is improved. With a focus on solving the problem of monitoring rapidly developing events and providing accurate earth surface information such as crop data crucial to farmers and policy makers, the Copernicus Sentinel-3 now has two sensors in orbit [2]. The Copernicus Sentinel-3, now with 3A and 3B in tandem, has in addition ensured the recording of quality measurements over inland waters, with 32,500 virtual stations that define lakes, reservoirs, rivers and glaciers worldwide [3]. Sentinel 3A is also the only sensor in space able to detect cyanobacteria and map in near real-time the presence of this serious threat to human and animal health [4]. By presenting the changes reflected in this type of data map in near real-time in specific localities directly to local farmers and policymakers based on DLT, decisions on farming techniques and adaptation to the changing conditions can be made. These contributions, together with data gathered and recorded by farmers, citizen scientists, distributors, logistics providers and customers, make it possible to find smarter ways of managing our food systems, test and implement far sooner [5].

CO-AUTHORS: Mohammed Chunggaze, Genevieve Leveille, Amanda Risi

CÉLIA LOINTIER

R&D environmental chemistry - VTL GmbH – Austria

TOPICS: BIOMATERIAL, MICROBIAL SAFETY

Biocidal Advanced Dyeing Technology to Reduce Microbial Activity

Microbial dyes are presented as a circular and sustainable solution to antibacterial agents to reduce the microbial burden on spaceflight textiles. Microbial growth in isolated environments poses both an engineering and medical risk to long duration spaceflights. The space station offers suitable conditions for commensal microorganisms to thrive despite microgravity: spacesuit underwear, electronic devices and surfaces are negatively affected. It is also known that under space conditions, the astronauts immune response decreases resulting in a higher risk of infections. Projected missions have limited maintenance capabilities and the microbial load e.g., biofilms must be controlled in a more efficient way. Metal nanoparticles, quaternary ammonium and iodine are current antibacterial agents in use. Despite a diminished lifetime under space conditions, they also pose health concerns. In vitro studies have revealed that silver nanoparticles cause toxic effect in the cells of mammals (brain,

Objectives: Role of the ESA and AgriLedger: Through the implementation of AgriLedger's solutions starting with Haiti, making use of citizens mobilised and connected to the ESA network of earth observers, there is an opportunity to deliver insights based on high-quality and analysis-ready data, making better decisions about local and global food systems. The long-term opportunity is the ability to respond to climate change challenges as these arise, with data delivered through AgriLedger's applications in near real-time. This will enable the support of global supply chain stakeholders in embedding the knowledge system required for effective ongoing deployment of the AgriLedger solutions.

Impact and benefits of this research: Establishing a reliable, distributed system that directly supports a diversified community of problem solvers [5] including food producers and supply chain stakeholders with useful data sets from ESA satellites and localised ground-truth in near real-time while linking this to better decision-making systems (human, AI & ML) can both transform the limitations of current food traceability and monitoring systems and provide a forum for ongoing R&D that improves response times in our adaptation to changing climate conditions. Supported by an immutable and trustworthy distributed-ledger-based record, each item is traceable and can be connected to specific microclimate data influencing its journey from farm to table, along with production data. End buyers are thus able to make better decisions regarding purchase within commodities markets. ●

liver, lung, skin). Moreover, such agents cannot be remanufactured in space which make them unsustainable for the use in long stay missions (e.g., mars). Biogenic secondary metabolites, on the other hand, have the advantage to exhibit high biocompatibility, low toxicity, and can be produced from a space station by recycling organic matters available onboard. The focus was set on space textiles and especially on the innermost layer of the Liquid Cooling Ventilation Garment (LCVG). This research investigated the current state of the art on bacterial dyes in biocidal textile applications, cross-referenced to actual needs of human spaceflight activities. Selected biogenic microbial dyes were investigated for their antibacterial properties using Disc diffusion assay and quantification of Minimum Inhibitory Concentration. The most performant dyes were impregnated on textile fibers (natural, cellulosic, and synthetic). Their antibacterial activity was further determined with parallel streak method (AATCC147) and

quantitative antibacterial activity of textile products (ISO 20743). The textiles were subjected to specific spatial challenges such as radiation, abrasive regoliths and were tested for their mechanical properties. Furthermore, a solid safety evaluation with focus on biocompatibility and toxicity was conducted. Reduction of the microbial load up to 98% has been showcased on one type of

CO-AUTHORS: DI Mascha Rauscher, Dr. Karin Fleck

FELICE MASTROLEO

Senior Scientist – SCK CEN – Belgium

TOPIC: SOCIETAL IMPACTS AND EDUCATION

Space exploration inspires food project in the Democratic Republic of the Congo

In the frame of long-haul manned space exploration missions, 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 microorganisms 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. For 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

textile, which has been tested non-toxic and biocompatible. The textile treatment had a positive impact on flammability, and mechanical properties were not altered with radiation and regoliths. For the first time, microbial textiles are shown to be promising novel antibacterial textiles for spaceflights ●

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 wastewater treatment, has been tested as alternative nitrogen and phosphorus source. Altogether, the INSPIRATION project aims to promote and support a circular economy approach helping to establish a framework for an economy that is restorative and regenerative by design. With this presentation, we want to share our experience in transforming the high tech lab experience into low tech field applications and education that can be useful in Equatorial Africa. ●

Keywords: *Limnospira*, *Arthrospira*, *Spirulina*, low tech, Congo, circular economy.

TOPICS: AIR RECYCLING AND CO2 VALORIZATION

Investigating Photoheterotrophic Conversion of Volatile Fatty Acids into Carbon Dioxide by the Bacterium *Rhodospirillum rubrum* S1H

This work focuses on the second compartment (C2) of the MELISSA loop, operating in photoheterotrophic conditions and inhabited by the purple spiral-shaped bacterium *Rhodospirillum rubrum* S1H. To function optimally, C2 must convert all volatile fatty acids (VFAs) in the effluent of C1 into CO2 for use by the plant and cyanobacteria compartments, using as little oxygen as possible. In our experimental setup, *R. rubrum* S1H cultures were inoculated in aerobic conditions, and incubated in an airtight container for 12 days at 30°C and exposed to $80 \pm 8 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ halogen illumination, until all oxygen was consumed. Thereafter, the cultures were monitored for 4 weeks. This included the follow-up of growth parameters including cell viability as well as medium composition and daily CO2 production. *R. rubrum* S1H cultures were grown using either acetate as a sole carbon source, or using a mix of VFAs (i.e. acetate, propionate and butyrate, 124mM net C concentration) replicating the VFAs composition of the effluent of the current C1 running at the University of Ghent. In addition, an experiment was conducted where the growth medium was spiked with VFAs on day 19, when anaerobic conditions had been present for 7 days and all carbon sources had been depleted. The results of these experiments confirmed previous findings regarding CO2

production on acetate as sole carbon source, and also showed that *R. rubrum* S1H produced CO2 while consuming virtually all VFAs in the setup used in this study. A total CO2 production of 90,000ppm in approximately 1.4 L of headspace was observed over the course of 24 days, although it should be noted that the environment was anaerobic for approximately half of this time frame. Spiking of cultures in stationary phase with VFAs resulted in a lower CO2 production; after brief introduction of ambient air, CO2 production was restored once again. The data suggests that stockpiling of a source of CO2 occurs in the medium or in the cells during the first 12 days of incubation, which is released steadily once anaerobic conditions are reached. Several mechanisms that explain this observation will be discussed. Taken together, the results showed that *R. rubrum* is able to produce CO2 using the C1 VFA mix composition as carbon source, and minimal, yet still to be quantified, oxygen is required for this process. Finally in order to get a glimpse into the active metabolic pathways, whole proteomic analysis of *R. rubrum* S1H at several time points has been initiated and first results will also be discussed during this presentation. Keywords: volatile fatty acids, CO2 production, *Rhodospirillum rubrum*. ●

CO-AUTHORS: Thomas Roersma1,2, Hugo Moors1, Surya Gupta1, Natalie Leys1, Felice Mastroleo1*

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

Microbially produced oxygen and microbial edible biomass are very interesting sustainable resources for future space travelers. Arthrospira-C (ArtC) is a space biotechnology flight experiment, to transplant cyanobacterial oxygenic photosynthetic bioprocess to space. ArtC is the follow-up and step-up of the pioneering ArtB flight experiment which flew in Dec. 2017 to ISS, this time allowing continuous culturing and variable light settings. A space compatible photobioreactor was built, allowing online measurements of both bio-oxygen production rate and microbial growth rate in space. They contain the cyanobacterium *Limnospira indica* PCC8005 (aka *Arthrospira* sp. PCC8005, or under the product name *Spirulina*). Four of such bioreactors are to be integrated in the ISS Biolab incubator in 2023 and will be operated in turbidostat mode by continuous feeding for a duration of 2 months, to test production kinetics at 4 different light settings. The experiment will be performed in parallel on ISS and on ground. The bioprocess will be monitored and steered, using a novel and dedicated model for the growth of *Limnospira* in membrane photobioreactors, and the space grown biomass will be analysed for its nutritive value in detail postflight. In this presentation we will update you on the current development of the ArtC flight experiment and we will address following challenges: (1) to build, qualify, and operate a

turbidostat photobioreactor in space, with special attention to liquid mixing and CFD modelling of reactor designs and operation states(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 the fitting to the predictive simulation, and (4) to implement successfully ground commands to adapt bioreactor conditions and allow several crew interventions. In addition, some reference data obtained pre-flight on ground will be presented and discussed. 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 the reactor conditions on ground. These data show it is feasible to design and operate a space-compatible continuous microbial photobioreactor, which is ready to be tested in ISS. Keywords : MELiSSA, photobioreactor, flight experiment, *Limnospira*, *Arthrospira*, model. ●

CO-AUTHORS: Thomas Roersma^{1,2}, Hugo Moors¹, Surya Gupta¹, Natalie Leys¹, Felice Mastroleo^{1*}

SANDRA ORTEGA

ECLSS Engineer – ESA – Netherlands

TOPICS: WASTE RECYCLING, MICROBIAL SAFETY

Identification of chemical contaminants in MELiSSA C1 bioreactor off-gas samples by Gas Chromatograph/Mass Spectrometry (GC/MS)

The desired sustained human presence in space requires the development of new technologies to maintain environment control, to provide water, oxygen, food and to keep astronauts healthy. Furthermore, the current Environmental Control and Life Support Systems (ECLSS) are heavily dependent on Earth resupply and on the logistics of mission resupply which limits human exploration in space. Regenerative Life Support Systems in conjunction with space resource utilization will initially reduce and ultimately eliminate consumables from the logistics chain. Minimizing this need for resupply while ensuring human safety will allow astronauts to travel further and stay longer in space than ever before. The European Space Agency (ESA) has been a pioneer in the development of highly regenerative ECLSS with the implementation 30 years ago of the European Project: Micro-Ecological Life Support System Alternative (MELiSSA). The driving elements of MELiSSA is a closed life-support system based on a circular approach focusing on a global overview of the complete life-support system aiming for the production of food, water (H₂O), and oxygen (O₂) from the organic wastes of the mission. The MELiSSA loop consists of several compartments, colonized with specific bacteria or higher plants which perform a specific function, providing all together edible material production, atmosphere regeneration and water recovery with a concomitant use of wastes, i.e., CO₂ and organic wastes. The first compartment (C1) of the MELiSSA loop is a liquefying compartment which gathers the pool for the organic waste

collected during mission (food waste, urine, paper, the non-edible output of the higher plant compartment, etc) and determines the fraction of organic wastes that can be recycled in the rest of the loop. MELiSSA C1 consists of a bioreactor and for biosafety reasons, as well as degradation efficiency, the compartment operates in thermophilic conditions (i.e. 55°C), acidic and anaerobic conditions. The main output products of the fermentation process by the microbial consortium present in MELiSSA C1 are ammonium, CO₂, volatile fatty acids and minerals. The aim of this work is to conduct an analysis of the composition of the off-gas produced upon MELiSSA C1 operation in steady-state conditions targeting the identification of chemical contaminants which are considered unsafe and/or unacceptable in spacecraft air. To fulfil this objective, two samples were collected: 1) low load (15 ml) sample aiming for the quantitative determination of compounds at higher concentration; and 2) high load (100 ml) sample to determine the concentration of low concentrations compounds. Gas Chromatography/Mass Spectrometry (GC/MS) analysis revealed a number of compounds which are considered toxic according to Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants (SMAC) including, acetaldehyde, dimethyl sulfide, methyl thiolacetate, dimethyl disulphide, methyl thiobutanoate, octamethyltetracyclosiloxane and hexanoic acid, being most of them sulfur organics. ●

CO-AUTHORS: Alex Bolkhovitinov, Christophe Lasseur

KATHARINA OST

Doctoral student – University of Applied Sciences Osnabrück - Germany

TOPICS: WASTE RECYCLING, FOOD PRODUCTION AND PREPARATION, PROTEINS PRODUCTION, CIRCULAR ECONOMY

Modular screening system for protein production in *Aspergillus niger*

The filamentous fungus *Aspergillus niger* is one of the most important production host used in the biotechnological industry to yield organic acids, proteins and enzymes. These production processes are not only economical but also resource-saving, since fungi are naturally able to grow on several waste flows and side streams of food and other industries (Cairns et al. 2018; Taddia et al. 2019). Industrial enzyme and protein production with filamentous fungi can lead to high yields using robust strains, which are greatly resistant to extreme environmental conditions such as nutrient-poor, acidic or cold habitats or even cosmic radiation during space travel (Meyer et al. 2011; Krijgsheld et al. 2013; Cortesao et al. 2020; Romsdahl et al. 2020). Making advantage of the highly efficient secretion system of *A. niger*, no downstream processing is necessary since the wanted products usually end up in the media and can be easily harvested with simple set-ups, low costs and in a time-efficient manner (Punt et al. 2002; Fleißner et al. 2010; Krijgsheld et al. 2012). In particular, the production of food-grade substances and complex biocatalysts used as additives or active ingredients mainly for food applications can be achieved in the eukaryotic expression system of *A. niger* (Abarca et al. 2004; Crains et al. 2018; Li et al. 2020). Food proteins or food enzymes such as casein, ovalbumin, lectin, lipase, phytase or glucoamylase are highly complex polymers that show different functional and structural characteristics. Most of them could be used as

nitrogen or energy source for animals and humans, while others are industrial relevant biomass-degrading enzymes used for biological waste processing and food production (Meyer et al. 2011; Damodaran et al. 2017; Li et al. 2020). However, the success of producing novel recombinant proteins can be challenging, resource- and time consuming. Therefore, *A. niger* mutant libraries are needed to understand the adjusting screws to produce high yields of recombinant proteins, preferably even in a kind of transferable system: A multipurpose microbial cell factory. In order to establish such an innovative and self-sustained expression platform e.g., for long-term space exploration, there is a need to overcome the lack of high throughput research (Hendrickx et al. 2006; Clauwaert et al. 2017; Cortesao et al. 2020). To tackle this problem, we designed a modular, quantitative and feasible high-throughput screening system to express and screen functional and stable recombinant proteins in *A. niger*. For this purpose, a dual-luciferase reporter gene system, which is applicable in small scale will be established for *Aspergillus niger*. After the generation of an *A. niger* secretion mutant library, the system will be transferred and tested to other proteins of interest. The technology can be integrated into bio-regenerative life support systems for the autonomous production of food, food ingredients and food enzymes for future deep-space missions. ●

CO-AUTHOR: Prof. Dr. Mareike Dirks-Hofmeister

YASH PARDASANI

MRes student - Scottish Association for Marine Science (SAMS-UHI) - United Kingdom

TOPICS: AIR RECYCLING AND CO₂ VALORIZATION, WASTE RECYCLING, FOOD PRODUCTION AND PREPARATION, LIFE SUPPORT ARCHITECTURE FOR FUTURE MISSIONS

Survival potential of algal species for long term space missions

Microalgae have the ability to provide essential life support functions on short and long terms missions. Applications include oxygen production, soil improvement, bioremediation of waste and as a source for human nutrition. However, we need species of algae that can be stored for extended periods of time and grown whenever required as re-stocking of fresh supplies or continual growth during transit may not be possible or if actively growing samples fail. Algae species such as *Haematococcus pluvialis*, *Dunaliella salina* and cryophilic snow algae may be suitable species for such long-term missions as they can produce high concentrations of antioxidants such as the red astaxanthin or the orange ²-carotene pigments. This accumulation also coincides with a period of dormancy in their encystment phase, after which the cells can be easily revived back to their vegetative forms when given favourable conditions. Another advantage of the physiology of these cells is that they may be more resistant to chronic doses of radiation when in space (due to the high antioxidant properties of the pigments). They may also be more tolerant of the effects of desiccation (as experienced in their dormant phase), and this would be a more efficient way of

storing such algae for space missions as it reduces the cost of launching and carrying algae in their liquid cultures to space. I will present the design concept, experimental design and current findings from my MRes student research project on algal dormancy and revivability in space. My experiments focus on which species can tolerate desiccation and whether this is correlated to their life cycle stage and pigment concentration. I have also exposed the algal cells to radiation doses similar to those reported on the International Space Station, at SCK Belgium. I have done the growth curves analysis, microscopic images, and lipid and pigment analysis on both radiated and non-radiated algal cultures to observe the effects of space radiation on the cells. In addition, the algal cells have been desiccated in well plates and revived after two weeks and two months to observe the effects of desiccation on the cells. Growth and metabolomic analysis will be undertaken on the revived desiccated cells. The findings from the desiccation experiments will provide insights into potential ways of storing algae for long-term space missions to the moon, Mars and beyond. ●

CO-AUTHOR: Dr Matthew P. Davey

CLARA PLATA RÍOS

Manager of Operation and Technologies - SEMILLA IPStar – Spain

TOPIC: CIRCULAR ECONOMY

SIMBIOPARK: Contributing to the transition to a Circular Economy through Industrial Symbiosis

The current economic system is based on a growth model that is heavily dependent on the consumption of raw materials. This linear 'take - use - dispose' model has resulted in excessive pressure on the environment that has led to some of the most serious manifestations of the environmental crisis we are suffering, such as climate change or the loss of biodiversity. In the industrial environment, the circular economy has applications at many levels, with industrial symbiosis being one of the most successful. Symbiosis is defined as the association between animals or plants of different species in such a way that both benefit from it. Inspired by symbiotic relationships in nature, the concept of industrial symbiosis reflects the symbiotic connections between companies that traditionally operate independently. These connections are based on the exchange or sharing of resources (materials, water or energy). Industrial symbiosis has proven to be effective in reducing material needs, reducing energy losses, improving the economic performance

of companies as well as improving the ecological footprint of industrial processes and the adoption of eco-innovation; creating added value for the actors involved. Following this line, SEMILLA IPStar has participated in the SIMBIOPARK project, funded by the Ministry of Ecological Transition and Demographic Challenge of the Government of Spain through the call for Innovative Business Associations and led by the Smart City Cluster. The aim of the project was to carry out a feasibility study to consolidate a methodology for the emergence of industrial symbiosis in technology and industrial parks inspired by the process approach of the MELISSA project, as well as to identify the technologies and digital tools to optimise the process. The methodology has been tested in a real environment, the Almeria Technology Park (Spain). The digital tools identified will be the subject of future projects that will consolidate a means to support the generation of symbiotic industrial parks. ●

CO-AUTHORS: Clara Plata Ríos, Ralph Lindeboom

LUCIE POULET

University Clermont Auvergne – France

TOPIC: SOCIETAL IMPACTS AND EDUCATION

AstroPlant: preliminary results from assembly and technical validation at UCA

The AstroPlant project is aimed at developing a distributed network of simple, instrumented and standardised plant growth chambers or experimental kits for researchers in the field of closed loop artificial ecosystems and circular systems as well as engage and connect citizen scientists, makers, students, and schools in this research. The focus of this project is on validating this objective within the context of the MELISSA project. Indeed, a challenge of plant production in space is the lack of data on plant growth in reduced gravity and closed environments. Data generation on plant growth behaviour under varying and non-standard (e.g., spaceflight) environmental conditions can be accelerated by developing simple plant growth facilities like AstroPlant and deploying a high number of them. Several

MELISSA partners have received the kit in 2020 to participate in the scientific validation of the system, among them University Clermont Auvergne (UCA). This poster summarises the activities performed at UCA, from setting up the hardware and configuring the information technology system, to the first tests for the technical validation. Different groups of students have used the hardware and have faced technical challenges, on the hardware and the software side. Solving them was a great learning experience for them and this demonstrates that AstroPlant can also be a wonderful educational tool, on top of being used for big data generation. The next step will be the scientific validation of the AstroPlant kit that is assembled at UCA. ●

CO-AUTHORS: Laurent PUGHON, Lucie POULET, Thieme HENNIS

BORJA POZO

Space technology leader – Tekniker – Spain

TOPICS: AIR RECYCLING AND CO2 VALORIZATION, GREY, YELLOW, BLACK WATER RECYCLING, LIFE SUPPORT ARCHITECTURE FOR FUTURE MISSIONS, SPACE EXPERIMENT

Photoelectrochemical reactor to convert Mars CO2 into space propellant using astronauts greywater

As human space exploration evolves toward longer journeys to Mars, ISRU will become increasingly important. Resupply missions are expensive, and as astronaut crews become more independent of Earth, sustained exploration will be necessary and more viable. The mission capabilities and net value will multiply when useful products will be created from Mars resources. As an example, in Mars, in which the materials can be scarce, CO2 appears as a valuable resource (95 % of the atmosphere composition) to yield chemicals and fuels. The main objective of this work is to develop an efficient and robust photoelectrochemical system (PEC) for CO2 reduction to produce space propellants based on CH4 using grey water as the electrolyte to strengthen ISRU activities of future Martian habitats and its operational properties under Mars solar conditions, where the subtracted electrons from this process would reduce CO2 in the cathode producing space propellant by direct conversion using solar light as energy source. This work is making signs of progress for a PEC system, related to reactor design, process control, and development of innovative photoelectrodes (photoanode-dark cathode configuration) with high-efficiency catalytic materials processed by magnetron sputtering and sol-gel technology due to their possibility to be an industrial-scalable technology. The catalyst is a fundamental component of a PEC cell because is responsible for the selectivity and catalytic activity of the photoelectrodes and is decisive for efficiency. Also, the synthetic method is a crucial point because it affects the properties and structure of the catalyst, and

magnetron sputtering allows for manufacturing nanostructures with accurate morphology, size, and composition maximizing the catalytic activity and the efficiency of the systems. Catalysts for the photoelectrode, using semiconductors like TiO2 or BiVO4 with nanostructured, plasmonic metals like Ni or Cu are being used to develop the cathode. Furthermore, it is carrying out the sewage treatment of the greywater produced by astronauts to the production of value-added materials from CO2, which would compensate for any lack of cell efficiency. A compact prototype gas-liquid two-chamber cell is being designed and will be manufactured with two different compartments for the two reactions separated by an anion exchange membrane. The results of this work will have an impact not only on the space technology but also on the Earth and on tackling climate change, as it could provide key information on the path to decarbonising our atmosphere. The technology could be applied for example to carbon dioxide reduction solutions in industry or to develop similar new products that reuse grey water produced by companies or means of transport such as boats and motorhomes. Summarizing in this work is developed an operative and efficient (5 %) photoelectrochemical system breadboard with an electrode area of 100 cm2, integration of a PV for the photoanode, achieving 1000 hours of lifetime, using grey water as electrolyte with a flow rate of 100-500 mL/min and producing CH4 from CO2 for future use as space propellant. Thus, promoting the ISRU concept for future Mars habitats. ●

CO-AUTHORS: Jonathan Albo (2), Jean Christophe Berton (3), Antia Villamayor (1), Itziar Azpitarte (1), Ivan Merino (2), Amaia Martinez (1)

CÉCILE RENAUD

PhD Student – UMons – Belgium

TOPICS: WASTE RECYCLING, FOOD PRODUCTION AND PREPARATION

Biostimulant effect of *Limnospira indica* polysaccharides on edible plants for future space GreenHab

Long-term manned space exploration missions to the Moon, Mars, and beyond, can't be considered without a reliable and efficient Life Support System. Fully autonomous technologies seem necessary to fulfil this goal and get rid of resupply from the Earth. By using bioregenerative systems with living organisms into a closed loop to regenerate organic waste into freshly produced oxygen and food, a fully autonomous system as required is foreseen. Within the fourth compartment, a GreenHab is developed to grow edible plants for crew consumption. For a truly efficient system, the growth of edible

plants must be optimized, the study of biostimulants seems necessary. This research is focused on a cyanobacteria strain, *Limnospira indica*, and a commercial *Spirulina* to deliver an overview of the biostimulant effects and defence on plants. To this end, evaluation of germination and morphological characteristics were analysed through a variety of analysis (PAL (Phenylalanine Ammonia Lyase) activity, free proline and anthocyanine content). Environmental stresses impact on germination and growth were also studied. ●

CO-AUTHORS: Camille Van Camp, Cécile Renaud, Ruddy Wattiez

GABRIELA SOREANU

Assoc. Professor - Gheorghe Asachi Technical University of Iasi – Romania

TOPICS: AIR RECYCLING AND CO2 VALORIZATION

Tillandsia (Xerographica) plant: a new challenge for air revitalization

This study investigates a new option for air revitalization based on Tillandsia plant, particularly Tillandsia Xerographica. For instance, biofiltration of carbon dioxide from ambient air was assessed in an illuminated column reactor operated under dynamic regime for the gas phase. Over the course of the test period, T. Xerographica exhibited a facultative crassulacean acid metabolism (CAM) and its CO₂ capture performance was depending on the experimental conditions (e.g. temperature, intensity/wave length of light). The obtained results indicate that this exotic aerial plant can overcome the drawbacks of other classical plants (e.g. soil-based) involved in air purification

devices, having the following advantages: no soil needs (no dirt related issues), minimum water supply and nutrients, light weight, easy to manipulate, easy maintenance, aesthetical look, competitive CO₂ uptake, zero dirt waste, adaptability to various designs. Moreover, simultaneous other gaseous pollutants capture is expected to also occur. These advantages make this plant emerging as a suitable candidate for air revitalization in controlled or other specific environments involved in space or terrestrial applications (e.g. underground urban automotive underground spaces). ●

Keywords: Tillandsia Xerographica, biofiltration, air revitalisation

CO-AUTHORS: Gabriela Soreanu*, Constantin Mardari, Catalin Tanase, Igor Cretescu

TOM VERBEELEN

PhD student - Belgian Nuclear Research Center – Belgium

TOPICS: GREY, YELLOW, BLACK WATER RECYCLING, WASTE RECYCLING, SPACE EXPERIMENT

Optimization of RNA Extraction from Nitrifying Bacteria in the Context of Urine Treatment in Space

Nitrification is an important microbial process on Earth and will also be key in space exploration as part of bio-regenerative life support systems. The Urine Nitrification in Space project aims to achieve full microbial nitrification from synthetic urine during a flight experiment in Low-Earth Orbit related to CIII of the MELISSA program. Nitrification from synthetic urine will be achieved through a gnotobiotic community composed of three axenic culture constituents: i) a ureolytic heterotroph that hydrolyses urea to ammonium; ii) a ammonium oxidizing bacteria (AOB) producing nitrite; and iii) a nitrite oxidizing bacteria (NOB) producing nitrate. The selected bacterial strains for these processes are Comamonas testosteroni, Nitrosomonas europaea and Nitrobacter winogradskyi, respectively. To characterize the impact of space conditions such as microgravity and ionizing radiation, samples from the spaceflight experiment will undergo phenotypical characterization, biofilm analysis, as well as transcriptomic, proteomic and metabolomic analysis. Here, we focussed on the optimization of the RNA extraction procedure for N. europaea and N. winogradskyi. These strains are characterized by a slow growth rate and low biomass production in axenic lab cultures. This represents challenges for transcriptome analysis in terms of the amount of available RNA for sequencing (RNA-Seq). Moreover, since space flight experiment set-up only allows the use of millilitres range of cultures during the experiment, increasing RNA yield by expanding the cultivation volume is not possible. Hence, alternatives to increase RNA yield were explored while retaining

quality of the RNA. In a first step, the effect of mechanical lysis through ultrasonication was assessed on several silica-column-based commercial kits. For one of the tested kits, the total RNA yield increased by a factor of almost 10 ($0.52 \pm 0.20 \text{ ng} \cdot 10^{-6}$ cells vs $4.90 \pm 1.41 \text{ ng} \cdot 10^{-6}$ cells). However, RNA in these elutions was strongly degraded (RIN = 3.6 ± 1.3). Efforts to improve sample quality by using varying amounts of lysis buffer, reducing agent, amplitude and interval of the ultrasonication process did not have an improved effect on the RNA yield and/or quality. Addition of an enzymatic lysis step with a lysozyme solution instead of ultrasonication lysis also highly increased RNA yield ($4.83 \pm 0.33 \text{ ng} \cdot 10^{-6}$ cells) and also provided high-quality RNA samples (RIN = 9.7 ± 0.5). By varying the lysozyme concentration, lysis buffer quantity and incubation time, a protocol was established that ensures optimal RNA yield and quality for N. europaea cultures. This protocol proved effective to reach threshold levels of RNA during validation on N. winogradskyi cultures as well ($1.01 \pm 0.27 \text{ ng} \cdot 10^{-6}$ cells, RIN = 8.1 ± 0.2). The procedure was also performed on C. testosteroni. It demonstrated its effectiveness on the high-biomass producing strain ($6550.00 \pm 905.15 \text{ ng}$, RIN = 8.6 ± 2.0). The protocol can also be applied to extract RNA from the gnotobiotic community. This novel RNA extraction procedure will allow to get insights in gene expression and regulation in autotrophic nitrifiers, in situations with limited biomass availability, like spaceflight experiments and samples from terrestrial experiments were RNA extractions have proven difficult. ●

CO-AUTHORS: Tom Verbeelen, Natalie Leys, Ramon Ganigué, Felice Mastroleo

ANGELO VERMEULEN

Researcher - Systems Engineering and Simulation, Faculty of Technology, Policy and Management, Delft University of Technology – Netherlands

TOPICS: PROCESS MODELING, SIMULATION AND CONTROL, LIFE SUPPORT ARCHITECTURE FOR FUTURE MISSIONS

Simulating bioregenerative life support for deep space missions without resupply: development of a fully closed stoichiometric model

For long-duration space missions it is imperative to use (bio) regenerative life support systems (BLSS) to reduce mass and volume. In most BLSS studies, only a fraction of the supplies is provided by the system (e.g., 40% of the food supply). The rest is considered to be taken on board at departure, or provided through resupply missions. In the E|A|S (Evolving Asteroid Starships) research project, autonomous long-duration space missions are envisioned without the possibility of resupply. In such a scenario, a fully closed BLSS, with minimal or no material loss is vital. A stoichiometric model is presented, based upon previous work (Vermeulen et al., 2019), updated with a detailed review of publicly available MELISSA literature from 1989-2022. A total of 23 studies provide detailed descriptions of the stoichiometry of the MELISSA BLSS. Eleven of these studies describe the entire loop, while the others describe one or two of the five MELISSA compartments. None of these studies provided a model for a fully closed stoichiometry, either because the goal was to only provide a part of the food or oxygen supply, or because some part of the stoichiometry was open, and outside resupply was deliberately integrated. The resulting stoichiometry of the presented study describes the cycling of the CHON elements through all five MELISSA compartments and one auxiliary compartment. This is done through a compact set of chemical equations with fixed coefficients. No kinetic limitations were integrated because this exceeded the level of detail

deemed necessary for further agent-based modeling studies. A spreadsheet model was developed that simulated the flow of all relevant compounds for a crew of six. The results showed that at steady state a very high degree of closure was attained, with 12 out of 14 compounds exhibiting zero loss in between iterations, and oxygen and CO₂ displaying a minor loss that amounted to respectively 0.85 g and 2.31 g per person per year. At steady state, the VFA spectrum that was produced by the liquefying Compartment 1 consisted of 48.88% acetate, 13.25% propionate, 37.53% butyrate and 0.34% valerate, a proportion that is in line with observations of anaerobic digestion in literature. This stoichiometric model is used as the foundation of an ABM of the MELISSA loop. In this ABM, humans, plant plots and bioreactors are all modeled as virtual agents that interact with each other. The ABM approach enables the introduction of heterogeneity at the level of agent attributes, and allows for the study of complex interactions that cannot be observed in more common equation-based modeling techniques. The most recent version of the ABM will also be presented with a range of results from different simulation experiments. Vermeulen, A. C. J., Papic, A., Kiem, J., Hallak D., & Brazier, F. (2019). Modeling and Simulating a Regenerative Life Support System to Understand the Effects of System Interaction on Survivability During Deep Space Missions: An Agent-Based Approach. IAC Archive. <https://iafaastro.directory/iac/archive/browse/IAC-19/A1/7/52461/> ●

CO-AUTHORS: Alvaro Papic, Jason Kiem, Igor Nikolic, Frances Brazier

CYPRIEN VERSEUX

Researcher – ZARM - University of Bremen – Germany

TOPICS: AIR RECYCLING AND CO2 VALORIZATION, LIFE SUPPORT ARCHITECTURE FOR FUTURE MISSIONS

The MaMBA facility as a testbed for bioregenerative life support systems

The Moon and Mars Base Analog (MaMBA) is a concept for an extraterrestrial habitat developed at the Center of Applied Space Technology and Microgravity (ZARM) in Bremen, Germany. The long-term goal is to create a technologically functioning prototype for a base on the Moon and on Mars. One key aspect of developing such a prototype base is the integration

of a bioregenerative life support system (BLSS) and its testing under realistic conditions. We will describe how the MaMBA-facility can be used for the integration, evaluation and comparison of different BLSS according to established engineering criteria and human factors criteria. ●

CO-AUTHOR: Dr. Christiane Heinicke

ANNA VERSHININA

Master student - KU Leuven – Belgium – poster

TOPICS: AIR RECYCLING AND CO2 VALORIZATION, GREY, YELLOW, BLACK WATER RECYCLING, WASTE RECYCLING, BIOMATERIAL, FOOD PRODUCTION AND PREPARATION, MICROBIAL SAFETY, LIFE SUPPORT ARCHITECTURE FOR FUTURE MISSIONS, CIRCULAR ECONOMY, SOCIETAL IMPACTS AND EDUCATION

Microbial Gardens: A design-led proposal for the creation of a human-bacteria cohabitation unit for inhabiting extreme environments

No creature can thrive in complete isolation from other living things. Life is innately interdependent on its interactions with all kinds of other life forms from reproductive strategies to substances produced by metabolism, the construction of niches and, in deep time, geological changes generated by the day-to-day actions of living and dying. By far the oldest, most multitudinous, and diverse organisms on our planet are microbes that have invested the most in this process, upholding the fabric of life on our home planet. A range of 21st century biotechnological developments (e.g., 16S rRNA screening, metagenomics) provide insights that reveal how fundamentally microbial our living world is. It is now known that ecosystems of microbes, known as microbiomes, are foundational to all ecosystems, raising their importance in the construction and maintenance of habitats. Microbiomes are not only found in the spaces around us, but also exist within many complex multicellular organisms, including humans. Forming an essential organ, these microbiomes provide the key interface between the body and the environment. While our knowledge of microbiomes is still emerging, the spatial understanding and 'gardening' of microbial populations, and the construction of different kinds of microbiomes (bodily, environmental, built environment) are not well defined, or fully understood. However, the incorporation of microbiomes into the construction of our living spaces is crucial for the survival of terrestrial life in off-world scenarios, and in extreme circumstances. This project explores how gardening microbial colonies and microbiomes within a space designed for human occupation can generate stable foundational microbial ecosystems that support the daily activities of (human) life, by enabling a foundational biodiversity that confers resilience on the participating communities. Set in Australia in 2070, which is no longer habitable owing to rising average temperatures and widespread bush fires, residents migrate to colder climates, like Antarctica. Here, they must establish new protocols and rituals to support their new lifestyles under extreme conditions (Armstrong, 2017). Forming alliances with microbes through the practice of microbial

gardening, the Australian migrants integrate them into all the daily rituals of life by constructing a novel life-supporting infrastructure for human settlement. Cultivated within the domestic realm, microbes are selected for their desired functions and in the specific example presented, they produce bioelectricity, filter air and clean water. These productive spaces are also sites for intense study, where detailed knowledge about the interdependence between human and microbe is established through extensive empirical observations. Additionally, these microbial gardens generate biomass and minerals for healthy nutrition, as well as supplying raw materials for the repair and upkeep of the home (Armstrong et al., 2017). By using new living materials in the overall structure, a new philosophy of the built environment is developed where dwellings possess some of the characteristics of living things i.e., they are impermanent, growing, evolving and decaying along with the people and the site. Thus, the unit s human and microbial residents, co-constitute one another to eventually become part of the living landscape of Antarctica, enriching the land for the next generation of settlers. ●

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CO-AUTHOR: Anna Vershinina, Rachel Armstrong Tel.: +32456300193 anna.vershinina@student.kuleuven.be anna.vershinina52@gmail.com rachel.armstrong@kuleuven.be Organization: KU Leuven

ANA VILLENA GIMÉNEZ

Predoctoral Student - Institute for integrative systems biology - CSIC-UV – Spain

TOPIC: MICROBIAL SAFETY

Dynamics of viral infection in simulated microgravity and low radiation conditions

The environment is a key factor in virus-host interactions, eventually modulating the infection outcome and its severity. Indeed, it has been shown in different organisms that microgravity affects their immunity (Ryba-White et al., 2001, Gilbert et al., 2020). Therefore, future long-term space missions will need to consider a variety of scenarios spanning different radiation backgrounds and gravities affecting the astronauts, as

well as the extent in which the associated changes in host physiology might impact the pathogen s evolution and virulence. In this project we use the nematode *Caenorhabditis elegans*, a model organism already being used in space research (Liu et al., 2019), as a host to study the effects of spatial conditions may have in the progression of viral infections. *C. elegans* is a millimetric nematode with a short lifecycle with

most of their genes having homologs with human genes. The discovery of the first virus naturally infecting *C. elegans* (Orsay nodavirus, OrV) has made the nematode an emergent model in the study of virus infection and immunity (Félix et al., 2011). It is known that simulated microgravity conditions (SMC) induce intestinal damage in *C. elegans* (Liu et al., 2019) and that OrV replicates in the intestinal cells of the nematode (Franz et al., 2014). Therefore, it seems conceivable that microgravity will have an effect on the progression of OrV infection. Furthermore, it is acknowledged that radiation has a strong influence on living systems and all biological processes, for example below background radiation reduces *Drosophila melanogaster* lifespan

and fertility (Morciano et al., 2018). However, it is still unknown how it affects the fly s immune system. Microgravity and low radiation conditions would have relevant consequences on the virus pathogenesis, conditioning infection outcomes and virulence evolution. In summary, the goals of the project are three-fold: (1) to characterize OrV infection progress of *C. elegans* under microgravity and/or low radiation conditions; (2) to identify the transcriptomic and physiological responses of the animal to such stressful conditions with and without infection; and (c) to evaluate how these stressful conditions affect the evolution of OrV fitness and virulence. ●

CO-AUTHORS: Rubén González and Santiago F. Elena

VIRGINIE BLANC

Montfermeil (Seine Saint Denis) France - Éducation nationale

TOPICS: LIFE SUPPORT ARCHITECTURE FOR FUTURE MISSIONS, SOCIETAL IMPACTS AND EDUCATION, SPACE EXPERIMENT

FOCALE SUR LA PLANÈTE : Mission AstrODD

Les élèves mènent l'enquête ! Objectif de développement durable et Espace ? Vous vous demandez quel lien il y a ? Les élèves du Cm2 et leur enseignante Virginie BLANC de l'école Christiane COULON de Montfermeil (Seine saint Denis) vous répondent à travers la mission AstrODD. À la suite de recherches sur la toile, d'interviews exclusives d'éminents experts venus en classe, les élèves parviennent à interroger des problématiques sociétales et environnementales. C'est ainsi que Christophe LASSEUR, chef du projet MELISSA, travaillant à l'agence spatiale européenne explique comment le projet peut permettre à des

spationautes de recycler les différentes eaux dans l'espace mais également de quelle manière MELISSA est utilisée sur Terre. Le poster, réalisé dans le cadre du projet FOCAL SUR LA PLANÈTE par les élèves, informe et propose des pistes d'amélioration pour répondre à l'agenda 2030 lancé par les Nations Unis. Vous pouvez retrouver les 17 posters ODD/espace ainsi que le webdocumentaire avec les 14 interviews d'experts menées par les enfants, introduites dans une narration interactive sur le site focalesurlaplanete.com ●

CO-AUTHORS: Les élèves du CM2 année 2021/2022

LAIA VULART

PhD student - Universitat Autònoma de Barcelona / Universiteit Gent – Belgium

TOPICS: WASTE RECYCLING, LIFE SUPPORT ARCHITECTURE FOR FUTURE MISSIONS

Organic waste degradation architecture for the MELISSA Pilot Plant

In the context of long-term space missions, life support systems (LSSs) become a mandatory need to satisfy human requirements and achieve mission success. The MELISSA (Micro-Ecological Life Support System Alternative) concept established within the European Space Agency is a closed loop designed to produce food, potable water and a breathable atmosphere from the wastes generated by the crew. The MELISSA loop is divided in six integrated compartments incorporating the main functions of the regenerative life support system. Currently, closing the carbon-cycle, by oxidizing the carbon in the organic waste into CO₂, is one of the main challenges in the MELISSA loop. It is done by the combination of two compartments, named C1 and C2. The C1 compartment is a thermophilic stirred-tank fermentation reactor coupled to a crossflow membrane where the biodegradation of the organic waste generated by the crew (e.g. feces and non-edible parts of plants) takes place, producing carbon dioxide (CO₂) and a liquid effluent rich in volatile fatty acids (VFA), ammonium and minerals. The C2 compartment is based on a microbial electrolysis cell (MEC), and it is intended to further oxidize the VFA of the C1 effluent to produce CO₂. This

contribution summarizes the current know-how on C1 and C2 compartments, their challenges and future perspectives in the MELISSA loop. The ensemble of these compartments will determine the fraction of organic matter that can be recycled in the full loop. Despite the progress previously made in their development, their performance still needs to be optimized in order to obtain a higher conversion of the organic wastes. Accordingly, the C2 compartment should achieve long-term stable operation, using the C1 permeate as feed and avoiding diversion of electrons to alternative electron acceptors. The integration of both compartments is another major step to take into consideration. Until now, the current MELISSA activities have demonstrated the feasibility of a C1-C2 integration in series, to drive carbon recovery to CO₂. However, the study of alternative strategies could be of great interest. For example, the recirculation of part of the outflux from C2 to the C1 compartment could be a promising operation strategy, since it could reduce the concentration of VFA and other inhibitory compounds in the C1 compartment, potentially decreasing VFA-related toxicity and enhancing biomass conversion. Furthermore, the integration of

the C1 and C2 compartments in the MELiSSA Pilot Plant (MPP), the ground demonstration and integration facility of the MELiSSA loop, is expected in the upcoming years. The composition and management of the microorganisms used in C1, the architecture of the C1 and C2 compartments, and the complexity in process monitoring and control of these compartments are some of the

CO-AUTHORS: Ramon Ganigué, Francesc Gòdia

DANIEL YEH

Professor of Environmental Engineering - University of South Florida - United States

TOPICS: GREY, YELLOW, BLACK WATER RECYCLING, CIRCULAR ECONOMY, SOCIETAL IMPACTS AND EDUCATION

The NEWgenerator Resource Recovery Machine for off-grid wastewater treatment: Case studies for global sanitation in India and South Africa, and implications for space colonies.

Water, sanitation and hygiene (WaSH) is at the core of the Sustainable Development Goals (SDGs). Yet, billions of people worldwide, many in marginalized communities, suffer from poor sanitation stemming from lack of wastewater infrastructure. Due to high CAPEX and OPEX, the conventional approach of centralized wastewater treatment plants served by an extensive sewer system is not an option for many communities. Accordingly, a new classification of modular and pre-fabricated non-sewered sanitation systems (NSSS) have been proposed as a micro-infrastructure alternative. Developed at the University of South Florida (USA) through the Bill and Melinda Gates Foundation's Reinvent the Toilet Challenge (RTTC), the NEWgenerator is a solar-powered, modular, automated, wastewater treatment and recycling system capable of operating completely off-grid from energy, water and sewer. The NEWgenerator is designed to recover nutrient fertilizer, clean water, and renewable energy from wastewater and organic wastes. The core technology stages within the NEWgenerator are the anaerobic membrane bioreactor (AnMBR) (for solids, COD and pathogen removal), nutrient capture bed (for N and P removal/recovery), and

key challenges in the implementation of the MPP roadmap. In conclusion, the optimization of both compartments and their integration in the MPP would be a major step in the development of this technology, determining not only the future of compartments C1 and C2, but also the performance of the whole MELiSSA loop. ●

electrochlorination (for pathogen removal and polishing). The NEWgenerator is capable of handling a wide range of wastewater strengths (black, yellow, grey), intermittent flows, and prolonged shutdowns/dormancies. This presentation will follow the two-decade journey of the NEWgenerator from concept to development to commercialization, including multi-year field trials in India (school in Kerala) and South Africa (informal settlement community in KwaZulu-Natal) with the NEWgen 100, which is capable of providing service to hundreds of users per day. Focusing on the water-energy-food nexus, horticultural feasibility studies have been conducted on the direct fertigation of crops (such as Swiss Chard) using NEWgenerator membrane permeate. The NEWgenerator is the recipient of the 2014 Cade Museum Prize for Innovation and the 2020 USPTO Patents for Humanity Award. Because the NEWgenerator can establish a closed-loop, circular-economy, micro-infrastructure in resource-constrained environments, the technology has the potential to be adapted for space colonies. The presentation will describe potential future applications in surface habitats on the Moon and Mars. ●

CO-AUTHORS: Robert Bair, Cynthia Castro, Hsiang-Yang (Gary) Shyu, Lindelani Xaba

IZABELA ZWICA

PhD student - University Warmia and Mazury in Olsztyn – Poland

TOPIC: CIRCULAR ECONOMY

Project of algae-bacterial reactor for the extraction of iron from lunar regolith

Biomining, the use of microorganisms to recover precious and base metals from mineral ores and their concentrates, is a process that has developed rapidly in recent years. Currently, about 15% of copper, 5% of gold as well as smaller amounts of other metals such as nickel or zinc are produced worldwide using biomining technology (Barrie Johnson & Hallberg, 2008), (Johnson, 2013). Research to date has largely focused on the design and engineering aspect of bioprocessing. Addressing the topic from a microbiological perspective has received much less in-depth analysis and scrutiny. The process of iron extraction from lunar regolith will allow the magnetic separation process of lunar regolith to be intensified. From previous considerations, the most effective bioleaching and biooxidation processes are carried out by consortia of acidophilic prokaryotes (Rawlings &

Johnson, 2007). These bacteria are capable of reducing metal ions and living in an environment with or without oxygen. However, they require the provision of adequate nutrients to carry out the bioreduction processes properly. This requirement can be met by using microalgal biomass. Algae contain all the necessary elements to provide an alternative growth medium for bacteria. In addition, microalgae help to recycle waste streams such as wastewater. What is more, microalgae convert the carbon dioxide produced during the bioextraction process into oxygen (photosynthesis process), which is also a form of recycling. For example, *Shewanella oneidensis* can utilize Fe³⁺, which is embedded in mineral structures, reducing it to aqueous Fe²⁺ while consuming lactate. A theoretical reactor design for mining activities using bacteria and algae on Mars, as described

by scientists, would be capable of producing 100 kg of iron per year (Volger et al., 2020). The end result is the creation of an iron-rich material. This can be used to manufacture, among other things, structural or machine components. The second product of the reaction is iron-free regolith, which can make a good growing medium for plants. When the material on which one intends to grow any crop contains a lot of toxic compounds (which is what iron has if it is present excessively), it is difficult to have abundant yields. Magnetic segregation followed by bacterial extraction of iron ensures the creation of a substrate suitable for cultivation. These modifications should enable plants to grow better on the substrate provided and, in the long run, make space exploration more sustainable. The iron extracted from the bioprospecting process will also help to reduce costs when designing extraterrestrial missions. ●

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