University of Bremen

Bioproduction
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Humans on Mars

Sustainable Bioproduction on Mars

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Scientific goals

Overarching goal: Providing a basis for sustainable bioproduction processes that rely on Martian resources. This entails, notably:

- Determining the **fitness level and coping mechanisms of cyanobacteria** under on-site cultivation conditions. *Methods: standard microbiology assays; adaptive laboratory evolution; proteomics; genome resequencing; low-pressure experiments.*
- Developing and characterizing **microbial-based processes that convert cyanobacterial biomass into a substrate** for secondary producers (here, **plants and plastic-producing microbes**) and **recover chemical energy**. *Methods: microbial reactor-based experiments; various analytical chemistry methods; in vitro plant cultivation; system analyses.*
- Developing and characterizing **novel plant irrigation technologies** that improve the consumption and application efficacy of a nutrient solution. *Methods: system analyses; hardware prototyping and testing; soilless plant cultivation.*

Achievements

Photobioreactor module

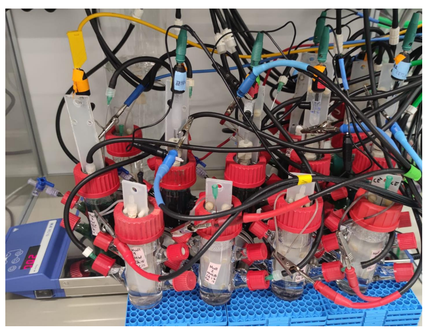
- Selected and characterized a model cyanobacterium.
- Over a year and a half of adaptive laboratory evolution (ALE); increase in fitness.
- Validated proteomics workflow.
- Acquired proteomics data, and related physiological data, pertaining to regolith and perchlorates.
- Acquired most physiological data pertaining to low pressures, and low pCO_2 and pN_2; lipidomics and proteomics pending.
- Mathematical modelling of the cyanobacterium compartment and associated ESM analysis: ca. 90% completed.

Microbial modules

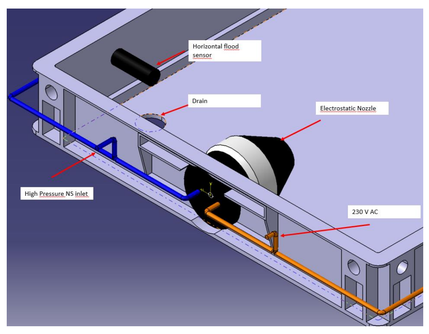
- Characterized cyanobacterial biomass as feedstock: ca. 80% completed.
- Conducted the anaerobic digestion of the biomass and selected a promising microbial community. Started process optimization: ca. 15 % completed.
- Started tests for the processing of biomass using microbial electrolysis cells (MEC).
- Identified triggers for bioplastic production in our study bacterium.
- Conceptualization of the potential roles of microbial electrochemical systems for Mars ISRU: ca 20 % completed.

Greenhouse module

- Developed and validated a protocol for axenic, *in vitro* plant cultivation to be used as a model for the first series of growth experiments.
- Performed first tests where lettuce is grown using outputs from the microbial modules.
- Defined methodology for, and conducted, plant production hardware systems analysis.
- Developed next-generation hardware design.
- Prepared laboratory for planned integrated hardware, nutrient solution, and crop testing



First run of MEC processing of cyanobacterial biomass



Plant growth trays design models

Status of planning of joint project(s)

We intend to apply for a DFG Refocusing on the development of sustainable production and recycling systems for settlements each Unit beyond Earth, which will include a strategy for technology transfer to terrestrial applications. The proposal will be submitted in 2024.

Next steps

Nov 23
- Completion of tests related to artificial atmospheres.
- Optimized anaerobic digestion of cyanobacterial biomass.
- Completion of nutrient solution analyses and replications

Dec 23
- Concluded modelling of the photobioreactor module.
- Finalized cyanobacterial biomass processing with MEC.
- Hardware validation testing, phase 1, finished

Mar 24
- Continuation and termination of ALE.
- Characterization (omics, cross-resistances,) of evolved strains.
- Systems analysis manuscript in publication

May 24
- Completion of in-vitro cultivation of plants in output of microbial modules
- Integrated hardware, nutrient solution, and crop testing

Cooperation

Collaborations are ongoing with:

- The “Living habitat” Seed project team, on the air revitalization system of their Doctoral sub-project 1.
- MARUM (Uni. Bremen) and the Robert Koch Institute (Berlin) on lipidome and proteome analyses.
- The University of Montreal (Canada) on adaptive laboratory evolution.
- University of Arizona Controlled Environment Agriculture Center (CEAC)

Publications

Published
- Ramalho, T.P.,. et al. (2022). Selection of *Anabaena* sp. PCC 7938 as a cyanobacterium model for biological ISRU on Mars. *Appl. Env. Microbiol*. 88 (15) [selected by the editors as an *Article of Significant Interest*].
- Ramalho, T.P., et al. (2022). On the growth dynamics of the cyanobacterium *Anabaena* sp. PCC 7938 in Martian regolith. *npj Microgravity 8*(1).

Planned for submission in the coming months
- Bunchek, J.B., et al. Current understanding and challenges of regolith as a crop production medium on Mars.
- Ramalho, T.P., et al. Tentative title: Modelling the *in situ* production of cyanobacterial biomass to support a sustainable human presence on Mars.
- Verseux, C., et al. Tentative title: Effects of reducing pressure on the mass-efficiency of an ISRU photobioreactor on Mars.