**Background**

Mars colonization will not be possible without the ability to acquire needed resources on-site in addition to remediating resultant waste. The ability to grow crops on Mars is imperative, but the planet lacks bioavailable nitrogen to enable plant growth. *Rhodopseudomonas palustris* (R. palustris) NiFA*, a strain of genetically engineered nitrogen-fixing bacteria, possesses characteristics making it ideal to generate bioavailable nitrogen on Mars. Its ability to remediate wastewater and use it for nitrogen production for colonial agriculture is evaluated.

**Research Goals**

1. Demonstrate *R. palustris* ability to utilize wastewater as a carbon source
2. Determine inhibitive components of wastewater
3. Develop a bioreactor system featuring accurate temperature control, exposure to infrared and fluorescent light, and an anaerobic environment to maintain *R. palustris* growth
4. Scale up wastewater trials of *R. palustris* in bioreactor system to demonstrate growth capacity in a controlled environment at a larger scale
5. Quantify nitrogenous biomass harvestable from bioreactor wastewater trials
6. Determine effectiveness of biomass samples to provide ammonia for plant growth

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**Methodology**

**Initial Trials:**

The control group included 0.03 M sodium acetate, a high-quality carbon source for *R. palustris*, and nitrogen-fixing media to optimize growth. Wastewater samples were tested for carbon content and growth inhibition. 0.03 M sodium acetate was added to an Acetate Dependent group to demonstrate capacity to grow relying solely on wastewater carbon compounds (prevalent ones being methanol, ethanol, propionic acid, lactic acid, acetic acid, formic acid, and acetone)

Simulated wastewater ersatz recipes were used which were derived from research conducted at the Johnson Space Center (JSC). [1]

All samples were treated with a phosphate buffer (0.0125M Na2HP04, 0.0125M KH2P04, and 1% V/V concentrated base solution). [2] All samples were sparged with N2 gas for 25 minutes prior to inoculation. All samples were provided with 0.005% V/V Wolfe’s vitamins. Samples were given approximately equal exposure to IR/Fluorescent light in a growth chamber held at 28 °C. Growth was measured by increases in optical density (O.D.) every 24 hours for six days. O.D. samples were diluted 10x with media, and O.D. readings were multiplied 10x accordingly.

**Bioreactor Trials:**

Bioreactor trials are ongoing. Current design incorporates a 3L glass tank modulated to include an N2 diffusion stone, an LED encasing, extraction and injection ports for media sampling and replacement, and temperature control devices.

**Outcomes**

Significant inhibition from wastewater components was observed in earlier trials. Upon removal of ammonium bicarbonate, drastic improvement in both wastewater-dependent and independent samples was observed.

Acetate-containing samples were observed to have highly accelerated initial growth in comparison to control groups, up to 105% (Figure 2).

Acetate-dependent samples had less growth than both the control and acetate-dependent groups, which was predicted to be the case. Notwithstanding this, it is believed that the growth is sufficient to proceed with upscaling tests to determine and quantify biomass output without using acetate.

**Continuing Research**

Upscaling acetate-independent *R. palustris* growth in bioreactors is an essential next-step in this research and will play a role in advancing the Technology Readiness Levels (TRL) of Mars biotechnology such as has been described in this research.

Upon successful bioreactor trials, biomass will be harvested and tested for its ability to provide bioavailable nitrogen to plant samples.

**References**
