12-9-2021

Establishing a Framework of Nitrogen Acquisition for Martian Agriculture

Tyler Wallentine
Utah State University, wallentinetyler@gmail.com

Establishing a Framework of Nitrogen Acquisition for Martian Agriculture

The evaluation of various microbes to produce nitrogenous fertilizer for an agrarian Martian colony

Tyler Wallentine | David Merkley | Lance Seefeldt

Introduction

Nitrogen (N) forms a crucial part of DNA, proteins, and other biomolecules and is an essential element to life. Luckily, N is abundant in Earth's and Mars' atmospheres in its atmospheric form (N_2); however, plants and humans are unable to metabolize it in this state. N_2 gas is only able to be consumed by undergoing nitrogen fixation, an intensive process that breaks the extremely-stable N ≡ N bond in order to form bioavailable ammonia (NH_3). Many prokaryotes are capable of nitrogen fixation. Plants may uptake fixed N from these, which are then consumed by other lifeforms including humans as a source of nitrogen.

Due to an apparent lack of biological activity on Mars, it is estimated that N will be overwhelmingly present as N_2. If humans want to permanently settle Mars, which demands in situ food production, they must devise a means to efficiently fix nitrogen to enable agrarian success. Industrial nitrogen fixation is infrastructurally intensive, and this work therefore elects to evaluate biological nitrogen fixation as an avenue to Martian cultivation. Three different microorganisms are evaluated for their capacity to fix nitrogen: Rhodopseudomonas palustris (R. palustris), Azotobacter vinelandii (A. vinelandii), and Azospira suillum (A. suillum). Initial efforts to culture these in-lab are detailed. An outline for a modular system in which these organisms may be advantageously used is proposed to be evaluated with further research and studies.

Comparing Nitrogen Fixing Species
for the development of a modular biological fixation system

	Rhodopseudomonas palustris NifA*	*Azotobacter vinelandii*	*Azospira suillum* PS
Metabolism	Photoheterotrophic / Photoautotrophic	Chemoheterotrophic	Chemoheterotrophic
Light	Yes	No	No
Electron Acceptor	N_2 / H^+	Molecular Oxygen	Perchlorate
Aerobic	No	Yes	No
Gas Evolution	H_2	Carbon dioxide, H_2	Carbon dioxide, H_2

Table 1. A comparison of the metabolic modes, as well as light, electron acceptor, aerobic requirements of the three organisms of interest for this study

Research Goal

Quantify, with respect to both time and resource demand, the effectiveness of R. palustris, A. vinelandii, and A. suillum at producing nitrogen-rich biomass using N_2 as a sole N source.

Methodology

R. palustris has been cultured under photoheterotrophic, anerobic conditions. In early research, these were typically small-scale (60 mL) but have since progressed into much larger scale volumes using photobioreactors (3000 mL) (Figures 1 & 3). In all cases, 30 mM (sodium) acetate has been used as the sole carbon source for standard cultures.

A. vinelandii was grown under chemoheterotrophic, aerobic conditions in the absence of light. Samples were cultured with 30 mM and 180 mM sodium acetate. These were grown in mid-scale volumes (500 mL) (Figure 2). A photobioreactor to optimize growth is under construction.

A. suillum has been cultured under chemoheterotrophic, anaerobic conditions, shielded from light. All culturing so far has been done in small-scale conditions (Figure 3). Originally, a variety of carbon sources were included in A. suillum media (10 mM sodium pyruvate, 10 mM sodium acetate, and 40 mM sodium lactate). Cultures have since been standardized and grown using 30 mM acetate. 5 mM potassium perchlorate was added to each growth to act as a final electron acceptor.

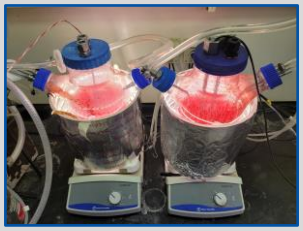

Figure 1: Photobioreactors for upscaled R. palustris growth

Figure 2: A. vinelandii culture (60 mL)

Figure 3: Small-scale R. palustris (left) and A. suillum (right) cultures

Results & Discussion

R. palustris has been successfully cultured in and harvested from both small and large-scale systems. Work has primarily focused on optimizing the output of photobioreactors and testing growth as a function of variables such as pressure, lighting, and heating for system modelling (in collaboration with the University of Florida)

A. vinelandii has been successfully cultured and harvested in mid-scale systems. It proved to use acetate more efficiently at a concentration of 180 mM, and is more time-efficient than R. palustris.

A. suillum has successfully grown, though to a much lower optical density than R. palustris and A. vinelandii. Significantly, its ability to grow while using perchlorate as a final electron acceptor has significant implications for Martian agriculture as a potential means of removing regolith perchlorate, which is present in high concentrations and toxic to plants and many microorganisms.

Efficiency values have been calculated using mass harvest and optical density values with respect to time or substrate mass required to reach said values. A comparison of efficiencies between these microbes has been included (Table 2).

Organism	Time Efficiency ($\frac{\text{mg N}}{\text{L day}}$)	Substrate Efficiency ($\frac{\text{mg N}}{\text{mg Acetate}}$)
R. palustris	6.03	14.2
A. vinelandii	53.9	7.29
A. suillum	1.47*	0.00**

*Hypothesized value based on small-scaled tests, highly subject to change after upscaled growths
**Extremely small based off of downscaled results and must be determined after upscaled trials

Table 2. Current results for organism time and carbon substrate efficiency

Future Work

Efficiency estimations for A. suillum have yet to be collected experimentally and doing so will form the immediate next steps for this research. Work will continue for all three organisms as biomass and nitrogen yields seek to be optimized. Bioreactors will be constructed for each organism in order to do this. Harvested samples will be tested for agricultural efficacy by undergoing downstream treatment for integration with plant soil in collaboration with Utah State's Crop Physiology Laboratory. Other nitrogen fixing organisms will be studied for their potential to perform similar functions and may be added to this study.

Utah State University | NASA | CUBES Center for the Utilization of Biological Engineering in Space | College of Engineering Utah State University | College of Science Utah State University