EFFECTS OF LOW GRAVITY AND COSMIC RADIATION ON MICROALGAE GROWTH AND POLYMERE PRODUCTION. T. Granata[1], F. Ille[2], B. Rattenbacher[3], and M. Egli[4]. [1]Lucerne University of Applied Sciences and Arts, Institute of Medical Engineering, Space Biology Group, Obermattweg 9, 6052 Hergiswil, Switzerland, timothy.granata@hslu.ch; [2]University of Applied Sciences and Arts, Institute of Medical Engineering, Space Biology Group, Obermattweg 9, 6052 Hergiswil, Switzerland, fabian.ille@hslu.ch; [3]University of Applied Sciences and Arts, Institute of Medical Engineering, Space Biology Group, Obermattweg 9, 6052 Hergiswil, Switzerland, bernd.rattenbacher@hslu.ch; [4]University of Applied Sciences and Arts, Institute of Medical Engineering, Space Biology Group, Obermattweg 9, 6052 Hergiswil, Switzerland, marcel.egli@hslu.ch.

Abstract: Crewed long-duration space missions and especially sustained human presence on the surface of celestial bodies are linked inevitably to a reliable life support system like wastewater treatment or reconditioning the air. The logistics necessary to secure the life support, however, increases substantially the farther and longer the crew is travelling in space. Indeed, the ultimate goal to reach in the future is to establish a certain independency of the space-travelers from the earth-bound supply chain.

We propose to reach this goal by implementing a cradle-to-cradle approach via operating on-site, microbial factories consisting of autonomously running photo-bioreactors. Microalgae cultivated in bioreactors can take-up CO_2 from the atmosphere and release O_2. Furthermore, microalgae can serve as sources for various biomaterials like proteins, lipids and carbohydrates. Such raw materials can be post-processed to obtain food supplements, oil or fuel, carbon fiber and bioplastic for 3D-printing. But because the concept is based on living cells that respond to low gravity as well as cosmic radiation, as previous studies have shown, the extend of the changes to the extraterrestrial conditions is uncertain. Thus, aim of the project will be to deploy biological experiments on the lunar surface to determine the long-term effects of low gravity and cosmic radiation, and how it will modify cell growth, biomass production, cell motility, and the cells' biochemical composition.

The backbone of the system is a modular bioreactor concept comprising two sections; one section is lit by LEDs for the cultivation and the other is a dark section used for the biochemical analyses.

Introduction: As part of ESA's sustainable material concept, Granata and Egli [1] proposed the idea of converging microbiology and materials science by using microbial factories to produce a wide variety of biomaterials in space. It turned out that ESA has a vested interest in life science in space, particularly microbial processes.

The proposed experiment is in line with a long tradition of the Space Biology Group projects concerning space bioreactors. which started in 1984 with the realization of a cell incubator for cultivating blood cells on board Spacelab1 [2] and continued with space experiments called "LYCIS", "PADIAC", "Yeast Bioreactor" etc. Our proposed photo-bioreactor for microalgae experiments thus profits from a long history in building space-proven bioreactors. In addition, the Space Biology Group maintains the ESA User Support and Operations Center BIOTESC that is responsible for the implementation and conduction of biological and biotechnological experiment on board the International Space Station ISS. BIOTESC has profound knowledge in planning and realizing space experiments through the interaction with ESA and NASA.

Project description: As a first step in realizing the proposed microbial bioreactor experiment, a series of pre-investigations have been designed and implemented already with the green algae, *Tetraselmis sp. Tetraselmis sp.* was selected because: 1) it is a marine species (33 ppt salinity) that does not tolerate low salinity, so fluidics can be decontaminated using distilled water; 2) it is motile and only requires light, so no stirring is needed to maintain a homogenous distribution; 3) it is a robust cell with moderate growth rates; 4) optically, it only needs moderate irradiance in specific wavebands [3]; and 5) it produces measurable amount of proteins, pigments, and carbohydrate. Furthermore, it can concentrate large lipid bodies for harvesting. Preliminary experiments were conducted under simulated microgravity conditions in the laboratory (by using a Random Positioning Machine RPM [4]). In one set of experiments, geotaxis was compared to phototaxis for a flagellated, green algae (*Tetraselmis sp.*) cultures maintained under simulated microgravity (0.05 g) and 1 g (ground control) conditions. By simulating microgravity conditions on the RPM while lighting half the *Tetraselmis sp.* cultures (top or bottom, or middle of the culture flask) on the RPM, it was found that algae could swim to the light source in both 0.05 g and 1 g, with response times of 1 hour. Cells could maintain their distribution in the lit portion of the culture flasks for weeks. Non-flagellated control cells (*Emiliania huxylei*), sedimented in 1 g, and were less evenly distributed at 0.05 g regardless of the light zone. This confirms that flagellated cells have an advantage over non-flagellated cells in the capability of using light as energy source.

Flagellated and non-flagellated cells showed similar grow rates under 0.05 g and 1 g in long-term (weeks) experiments. No differences were detected in chlorophyll fluorescence or lipid content between steady state cultures maintained in 1 g or simulated microgravity conditions. This indicates that *Tetraselmis sp.* can be grown in bioreactors in space (e.g. on the lunar surface or on board the ISS).

Tetraselmis cells are ideal as microbial factories for space since they produce a number of biopolymers and are motile, requiring no mixing in growth chambers. In addition to the experiments described above, many of the biochemical components of biomass in these algae has been extracted and some post-processed into biomaterials (e.g. bioplastic).

Hardware description: The proposed *Tetraselmis* photo-bioreactor will consist of a lit (bioreactor) section and a dark (analytical) one integrated into an Experiment Container (see Fig. 1). Its total mass is estimated to be not more than 10 kg, occupying a volume of roughly 300 mm x. 300 mm x 300 mm.

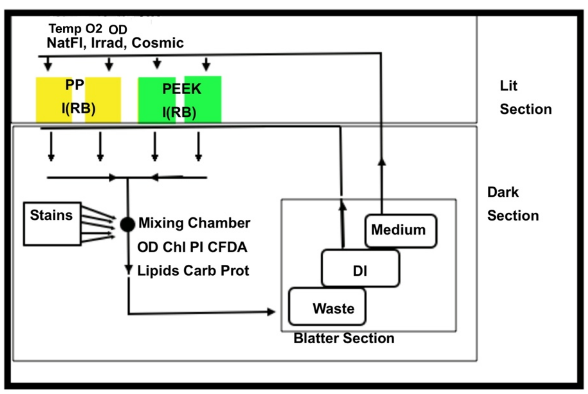

Fig.1: Photo-bioreactor concept: A modular bioreactor concept is foreseen comprising two sections; one section is lit by LEDs to drive photosynthesis in four, 4 ml growth chambers and the other section is dark and contains reservoirs for stains, growth medium, rinse water and waste, as well as a mixing chamber for biochemical analyses.

This Experiment Container will require connections to power it (estimated >150 W). Once powered the hardware will run autonomously and thus, no further crew time is needed. Additional connections to and from the Experiment Container are required to command and to receive telemetry data from it (preferred system would be an Ethernet based system comparable to JSL on the ISS). Actions for the crew are minimized to retrieve it from the vehicle and deploy it on the lunar surface in a radiation exposed area (the only requirement on the landing site). At the end of the experiment period, additional crew time is required for deinstallation and stowage for return (TBC) or trashing. These activities are estimated, all together, to be between 60 - 80 min.

The costs estimated to develop and operate the investigation (ground-based activities only) are € 2’000’000. Fortunately, some of the photo-bioreactor elements are existing already and have been tested under ground-based as well as simulated microgravity conditions. Thus, the development costs will not as high as if we would have to develop the hardware from scratch. This is also the reason why we are confident to make the 2024 flight deadline.

References:

[1] Granata, T. and M. Egli. 2016. Biological Nutrients: In: Sustainable Materials Concept. 2016 ESA Report (AO/1-7707/13/NL/R), T324-001QT. [2] Cogoli A, Tschopp A, Fuchs-Bislin P (1984) *Science 225:228-30*. [3] Granata T, Habermacher P, Härri V, Egli M (2019) *Nature Applied Science 1:524*. [4] Wuest SL, Richard S, Kopp S, Grimm D, Egli M (2015) *Biomed Res 2015:9714-74*.