PIONEERING MARS: LESSON THREE

HYPOTHESIS: Cyanobacteria, a microscopic algae found in extreme conditions in Antarctica, could grow in similar extreme conditions on Mars.

INSTRUCTIONAL GOAL: The third lesson covers practical aspects of planning experiments to determine whether or not the hypothesis can be proved.

There are two ways to culture cyanobacteria in a lab. BATCH CULTURE involves using sealed beakers or flasks as a "closed-system incubation", where cultures of cells are grown under strictly-controlled environmental conditions and analyzed at the end of the growth experiment as a batch. CONTINUOUS CULTURES are grown in beakers, flasks, or tanks as an "open-system incubation," where the culture is continuously provided new nutrients and grown in perpetuity. These experiments will be batch cultures, since the environmental conditions of Mars can be more easily controlled in a closed (sealed) system.

Students will culture aquatic algae in individual flasks. Each flask will contain LIQUID GROWTH MEDIA and HEAD SPACE.

- LIQUID GROWTH MEDIA—This is the aquatic medium in which the cyanobacterial cultures shall grow. Predominantly made from ultrapure water, students will add salts, minerals, and vital nutrients in precise amounts, using what they have researched about the Martian soil chemistry.
- HEAD SPACE—This is the space reserved in the growth chamber to mimic the Martian atmosphere. Students will mix the gases in precise proportions using what they have researched about the types and percent abundance of Martian atmospheric gases. Once the gas blend is similar to that of the Martian atmosphere, the students will administer vacuum suction to each growth chamber to reduce the overall pressure to ~1% that of earth's, in order to mimic the thin Martian atmosphere.

Once students prepare each of the sealed flasks, each will be sterilized in an autoclave to prevent contamination from unwanted bacteria or fungal spores that could compete with the cyanobacteria for the water and nutrients present, or could infect and cause direct harm to the cyanobacteria the students are attempting to culture.

The cyanobacteria cells will be ordered from research institutions based in Antarctica and will be stored frozen in liquid nitrogen until it is time to use them. They must always be handled with cold-protective gloves and in as sterile an environment as possible.

An important factor to note is that all of the cyanobacteria must come from the same parent culture to rule out any genetic variations that might alter results.

Before introducing the cyanobacteria to the flasks, the parent culture will be thawed and put into a centrifuge to separate the cyanobacteria cells from the liquid growth media in which they were stored for shipping. Because of the small size of the flasks and the rate of reproduction of the cyanobacteria, only a few cells are needed to inoculate each flask for culture.

Once the flasks are ready, they will be housed in special incubators that will be set to strictly control light and temperature to closely mimic the Martian environment.

The flasks will be monitored continually throughout the incubation in a variety of ways:

- A simple change in color of the liquid growth media—from clear to greenish—will indicate the cyanobacteria are multiplying.
- The specialized flasks used, called micro-respiratory chambers, have an electrode that extends down into the liquid growth media. This electrode can be used to measure the consumption of CO2 and/or the evolution of O2, both of which will occur during photosynthesis. Based on the results, the changing amount of CO2 and/or O2 in the growth chambers will allow students to calculate rates of photosynthesis and growth, if any.
- A fluorometer is a device that measures the emission of light, an indicator of the presence of chlorophyll. If repeated measures indicate an increase in fluorescence, this will also indicate an increase of chlorophyll concentrations: strong evidence that the cyanobacteria are multiplying.

Students will perform multiple batches of experiments to adjust for potential conditions on Mars. When planning the experiments, the following factors must be considered:

- The type and concentration of dissolved salts in the liquid growth medium
- The vitamins, minerals, and/or nutrients introduced into the flasks
- UV and visible light quality and quantity
- Pressure and temperature consequences
- If cyanobacteria prove to be unsuitable for Martian culture, are there other micro-organisms, most likely from the group of hardy stock known as extremophiles that might be able to live on Mars?