ALGAE CULTIVATION FOR LONG-TERM FOOD AND OXYGEN PRODUCTION ON MARS.

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Introduction: Long-term space exploration missions to Mars and beyond will require significant amounts of both oxygen and food, and the ability to produce some of these on Mars would significantly facilitate such exploration. *In situ* production of oxygen and food, such as by algae, would be an important step forward in the exciting field of human space exploration.

However, very little literature exists on the growth of microorganisms at pressures close to the conditions found on the surface of Mars (range 1–14 mbar) [2]. Experiments examining the possibility of algae growth in space date from the 1960s, including some studies examining plant growth under low-pressure conditions [10, 16]. However, to the best of our knowledge from existing literature, including a 2018 review of over 50 space studies examining algal growth [9, 15], no studies have examined algal growth under low atmospheric pressure conditions relevant to growth on Mars.

Algae, which produce much of the oxygen on Earth, have also adapted to extreme terrestrial environments. Snow algae occur in the Polar Regions and mountains and are considered primary oxygen producers in snow ecosystems [11]. Under favorable conditions, they could also be considered an important CO_2 sink [18]. Snow algae grow very well in challenging high UV, low temperature, and low nutrient snow environments, reaching concentrations of over one million cells per ml [6].

Some species of algae are also edible, and are increasingly utilized as food sources [14, 17]. Growth of such cold-adapted and edible algae species, therefore, has the potential to provide both oxygen and nutrients for long-term Mars exploration.

Methodology: The primary goal of this research is to discern the potential of algae for oxygen and food production on Mars by determining their growth under low pressure conditions. Our lab has previously grown snow algae under challenging low-nutrient conditions[4, 12, 13]. Here we expanded on previous work and set up low-pressure growth experiments using two species of snow algae. *Chloromonas brevispina* and *Kremastochrysopsis austriaca* [5, 8] were cultured in a low-pressure vacuum desiccator at initial pressure of 0.67 bar (+/-~ 15 mbar). Temperature was maintained at 4°C and culturing occurred under a T5 High output fluorescent grow light lamp (2500-3000 lux). Atmosphere was evacuated and purged with CO_2 three times, every three days to refresh the pure CO2 atmosphere and pressure which could increase (up to 0.85) over time, presumably due to media vaporization. The cultures were set up in duplicate in M1 medium [7], and the atmosphere was evacuated and purged with CO_2 three times, every three days. For the duration of the experiment, sampling was performed once a week for growth measurement. We are monitoring the growth qualitatively by taking the optical density (OD) at 750 nm of the cultures (Figure 1). 750 nm is out of the absorbance range of algal pigments and hence is a preferred choice for OD measurement [3]. We are also in the process of measuring algal growth quantitatively by collecting the cell count using disposable hemocytometer chambers (Incyto C-Chip, Neubauer Improved, Model # DHC-N01) under 400× magnification (Olympus BH microscope).

Figure 1. *The low-pressure vacuum desiccator holding a pressure of 0.67 bar. The snow algae Kremastochrysopsis austriaca and Chloromonas. brevispina in liquid M1 growth medium are set up in duplicate at 4°C under 2500-3000 lux.*

Results and discussion: Our preliminary results show steady growth of the snow algae *Chloromonas brevispina* and *Kremastochrysopsis austriaca* under low pressure as demonstrated by increasing OD_{750} with time (Figure 2). Despite the slow growth at low pressure, the rate of growth is steady, and the cultures are still in the exponential growth phase after a month since the experiment started.

Next, we plan to set up another set of growth experiments with cultures from our ongoing experiments at a pressure that is 10% (0.067bar) of the current low pressure experiments (0.67bar), to evaluate their growth. We will similarly decrease atmospheric pressures until we reach Mars relevant pressures (6 – 14 mbar) or pressures at which the algae will not grow. To allow water to be liquid at these low pressures, we will also decrease the temperature to close to 0ºC [1]. We are also in the process of growing edible algae, *Dunaliella salina and Chlorella vulgaris* for use in low pressure experiments to determine the best candidates for long-term oxygen and food production on Mars.

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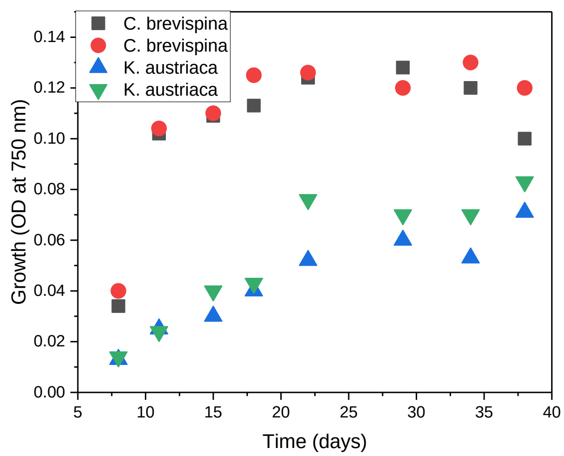

Figure 2. *Growth curve of duplicates of snow algae C. brevispina and K. austriaca as demonstrated by* OD_{750} *at 4°C under 0.67 bar at 2500-3000 lux.*

Conclusions: Our preliminary results show that snow algae can grow and survive at 0.67 bar. In addition to growing at low pressure, snow algae are also known to grow very well in challenging high UV, low temperature, and low nutrient snow environments, making them ideal candidates for potential oxygen and food production on Mars. Future work will include a detailed set of experiments to test the lower limits of pressure that allow growth, as well as the effects of gravity, light, UV, and nutrients.

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