MELiSSA WORKSHOP

8. – 9. June 2016
Lausanne

Workshop on Regenerative Life-Support
Book of Abstracts

European Space Agency

Organizers

Dr Christophe Lasseur (ESA), Dr Stephanie Raffestin (ESA), Prof. Suren Erkman (UNIL), Theodore Besson (UNIL/Earth Space Technical Ecosystem Enterprises SA)

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Lorenzo Bucchieri (Enginsoft)

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Prof. Ruddy Wattiez (University of Mons, U MONS)

Prof. Suren Erkman (University of Lausanne, UNIL)

Prof. Stefania De Pascale (University of Naples)

Prof. Mike Dixon (University of Guelph, UoG)

Dr Heleen de Wever (VITO)

Sessions Chairs

Session 1: Prof. Francesc Godia (UAB)

Session 2: Dr Heleen De Wever (VITO)

Session 3 : Dr Baptiste Leroy (U MONS)

Session 4 : Brigitte Lamaze (ESA)

Session 5 : Dr Natalie Leys (SCK.CEN)

Session 6 : Prof. Suren Erkman (UNIL)

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PROGRAM

Wednesday, June 8, 2016

08:00 Registration / Amphimax 410

08:30 Introduction & goals of the workshop

- Franco Ongaro (ESTEC Director, ESA/TEC Directorate Director)_ **Amphimax 414**
- **Prof. Philippe Moreillon (UNIL's vice rector "Research and international relations")**
- **Dr Oliver Botta (Swiss Space Office)**

Session 1: Waste Processing

Chair: Prof. Francesc Godia (UAB)

09:00 ***MELiSSA State of art: Waste recovery as a prerequisite for long term Space missions*. Dr Peter Clauwaert** (U GENT), **Prof. Ruddy Wattiez** (U MONS)

09:30 ***How to discover and apply "impossible" anaerobic micro-organisms to recycle carbon and nitrogen in a sustainable way*. Prof. Mike Jetten** (Soehngen Institute of Anaerobic Microbiology, Radboud University, Nijmegen, Netherlands)

10:00 *Integrated omics provides unprecedented insights into microbial community structure and function*. Shaman Narayanasamy, University of Luxembourg

10:15 *Engineering microbial communities for understanding and applications*. Prof. Dr Orkun Soyer, University of Warwick (UK)

10:30 *Combination of a bioanode and a biocathode in a bioelectrochemical system allows biowaste oxidation and microbial electrosynthesis of carboxylic acids with a more than two-fold increase in energetic efficiency*. Dr Theodore Bouchez, IRSTEA-HBAN (France)

10:45 *Pyrolytic conversion of human waste: an element of a closed-loop system?* Andreas Schoenborn, Zurich University of Applied Science ZHAW

11:00 Coffee break/Poster Session

11:30 *From living cells to stable isotopes: an interdisciplinary approach for unravelling microbial interactions in ammonia-overloaded anaerobic digesters*. Dr Francesc Prenafeta, GIRO Joint research Unit IRTA-UPC (Spain)

11:45 *Short and long term road map for the development of a robust mechanistic and dynamic model of the MELiSSA C1 compartment based on microbial community characterization*. Dr V. Nolla-Ardèvol, KUL (Belgium)

Session 2: Water Recycling

Chair: Dr Heleen De Wever (VITO)

12:00 ***Water cycling for manned Space missions: State-of-the-art within the MELiSSA concept***. **Prof. Siegfried Vlaemick** (U GENT)

12:30 ***How to cope with so much food: challenges for nitrifying bacteria in urine***. **Dr Kai M. Udert** (EAWAG, Swiss Federal Institute of Aquatic Science and Technology, Switzerland)

13:00 Lunch

14:00 *Electrochemical systems as core engines of the MELiSSA loop*. Prof. Korneel Rabaey, Ghent University (Belgium)

14:15 *Closed loop hydroponics for a novel crop cultivation system on the EMCS rotor*. Silje Wolff (CIRIS, Norway), Sander van Delden, Wageningen University (The Netherlands)

14:30 *A certainty of bacterial growth on pipe materials in contact with potable water*. Frederik Hammes, EAWAG (Switzerland)

Session 3: Air Recycling

Chair: Dr Baptiste Leroy (U MONS)

14:45 ***Photobioreactor in space habitat: status and challenges***. **Christel Paille** (ESA), **Steven Hens** (QinetiQ Space), **Samuel Gass** (RUAG SPACE)

15:15 ***Some insights on photobioreaction engineering***. **Prof. Jack LEGRAND** (GEPEA, -UMR CNRS 6144, University of Nantes – St Nazaire, France)

15:45 *Cultivation of microalgae for advanced closed life support systems as a technical and biological challenge*. Dr Stefan Belz, University of Stuttgart, Institute of Space Systems (Germany)

16:00 *ModuLES-PBR – lessons learned through parabolic flight tests*. Dr Klaus Slenzka, OHB system (Germany)

16:15 *Heat Ventilation and Air Conditioning of a Greenhouse in Antarctica*. Lorenzo Bucchieri, EnginSoft (Italy)

16:30 Coffee Break/Poster Session

Session 4: Food production & Preparation

Chair: Brigitte Lamaze (ESA)

17:00 ***MELiSSA State of art*. Dr Roberta Paradiso** and **Prof. Stefania de Pascale** (University of Naples)

17:30 ***Climate factors and crop growth: physiology and mechanistic modelling*. Prof. Ep Heuvelink** (Wageningen University, the Netherlands)

18:00 *Emerging bioprocessing concepts for healthy and sustainable foods*. Prof. Dr.-Ing. Alexander Mathys, ETH Zurich (Switzerland)

18:15 *PFPU-Main Scientific criticalities of the Precursor of Food Production Unit in the MELiSSA framework*. Giorgio Boscheri, Thales Alenia Space (Italy)

18:30 *NEMO'S garden: growing plants underwater*. Dr Elisabetta Princi, OCEAN REEF GROUP

18:45 *Lessons learned from commercial scale urban Spirulina production*. Saumil Shah, EnerGaia

19:00 End

20:00 Dinner

Thursday, June, 9, 2016 (UNIL, Amphimax 410)

Session 5: Chemical & Microbial safety

Chair: Dr Natalie Leys (SCK.CEN)

08:*00* ***Closed loop system: a safety challenge for the crew as well as for the life support processes***. **Dr Christophe Lasseur** (ESA)

08:15 ***About Microbial hazard and risk for Humans in spatial habitats***. **Prof. Jean-Pierre Flandrois** (University of Lyon 1, LBBE-UMR 5558, France)

08:45 *Application of ultraviolet-LED systems for microbial control in air and water loops*. Richard Simons, AquiSense technologies (Europe) Ltd/BIOWYSE

09:00 *Automated high-frequency flow cytometry enables in-situ process monitoring, control, and optimization of engineered water systems*. Michael Besmer, EAWAG/ETH Zurich (Switzerland)

09:15 *The Characterization of Humidity Condensation and Associated effects using Image Processing Techniques*. Dr Akhilesh Tiwari, IIIT Allahabad (India)

09:30 *Understanding and Modelling of Airborne Bio-Contamination Process in Manned Spacecraft*. Aku Karvinen, VTT Technical Research Centre of Finland

09:45 *The peak of harvest- a non-destructive identification method. Streamlining the crop production for planetary missions by nutritional value indexes.* R.M. Giurgiu, University of Agricultural Sciences and Veterinary Medicine (Romania)

10:00 Coffee Break/Poster session

Session 6: System tools

Chair: Prof. Suren Erkman (UNIL)

10:30 ***Comprehensive modelling and simulation, reliable sensors and multilayer control strategy: the essential system tools for a sustainable bioregenerative LSS***. **Prof. Gilles Dussap** (UBP)

11:00 ***Model Predictive Control: State of the Art and Possible Opportunities for Life-Support Systems***. **Prof. Alberto Bemporad** (IMT School for Advanced Studies, Lucca-Italy)

11:30 *A thermodynamic theory of microbial growth and its perspectives for modelling environmental biotechnology processes*. Dr Theodore Bouchez, IRSTEA-HBAN (France)

11:45 *EnRUM-Space and Energy Resources utilization Mapping*. Gino Perna, Enginsoft (Italy)

12:00 *Marine fish aquaculture in closed system : the couple Fish/algae*. Dr Cyrille Przybyla, IFREMER (France)

12:15 *European Platforms for Life-Support System development and validation*. Dr Peter WEISS, COMEX (France)

12:30 *Oïkosmos research agenda: relevance of manned interplanetary missions to terrestrial sustainability*. Théodore Besson, UNIL (Switzerland)

12:45 *How Space technology empowers the Transition towards a circular economy*. Rob Suters, IPSTAR (Netherlands)

13:00 *Workshop Conclusions*, Dr Marc Heppener (Head of Science Department, Directorate of Human Spaceflight and Robotic Exploration, ESA)

13:30 End of the MELiSSA Workshop

SESSION 1 – WASTE PROCESSING

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MELiSSA State of art: Waste recovery as prerequisite for long term space missions

Dr Peter Clauwaert[a], Prof. Ruddy Wattiez[b] peter.clauwaert@ugent.be

Amanda Luther[a], Frederik Ronsse[a], Diego López Barreiro[a], Dongdong Zhang[a], Litse Huyghe[a], Heleen De Wever[c], Ilse Smets[c], Ralph Lindeboom[a], Benedikt Sas[a], Korneel Rabaey[a], Dries Demey[d], Vimac Nolla Ardevol[d], Dirk Springael[d],

a – Gent University, Belgium; b – Mons University, Belgium; c – VITO, Belgium; d – KU Leuven, Belgium; e – Qinetiq Space, Belgium

Long term manned space missions such as the establishment of a base on Mars present the daunting challenge of supplying basic resources necessary to support human life: water and food. For 25 years, the European Space Agency has been developing a Micro-Ecological Life Support System Alternative (MELiSSA) to address this challenge. The MELiSSA-loop is conceptually a closed loop compartmentalized artificial aquatic ecosystem designed to recover water, carbon, and nutrients from solid wastes (food waste, urine and feces) for the regeneration of food and oxygen. This review will highlight some of the extensive research activities that have contributed to the development of the waste treatment compartment, including lab and pilot scale studies. We will focus in depth on current challenges, including the microbial characterization of a thermophilic fermentation reactor and on the development of a fibre degradation unit (FDU) to achieve a fast and complete oxidation of recalcitrant organic compounds to CO2. Applying a thermochemical step allows obtaining a clear aqueous product while simultaneously recovering nutrients in dissolved form and CO2.

Biography: Prof. Mike Jetten

Prof. Jetten is a world leader in the field of environmental microbiology and he received the prestigious ERC Advanced Grant in 2008 for his research on anammox bacteria, the Spinozapremie in 2012, and a second ERC Advanced grant in 2013 to study the ecology of methane oxidizers. In 2013 he won two Gravitation awards. He is a member of the Royal Netherlands Academy of Sciences, the European Academy of Science and EMBO. In 2013 he was bestowed with a knighthood for his exceptional services to science. He has published more than 400 papers that have been cited more than 34000 times. His H index is 91. He has supervised 35 PhD theses and 32 post docs, and 19 PhD students and 8 post docs are currently working in his laboratory. He has been invited more than 250 times for keynote lectures and organized many international meetings and conferences. He holds several patents. A list of video interviews is provided below.

Chronology

PhD study 1987-1991 Wageningen University
Post doc 1991-1994 MIT Cambridge USA
KNAW fellow 1994-1998 TU Delft
Assistant professor 1998-2000 TU Delft
Full professor Ecological Microbiology 2000-now at Radboud University
IWWR Director of Research 2004-2010
Deputy Dean Faculty of Science 2010-2014
Scientific director Soehngen Institute of Anaerobic Microbiology

Websites

Google Scholar Citations	http://scholar.google.com/citations?user=iXjCKTgAAAAJ
LinkedIn	http://www.linkedin.com/in/mikejetten
Radboud University	www.ru.nl/microbiology
Anaerobic Microbiology	www.anaerobic-microbiology.eu

Video Interviews

http://vimeo.com/86984216
http://horizon-magazine.eu/media/methane-munching-bacteria-can-help-save-environment_en.html
http://vimeo.com/84208443
http://vimeo.com/84402044
http://www.oogomoog.nl/promotiefilms/topwetenschapper-mike-jetten-een-portret/

How to discover and apply "impossible" anaerobic micro-organisms to recycle carbon and nitrogen in a sustainable way

Prof. Mike Jetten; Radboud University, Netherlands m.jetten@science.ru.nl

The world is a microbial planet, where microbes fulfil many beneficial roles, and only very few are pathogens. The strategy of the Radboud Microbiology group to discover and apply new anaerobic microbes will be explained and several examples of new discoveries in the nitrogen and methane cycles will be highlighted. Anaerobic oxidation of ammonium by anammox bacteria is such a recent discovery in the nitrogen cycle catalyzed by novel so-called impossible microbes. The anammox processes was once deemed to be biochemically impossible and non-existent in nature, but has now been identified as important player in global nitrogen cycling. Molecular studies showed that anammox bacteria can make the rocket fuel hydrazine by novel protein complexes that are located in a unique bacterial organelle surrounded by ladderane lipids. Anammox is applied both in side stream and main stream waste treatment, circumventing the use of oxygen and organic carbon and preventing the emission of greenhouse gasses. Another example is the anaerobic oxidation of methane (AOM) by so called Methylomirabilis. oxyfera bacteria that turned out to have a new intra-aerobic metabolism. They are able to produce their own oxygen by conversion of 2 NO into O2 and N2 by a putative NO dismutase. Also AOM can be applied in waste treatment in combination with anammox or oxygen limited nitrification. The carbon that is not used in the processes can be converted into biogas or into bacterial storage material that can be used in the production of biodegradable plastics. Molecular surveys have indicated that both these organisms are wide spread in anaerobic ecosystems around the globe where they most probably interact in an intricate anaerobic food chain.

Furthermore these new microorganisms are investigated within the center of excellence in anaerobic microbiology (www.anaerobic-microbiology.eu) funded by the Netherlands Gravitation program 024.002.002 SIAM and ERC AG EcoMOM 339880.

Integrated omics provides unprecedented insights into microbial community structure and function

Shaman Narayanasamy; University of Luxembourg, Luxembourg shaman.narayanasamy@uni.lu

Microbial communities are ubiquitous and underlie various biotechnological, biogeochemical and biomedical processes. In situ characterization of such communities is challenging due to their inherent complex structures and dynamics, rendering classical microbiology methods ineffective. We recently developed a suite of methods to study such consortia, and applied them to numerous microbial ecosystems of varying complexities, including wastewater sludge microbial communities, human gut microbiome and acidophilic bio-mining cultures. Developed methods encompass wet-lab and bioinformatic procedures, allowing high-throughout, high-fidelity and high-resolution molecular analyses. The foundation for such surveys is the extraction of high-quality biomolecules; DNA, RNA, proteins, and metabolites, isolated from a single unique sample enabling downstream integrative analyses. The protocol is further enhanced by automation using a robotic platform, which also minimizes technical variability. These biomolecular fractions undergo systematic high-throughput measurements, such that DNA and RNA sequencing yields metagenomic and metatranscriptomic data, respectively. Chromatography followed by mass-spectrometry methods are applied to proteins and metabolites yielding metaproteomic and (meta)metabolomic data. The integration of the resulting data provides a detailed view of microbial community composition whereby metagenomic data provides an overview of community structure and functional potential, whereas metatranscriptomics, metaproteomics and metabolomics enable functional characterization of the community or single populations. Integrative large-scale bioinformatic analyses produces a molecular-level overview that enables discovery of microbial community- and population-level characteristics and dynamics, ranging from the resolution of lifestyle strategies of various bacterial populations to the identification of keystone-genes and -species. The complementary application of high-resolution microscopy (nanoSIMS) provides a means of hypothesis testing, for example providing a high-resolution view of population resource usage on a cellular-level. In addition, time-resolved studies allow the extension towards inferences on community dynamics, including the influence of physico-chemical parameters on the community and the interaction of biotic factors within the community. The knowledge from the aforementioned interactions enables the formulation of mathematical/probabilistic models that elucidate and potentially predict the behavior of a given system. In summary, the systematic study of microbial communities using integrated-omics enables unprecedented insights within the scope of microbial ecology, which potentially allows targeted perturbation of microbial systems to optimize biotechnological, biogeochemical and biomedical processes in the future.

Engineering microbial communities for understanding and applications

Orkun Soyer; University of Warwick, United Kingdom o.soyer@warwick.ac.uk

The key mantra of synthetic biology to engineer biology is applicable across biological scales. Thus, engineering of multi-species, synthetic microbial communities is increasingly being recognised as a new frontier in synthetic biology. The resulting systems can allow fully exploiting the biochemical potential of the microbial world, and that can allow novel applications such as artificial gut, synthetic soil, waste-to-value conversion, and ecosystem functions in space missions. Despite this potential, the progress in engineering synthetic communities is slow paced. In particular, it is unlikely that the current focus on engineering genetic circuits in model organisms on its own can achieve these high level applications of synthetic microbial communities composed of multiple microbial species that are biochemically and industrially relevant. To successfully carry out this kind of complex engineering, synthetic biology will need to establish the design principles governing microbial interactions and develop the experimental tools for their characterisation and manipulation. Here, I will describe our ongoing efforts in discovering the reasons for metabolic interactions among microbes. I will argue that these metabolic interactions can be understood as an emergent property of the biochemical environment and associated thermodynamics within a multi-species system. Particularly, results from our recent research has shown that the thermodynamic basis of microbial growth can lead to co-existence, even under conditions that are kinetically predicted to lead to competition-driven exclusion. This thermodynamic inhibition can have significant consequences for the engineering of multi-species system. I will illustrate these with experimental systems that we are developing in our group, where we aim to establish key metabolic interaction motifs among biochemically and biotechnologically relevant microbes and extending to inter-kingdom interactions with fungi and plants.

Combination of a bioanode and a biocathode in a bioelectrochemical system allows biowaste oxidation and microbial electrosynthesis of carboxylic acids with a more than two-fold increase in energetic efficiency

Dr Theodore Bouchez; IRSTEA-HBAN, France theodore.bouchez@irstea.fr

Theodore Bouchez, Elie-Desmond-Le Quéméner, Arnaud Bridier, Lenaïck Rouillac, Céline Madigou, Laurent Mazeas

Microbial electrosynthesis (MES) is a promising technology to produce organic chemicals or fuels from carbon dioxide and electricity. Moreover, cathodic carbon dioxide reduction reactions might be coupled to biological oxidation of organic substrates thus allowing to leverage the chemical energy contained in residual waste streams. Here we show how the coupling of a bioanode with a biocathode allows a dramatic increase in energy efficiency of MES. We monitored 3 dual-chamber reactors with both biotic anode (carbon cloth) and cathode (stainless steel) separated by a cation-exchange membrane. A specific strategy was developed with successions of potentiostatic and amperometric controls to let the biofilm develop on the anodes at the beginning of the experiment, and then let deliver a stable 5 A/m^2 current density at the cathodes until anodic substrate exhaustion patent (FR 3 026 413). After a first substrate injection, acetate was efficiently used at the anodes with a mean coulombic efficiency (CE) of 78%. However 53% of the produced electrons were used at the cathodes for methane production and only traces of VFAs were detected. After a second substrate injection, the CE reached 89% at the anodes. Methanogenesis was inhibited in cathodes using 2-bromo-ethane sulfonate (BES) and VFAs therefore accumulated in the catholytes with a production rate of acetate reaching 11 $g.m^2.d^{-1}$ and a CE of 29%. 16S ribotags pyrosequencing showed a dramatic switch of microbial populations on cathodes after BES injection with an inhibition of Methanomicrobiaceae (from 43% of total diversity to less than 1%) and a development of Clostridiaceae (from 2% to 57%). The electrons used for VFAs production were extracted with a potential of -0.24 V (vs. standard hydrogen electrode) thus lowering the required anodic voltage from around 1 V in comparison with water electrolyzing anodes. The combination of a biocathode with a bioanode allowed thus a more than two-fold increase in energetic efficiency compared to systems electrolyzing water at the anode and promise higher energy savings with optimized cathodic potentials. Such bioelectrochemical reactors oxidizing biowaste at the anodic compartment and producing carboxylic acids at the cathodic compartments were successfully operated during several months.

Pyrolytic conversion of human waste: an element of a closed-loop system?

Andreas Schoenborn; Zurich University of Applied Science, Switzerland andreas.schoenborn@zhaw.ch

Andreas Schoenborn, Mira Bleuler, Nicola Bulant

A critical step for the "total conversion of organic wastes and CO2 to oxygen, water and food", as envisioned by MELiSSA is the 100% hygienically safe conversion of human excreta. At ZHAW we are developing a sanitation concept built around the pyrolytic conversion of human fecal matter to fecal biochar. We were able to demonstrate that most micronutrients and nutrients (with the exception of nitrogen) can be found in the resulting fecal biochar. In combination with a suitable procedure for N-recovery from urine, this concept can point a way to a sustainable closed-loop system. In current research we are testing the effects of this fecal char on germination (with cress and salad seeds) and on plant growth (in pot experiments). If the feedstock is not too wet, pyrolysis can be net-energy positive. In the presentation I will report about these experiments.

From living cells to stable isotopes: an interdisciplinary approach for unravelling microbial interactions in ammonia-overloaded anaerobic digesters

Francesc Prenafeta; GIRO Joint Research Unit IRTA-UPC, Spain francesc.prenafeta@irta.cat

J. Ruiz-Sánchez, B. Fernández, M. Viñas, M. Guivernau, L. Tey, V. Riau, F. X. Prenafeta-Boldú

Anaerobic digestion (AD) is a widely used technology for the treatment and bioenergy valorisation of organic wastes and wastewaters. However, the AD of substrates rich in nitrogen-containing compounds (i.e. food waste, slaughterhouse by-products, and animal dejections) results in the accumulation of free ammonia inside the bioreactor. Such relatively high ammonia concentration levels impair the activity of acetoclastic methanogenic archaea (AMA) and, thus, compromises the feasibility of the AD process. Alternatively, the rather ammonia-tolerant syntrophic acetate oxidizing bacteria (SAOB) and hydrogenotrophic methanogenic archaea (HMA) might form a consortium that allows the full mineralization of organic matter under high nitrogen-content conditions. SAOB are characterized by low growth rates and its enrichment and retention in bioreactors is a challenging process. On-line monitoring of SAOB and HMA is therefore fundamental for an effective bioprocess control that leads to an increased AD efficiency and robustness. The present ongoing study is aimed at the better understanding of the microbial interactions in AD reactors when subjected to high concentrations of acetate and ammonia. An interdisciplinary approach has been implemented which combined batch methanogenic activity tests (both under mesophilic and termophilic conditions), microbiome characterization by next-generation sequencing (NGS), and the quantification of the expression level of relevant genes by reverse transcription quantitative PCR (RT-qPCR). Furthermore, the obtained physiological and molecular data were validated by determining the predominant methanogenic pathway (AMA versus HMA) via the fractionation of stable carbon isotopes in biogas samples (13C/12C of CO2 and CH4). The obtained results will support the development biosensors for advanced process monitoring and control.

Short and long term road map for the development of a robust mechanistic and dynamic model of the MELiSSA C1 compartment based on microbial community characterization.

Dr Vimac Nolla-Ardèvol; KU Leuven, Belgium vimac.nollaardevol@kuleuven.be

V. Nolla-Ardèvol[a], A. Luther[b], K. Bernaerts[a], J. Ryckeboer[a], K. Rabaey[b], R. Wattiez[c], O. Gebri[d], L. Poughon[e], P. Wilmes[f], P. Simonet[g], T. Bouchez[h], K. Faust[a], I. Smets[c] , D. Springael[a]

a – KU Leuven, Belgium; b – Gent University, Belgium; c – Mons University, Belgium; d – Sherpa Engineering, France; e – Université Blaise Pascal, France; f – University of Luxembourg, Luxembourg; g – Lyon University, France; h – IRSTEA-HBAN, France;

As is the case for all of the MELiSSA compartments, the control of the C1 compartment relies on the design of a comprehensive mechanistic mathematical model. Such a model can only be developed on the basis of a thorough understanding of the C1 microbial community composition, the metabolic reactions involved and their kinetics, and the impact of operational and environmental conditions on them. Today, achieving such a deep understanding of the C1 compartment is still a daunting task and crucial questions remain such as which biological information is needed and at which resolution. Moreover, up to date technologies and approaches, mainly meta-omics combined with other culture independent techniques, are available to acquire an in depth knowledge on the composition of a microbial community and its functionality but still suffer from several issues such as (i) lack of standardization, both technical and experimental (ii) lack of knowledge about reproducibility and (iii) in some cases the techniques are not fully developed or have a low resolution. To bring light into this challenging task, ESA and KU Leuven organized a one day workshop in which renowned experts in the field of microbial systems biology, environmental meta-omics, microbial populations dynamics and process modeling discussed the challenges involved in the C1 microbial (community) characterization for modelling purposes and how to overcome them. In this presentation we will give an insight into the proposed short and long term roadmap and experimental design for the thorough characterization of the microbial community in the MELiSSA C1 reactor in view of mechanistic model development.

SESSION 2 – WATER RECYCLING

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Water cycling for manned Space missions: State-of-the-art within the MELiSSA concept

Prof. Siegfried Vlaeminck; University of Antwerpen/Gent University, Belgium siegfried.vlaeminck@uantwerpen.be

S.E. Vlaeminck, P. Clauwaert, B. Alonso Farinas, M. Vanoppen, A. Alloul, W. Coessens, J. De Paepe, H. Beckers, C. Dotremont, A.R.D. Verliefde, K. Rabaey, B. Sas, D. Geelen, D. Demey, R. Lindeboom

Water supply becomes a technical challenge for manned missions in and beyond Low Earth Orbit (LEO), burdened by high transportation costs or even the impossibility of re-supply for long-distance Space missions. Therefore in-situ water recovery is vital, however, quantity and quality of available streams is quite divergent, with a spectrum stretching from condensate to urine. Vapor Compression Distillation (VCD) is currently used in the International Space Station (ISS), but is facing some technical challenges. Within MELiSSA, the bio-physicochemical Water Treatment Unit Breadboard (WTUB) is, among others, derived from the greywater treatment at Concordia Station (Antarctica), complemented with a controlled crystallization unit, a bioreactor and an electrodialysis unit. Starting from condensate, grey water and urine, the WTUB installation could achieve beyond 90% water recovery. The plant production compartment, based on hydroponics, presents another considerable flow of water, where recycling is crucial, yet not without challenges. This talk aims to provide a comprehensive overview of the state-of-the-art (re)use of water for MELiSSA.

Biography: Dr Kai Udert

Dr Udert has a background in environmental engineering. He received his PhD from the Swiss Federal Institute of Technology (ETH) in Zurich in 2003. After a postdoctoral appointment at the Massachusetts Institute of Technology (MIT), he joined the Swiss Federal Institute of Aquatic Science and Technology (Eawag) in 2006. He leads a research group on decentralized wastewater treatment and source separation. His main research focus lies on processes for on-site treatment of wastewater. Dr. Udert led the transdisciplinary research project VUNA (www.vuna.ch). In this project, an international team of researchers and practitioners developed processes and management tools for nutrient recovery from urine. Currently, he is leading the project Autarky (www.autarky.ch), which is part of the Reinvent the Toilet (RTT) initiative of the Bill and Melinda Gates Foundation. Besides working as a researcher, Dr Udert is also a lecturer at ETH Zurich for process engineering in water and wastewater treatment.

How to cope with so much food: challenges for nitrifying bacteria in urine

Dr Kai M. Udert; EAWAG, Switzerland udert@eawag.ch

Separate collection and treatment of urine simplifies the recovery of nutrients from human excreta and the removal of micropollutants. After diverting the urine in NoMix toilets or waterless urinals, urea and other organic substances are spontaneously degraded by microorganisms. As a result, stored urine is malodorous and the concentrations of ammonia as well as the pH value are high. To prevent the loss of ammonia and to reduce the malodor, stored urine has to be treated before nutrients and water can be recovered. This treatment, also called stabilization, can be achieved by nitrification: with the help of ammonia-oxidizing bacteria and nitrite-oxidizing bacteria half of the ammonia can be converted to nitrate and the pH is lowered to values around 6. Concomitantly, heterotrophic bacteria oxidize the malodourous organic substances. This stabilized solution can then be treated with distillation to gain two products: a highly concentrated fertilizer and distilled water. This process combination has been successfully used at Eawag to produce the fertilizer "Aurin". However, the nitrification must be well controlled as the nitrifying bacteria are challenged by the high concentrations of salt and their actual substrate, free ammonia (NH3). We identified three major causes for failures. First, ammonia-oxidizing bacteria are inhibited by high substrate concentrations, when the inflow is too high. The second failure occurs, when the inflow rate is sufficiently low to support a strong growth of ammonia-oxidizing bacteria, but they produce too much nitrite for the nitrite-oxidizing bacteria. The unwanted intermediate nitrite accumulates and the nitrite-oxidizing bacteria are washed out of the reactor. Finally, a strong decrease in the influent fosters the growth of acid-tolerant ammonia-oxidizing bacteria. The pH will decrease below the typical limit of 5.4. In the laboratory, pH values as low as 2.2 were reached. At very low pH values, no nitrite-oxidizing bacteria are active, but the nitrite is oxidized chemically to nitrate and volatile nitrogen oxide compounds such as nitrous acid (HNO2), nitric oxide (NO) and nitrous oxide (N2O) are released. All three failures can be prevented with careful process control. If the urine inflow is regulated so that the pH stays in a narrow range, for example between 6.3 and 6.35, the activities of ammonia-oxidizing and nitrite-oxidizing bacteria are well tuned. The growth of acid-tolerant ammonia-oxidizing bacteria can be prevented, if both the inflow and the aeration are switched off during short periods of low urine supply.

Electrochemical systems as core engines of the MELiSSA loop

Prof. Korneel Rabaey; Gent University, Belgium korneel.rabaey@ugent.be

Korneel Rabaey, Amanda Luther, Peter Clauwaert

Electrochemistry has in the past years known an upsurge. For terrestrial applications, the use of electrochemical processes is emerging for disinfection, removal of emerging contaminants, acid/base regulation, production of energy vectors and many more. This versatility is even enhanced when a single electrochemical system is used for multiple processes simultaneously such as production and extraction of chemicals. The technology is sufficiently mature to be deployed in space settings, with system lifetimes well over 5 years even in waste flows. In my presentation I want to highlight several key processes that can contribute to an effective life support system. Firstly, I will discuss a potential approach for urine treatment whereby nutrient recovery is coupled to neutralization and organics removal. Second, I will discuss the benefits of electrochemistry for acid/base production within the loop, using actual streams, as well as for disinfection and trace contaminant removal. Lastly, I will make a sidestep to possibilities for bioelectrochemical systems, enabling organics conversion without need for oxygen and with low cell yield, potentially coupled to e.g. base production.

Closed loop hydroponics for a novel crop cultivation system on the EMCS rotor

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The TIME SCALE project will bring fundamental research on plant, crop and algae growth in space to the next level by further development of the European Modular Cultivation System (EMCS). The EMCS contains rotors allowing scientific research under a range of gravitational conditions. A state of the art crop cultivation system (CCS) for the EMCS will be developed and tested. Current water and nutrient supply systems in space are not free from complications. Both in terms of operation and system scalability for extra-terrestrial cultivation systems. One of the focal points of the project is to develop a water and nutrient recirculation supply system for the new CCS. The new system intends to grow plants in water with dissolved nutrients (without solid substrate) and includes early warning systems that involve mathematical model predictions and usage of optimized sensor technologies to monitor plant nutrient availability. Our design aims to ensure a safe and reliable food supply in future space exploration. Gained knowledge and concepts will also be explored for terrestrial research and commercial plant cultivation practices.

A certainty of bacterial growth on pipe materials in contact with potable water

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Frederik Hammes, Caitlin Proctor, Romina Sigrist, Franziska Rölli, Stefan Kötzsch

Water supply systems aim to deliver hygienically safe and aesthetically acceptable water. Despite best efforts to control growth, potable water distribution systems are far from sterile, and building plumbing (including closed-loop or contained systems) offer conditions particularly favorable to microbial growth, including warm temperatures, large surface-to-volume ratios that increase the importance of biofilms and bio-friendly materials that essentially offer food to bacteria. Excessive microbial growth leads to biofouling, system malfunctioning, taste & odor problems and even enrichment of opportunistic pathogens. Our group's research investigates the growth potential of materials in contact with water (pipes, sealing rings, water reservoirs, coating materials, etc.). Particular emphasis is placed on synthetic polymeric materials that leach biodegradable organic carbon into the water. In a series of pilot experiments we demonstrated microbial growth ranged from 2 x 10^6 to 2 x 10^8 cells/cm2 depending on the hard and flexible pipe materials used. We showed that rapid 2-week microbial growth potential tests are highly predicative (R2 = 0.77) of microbial growth on materials over 8 months. Moreover, 16S amplicon sequencing of biofilms showed that only five dominant phyla (Proteobacteria, Bacteroidetes, Planctomycetes, Actinobacteria, and Acidobacteria) accounted for 94% of all sequences. Communities clustered with both material (R2 = 0.31) and time (R2 = 0.25), indicating a possibility to control the biofilms via material choice. Interestingly, we found that genera containing opportunistic pathogens were more common in low-biomass pipes. In conclusion, this research shows the critical importance of material choice with respect to microbial community development on pipe material in contact with potable water, and the possibility to control biofouling through smarter material selection.

SESSION 3 – AIR RECYCLING

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Photobioreactor in space habitat: status and challenges

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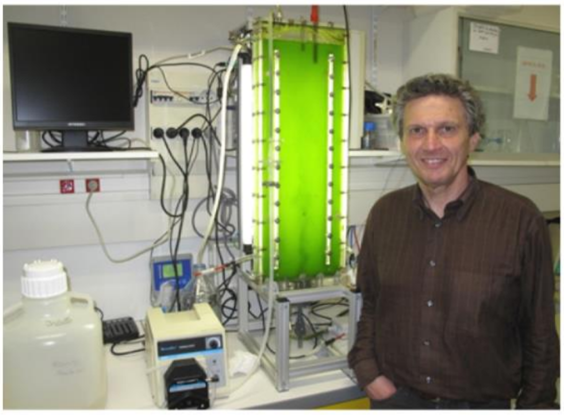
Steven Hens ; QINETIQ Space, Belgium
Samuel Gass, RUAG Space, Switzerland

Since the early 90's, ESA has initiated R&D activities in life support to prepare long duration manned missions. For obvious logistics and cost issues associated to those missions, ESA has focused on the development of closed regenerative systems fulfilling wastes management, air revitalisation, water recycling and food production. Due to safety and robustness requirements, the accurate control of the closed regenerative system is a condition sine qua non. However, such systems are based on the interconnection of sub-systems, which have distinct characteristics such as kinetics and dynamics. Hence the control of the system results from a deep knowledge and prediction capability of each sub-system.

Therefore, for such developments, ESA has followed a very progressive approach to i) understand, predict and control processes involved in the system, ii) validate individual processes in space environment and finally iii) test and demonstrate integrated processes with a consumer both on ground and in space environment.

Within this context, the particular case of air regeneration using photosynthetic process, here photo-bioreactor technology, will be addressed. The presentation will introduce the status of the research phase dedicated to the development of robust dynamic model describing the photo-autotrophic growth. Today, such model links the biomass growth to the radiant light energy transfer, and the effort being made to include selected physiological parameters will be mentioned. If such model constitutes a good basis for photo-bioreactor design and performances prediction as well as process control, the validity of the model remains to be verified in environmental conditions which are relevant for space applications, namely micro-gravity environment. As far as performances prediction is concerned, the space experiment ArtEMISS was conceived to collect in-flight data necessary to the model verification. As far as process control is concerned, the space experiment BIORAT was conceived to provide in-flight demonstration of the predictively controlled air recycling between a photo-bioreactor and a consumer. The presentation will also introduce the status and engineering challenges of the ArtEMISS and BIORAT development.

Biography: Prof. Jack Legrand



Prof. Jack LEGRAND is Professor in Chemical Engineering at the University of Nantes since 1988. He is specialist in transport phenomena and mixing in processes and (photo) bioreactors. He has ca 230 publications in international journals and is co-author of 16 patents. He is Director of the Joined Research Unit (UMR), constituted by about 220 people, GEPEA (Process Engineering for Energy, Environment and Food) between CNRS, University of Nantes, ONIRIS and School of Mines of Nantes. Since 2009, he is co-coordinator of the thematic group "Biomass for Energy" of the French Alliance for the Research Coordination for Energy (ANCRE). Member since 2010 of the European Science Foundation in the EUROCORES (EUROpean COllaborative RESearch) programme EuroSolarFuels; Molecular Science for a Conceptual Transition from Fossil to Solar Fuels. Since 2014, he is the CNRS representative in the Joint Programme Bioenergy of the European Energy Research Alliance for Bioenergy. Member of the board of the French Society of Chemical Engineering (SFGP) since 2010 and President of Scientific and Technological Council of the SFGP since 2015.

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Some insights on photobioreaction engineering

Prof. Jack Legrand; GEPEA, Nantes University/St. Nazaire, France jack.legrand@univ-nantes.fr

Jack Legrand, Jérémy Pruvost

Microalgae have much to offer to industry, not only for their proteins, lipids, sugars and pigments useful in diverse areas ranging from human nutrition, animal feed, cosmetics and health to biofuels or green chemistry, they can be also used as bioremediators for CO2 capture or for removing pollutants from wastewater. In spite of the interest of photosynthetic microorganisms in various domains, industrial applications remain limited, mainly owing to the difficulty of proposing intensive cultivation systems with high biomass concentration and productivity. Light is known to be the principal limiting factor of closed photobioreactor efficiency, making biomass productivity only dependent on the light intercepted by the process and of its photosynthetic conversion in the culture volume. Due to cells absorption and scattering, the radiation field is heterogeneous inside the culture. From the engineering point of view, in order to reach high culture densities, light input has to be increased, by working under high photons flux density, or by maximizing the illuminated surface for a given culture volume. The biomass production or of a given metabolite of interest requires controlled photobioreactors for the microalgae cultures. Theoretical approaches as modeling allow to link irradiation intercepted by the process, radiant light energy transport inside the culture volume, and its local coupling to photosynthetic growth. Based on a long-term effort devoted to the development of the theoretical framework for artificially lightened systems, the models have been recently extended to simulate the dynamic functioning of solar photobioreactors as a function of meteorological conditions. Those models were also used to define engineering rules which were used to intensify solar culture performances. In our lab, we have developed an innovative photobioreactor, AlgoFilm, with breakthrough productivities in terms of volume productivity by reducing the culture medium thickness down to ultra-thin culture. Models were used to determine key-aspects of its design. Biomass productivities were then simulated for the systematic optimization of its performances in solar conditions, emphasizing the direct influence of irradiation conditions, but also effects of day-night cycles, culture harvesting strategies. Moreover, the dynamic model for the photoautotrophic growth of microalgae in photobioreactor, describing principal variables of the system, allows obtaining the precise prediction of the pH in the medium. This example of bioprocess development emphasize the interest of developing knowledge robust models. Such models, which derived from the research developed in the framework of ESA's MELiSSA – Micro-Ecological Life Support System Alternative, have direct benefits for the setting of industrial production of microalgae. This allows applying refined physiological and radiative models either for the design of new photobioreactors technologies, the optimization of the production and for process advanced-control.

This effort will be pursued at preindustrial scale. This will be conducted especially in the AlgoSolis R&D facility (www.algosolis.com), which is devoted to investigations in representative conditions of industrial applications of microalgae (outdoor production, large-scale) and to the integration of technological bricks defining an industrial exploitation of microalgae. This core-facility proposes external surfaces of production, a technological hall for biomass harvesting and refining (production of extracts and ingredients), and an appropriate piping to interconnect platform devices in order to ensure the transfers of biomass or medium between the different operating stages (production-harvesting-biorefining-culture medium recycling). A pilot simulating a building biofacade for microalgae culture is also set-up in AlgoSolis. Such technology is adapted for an integration into urban environment, by combining algal culture to façade of public or private-sector buildings. An optimal integration also improves the building's green credentials and offers an alternative and cost-effective solution to algae culture.

Cultivation of microalgae for advanced closed life support systems as a technical and biological challenge

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Life Support Systems (LSS) are essential for human spaceflight. Ambitious goals of human space exploration in the next 40 years like a permanently crewed surface habitat on the Moon or a manned mission to Mars require technologies which allow for a reduction of system and resupply mass. Enhancements of existing technologies, new technological developments and synergetic components integration help to close the O2, water and carbon loops. Based on photosynthesis and able to be combined with physico-chemical systems, microalgae are able to generate edible biomass and release O2. Algae cultivation in aquatic reservoirs reaches up to ten time higher growth rates and significant lower energy and volume investments than higher plants. Up to 30% of human food can be covered by algae biomass, limited by the high protein content of edible algae species. Research on the biology is important, but it is also challenging from a technical point of view to provide an efficient photobioreactor (PBR) system. Mass und energy benefits strongly depend on cultivation techniques, illumination and gravity level (μg in space or partial g on planetary surfaces), but also on the mission scenario (transfer, orbit, surface). Since 2010, microalgae are cultivated and investigated at the Institute of Space Systems (IRS), University of Stuttgart. Especially the species Chlorella vulgaris is selected as a promising candidate for future use in LSS, but other species like Spirulina or Scenedesmus are considered as well. The presentation will give an overview about of the different technological steps from on-ground test-beds and parabolic flight experiments to a spaceflight experiment, in order to demonstrate the technological readiness of photosynthetic conversion of concentrated carbon dioxide into biomass and oxygen in a long-term operating PBR system. A continuous cultivation of over 5 years is running at IRS. Additionally, the connection to physico-chemical systems for CO2 concentration is essential to get access to CO2 in the cabin.

ModuLES-PBR – lessons learned through parabolic flight tests

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Klaus Slenzka, Sandra Podhajsky

Based on requests from the scientific community, the German Space Agency, DLR Space Management, has initiated a programmatic approach to gather a team of scientists, ensuring the research-characteristics of ModuLES (Modular Life Support and Energy Systems), the scientific and technical development as well as understanding of Life Support & Energy Systems being based on ecological, sustainable processes. ModuLES is the basis for future applications in space (exploration) as well as on ground in the lab and/or in extreme habitats. The first module on the ModuLES concept is a Photobioreactor (ModuLES-PBR) designed and tested on ground and in parabolic flights. A Photobioreactor was chosen as first module, because microalgae serve with their photosynthetic activity for up to 50% of the oxygen supply on our planet and thus each of our breaths. The overall goal of this ModuLES-PBR is the development of an energy-efficient and highly effective Photobioreactor-system with clearly defined in- and outputs. The PBR is designed for a maximum efficiency with respect to oxygen production and carbon dioxide uptake as well as the optimization of closure-level of the nutrient loop during operation for various environmental conditions. The core unit consists of a bioreactor that allows the cultivation of the microalga Chlamydomonas reinhardtii with highly efficient photosynthetic gas exchange rates. The efficiency of the system depends upon the quality and quantity of light, liquid mixing, gas supply and mixing, gas exchange for optimization of dissolved oxygen concentration in the algae solution to prevent bubble formation, medium composition and the growth phase of the microalgae. A second unit, which was added to the system after PBR-verification, is used for media recycling and algae filtration and is a first step for optimizing the nutrient-loop closure, which is essential for long duration operations. A consumer module is under evaluation. The technical complexity of the system has kept growing with the project duration and new units will be added step-by-step to close the loop appropriately. Presently, media recycling and enhancement of gas exchange capabilities are under testing, as well as adaptations for a design based on the lessons learned. A summary will be given in the presentation. Acknowledgement: Specials thanks are given to Dr. Katrin Stang and Dr. Markus Braun from the DLR Space Management for continuous discussions and support. The German Ministry of Economics via the DLR Space Management funds ModuLES (FKZ: 50 WP 1211 & 1511).

Heat Ventilation and Air Conditioning of a Greenhouse in Antarctica

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The development of plant cultivation technologies for safe food production in space is a research filed which intends to increase the possibilities of human future long-term space missions. EDEN ISS project (Evolution and Design Environmentally-closed Nutrition-Sources), which is funded by the European Union's Horizon 2020 research and innovation program, is embedded in this context to provide a ground demonstration of a bio-regenerative life support system in an highly-isolated environment. In fact, during this project, a greenhouse will be built, tested and then placed in Antarctica, close to the German base "Neumayer Station III", to collect experimental data on plant growth. In detail this work shows the preliminary design phase of the air distribution system of this greenhouse. The optimization process of the air distribution system has followed this is main aim: ensure the proper climate conditions for plant growth. Thus this means homogeneous temperature, relative humidity and velocity in the growth chamber. To achieve this goal CFD analyses are performed, checking global and local aspects. This part of the project is developed in close collaboration with the German Aerospace Center (DLR), as EDEN ISS project coordinator, and AeroSekur, as responsible for the air management system.

SESSION 4 – FOOD PRODUCTION & PREPARATION

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MELiSSA State of the Art

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Roberta Paradiso, Veronica De Micco, Giovanna Aronne, Stefania De Pascale

The project MELiSSA aims to conceive a closed-loop artificial ecosystem, based on recovering food, water and oxygen through the complete recycling of waste (carbon dioxide, urine and faeces).

The MELiSSA loop was originally conceived without higher plants and utilized a photosynthetic microorganism, the cyanobacterium Arthrospira platensis (also known as Spirulina), as source of food. However, this strategy entailed low rate of air regeneration and limitations in the diet nutritional value and composition.

Higher plants were introduced as bioregenerative component in 1997, and research on a plant chamber to be integrated in the loop started. The MELiSSA Pilot Plant (MPP), a sealed chamber with gas, liquid, and solid connections from/to the other compartments, was built and the study of its interface with the system, in terms of quantity and quality of fluxes, was introduced as a research topic. Three crops, wheat, beet and lettuce, were selected for initial hydroponic cultivation tests, in a staggered plantation system, with the target of an edible biomass production of 20% of the crew needs. Results on crop yield and carbon exchange modeling, even in response to variable CO2 concentration and light intensity, demonstrated the potential of plants in closed environment systems.

Based on these results, more challenging goals were introduced in Food Characterization Phase 1 (years 2009 - 2010), which aimed to the characterization of 4 crops, potato, soybean, bread wheat and durum wheat (4 cultivars each) in hydroponics under controlled environment, and to the design of a next generation chamber. On the same crops, in Phase 2 Cultivar Selection - CulSel (2013 - 2015), further objectives were to improve the criteria of cultivar selection for Space cultivation and to assess the impact of beneficial microbes in the rhizosphere on plant performance. Beside, within the project HYdroponic SubSystem Engineering - HYSSE, studies to model and develop, with an engineering approach, a modified hydroponic system to address critical modeling requirements in closed chamber were carried out.

The lecture will present an overview on fundamentals of the food production and preparation chain in MELiSSA. Focus will be on the relevant achievements in controlled environment technologies for higher plants and crop productivity, and the related current technical and scientific issues. The contribution of fresh food to preserve the physiological and psychological wellbeing of the astronauts during long distance flights will be also considered. Finally, future directions, including prospective terrestrial exploitation of developed technologies and missing space application will be summarized.

Biography: Prof. Ep Heuvelink

Dr. Ep Heuvelink is Associate Professor at the Horticulture and Product Physiology group of Wageningen University, The Netherlands. He has extensive experience and expertise in both academic research and education on ecophysiology and simulation models for crop growth, development and yield. Dr Heuvelink is frequently invited as a keynote speaker in international scientific symposia and teaches advanced intensive courses on greenhouse production, crop physiology and crop modelling all over the world. He published more than 80 scientific papers in refereed journals and his H-index is 37 (Google Scholar).

Selection of 10 journal papers

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Climate factors and crop growth: physiology and mechanistic modelling

Prof. Ep Heuvelink; Wageningen University, Netherlands ep.heuvelink@wur.nl

Ep Heuvelink, Leo F.M. Marcelis

The Melissa project aims at developing an artificial ecosystem for regenerative life support systems for long-term space missions to lunar bases or flights to Mars. The higher plant compartment in this system on the one hand produces food and non-edible plant material and oxygen, on the other hand it uses macro- and micronutrients, carbon dioxide and water. Knowledge on how climatic factors influence plant development and growth, and mechanistic modelling of these processes is essential for the development of such an artificial ecosystem. A lot can be learned from greenhouse horticulture and production in climate rooms (plant factories).

Plant development, i.e. the systematic movement along the genetically programmed sequence of events during its life cycle, is mainly controlled by temperature, whereas plant growth (increase in size and biomass) is primarily influenced by light intensity and to a lesser extent by carbon dioxide concentration. Within a range of temperatures, development rate depends linearly on temperature, which means that a temperature sum defines the developmental stage. Light intensity and carbon dioxide influence plant growth via photosynthesis. Light spectrum plays an important role in plant morphology (e.g. elongation, leaf thickness) and therefore plant growth, especially in young plants.

A model is a simplified representation of part of reality, i.e. a system. Mechanistic models for crop growth typically are a set of equations, which represent the behaviour of a crop. Models are powerful tools to test hypotheses, to synthesize knowledge, to describe and understand complex systems and to compare different scenarios. A large number of mechanistic models exist varying in degree of complexity and number of processes considered. Relatively simple mechanistic models are for instance those using a direct relation between intercepted light and biomass production, i.e. a (constant) light use efficiency. Probably the most common are those models that have functions or modules for leaf area development, light interception, photosynthesis and respiration, biomass partitioning and dry matter content of the plant organs. These models can also include the uptake of water and nutrients.

We conclude that for many crops models have been developed that can accurately predict crop growth, needed for developing an artifical ecosystem. Focus has been on the edible parts, with much less attention for the non-edible biomass (amount and quality). The latter is however equally important for closing the loop in regenerative life support systems.

Emerging bioprocessing concepts for healthy and sustainable foods

Prof. Dr-Ing. Alexander Mathys; ETH Zurich, Switzerland alexander.mathys@hest.ethz.ch

Emerging and sustainable food processing is based on the detailed understanding of process-product-operation interactions, where selected examples of innovative thermal, electro-magnetic, mechanical and combined processes will be presented and are introduced hereafter. Modular thermal micro process engineering was effectively applied to improve upscaling of microbial inactivation processes, but its mechanical process elements could also be used for tailored structure formation and synthesis. Upscaling is based on numbering-up approaches to keep the beneficial mass and heat transfer dimensions. Electro-magnetic based pulsed electrical field PEF processing enables an efficient use of biomass and energy within different value chains. Selected implementations within potato, fruit juice and algae processing will be discussed with maximum capacities for cell disintegration up to 80 tons per hour. During mechanical high pressure processing in batch, focused investigations on the property changes within pure water and more complex systems, such as proteins and microorganisms, enabled a detailed understanding of the respective process-product-operation interactions. Special focus was laid on bacterial spores, the target of sterilisation. After studying spore inactivation in very detail, classical high pressure preservation could be optimized through combined thermal and mechanical processes such as high pressure thermal sterilisation as well as continuous ultra-high pressure processing up to 400 MPa as innovative multi hurdle technologies for gentle sterilisation of healthy and high quality food. Advanced approaches relying on innovative raw materials and biorefinery concepts to create new and innovative value chains could even increase the impact of sustainable food processing. Such innovative value chains could be linked to novel opportunities to value alternative protein sources. By using novel proteins from algae and insects, food security and sustainability of the protein supplies can be significantly improved. Connected biorefinery approaches within these innovative value chains realise the sustainable material and energetic utilisation for a valorisation of all side streams by applying combined processes. Holistic life cycle sustainability assessment, aligned with the introduced process innovations, can evaluate the suggested solutions on a multi parameter base, in terms of improved food production sustainability. A focused knowledge transfer via food processing workshops as well as student and expert exchanges will assure the mid and long term impact of the presented solutions.

PFPU-Main Scientific criticalities of the Precursor of Food Production Unit in the MELiSSA framework

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So far, several scientific issues related to the development, implementation and operations of food production system for space applications in microgravity were identified and include the food quality prediction, food safety, microbial population management, multi-phase flow understanding and management. Thus, considering the number of issues and their respective criticality, the cost-conscious development of a food complement production unit for space application requires a step-by step approach. PFPU is a study of a modular food complement production unit demonstrator, aiming at a statistically representative production of edible tuberous plants in micro-gravity. The study is performed within the MELiSSA framework under contract with the European Space Agency, carried on by an Italian consortium led by Thales Alenia Space Italia. The PFPU preliminary main identified scientific issues are presented and discussed, focusing on microbial population management and multi-phase flow understanding and management within the root and tuber zone and related components. Moreover, understanding and control of the tuberisation mechanisms in microgravity is a key issue that is being addressed.

NEMO'S garden: growing plants underwater

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E. Princi, L. Gamberini, S. Gamberini, G. Cucchia, G. Fontanesi, D. Piombo

After the positive experiences collected in the period 2012-2015, Nemo's Garden Project starts again in 2016 trying to improve the knowledge not only about the engineering and installation underwater of structures dedicated to the growth of vegetables for human consumption, but also about the physical-chemical-biological processes occurring during the plants development under sea. The project goal is to create alternative ways of agriculture, especially dedicated to those areas where environmental conditions make the plants growth extremely difficult if the conventional farming would be applied. Regions where both lack of fresh water and fertile soils and extreme temperature changes occur may be interested in. Technology developed in the framework of Nemo's Garden Project might help to create efficient plants productions in an alternative and economical way in respect to standard greenhouses, looking at the new branches of green and blue economy. Nemo's Garden Project may be described with three keywords:

- Eco-friendly: the underwater farm determines very minimal harms to the marine environment and related ecosystems.
- Self-sustainable: the underwater agriculture represents an alternative solution to the existing methods (sustainability). The use of renewable energy, the resource saving, the fresh water creation and its possible reutilization make Nemo's Garden as a self-sustainable system. Indeed, once the crop system has been activated by using fresh water obtained by desalination of seawater, it continues to sustain itself without any external support.
- Ecological: no pollution and no damage in the seawater occur. Inside each underwater farm an efficient ecosystem, composed of dynamically interacting parts including organisms (plants) and non-living components of their environment, is developed. Preliminary analyses performed on basil and salad growth underwater demonstrated that the whole content of essential oils is not far from that of terrestrial plants; again, taste and appearance of the specimens showed no differences.

Lessons learned from commercial scale urban Spirulina production

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I would like to present about our real world findings from 4 years of commercial scale, food-grade spirulina cultivation using low cost closed-system tanks in Bangkok, Thailand. More information about us can be found on our website at www.energaia.com.

SESSION 5 – CHEMICAL & MICROBIAL SAFETY

Table of Content

Closed loop system: a safety challenge for the crew as well as for the life support processes

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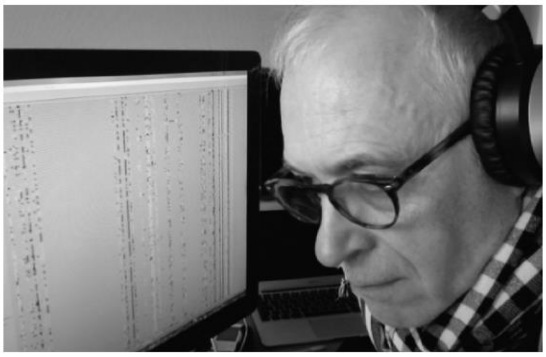
Christophe Lasseur, Christel Paillé, Stephanie Raffestin, Pierre Rebeyre, Brigitte Lamaze

Today, on board ISS the safety requirements concerned the risk to the crew. These requirements are mainly organised in three part: physical, chemical and biological. For physico-chemical beyond LEO the main challenge will probably come from the risk exposure duration and some standard values may have to be revisited for a 3 years mission. Regarding the biological risk, and mainly due to technical improvement there is a fair chance that standard will progressively evolved from total counting to pathogen identification and quantification. However, once may wonder if the risk evaluation can be limited to "full organism" and if a progressive move to sequences of high virulence, pathogenicity associated to the host characterisation will not have to be considered. This issues being reinforced by the high radiative environment once the space vehicle is not protected by van Allen belts.

In parallel of this almost natural evolution of risk evaluation, once may remark as well the increasing importance of the biological based technologies within the space system. Far from the 90' discussion, where a choice between physico-chemical technologies and biotechnologies had to be done, it is not questioned anymore that biological processes will be part of the technology panel of the mission. These evolution raises however challenges in the reliability and robustness characterisation of bio-based technologies. Looking more specifically to closed loop regenerative life support, it is rather clear that such biotechnologies can be involved at many steps of the recycling cycle: from waste degradation, urine transformation, contaminants removal, food production and preparation. To fulfil the ESA ALiSSE criteria the reliability of these processes will have to be studied and determined. This paper is aiming to a preliminary identification of the key challenges to quantify robustness of biological based processes.

Biography: Prof. Jean-Pierre Flandrois

Education



1971 Master degree in Animal Physiology

1972 Post-graduate degree (« Grand Cours ») in Pasteur Institute ; *Medical Doctor* (Lyon)

1975 Master degree In Human Biology (Microbiology) Institut Pasteur de Paris and Université Paris V

1976 Doctoral studies in Lyon Claude Bernard University and Gade Institute, Bergen, Norvège.

1978 PhD on immunochemistry and sub-typing of Staphylococcus aureus

1979 *Professor of Microbiology*, Lyon, Claude Bernard University

1991-2013 Head of Bacteriology Laboratory, University Hospital Lyon.

National responsibilities

President of the French Microbiological Society from 1995 to 2001.

Councilor in the French Research Council at Ministry for Research from 1997 to 2002.

President of the Microbiological Expert Committee of the french food protection agency (AFSSA) from 1999 to 2003 and management of food safety, risk analysis, safety alerts and impact on regulation.

Member of the French Planetary Protection Committee (CNRS and Ministry of Research) from 1999 to 2003 as clinical microbiologist and statistician in the frame of the Mars Sample Return Project (NASA-CNES).

European Expert Group

ERC Projet Theseus] European Research Council - Theseus project 2010-2012. Cluster 4: Habitat Management. Head of Subgroup 1: Environmental Microbiology.

Consulting

bioMérieux 2014-2016
CNES-MEDES, 2014

University responsibilities

Director of the Doctoral School "Evolution, Environment, Microbiology, Mathematical Modelling", University of Lyon, 2000-2012.

Director of the Biomath-health department, UMR 5558 CNRS-INRIA-LYON1 "Biometry and Evolutive Ecology" 2010-2014

Scientific activity

I have had 4 mains sequences with overlapping and I have published 171 papers. Here are only the milestones steps.

1) Immunochemistry of Staphylococcus aureus cell walls

   - The isolation and characterization of various protein (Flandrois, Fleurette, & Modjadedy, 1975; Grov, Flandrois, Fleurette, & Oeding, 1978) or polysaccharides (Ndulue & Flandrois, 1983) was the following of my PhD Thesis. I have also described the affinity of the cells to human proteins (Carret et al., 1985) .
   - A side effect of this research has been a world-wide used affinity reagent (Carret, Flandrois,

Bismuth, & Saulnier, 1982) ("Staphyslide") that remains, in a revised version, the basis of most of the immediate identification reagents for Staphylococcus aureus.

2) Modelling of bacteria growth and applications to antibiotics sensitivity and food safety

- Modelling of bacteria growth was made possible by the early development of informatics. My team was involved in the mathematical approach and optimal production of biological datas. we have demonstrate the change of growth model occuring when bacteria are exposed to the drugs and described the mathematical law linking growth rate and other parameters and antibiotics concentration. This was done both for sub-inhibitory concentrations (Comby, Flandrois, Carret, & Pichat, 1988) and lethal concentrations (Guérillot, Carret, & Flandrois, 1993) . A major paper describe the interpretation of the antibiotic effect in term of maintenance energy for the cell (Lobry, Carret, & Flandrois, 1992).
- The applications were not possible in real life due to the need of a dedicated high throughput performance photometer. We described also how photometry combined with mathematical modelling is able to study the swelling of the micro-organisms during the lag phase. The possibility to use our algorithms is now studied by research teams using the new "digital microbiology" concept.
- Aside this extensive model approach, I have developed the first "expert systems" to analyse and automatically correct the result of antibiotics susceptibility testing (Comby, Flandrois, & Pave, 1988) . These "partially intelligent" programs are now included in each antibiotic susceptibility systems and are all derived from my original work.
- I oriented in parallel the team on the basic physiology of bacteria and we described then the biological-mathematical laws linking growth rate and latency to pH and temperature (Rosso, Lobry, Bajard, & Flandrois, 1995) . More, we have discovered a fundamental law in the bacteria linking the optimum, minimum and maximum temperature of growth (Rosso, Lobry, & Flandrois, 1993) . A lot of work was also done to model interactions between bacteria (especially those found in food, pathogens vs non pathogens) (Cornu, Kalmokoff, & Flandrois, 2002) .
- There results and more works (Bréand, Flandrois, Rosso, Tomassone, & Fardel, 1997) were applied to the prediction of growth of bacteria in food in cooperation with Danone. The prediction of growth of pathogens in food is good at months level (up to 3 months). This "predictive microbiology" is now widely used in agro-food industry.
- Predictive microbiology is a part of the risk-analysis process in food, environment...

3) Bioinformatics applied to bacteria identification

- The main result was the "BIBi" project (Devulder, Perriere, Baty, & Flandrois, 2003, Flandrois, Gouy and Perrière 2014) . I demonstrate that Bacteria Identification based on Bioinformatics (BIBi) was possible and easy to use in routine (S Mignard & Flandrois, 2006) if a convenient database was built. This program is available on the web and I recently release the mark 5 version using new algorithms to build the database and extract the pertinent information. Annually 50000 identifications are done by the system and it is cited in 92 papers.
- As the Mycobacterium genus was the model genus that I use to develop BIBi (Devulder, Pérouse de Montclos, & Flandrois, 2005) , I improve the phylogeny and taxinomic analysis of this genus by combining bioinformatics methods (Mignard & Flandrois, 2008, Guérin et al. 2014, Pin 2014) . I developped also an alternative approach to the use os 16S RNA with better discrimination power (Jauffrit et al 2015, 2016).

4) New methods of bacteria detection and enumeration

- This topic has been important all over my work, partly due to my position as Head of a diagnostic laboratory. I have been involved in patents concerning biomolecular detection of pathogens, new detection methods in food microbiology and use of digital microbiology in bacteria enumeration. This applied scientific research is my main preoccupation today: i) detection of pathogens in food and water in a sealable container (Junillon 2014a,b,c) ii) detection of pathogens and contaminants in water in ESS conditions by molecular biology (Bechy 2015). What is important for me is that the two project have similar probability issues : the binomial law is to take into account and statistical signification of a given level of detection (especially 0) has to be accurately computed to compare the results. Earlier studies of my team on blood culture results were dealing with the same problem (Lamy 2002, Leyssene, 2012).

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About Microbial hazard and risk for humans in spatial habitats

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On earth, Humans are living among microbes but also with their own microbes. There are microbiomes on and inside living entities but also in water, soils, machines. The interactions are globally neutral or positive for health, a minority of microbes being potentially pathogen. Most of these are known but infections are occasionnaly due to unknown ones. In a spatial habitat the microbial ecosystem is dominated by the Human microbiome in term of number and diversity, other colonized areas are comparatively poor in biodiversity and number. The subset of human microbes that colonize the environment is selected by the harsh ecosystem. The complexification of the environment may extend this in term of number and diversity. Among the environmental microbes some may be pathogens and some other may be responsible for degradation by biocorrosion. The hazards are potentially numerous but fortunately the risks are factually low. Risk is an expression of the probability to cause an illness for a given hazard. An risk level may be computed for an hazard if we know the exposition level, the growth kinetics of the hazard and the population susceptibility. Risk levels are rarely estimated. Is a given microbe a hazard for the crew ? Some limited lists of pathogens exists and may be helpful. For other species, the only way to suspect it as pathogen is to use the genome content and infer the hazard from the presence of genes linked to pathogenicity but this may led to overestimate the hazard. The next step is to compute the risk. The estimation of the exposition level to the hazard imposes quantitative analysis of microfloras, unfortunately gathering such information is technically difficult and costly in spatial conditions. Knowing the susceptibility of a Human being is a major issue to compute the risk, especially in the case of long stay in space where Human immunity is affected. This may in part solve the problem of the risk analysis in the case of an introduction of a great number of non-human microbes and extra growing niches as this will be done in the future of the MELISSA project.

Application of ultraviolet-LED systems for microbial control in air and water loops

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The inactivation effects of ultraviolet light on microbial contaminants are well established and UV systems have long provided chemical-free, reliable disinfection of fluids. Traditionally, these systems have utilised mercury vapour lamps to generate germicidal UVC radiation, though the toxicity of mercury has long posed a problem through the risk of lamp breakage. The development of UV-LEDs has opened the door to novel, high-efficiency treatment reactors. Whereas LED sources require less in the way of packaging and drive electronics than mercury systems, design of the reactor itself is more complex due to the inherently lower power sources, with trivial designs yielding poor treatment efficiencies. In UVC-LED disinfection every photon counts. AquiSense Technologies are the first to market with a commercial-scale UVC-LED system, the Peal Aqua. The Pearl utilises a proprietary design to maximise the disinfection capabilities of industry-leading LED lamps. AquiSense Technologies (Europe) recently joined the BIOWYSE consortium and intend to leverage their expertise gained in the development of the Pearl Aqua to design and build a custom UVC disinfection unit intended for use on the ISS. BIOWYSE is a Horizon 2020 funded action and managed by the European Science Foundation; the project aims to develop a rapid monitoring, control, and disinfection system for stored potable water aboard space habitations. UVC-LEDs are now well into the ascent phase of development, with many concerns over power, lifetime, and durability being met. As the technology matures and awareness increases demand is expected to grow significantly as novel applications are found for these new sources. UVC-LED systems have application in air, water and surface disinfection, though air and water systems offer the best opportunity to maximise overall device efficiencies. The massed benefits of solid-state devices make them ideally suited to use in both remote terrestrial communities as well as space habitations.

Automated high-frequency flow cytometry enables in-situ process monitoring, control, and optimization of engineered water systems

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Michael D. Besmer, Frederik Hammes

Detecting dynamics of any given timescale requires sampling at a frequency that is 1 to 2 orders of magnitude higher than the targeted timescale (e.g., monthly sampling for seasonal dynamics, hourly sampling for diurnal dynamics). For microbiological variables, sampling and measuring more than daily samples over weeks or even months is particularly challenging due to the laborious detection methods. Hence, high-frequency microbial datasets are scarce and knowledge on microbial dynamics in engineered water systems are lacking. We have developed a fully automated online flow cytometry system that overcomes the tedious and restricting practice of grab sampling and subsequent cultivation on agar plates. During the past three years, we opened a window on a myriad of short-term microbial dynamics in engineered and environmental aquatic ecosystems including river water, groundwater, springs, treated drinking water, and tap water. Measurements were done in situ, at high frequency (from seconds to hours), and in near real-time (10 minutes delay). This allowed for the detection of subtle changes in bacterial concentrations during measurement periods ranging from hours to months. We detected interesting and often unexpected microbial dynamics in every investigated system at time scales from minutes to weeks and bacterial concentrations levels between 104 and 107 cells ml-1. For example, we showed clear diurnal fluctuations in bacterial concentrations due to (1) intermittent operation of a groundwater extraction plant, (2) varying water production rates in a drinking water treatment plant, and (3) daily water usage patterns in a building. Such results boost the idea of advanced monitoring of microbial dynamics, which is critical for a better understanding of underlying causes of fluctuations (both in environmental and technical systems) as well as the ecological and operational consequences thereof. We expect that these findings will stimulate improvement of early warning systems, process monitoring, conceptual approaches to smarter sampling schemes, and system optimization. Direct, automated, high-frequency testing of new systems and/or operational and other measures to improve system performance with respect to microbiology (e.g. in production and disinfection processes) is now feasible.

Characterization of Humidity Condensation and Associated effects using Image Processing Techniques

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The condensation of water vapour plays an important role in a closed habitat, where plants and humans live together. The associated effects of humid air condensation such as the development of mold, rot, corrosion and also nosocomial infections will have a paramount effect in long duration space flights. The improvement of the characterization tools for the prediction of mass transfer coefficients due to condensation of water vapour from humid air will facilitate the development of mathematical models, which will further assist in simulation studies. The computer vision and image processing tools give imminent information into the nucleation of water condensates on small obstacle and walls. In this study we will discuss the tools and techniques of the algorithms developed using image processing tools for the characterization of such effects. The comparative results will be discussed with experimental data and some application areas have already been explored.

Understanding and Modelling of Airborne Bio-Contamination Process in Manned Spacecraft

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In manned missions astronauts are living in a hermetically sealed and artificially created environment. They are also in close contact with each other. These conditions, combined with microgravity, potentially enhance the microbe transmission between individuals and alter exposure routers. On earth, majority of the microbes shed by individuals such as microbe containing respiratory droplets, skin cells and other particles settle quickly due to gravity, reducing the risk of exposure. On the contrary, in microgravity these particles do not settle but follow airflows until removed by the ventilation system causing potential inhalation exposure of the fellow astronauts. The absence of settling also affects the deposition of the microbes. The aim of the ESA funded BIOMODEXO project is to develop understanding in microbe dispersion and deposition with the aid of a validated Computational fluid dynamics (CFD) model. In the work, a laboratory scale analogue has been constructed to validate the calculations and to assist in the selection of suitable turbulence modelling approach. A challenge is that an accurate representation of the case requires fine mesh, in the studied case of the order of 107 cells. This, combined with the fact that the studied case is unsteady by nature, means that the situation needs to be calculated as time-depending resulting in very long calculation times. So far, the microbes have been treated as discrete particles and their paths have been modelled solving the forces affecting on the particle. These simulations showed clearly the particle dispersion and the preferred locations of deposition. The developed model will be used to simulate the conditions in a full-size setup. For this, a ventilation system has been set up in a hermetically sealed environment to create controlled air flows and air exchange rates typical to space modules. The dispersion and deposition of microbes will be studied with the aid of harmless bacteria spores generated inside the spaces, and the accuracy of the simulations will be validated by air velocity, concentration and deposition velocity measurements.

The peak of harvest- a non-destructive identification method - streamlining the crop production for planetary missions by nutritional value indexes

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Human planetary missions are crucial in the space exploration discourse. Achieving sustainable plant production for assuring healthy diets and enhancing positivity among the astronauts is one of the main goals. Many studies have been conducted on different growing technologies, species and their adaptation to microgravity or lunar and mars like soils. Growing plants in space is challenging through the resource utilization, contamination and plant behaviour in an unpropitious environment. Higher plants are sensitive to environmental parameters, nutrition, irrigation etc. Among this factors, gravity is also involved in growth and development of the plants. Microgravity is linked to stress factors (elicitors) that promote microbial growth and so the plants are being susceptible to diseases. Secondary metabolism is known to have an important role in plant protection and in accumulating bioactive substances that have medicinal proprieties and that give the flavours and nutritional content of the products. The secondary metabolite dynamic is not always linear throughout the vegetation cycle, having regularly, an optimal moment for harvest and specific post-harvest processes that maximize the nutritional characteristics of the product. In a space exploration context, the astronauts should be able to identify the optimal moment of plant harvesting and then continuing to specific post-harvest processes, in order to take the most nutritional content of the crops and so, streamlining the crop production technology. This review aims to show non-destructive methods of plant evaluating in terms of quality, rather than quantity, which in space mission constraints, can have a greater importance. Different methods as (i) repeated sampling, (ii) real-time secondary metabolites determination, and (iii) mathematical model prediction, are being investigated and interpreted in a planetary mission context. Identifying the peak of harvest methods can lead to better understanding of plant metabolism in space and also to novel terrestrial capitalizing of the crops.

SESSION 6 – SYSTEM TOOLS

Table of Content

Comprehensive modelling and simulation, reliable sensors and multilayer control strategy: the essential system tools for a sustainable bioregenerative LSS

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Regenerative ECLSS, by contrast of non-regenerative ECLSS, are based on the assembly of a large number of intertwisted processes. The nature diversity, the dynamics are so different that they cannot operate successfully in stand-alone operation. A deterministic control is therefore a prerequisite of robust performance. This includes at the prime level, the triptych: (i) measurement by reliable sensors, (ii) scheme of control and (iii) regulation. But in any case, the deterministic control cannot be developed without a thorough understanding of each part of the system and of the interrelations between the different subparts. This mathematical deterministic modelling and simulation of the interacting parts of the system materializes the brain-level of the manmade ecosystem.

MELiSSA system is semi-closed loop aiming at recycling and recovering oxygen, water and food from waste with minimum on-board extra resources and minimum mass buffers. MELiSSA is composed of interacting bioreactors, separators, plant chambers and a crew compartment. On a systemic approach, MELiSSA must be considered as an integrated sum of interconnected unit operations, including biological compartments. On one hand, all unit operations in charge of the elementary functions constitutive of the entire loop must be understood, up to a thorough translation in mathematical models. On the other hand, the systemic approach of complex, highly branched systems with feedback loops is performed. This entails to study in the same perspective, with the same degree of accuracy and with the same language and concepts, waste degradation, water recycling, atmosphere revitalisation, food production systems...etc. prior integration of knowledge based control models, organised in several hierarchical levels with a decision system interface with human environment. Intelligence of the system is based on the adequacy of the model for representing each unit operation and their interrelations in a suitable degree of accuracy and adequate range of validity of the models for implementing a hierarchical strategy of control.

To face this challenge, mainly hierarchical control techniques are used ("Model Based Predictive Control techniques"). They are coupled with predictive estimators of disturbances and set points, using internal models, which are reductions of the physical / biological models and allowing anticipation.

This presentation aims at retracing the pathways and the challenges from bioprocesses modelling to the perspective of predictive control of a complex looped system such as MELiSSA. It is shown that this approach is applicable to bioprocesses, including bioreactors and higher plants chambers. The modelling challenges of the MELiSSA compartments are investigated. This include:

- Deep understanding and mathematical representation of microorganisms behaviours (including microbial communities or higher plants behaviours as well): this includes the description of the activity of the intracellular metabolism from the genetic characterization (metabolic engineering) to the knowledge of the adaptation of living cells in their environment; the actual scientific challenge stay at this level of understanding, in terms of 'omics' tools, with an as thorough level of integration.
- Thorough understanding of physics (and equilibrium properties) of transport and mixing processes, particularly for assessing microbial and living organisms environment;
- Fitting in the models of the different sub-systems (unit operations levels) in order to achieve an overall representation of the global MELiSSA loop.

The objective is to develop a system model for the robust design and validation of the multilevel control of MELiSSA. It is shown how this system model is parameterized from available data at each level. It must also permit simulating different working modes, both in normal and degraded situations and bringing support during all the phases of system life cycle. The following points are addressed:

- Global overview of the hierarchical control strategy of MELiSSA;
- Derivation of control requirements from the system requirements;
- Model based design process and hierarchic architecture of the control:
    - At functional level: multivariable management of system variable (flows, stocks of water, air, food, wastes, etc.)
    - Control of bioreactors as a system element in order to fulfil multivariable control of MELiSSA flows.
- Validation process of the system to test in virtual environment the control and assess the MELiSSA performances.

The overall objective is to show how the MELiSSA model constitutes an important and generic element for the knowledge management and capitalisation and to describe how the different milestones in modelling and control are constitutive of an efficient global strategy of LS operation.

Biography: Prof. Alberto Bemporad

Alberto Bemporad received his master's degree in Electrical Engineering in 1993 and his Ph.D. in Control Engineering in 1997 from the University of Florence, Italy. In 1996/97 he was with the Center for Robotics and Automation, Department of Systems Science & Mathematics, Washington University, St. Louis. In 1997-1999 he held a postdoctoral position at the Automatic Control Laboratory, ETH Zurich, Switzerland, where he collaborated as a senior researcher until 2002. In 1999-2009 he was with the Department of Information Engineering of the University of Siena, Italy, becoming an associate professor in 2005. In 2010-2011 he was with the Department of Mechanical and Structural Engineering of the University of Trento, Italy. In 2011 he became full professor at the IMT Institute for Advanced Studies Lucca, Italy, serving as director of the institute in 2012-2015. In 2011 he cofounded ODYS S.r.l., a consulting and software development company specialized in advanced controls and embedded optimization algortithms. He has published more than 300 papers in the areas of model predictive control, automotive control, hybrid systems, multiparametric optimization, computational geometry, robotics, and finance. He is author or coauthor of various MATLAB toolboxes for model predictive control design, including the Model Predictive Control Toolbox (The Mathworks, Inc.), the Hybrid Toolbox, and the MPCTool and MPCSofT toolboxes developed for the European Space Agency. He was an Associate Editor of the IEEE Transactions on Automatic Control during 2001-2004 and Chair of the Technical Committee on Hybrid Systems of the IEEE Control Systems Society in 2002-2010. He received the IFAC High-Impact Paper Award for the 2011-14 triennial. He has been an IEEE Fellow since 2010.

Model Predictive Control: State of the Art and Possible Opportunities for Life-Support Systems

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Model Predictive Control (MPC) is one of the most successful techniques adopted in industry to control multivariable processes in an optimized way under constraints on manipulated and controlled variables. In MPC, the manipulated inputs are computed in real-time by solving a mathematical programming problem that depends on a dynamical model of the controlled system, the performance index to optimize, and constraints on variables. In my talk, I will review the basic concepts of MPC based on linear and hybrid dynamical models, with an emphasis on how to design MPC controllers using toolboxes and how to implement them in embedded control systems. I will also show the capabilities of MPC in a selection of industrially-relevant applications, and discuss its potentials in automatic and optimized control of life-support systems.

A thermodynamic theory of microbial growth and its perspectives for modelling environmental biotechnology processes

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The study of microbes, the simplest form of life, constitutes a fertile thinking ground for a deeper interlinking between physical and biological concepts. In 1922, Alfred Lotka suggested that the similarity between individuals was an invitation to imagine a "statistical mechanics of living beings". As an extension of these ideas, we proposed a thermodynamic theory of microbial growth by showing how systems constituted by microbes in contact with molecules could be likened to ensembles described by the laws of statistical physics. Based on thermodynamic balances established by different authors, we defined the "activation energy" of a microbe and thus the probability for an elementary division act to be triggered. A growth equation could be proposed, which links a flux (the growth of microbes) to a force (the free energy density in the environment). The equation allows adequate modeling of experimental growth data. More importantly, it also allows making new predictions in relation to the microbial isotopic fractionation phenomenon which can be viewed as a kinetic consequence of the differences in energy contents of isotopic isomers used for growth. We showed how recent experimental data actually support these original predictions. We are currently using this thermodynamic framework for modelling microbial community dynamics in environmental biotechnology processes to assess the possibility to obtain models featuring increased predictive abilities. Our model aims at simulating the concentration of chemical species in a reactor along time together with several actively growing microbial populations, each defined by the specific metabolic reaction they undergo (microbial guilds). In the model, each microbial guild is represented as a chemical species with a given elemental composition corresponding to the average composition of microbial biomass. The stoichiometry of each metabolic reaction is dynamically adjusted in order to preserve both the elemental and energetic balance (according to the reactions' Gibbs energy variations). Strikingly, in first simulations performed on a simplified virtual activated sludge community with standard thermodynamic properties and the same defaults model parameters for all microbial populations, an emerging functional behavior well in line with activated sludge plants engineering knowledge was observed. These first results are encouraging and indeed suggest that, in the future, such types of approaches might be useful for a deeper understanding and control of more complex microbial communities such as those present in the Melissa C1 reactor.

EnRUM-Space and Energy Resources utilization Mapping

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The objective of the present work is to develop a comprehensive simulation and mapping tool of all involved energy sources in a given space mission, allowing the user to perform analysis and optimization of the energy balance.

Space missions require energy during their different phases. Depending on the type of mission, energy can be provided either by solar panels or nuclear power sources (i.e. juno spacecraft, landers, rovers) and it could be recovered, recycled and/or stored for later usage.

Moreover, during lifecycle of a project, there is a diversity of needs and models to be maintained, modified or refined: at early stages the concept is not still defined and people need to compare different concept models from the energy point of view; once the project progresses, the concept and its main operating principles are defined; then functional and operational architectures are progressively determined and finally physical architecture is onwards established. At each stage, data are not the same, neither the models: the proposed architecture, based on a polymorphic library and high degree of integration, is suitable to allow the user to define the right level of modelling.

As a result the user can analyze time-history of energy utilization and consumption as well as energy flow diagrams. A concept model is under development for 'crew-water-air-food' closed cycle.

Marine fish aquaculture in closed system : the couple fish/algae

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In the frame of research on sustainable aquaculture, the French Institute of Exploration of the Sea conducts projects for understanding and developing systems Integrated Multi Trophic Aquaculture (IMTA). The objectives are: (1) collect nutrient production from the aquaculture system using an algal biomass to avoid dissemination in the nature and conserve water availability for a safe environment for fish. (2) use algal biomass from bioremediation for fish feed and reduce wild pelagic fish catches for the fish meal and fish oil production . The laboratory IFREMER "Adaptation and Adaptability of Animals and Systems" located in Palavas les flots (south of France) focussing on the couple fish (European sea bass)/ marine micro algae using two closed aquatic loops. Fish are reared in closed system (bacteria biofilter). The dissolved nutrients in liquid effluent (nitrogen, phosphorus) and gaseous effluent (CO_2) are treated using a natural micro alga consortium cultivated in outdoor. Water is reused for the fish primary system. The valorization of marine algae is envisaged as proteins and lipids sources to formulated fish feed. This thematic also deals with in the primary fish loop: the fish stress (density), CO_2 bioremediation and oxygen availability, nitrification, heterotrophic and autotrophic bacteria interaction. For the algae loop for feed : the production orientation for high value lipids, the bacteria population modification in fish digestive tract. I propose to the MELiSSA workshop a presentation named of "marine fish aquaculture in closed system : the couple Fish/algae.

European Platforms for Life-Support System development and validation

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Future manned or robotic missions to the Moon and other destinations will require an intensive preparation work on Earth leading to simulations and testing in terrestrial facilities of Life Support Systems. The proposed presentation will present two European facilities that can be used for LSS test and development: The Self-deployable Habitat for Extreme Environment SHEE has been developed to address some of the complex problematics that will face astronauts during the coming phases of planetary exploration in terms of habitability and life support system. SHEE is a foldable habitat for a crew of two. Its first field use is foreseen in April 2016 and first results can be presented at the time of the workshop. The second facility that offers the possibility to test LSS is the COMEX' HYDROSPHERE which is an ESA Ground Based Facility. The HYDROSPHERE offers the possibility to simulate various aspects of human missions in the frame of the exploration of the solar system in the coming post-ISS era. Initially it was built to test procedures for deep-sea diving. Not used for this purpose anymore today, it offers the possibility to simulate lunar EVA (by astronauts and robots) in a large regolith testbed under vacuum conditions with an adjacent habitat linked to it. The facility is composed of three hermetically closed chambers and one control center. The main element of the facility is a 5m diameter sphere, which can be used as an EVA or robotic testbed. Linked to the sphere is a second chamber, which can be used in the future as an airlock to the EVA testbed and includes currently the hygiene facility. The third chamber is the habitat section that offers a space for a crew of maximal eight subjects. The facility can be used to simulate and test different scenarios of future planetary habitats, such as Life Support Systems, dust mitigation, habitability, contamination by dust or living organisms, and confinement psychology.

Oïkosmos research agenda: relevance of manned interplanetary missions to terrestrial sustainability

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Manned interplanetary missions imply the recycling of the whole organic waste produced by its crew members. Such quest for integral recycling aims at closing the entire material flows that are generated by an Artificial Closed Ecosystem (ACE). The challenge is therefore to design and build a self-sufficient habitat (excluding energy supply) that enables a small group of people to live (survive) in symbiosis with organisms such as bacteria, micro-algae and plants during a long period, by the means of life support systems functions that include air purification, water regeneration and food production. In order to prepare future human planetary exploration under the most realistic conditions, it is essential to build on Earth a technological demonstrator that simulates the strong constraints of space habitats. In addition to the applications dedicated to space, one can easily envision the ensuing “terrestrial” benefits that will emerge from R&D activities conducted in such ACE ground simulator. This presentation introduces a research agenda called Oïkosmos, that emanates from the convergence of space and terrestrial research on ACE. Based on synergistic and systemic approach, Oïkosmos encompasses a vast scientific and technological programme at the frontiers of several research fields such as industrial ecology (sustainable resource management, material loop closure, ecotoxicology, CO_2 valorisation and biorefinery), systems biology (omics sciences: health biomonitoring, genomic, proteomic, metabolomic, nutrigenomic and microbiomic tools), information and communication technology (human-machine interactions, embedded technologies, smart monitoring and telehealth). The combination of the above-mentioned research fields is crucial for providing the necessary quasi real-time monitoring, fine regulation and process control of both the organisms' health and environmental conditions within a closed habitat. The research on such sustainable habitats could also act as a catalyst for innovation and as an eco-innovation driver for the technological development of highly efficient recycling systems in the perspective of industrial ecology. This alternative and innovative testbed could then help face terrestrial sustainability issues, among others the depletion of material resources, the increasing pollutants dissipation in natural ecosystems and the of environmental impact of buildings.

How Space technology empowers the Transition towards a circular economy

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The world is witnessing a paradigm shift. The transition from a linear to a circular economy may be about to drive the biggest transformation in business since the Industrial Revolution 250 years ago through a radical departure from the traditional 'take, make, waste' production and consumption models. We are going through the evolution to a circular economy, a socio-economic system which is smart, cross-sectorial and full of opportunities and challenges. SEMiLLA is a multifunctional business & experience hub that is designed to attract the public, governments, educators and entrepreneurs to accelerate and demonstrate the use of both space and non-space innovations for circular economic models on earth and to foster the technology transfer of innovations from space to earth and reversed.

POSTER SESSION

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Integrated omics provides unprecedented insights into microbial community structure and function

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Microbial communities are ubiquitous and underlie various biotechnological, biogeochemical and biomedical processes. In situ characterization of such communities is challenging due to their inherent complex structures and dynamics, rendering classical microbiology methods ineffective. We recently developed a suite of methods to study such consortia, and applied them to numerous microbial ecosystems of varying complexities, including wastewater sludge microbial communities, human gut microbiome and acidophilic bio-mining cultures. Developed methods encompass wet-lab and bioinformatic procedures, allowing high-throughout, high-fidelity and high-resolution molecular analyses. The foundation for such surveys is the extraction of high-quality biomolecules; DNA, RNA, proteins, and metabolites, isolated from a single unique sample enabling downstream integrative analyses. The protocol is further enhanced by automation using a robotic platform, which also minimizes technical variability. These biomolecular fractions undergo systematic high-throughput measurements, such that DNA and RNA sequencing yields metagenomic and metatranscriptomic data, respectively. Chromatography followed by mass-spectrometry methods are applied to proteins and metabolites yielding metaproteomic and (meta)metabolomic data. The integration of the resulting data provides a detailed view of microbial community composition whereby metagenomic data provides an overview of community structure and functional potential, whereas metatranscriptomics, metaproteomics and metabolomics enable functional characterization of the community or single populations. Integrative large-scale bioinformatic analyses produces a molecular-level overview that enables discovery of microbial community- and population-level characteristics and dynamics, ranging from the resolution of lifestyle strategies of various bacterial populations to the identification of keystone-genes and -species. The complementary application of high-resolution microscopy (nanoSIMS) provides a means of hypothesis testing, for example providing a high-resolution view of population resource usage on a cellular-level. In addition, time-resolved studies allow the extension towards inferences on community dynamics, including the influence of physico-chemical parameters on the community and the interaction of biotic factors within the community. The knowledge from the aforementioned interactions enables the formulation of mathematical/probabilistic models that elucidate and potentially predict the behavior of a given system. In summary, the systematic study of microbial communities using integrated-omics enables unprecedented insights within the scope of microbial ecology, which potentially allows targeted perturbation of microbial systems to optimize biotechnological, biogeochemical and biomedical processes in the future.

Butyrate metabolism under photoheterotrophic conditions in Rhodospirillum rubrum S1H

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Rhodospirillum rubrum S1H has the ability to metabolize volatile fatty acids through photoheterotrophic metabolism. R. rubrum was subsequently selected by the European Space Agency to colonize the second compartment of its bioregenerative life support system and to remove VFA from the effluents produced by the first compartment. Recently we have highlighted new pathways in acetate assimilation in R. rubrum. Here we report based on proteomic, enzymatic and bacterial growth analysis, a better view of the photoheterotrophic metabolism of butyrate. According to quantitative SWATH-MS proteomic analysis, butyrate is first converted into crotonyl-CoA and then into acetyl-coA. Because R. rubrum is an isocitrate lyase lacking organism, an alternative anaplerotic pathway to glyoxylate cycle should be used to replenish the TCA cycle in intermediates for biosynthesis. We suggest here that the ethylmalonyl-CoA pathway and the valine biosynthesis and degradation pathways could be used to convert butyrate into succinate. Our bacterial growth analysis showed that assimilation of butyrate depends on the amounts of carbonate supplied. In accordance with the observed upregulation of RuBisCO, CO2 fixation is most probably used as a redox balancing reaction necessary to sustain butyrate assimilation. Finally, we also showed an inhibition of the butyrate assimilation by acetate when they are provided together at an equivalent net carbon concentration to feed R. rubrum culture. This effect could be the result of various factors: on one hand, reduced abundance of key enzymes of the butyrate assimilation pathway when acetate is used as a carbon source, and on the other hand shared pathways that cross each other in an opposite manner. Altogether our data allows better understanding of metabolic pathways involved in butyrate photoassimilation in the purple non-sulfur bacteria, R. rubrum. This research was supported by the European Space Agency and BELSPO (GSTP 'melgen-3').

Adaption of Arthrospira sp. PCC8055 to modification of the nitrogen sources in photobioreactor operated in continuous mode

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Non-toxic cyanobacteria Arthospira has been used as a food supplement since ages due to its high protein content, antioxidant and other therapeutic properties. The ability of autotrophic cyanobacteria like Arthospira sp. PCC 8005, to thrive and grow using waste nitrogen (both organic and inorganic) and release oxygen has made it a potential candidate for the production of food and oxygen for astronauts in the MELISA loop. Previous works on this cyanobacterium have been done using a single nitrogen source namely nitrate. The present case study evaluates the effect of different nitrogen sources namely ammonium and urea in addition to nitrate, on the growth kinetics and metabolism of Arthospira sp. PCC 8005 in photobioreactor, which could be used to pave the road for the use of human urine as a nitrogen source for the growth of Arthospira sp. in the CIVa compartment of MELISSA System. Since reported problems linked to ammonium use as nitrogen source, overwhelmingly depends on both the pH and the equilibrium between NH4+/NH3 (pKa 9.2), Arthrospira sp. was continuously cultivated in a photobioreactor with pH maintained/settled at 8.5. After bioreactor reached a steady state, NO3- ions were progressively replaced by NH4+ ions (28mM, Dr 180 - 360mL). Results showed no toxicity effect, but ammonium did not seem to be assimilated in respect with the theoretical model. Proteomic analysis would be further performed to evaluate and study, at the metabolic and molecular level, the response of our strain to the modification of nitrogen sources when growing in continuous mode. The study and quantification of the energy state parameters namely ATP, NADPH, Quinone pool etc. may further help in understanding the metabolic and stress response of cyanobacteria to the different nitrogen sources at cellular level. Finally these data will be useful to upgrade the so-called "photosym model" currently linking O2 production to light intensity in order to implement variation in nitrogen supply.

Functional characterization of N metabolism within Arthrospira sp. PCC 8005, and its application for space flight

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The main limiting factors in universe and planets exploration come from the current technologies, which do not enable to sustain long-term space flight without regularly providing supplies (e.g., oxygen & food). To overcome this constrain, the European Space Agency plans to develop an artificial closed system named Micro-Ecological Life Support System Alternative, hereafter referred as MELiSSA project. This project has been conceived to support the life of crews by using microorganisms as well as higher plants differentially located in 5 compartments. Among the microorganisms used in MELiSSA project, Arthrospira sp. PCC 8005 is a cyanobacterium devoted to produce both biomass and oxygen from CO2 and nitrogen sources generated by the other compartments. In this context, Arhtrospira sp. PCC 8005 was cultivated with the three typical nitrogen (N) sources present in this artificial ecosystem. Several concentrations of nitrogen were tested in order to detect potential detrimental effects as it was already reported for ammonia. The strain PCC 8005 was then cultivated in flask with no pH control. Results showed a lower growth and cell lysis at 6mM and 12mM NH4+, respectively. No toxicity effect was observed for nitrate and urea at mentioned concentrations. We also evaluated the response of this cyanobacterium at several level including the sedimentation index, microscopy, assimilation rate; this may help to establish whether several nitrogen sources can be used for the cultivation of Arthospira sp. in the MELISSA Loop. Ammonium is also known to induce repression in transcription of gene coding for the N assimilatory pathway. This repression phenomenon was then investigated at mRNA levels within Arthrospira sp. PCC 8005. It was cultivated with nitrate and/or ammonium or urea. Results clearly showed that NH4+ induced a repression of the aforementioned genes at short and longer term. This research was supported by the European Space Agency and BELSPO (GSTP 'melgen-3', Biorat-2).

Project Hydronaut Planned Use of Station Hydronaut H3 Underwater Experimental Facility

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The Hydronaut H3 Underwater Research Laboratory and Training Station will serve as a ground-based space analog research and training facility. It enables implementation of a wide range of scientific experiments focused primarily on issues of physiological responses and the adaptation of the human to extreme, confined and isolated environments. We will provide support and research in the related psychological stressors, like confinement, reduced interpersonal contacts, reduces spare-time activities, lack of privacy, sleep disturbances, changes between high workload and monotony, or continuous dependence on artificial life support system. The Hydronaut module is also suitable for practicing specific procedures in tactical medicine and rescue operations in difficult high-risk situations, such as flooded areas or during spaceflight. Due to its size, capabilities and specifications, the Hydronaut H3 module is adaptable for research in the field of human factors in emergency situations and for developing and practicing new emergency procedures for crews training for deep sea and deep space missions. An important part to the construction of our facility are some new types of life support and waste management systems. We hope that after completion the Hydronaut H3 will be available not only to scientists from space agencies around the world for which we want to prepare special physical and psychological training course based on knowledge from training of astronauts.

Microgravity liquid-gas phase separator for biological and industrial processes

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In the space related industrial, chemical and biological biphasic processes, liquid-gas separation is often matter of concern. Although on earth in such cases the phase separation is easily achieved thanks to the gravity force, in microgravity or in weightlessness the separation is non-trivial and must rely on other techniques based on other principles such as surface tensions and imposed accelerations. The project presented here focuses on a technique aiming at a fully passive liquid-gas separation for processes occurring both in gravitational and non-gravitational conditions. Such a device could be viable for bioreactors, where surface tension based separation is considered with caution, as well as in other industrial and chemical processes where the same constraint could apply.

Development of a synthetic plant-supporting microbial community towards understanding of soil plant-mutualist bacteria-fungi interaction

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The plant mutualist fungi and bacteria in the soil contribute a lot to plant growth. How these microbes interact with each other in such soil complex system remains elusive. To analysis of such interactions could help us better understand what is happening within the soil, and how to improve the soil microbial community to enhance plant growth. During our research, we found the fungus Piriformospora indica is auxotrophic to some vitamin. Based on this, we built a synthetic community consisting of a vitamin consumer, P.indica, and a vitamin producer, Bacillus subtilis, using a defined medium in order to mimic and understand their interaction in natural environment. In this system, we discovered that adjusting certain components of the medium would lead to two different behaviour interactions. This finding could help build a model system to analyse the relationship between plant mutualist fungi and bacteria, and could also be expanded to more efficient synthetic plant supporting community.

High throughput functional genomic analysis made possible on Rhodospirillum rubrum by the creation of a bar-coded transposon mutant library

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Understanding the bacterial metabolism requires multidisciplinary approaches most often referred to as the “omics toolbox”. If metabolomics, proteomic and transcriptomic allow studying what is actually occurring in an organism or a cell population, genomic mostly describes what can be achieved by the organism. Recently, functional genomic was introduced with the development of mutant fitness assay and TnSeq technologies. Functional genomic allows closing the gap between genomic and the other omics strategies by giving insight on the essentiality of genes rather than just their presence. In other words, mutant fitness assay experiments allow detecting which genes are essential for an organism to survive in defined conditions. These data are highly complementary to proteomic data. In order to better understand both carbon metabolism and stress response in Rhodospirillum rubrum S1H, we constructed a bar coded mutant library on this strain. Bar coding make it possible to replace the very expensive and time consuming full genome sequencing for mutant fitness assay by the cheaper and easier Bar-Seq technology. The produced library has mutant in more than 80% of the genome. Pool of essential gene has been compared to recently published data on Rhodobacter sphaeroides. A first mutant assay has been realized in order to analyze gene essentiality in the context of photoheterotrophic growth with different carbon sources. These data will first help confirming and validating proteomic derived hypothesis on assimilation pathways but also open new doors concerning regulatory aspect of carbon metabolism in purple bacteria. A major breakthrough in space related microbiology could also be reachable through the performance of such a mutant fitness assay on-board ISS. This research was supported by a mobility grant of FNRS, by the “fond Franeau” for mobility of UMONS and by the Research institutes for Biosciences of UMONS.

Aquaponic: A possible technology for closed-loop food production in space?

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Ranka Junge, Andreas Graber, Zala Schmautz, Alex Mathis

At ZHAW we are developing the aquaponic technology since 2004. We were able to demonstrate that production of a wide array of species of fish and vegetables of suitable quality for human nutrition is possible in one-loop recirculations system. Currently we study in detail the element cycling in the system, including the microbiological community with the aim of improving the system. We also investigate aspects of biocontrol as means of prevention of disease in Aquaponic. In the presentation I will report about these experiments.

Impact of Nutrient Availability on Wheat Root Plasticity for Higher Plant Modelling

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The European Space Agency (ESA) has initiated controlled life support system activities required for long-term human space manned missions. The ESA project, Micro-Ecological Life Support System Alternative (MELISSA) program was developed to design a closed regenerative life support system, based on microorganisms and higher plant processes. Higher plants are indispensable for these activities to provide a continuous supply of fresh food, oxygen and water for human consumption. The ability of plants to respond appropriately to nutrient availability by modifying their root architecture is crucial for their adaptation to the environment. Plant roots response to different nutrient deficiencies with a high variation of root morphological changes. The objective of the project is to investigate root plasticity in response to different nutrient supplies and levels. Total of seven root system architecture traits (e.g. length, area, volume, number of tips and diameter) are being measured using WinRhizo. The result of the project is to be used for drawing mathematical models which will describe predictive plant root response in the surrounding environment. The prediction of plant productivity and plant modifications in uncommon extraterrestrial environments highlight the importance of predictive models of plant growth. Additional to fulfill the need of predictable and reliable life support system, a deeper understanding of the adaptive responses of crop root systems on different nutrient growth environments will impact on nutrition for space plant production that better exploit fertilizer nutrients.

Microbial community dynamics and response to plant growth-promoting organisms in the rhizosphere of four common food crops cultivated in hydroponics

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S. Bahar Aciksöz, Emmanuel Frossard

Plant growth promoting organisms (PGPOs) have received an increasing amount of interest in recent years for the benefits they provide to plant growth, yield and resistance to pathogens. While largely described for soil-cultivated plants, the effects of PGPOs on the plant root zone microbiome have received limited attention in hydroponic cultivation systems. In the framework of a project aimed at the development of a biological life support system (BLSS) for manned missions in space, we investigated the effects of PGPOs on four common food crops (durum and bread wheat, potato and soybean) cultivated in recirculating hydroponic systems for a whole life cycle. Each crop was inoculated with a commercial PGPO mixture and the composition of the microbial communities associated with their root rhizosphere, rhizoplane/endosphere and with the recirculating nutrient solution was characterised through 16S- and ITS-targeted Illumina MiSeq sequencing. PGPO addition was shown to induce changes in the composition of these communities, though these changes varied both between crops and over time. Microbial communities of PGPO-treated plants were shown to be more stable over time. Though additional development is required, this study highlights the potential benefits that PGPOs may confer to plants grown in hydroponic systems, particularly when cultivated in extreme environments such as space.

A simple mechanistic model of higher plant growth in a reduced gravity environment linking mass balances and plant morphology

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Long-duration human space missions and the establishment of permanent off-Earth bases (e.g. on the Moon or Mars) is one of the main focuses of today's space exploration. This poses many severe challenges at the life-support level, which needs to recycle atmosphere, water and waste for crew survival. The European Space Agency (ESA) project Micro-Ecological Life Support System Alternative (MELiSSA) can ensure these functions. It is a closed-loop bio-regenerative life-support system functioning with microorganisms and higher plants and providing a circular cycling of mass, including O2 production, CO2 capture, water recycling and food production. The growth and development of higher plants are strongly influenced by environmental conditions (e.g. gravity, pressure, temperature, relative humidity, partial pressure of O2 or CO2) so bio-regenerative life support systems require a high level of control and management.

The goal is to develop a mechanistic physical model of plant growth to predict the effects of microgravity or of a reduced gravity environment (like on Mars or on the Moon) on plant growth at its morphological, physicochemical and biochemical levels. Current existing plant growth models are developed for agronomy and are therefore not adapted for modeling plant growth for applications in life-support systems, which require being able to extrapolate plants behavior for a wide range of environmental conditions. The first mechanistic plant growth model developed in the framework of the MELiSSA project has attempted to address these limitations. Based on this work, a preliminary structure of the model was defined.

In this presentation, the addition of gravity as a parameter is addressed. Indeed adding gravity involves the mechanism of gravitropism, which by nature provides a link to the plant's morphology. The link between mass balances and plant morphology in a mechanistic way is discussed.

Nitrosomonas europaea and *Nitrobacter winogradskyi* culture in a fixed-bed bioreactor: from modelling to a one-year experiment

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Nitrogen is a key element for the life and its balance on Earth is regulated by the nitrogen cycle. This loop includes several steps among which nitrification that permits the transformation of the ammonium into nitrate. The MELiSSA loop is an artificial ecosystem designed for life support systems (LSS). It is based on the carbon and nitrogen cycles and the recycling of the non-edible part of the higher plants and the waste produced by the crew. In this order, all the wastes are collected in the first compartment to degrade them into organic acids and CO2. These compounds are joining the second compartment which is a photoheterotrophic compartment where at the outlet an organic-free medium containing ammonium is produced. This solution will be the substrate of the third compartment where nitrification is done.

This compartment has to oxidize the ammonium into nitrate, and this biological reaction needs two steps. In the MELiSSA loop, the nitrification is carried out by two bacteria: Nitrosomonas europaea ATCC® 19718™ which is oxidizing ammonia into nitrite and Nitrobacter winogradskyi ATCC® 25391™ which is producing nitrate from nitrite in the third compartment. These two bacteria are growing in axenic conditions on a fixed bed bioreactor filled with Biostyr® beads using CO2 as their carbon source.

A model of the nitrification has been done in order to predict the nitrifying compartment in the MELiSSA loop. The fixed-bed bioreactor and the bacteria strains were characterized and the model was validated. This work deals with a coculture of the two strains in a packed-bed bioreactor in continuous mode which was running for one year. We worked with several nitrogen load or residence time in order to observe the bacteria response to these troubles and extreme conditions. The model was applied on the experimental data obtained in order to verify its efficiency in several culture conditions.

The nitrogen loop in the MELiSSA loop: Phd project and host laboratories

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Nitrogen, just as in any biological system, is a crucial element in the MELiSSA loop. This PhD project comprises several objectives: A critical step of the nitrogen in MELiSSA is the introduction of urea to the pilot plant. The crew's urine will be directed to the nitrifying compartment. Organic compounds from urine will probably cause growth of heterotrophic microorganisms. If the growth of heterotrophic microorganisms is inevitable, these should be monitored and controlled. Also, a new support for the packed bed reactor will be designed. This should be able to handle heat sterilization. Another objective is to recycle the N2 in gas phase exiting the liquefying compartment. In order to prevent nitrogen loss from the loop, N2 could be assimilated by nitrogen fixing bacteria. One possible task, not necessarily related to nitrogen, will be the recycling of acids and bases by electrochemical means. Currently these are added constantly to some of the compartments. The project will be performed in the Universitat Autònoma de Barcelona (UAB) and Ghent University (UGent). The Chemical Engineering Department of the UAB focuses on biotechnology and environmental engineering: biochemical engineering, enzyme engineering, biological wastewater treatment, fermentation and bioreactor systems modelling and rivers modelling. The department is involved in the design, maintenance and research of the MELiSSA pilot plant. The Laboratory of Microbial Ecology and Technology of UGent is specialized in the study and application of mixed microbial cultures or communities. Its main interests are: microbial ecology, functional food and feed, medical microbial ecology, risk assessment, biomaterials and nanotechnology, water treatment, aquaculture, bio-energy, and soils and sediments. It has been involved with MELiSSA developing complementary technologies for most of the compartments, but has been focused on the liquefying, fiber degradation and nitrifying compartments.

Probiotics beyond the terrestrial limits: transcriptomic and proteomic approach to investigate the effect of simulated microgravity on the probiotic Lactobacillus reuteri DSM17938

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The design of life support technologies is necessary in human space exploration to work in an adverse space environment. In this confined environment recycling of waste and the production of food will be of great importance and therefore an efficient microbial based life support system has been studied and developed for this purpose. The short generation time of microorganism makes them ideal for studies about the responses to altered environmental conditions. The spacecraft environment determines a particular ecological niche where parameters such as gravity, radiation and acceleration are different from those on Earth. In fact some environmental stresses could induce and select some microorganisms for physiological, metabolic and or/genetic variations. Therefore the study of microbial activities under spacecraft conditions are essential for the early identification of changes in microbial communities and microorganisms with medical, environmental, or life support consequences. The scientific objective of my PhD project is to investigate the microbial response of microgravity by using a random-positioning machine (RPM) that reproduce many characteristics of this environment. My activity is based at Division of Microbiology, who has expertise in both traditional and molecular manipulation of bacterial cultures. In particular, I will evaluate whether the simulated space flight conditions could affect the metabolism of probiotic bacteria and modify the probiotic features of cultures. We will focus the study on the probiotic strain Lactobacillus reuteri DSM 17938 whose genome have been recently sequenced as part of the Human Microbiome Project. Transcriptome of L. reuteri in microgravity conditions will be monitored using RNA sequencing technology and compared with the strain-specific genome in order to improve predictions of metabolic processes and enzymatic pathways present in a well-defined environmental condition. Furthermore, L. reuteri cultures will be subjected to proteomic analysis by high-throughput technology using a quadrupole Orbitrap mass spectrometer. These investigations could highlight the mechanisms of regulation and adaptation of this strain, which will be helpful in uncovering the interactions between lactobacilli and environments, including human and animal intestine.

Nutrient and water management for producing high quality functional vegetables for BLSSs

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Space exploration or remote research stations are always related to constraints; of which we mention food provision for astronauts or researchers since resupply is not an option for long duration missions. Maintaining astronauts or researchers physical and psychological health is a primary concern, and here lies the importance of the development of bio-regenerative life support systems. The cultivation of higher plants takes an essential role, as higher plants contribute to all major functional aspects e.g. food production,CO2 reduction, O2 production, water recycling and waste management. As a doctorate student working on the nutrient delivery and water recycling part of MELiSSA project, I will be working along with Prof. De Pascale and Dr Rouphael in order to optimize the nutrient solutions (composition, concentration or biofortification) that we will definitely need in order improve the quality and yield of the chosen crops specially when water and nutrient use efficiency, harvest index, time and shelf life play an important role in such missions. For my 3 years Ph.D period, we will be running our experiments in a growth chamber where light, temperature and humidity are controlled, using an NFT system. Lettuce will be the first tested crop, since it plays a major role as a fresh leafy vegetable in food diets, rich in dietary fibers, iron, folate, vit. C, beta-carotene and lutein and finally phenolic compounds but definitely depending on the variety. The second crop will be dwarf tomato, since also is an important vegetable to be inserted in everyday diet, it provides essential antioxidants, calcium, vit. K and lycopene. As second step, some experiments will be repeated but with simulated low gravity conditions (RPM) to monitor any change in plants at physiological and metabolomic levels as well as growth, yield and product quality. Also we might work on several functional vegetables that will be harvested at early stage using a multi-crop system. These target functional vegetables will be rich in phytochemical compounds crucial for the wellbeing of astronauts, but these vegetables will be grown in a different media and light conditions, that might be suitable for the lack of gravity in space

Improving plant physiological performance and growth by increasing the efficiency of lighting systems

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The objective of this doctorate project is to investigate on the interactions between light features and crop species selected for space cultivation. The main goal is to maximize plant morpho-physiological performance and optimize lighting efficiency, in terms of light quality and quantity, while satisfying growth parameters, productivity and nutritional values of the final product. The work is being carried out with a multidisciplinary approach including experimental methods for morphological, anatomical, physiological and biochemical traits. Some of the results will be used to model specific processes of the plant system. Research activities are based at the University of Naples Federico II, involving a close cooperation between researchers from the Departments of Agricultural and Biology. According with specific obtained results, international stages are planned for the second and third year of project to collaborate with other scientific groups, including MELiSSA partners. The working group has a strengthened expertise concerning research about plants in space, especially for microgravity and radiation topics, two factors that characterize space environments, as well as knowledge on the interactions between plants and other environmental factors in agronomical and natural ecosystems. The first year work started with a literature review to define the state of art and propose experiments that could help the scientific community in the field of interest. As a result of the literature survey, the effect of light quality on species characterized by different compositions of pigmentary endowment, such as red or purple leaves cultivars, has been identified as an innovative field of research that can lead to future applications in space. Results from this research may give the possibility of selecting species or cultivars that benefit from the modulation of growth parameters and capable of adapting to stress condition in order to optimize the production in terms of quantity and quality. Within this framework, specific experiments are ongoing. Cultivars characterized by green or purple leaves have been selected within species that can be potential candidate for cultivation in space. Comparing tests are being performed using ground-based growth chambers equipped with modular LED lighting systems and controllable environmental factors. First results are already being elaborated and interpreted.

Friends or foes? Microbes in confined habitats

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Indoor environments, are characterized by a specific microbial community, the indoor microbiome. Most indoor environments are connected to the natural environment by high ventilation, but some habitats are more confined: intensive care units, operating rooms, cleanrooms or the international space station (ISS) are extraordinary living and working areas for humans, with a limited exchange with the environment. The purposes for confinement are different: the patient has to be protected from infections (intensive care unit, operating room), the product quality has to be assured (cleanrooms), or confinement is necessary due to extreme, health-threatening outer conditions (ISS). The ISS represents the most secluded man-made habitat, constantly inhabited by humans since November 2000 – and, inevitably, also microorganisms.

All of these man-made confined habitats need to be microbiologically monitored and controlled. However, the application of microbial cleaning and disinfection measures increases the abundance of survival specialists and multi-resistant strains. Application of a constant selective pressure supports microbes with antibiotic resistance or resistance towards chemical and physical treatments.

Here, we discuss the available data on the microbiome of the ISS and other confined habitats on ground. By comparing the different operating, maintenance and monitoring procedures as well as microbial communities therein, we emphasize the importance to properly understand the effects of confinement on the microbial diversity, the possible risks represented by some of these microorganisms and by the evolution of (antibiotic) resistances in such environments - and the need to reassess the current hygiene standards.

Usage of PVA Gel bioreactor beads as a substratum for biofilm formation of co-cultures for urine degradation

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Food, water and fresh air (oxygen) are very important for life support in long-term Space missions with no opportunity for resupply. The Micro-Ecological Life Support System Alternative (MELiSSA) developed by ESA, aims at providing proteinaceous food via vegetable crops and edible algae. To produce this phototrophic biomass, it is planned to convert urine, a waste stream containing 85% of the crews' nitrogen intake, into a nitrate fertilizer. Conversion of urine to nitrate is extremely important for the photoautotrophic compartment, because nitrates are the main source of nitrogen for plants.

Nitrification of urine provides a pivotal role for nutrient and water recovery in Space. This is the main goal of URINIS (Urine Nitrification for Space) project. The achievement of conversion of urea to nitrate in one bioreactor is catalysed by a consortium of three functional groups of bacteria, i.e. (i) urease positive heterotrophs responsible for the hydrolysis of urea to ammonia and for aerobic oxidation of residual organic compounds, e.g. Cupriavidus pinatubonensis, (ii) ammonia oxidizing bacteria responsible for the aerobic oxidation of ammonia to nitrite (nitritation), e.g. Nitrosomonas europaea, and (iii) nitrite oxidizing bacteria responsible for the aerobic oxidation of nitrite to nitrate (nitratation), e.g. Nitrobacter winogradskyi. Due to an efficient collaboration of these subprocesses, urine nitrification can be performed in a single bioreactor system that contains a microbial biomass retention system, and is fed with urine and oxygen. The key purpose of urine nitrification is nutrient recovery.

The URINIS experiment encompasses the following specific main goals:

- Investigation how different relevant nitrifying bacteria grow and develop biofilms
- Demonstration the efficient collaboration between the three functional bacterial groups to transform urea into nitrate

One of the most significant aim of performed experiments is comparison of nitrogen compounds conversion rates in liquid cultures (suspension) and cultures with PVA gel bioreactor beads (biofilm). In both cases carried out experiments are co-cultures. PVA gel are applied for biofilm formation. These spherical, porous beads are made from polyvinyl alcohol. Porous structure provides huge amount of surface for biofilm formation. Usage of PVA gel enables to achieve high concentration of the biomass in the culture, which is crucial factor for increase nitrogen compounds conversion activity of nitrifying bacteria. Furthermore PVA gel are autoclavable, which is very important issue for maintain axenic cultures. The thickness of biofilm created on a surface of PVA-gel beads is analysed by using E-SEM (Environmental Scanning Electron Microscopy). This technique allows creating electron micrographs of a wet, uncoated samples. Furthermore CLMS (Confocal Laser Scanning Microscopy) will be applied for examination of biofilm structure and evaluating ratio of bacteria to EPS (extracellular polymeric substances)