

**INVESTIGATING ALGAE GROWTH UNDER LOW ATMOSPHERIC PRESSURES FOR POTENTIAL FOOD AND OXYGEN PRODUCTION ON MARS.** L. M. Cyclic<sup>1</sup>, E.M. Hausrath<sup>1</sup>, D. W. Ming<sup>2</sup>, C. Adcock<sup>1</sup>, J. Raymond<sup>3</sup>, D. Remias<sup>4</sup>, E. B. Rampe<sup>2</sup> <sup>1</sup>UNLV, Department of Geoscience, Las Vegas, NV 89154, <sup>2</sup>NASA JSC, Houston, TX 77058. <sup>3</sup>UNLV School of Life Sciences, Las Vegas, NV 89154. <sup>4</sup>University of Applied Sciences Upper Austria, Wels, Austria.

**Introduction:** Algal systems are promising candidates for human life support due to their fully edible biomass, ease of handling, and fast growth rates. Since the beginning of spaceflight, algae have been intensively studied for this purpose [1]. With long-term missions to Mars and beyond that would not allow resupply, a self-sustaining bio-regenerative life support system (BLSS) that would provide food and oxygen production is essential. Using algae in BLSS to generate food and oxygen would be an important step forward in the exciting field of human space exploration. To the best of our knowledge, and as reflected in over 50 prior space studies examining algal growth, very few studies have examined the growth of microorganisms at pressures close to the conditions found on the surface of Mars (range 1–14 mbar) [2, 4, 11]. Additionally, other than our ongoing work [3], no prior studies have examined snow algae and other extremophilic microalgae for use in BLSS.

Extremophilic microalgae such as snow algae and halophilic microalgae are naturally exposed to extreme conditions on Earth including low-temperatures, low nutrient conditions, high salinity, and high UV radiation. However, despite these challenging conditions, snow algae can reach very high concentrations of over one million cells per ml [8]. These microalgae are often the primary producers and an important CO<sub>2</sub> sink in cold and saline environments [13,16,17]. Some species of microalgae, including the extremophilic halophilic *Dunaliella salina*, are also edible, and are increasingly utilized as food sources [12]. Growth of such polyextremophilic and edible microalgae species, therefore, has the potential to provide both oxygen and nutrients for long-term human exploration of Mars.

**Methodology:** The overarching goal of this research is to determine the potential of algae for oxygen and food production on Mars by determining their growth under low-pressure conditions relevant to Mars. Our lab has previously grown snow algae under challenging low-nutrient conditions and low-pressures of 670 ± 5 mbar [3, 6,12]. Here we expanded on previous work and investigated the growth of microalgae under low-pressure ranges of 330±5mbar, 160±5mbar, and 80±5mbar. A total of 5 microalgae species were used for our growth experiments. Of these, the snow algae *Chloromonas brevispina* [7], *Krematochryopsis austriaca* [10] and the halophilic microalgae *Dunaliella salina* are extremophilic microalgae, and *Chlorella vulgaris* and

*Spirulina plantensis* are widely studied edible microalgae. The cultures were grown in duplicate in M1 medium [9] in a 3 gallon aluminum vacuum chamber (SlickVacSeal) with a clear tempered glass lid (Figure 1) under continuous light exposure from a T5 High output fluorescent grow light lamp (2500-3000 lux). The atmosphere was evacuated and purged with CO<sub>2</sub> three times using a 16g CO<sub>2</sub> threaded cartridge (ASURA) every three days. For the duration of the experiment, sampling was performed once a week for growth measurements.

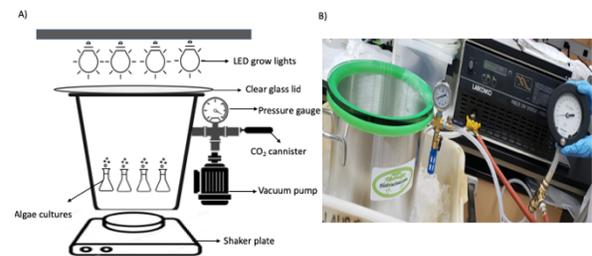


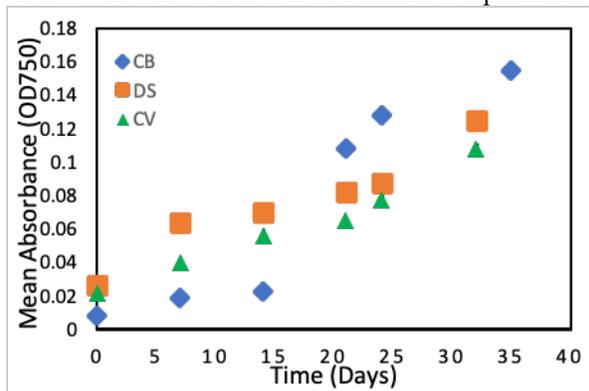
Figure 1: A) Design of low-pressure growth chamber, B) An experimental setup for low-pressure growth experiments using the SlickVacSeal Al vacuum chamber.

Temperature was maintained at 4°C for snow algae and at room temperature (25°C) for other microalgae at pressures of 670, 330, and 160 mbar. At lower pressure (80 mbar), microalgae other than snow algae were grown at 10°C to decrease vapor pressure [14]. The cultures showing the best growth at low-pressure are being further tested for their ability to grow on Mars regolith simulant. The Mars regolith simulant (Exolith MGS-1C) was heated at 160 °C for 3 hours to sterilize it, then solutions were prepared by adding 4g of regolith to 100 ml of autoclaved 18 MΩ water per reaction vessel and agitating them at 35°C for one week at 100 shakes/min [15]. The solution was decanted, filter sterilized (0.20µm) and used as media for growth experiments.

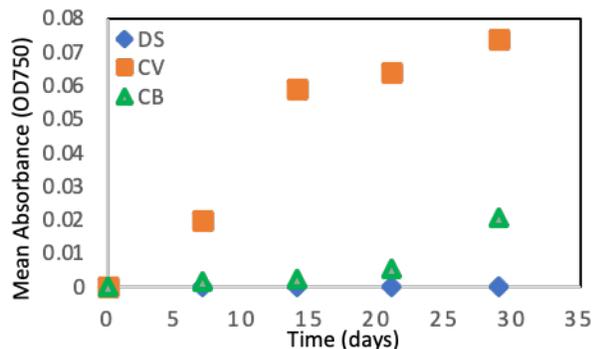
Algal growth was measured using optical density determined on a GENESYS 10S (UV/Vis spectrophotometer) at 750 nm [5]. Cell counts were also performed for candidate microalgae species *Chloromonas brevispina*, *Dunaliella salina*, and *Chlorella vulgaris* using disposable hemocytometer chambers (Incyto C-Chip, Neubauer Improved, Model # DHC-N01) under 400× magnification on an Olympus BH microscope.

**Results and discussion:** All 5 strains of microalgae showed growth at 670 mbar [3]. However, at lower pressures, only 3 species grew as demonstrated by increasing OD<sub>750</sub> (Figure 2) and cell counts with time. Since *Krematochryopsis austriaca* and *Spirulina plantensis*

did not grow at pressures below 670 mbar, these cultures were not used in additional experiments.



**Figure 2.** Growth curves demonstrated as a mean absorbance of duplicate  $OD_{750}$  measurements taken of candidate microalgae species *C. brevispina* (CB), *C. vulgaris* (CV) and *D. salina* (DS) growing at  $80 \pm 5$  mbar.



**Figure 3.** Growth curves demonstrated as a mean absorbance of duplicate  $OD_{750}$  measurements taken of candidate microalgae species *C. brevispina* (CB), *C. vulgaris* (CV), and *D. salina* (DS) growing in Exolith MGS-1C solution.

The candidate strains *Chloromas brevispina*, *Dunaliella salina*, and *Chlorella vulgaris* all showed significant growth at 160 mbar before reaching the stationary phase. Despite the slow growth rate at the low pressure of 80 mbar, the cultures are still in the exponential growth phase and the experiment is still in progress.

Due to the successful growth of *Chloromas brevispina*, *Dunaliella salina*, and *Chlorella vulgaris* at 80 mbar, we used these three candidate species for further growth analysis using Mars regolith simulant as a nutrient source. Our preliminary results indicate that both the snow algae *Chloromas brevispina* and the microalgae *Chlorella vulgaris* showed the ability to successfully grow in the Exolith MGS-1C solution. *Dunaliella salina* did not show any growth (Figure 3), which is likely due to the fact that it is halophilic and the regolith solution contained a lower salinity than the medium used to grow *Dunaliella salina*. Next, we plan to

add different salts to the Mars regolith simulant medium to assess the impact on algae growth. We will also include other Mars simulant samples such as JSC Mars-1 and other commercially available Mars regolith simulants. We will also test the maximum amount of Mars regolith simulant that algal species can tolerate before growth rates are decreased by growing the microalgae species in their respective media to the exponential phase and then inoculating with different concentrations of regolith solutions. We are in the process of conducting whole genome sequencing analysis of our candidate species growing at 80 mbar and their counterparts growing under normal atmospheric pressures to determine key genes involved in adaptations to extreme atmospheric conditions.

**Conclusions:** Our results demonstrate the ability of three microalgae species, *Chloromas brevispina*, *Dunaliella salina* and *Chlorella vulgaris* to grow at pressures as low as  $80 \pm 5$  mbar. In addition to growing at low pressure, two of our candidate algae species, *Chloromas brevispina* and *Chlorella vulgaris*, are also successfully growing in Exolith MGS-1C solution making them ideal candidates for potential oxygen and food production on Mars. These experiments are ongoing and the species are currently in the exponential growth phase. Future work will include a detailed set of experiments to test combinations of space conditions including low pressure, low light, and low nutrients on the growth of our candidate algae species.

**Acknowledgments:** The work was funded by NASA EPSCOR Grant # 80NSSC19M0171. We would also like to acknowledge the assistance of Richard Panduro and Paloma Marcos in data acquisition.

**References:** [1] Semenenko, V et al., (1961) *Physiol. Plants*, 8, 743–749. [2] Verseux, C. et al., (2016) *Int J Astrobiol*, 15, 65–92. [3] Cyclic, L.C et al., (2020) *LPI*, 2326, 1517. [4] Forget, F., (2009) *Eur. Phys. J. Conf.* 1235, 248. [5] Griffiths, M.J. et al., (2011). *J Microbiol Meth* 85:119–123. [6] Harrold, Z.R. et al., (2018). *Appl Environ Microbiol*, 84(7): 1–10. [7] Hoham, R.W. et al., (1979). *Phycologia*, 18: 55–70. [8] Hoham, R., Duval, B. (2001). Cambridge Uni Press 400. [9] Hoham R.W. et al., (2006) *Phycologia*, 45, 319. [10] Raymond, J.A. and Remias, D. (2019). *Front Microbiol.* 10, 2697. [11] Niederwieser, et al., (2018). *Acta Astronaut.*, 146, 359–367. [12] Phillips-Lander et al., (2020) *Geomicrobiol*, 1–10. [13] Gray, A., et al., (2020) *Nat Commun* 11, 2527. [14] Averill, B.A. et al., (2012). *Principles of General Chemistry*. Creative Commons. [15] Adcock, C.T., et al., (2020). *Resources from Water-Rock Interactions for Future Human Exploration of Mars*. [16] Oren, (2014) *J Biol Res-Thessaloniki* 21, 1–8. [17] Cyclic, L.M., et al., *Front Microbiol*, 11, 1567.