Utah State University

DigitalCommons@USU

Fall Student Research Symposium 2020 Fall Student Research Symposium

12-10-2020

Enabling Mars Farms Through Microbial Remediation of Wastewater

Tyler Wallentine
Utah State University, twwallentine@aggiemail.usu.edu

Follow this and additional works at: https://digitalcommons.usu.edu/fsrs2020

Part of the Chemistry Commons

Recommended Citation

Wallentine, Tyler, "Enabling Mars Farms Through Microbial Remediation of Wastewater" (2020). *Fall Student Research Symposium 2020*. 96.
https://digitalcommons.usu.edu/fsrs2020/96

This Book is brought to you for free and open access by the Fall Student Research Symposium at DigitalCommons@USU. It has been accepted for inclusion in Fall Student Research Symposium 2020 by an authorized administrator of DigitalCommons@USU. For more information, please contact digitalcommons@usu.edu.

UtahStateUniversity
MERRILL-CAZIER LIBRARY

ENABLING MARS FARMS THROUGH MICROBIAL REMEDIATION OF WASTEWATER

TYLER WALLENTINE

UNDERGRADUATE

BIOCHEMISTRY AND BIOLOGICAL ENGINEERING

COLLEGE of SCIENCE
UtahStateUniversity

UtahStateUniversity
CHEMISTRY AND BIOCHEMISTRY

NASA

CUBES

BUILDING A MARS COLONY

- Colonizing Mars is very demanding on resources
  - Transport takes a long time
  - Transport is expensive
  - Transport cannot be relied upon in an independent colony
- A Mars colony, independently, must be able to:
  - Utilize resources
  - Recycle resources
  - Acquire resources
- What kinds of resources?
  - Energy
  - Raw materials (i.e. for building)
  - **Food**

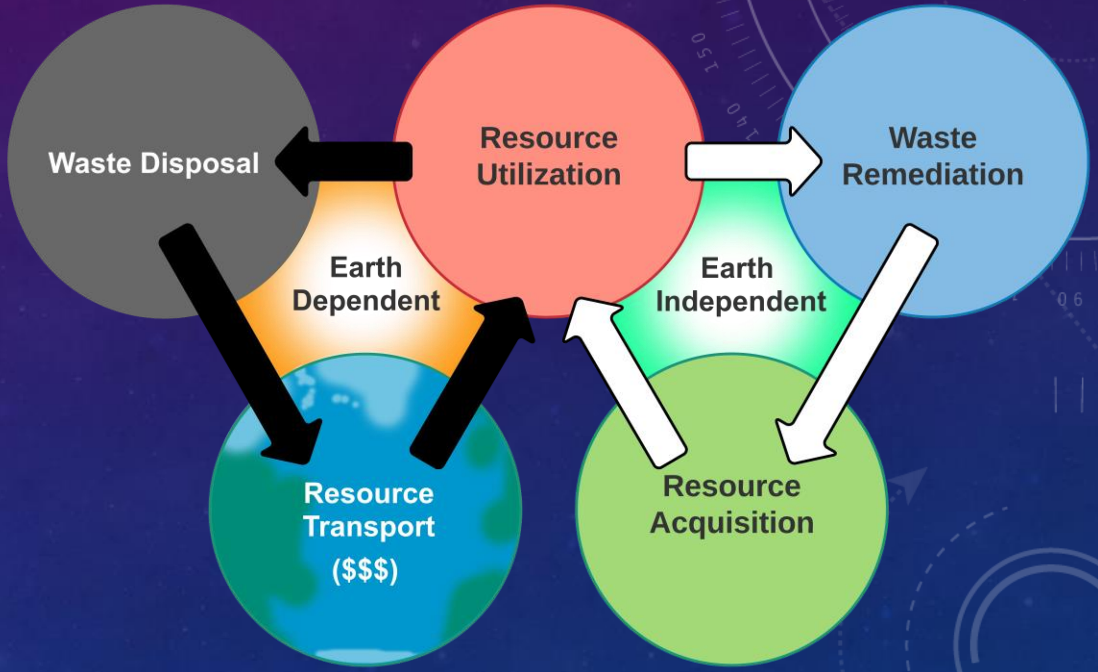

Figure 1: The Earth-Mars Resource Cycle. Improving utilization and remediation serve to reduce dependence on Earth

THE RISE OF MARTIAN AGRICULTURE

- Agriculture will be the primary means of food production
- As it stands, agriculture is not possible due to soil deficiencies
    - Soil lacks essential nitrogen
    - Plants cannot use atmospheric nitrogen

- Nitrogen fixation is required to make atmospheric nitrogen (N_2) bioavailable
    - This is performed industrially
        - Places demands of energy and industry on colony
    - Capable of being performed biologically
        - Energy independent in photosynthetic nitrogen-fixing bacteria

- Agriculture can be enabled through biological means

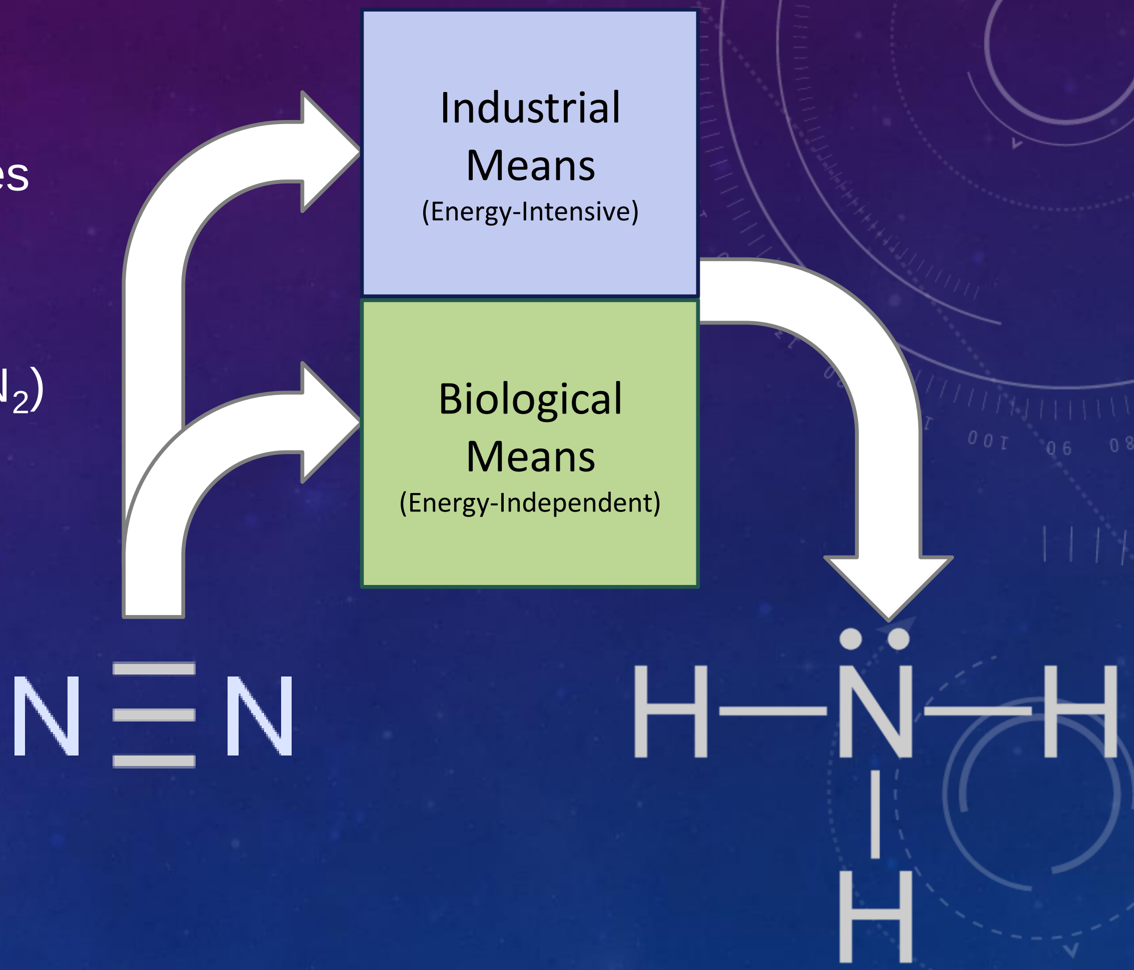

Figure 2: Nitrogen fixation can be performed industrially or biologically. Biological means are less weight and energy intensive.

NITROGEN FIXING BACTERIA

- Parameters for biological selection:
  - Can it be sustainably supported using available resources?
  - Can it produce nitrogen?

- *Rhodopseudomonas palustris* NifA*
  - Genetically modified bacteria
    - Reduced nitrogenase enzyme inhibition, increased nitrogen output
  - Photoheterotrophic Metabolism
    - Energy source: Sunlight
    - Carbon (Food) source: Various organic compounds (Acetate, Malate, Lactate, etc.)
  - Non-pathogenic
    - Safe for agricultural integration

- Carbon Source
  - Light is easily accessible, but a carbon source must be found that is not invasive on resource demands
  - Evidence suggests a capacity for **wastewater** to act as such
  - Success means nitrogen production **and wastewater remediation**

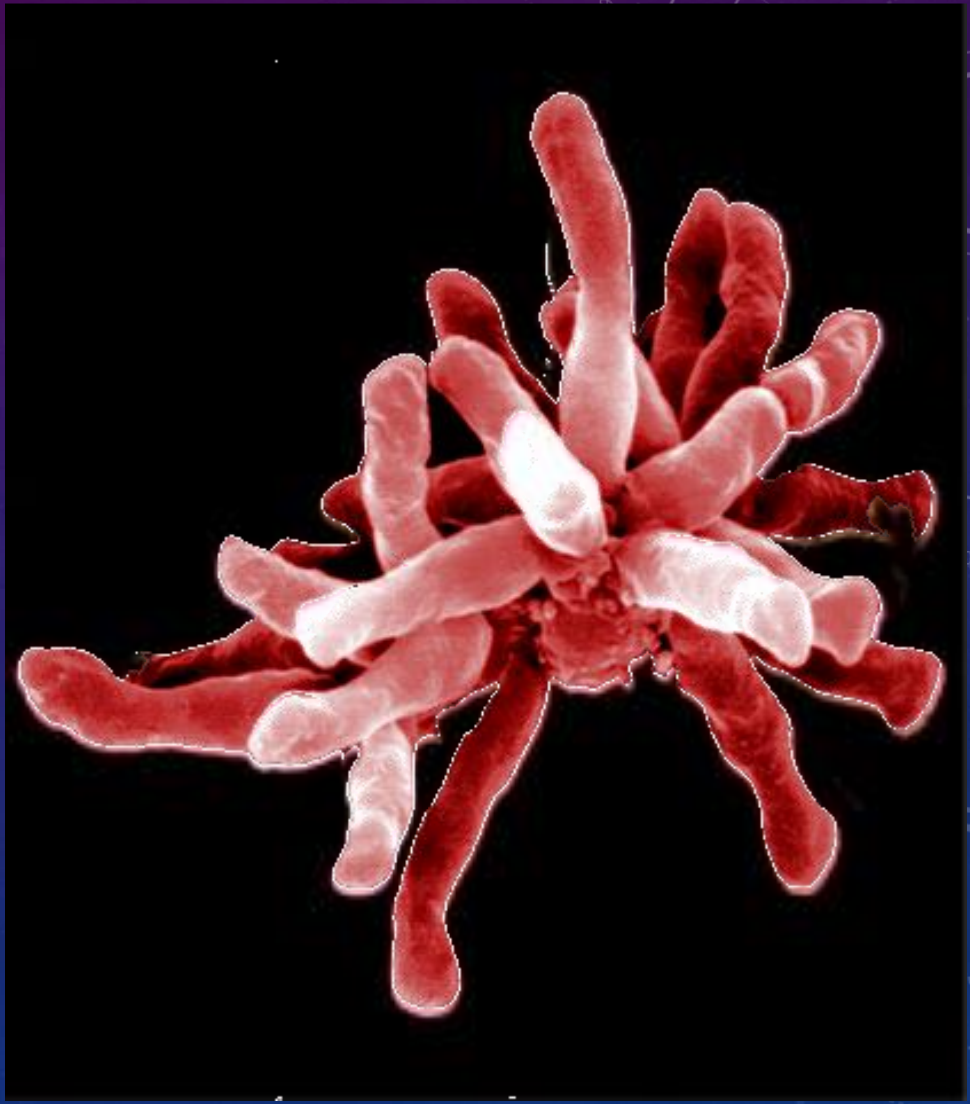

Figure 3: *Rhodopseudomonas palustris*[1]

RESEARCH GOALS

- Three key questions must be answered in the course of this research:
  - **Can *R. palustris* grow significantly, relying only on wastewater carbon content?**
  - **Can this be done in spite of inhibitory components present?**
  - **Is this system capable of being upscaled to meet the agricultural demands of a colony?**
- **My hypothesis has been that all three of these questions can be answered with a "Yes"**

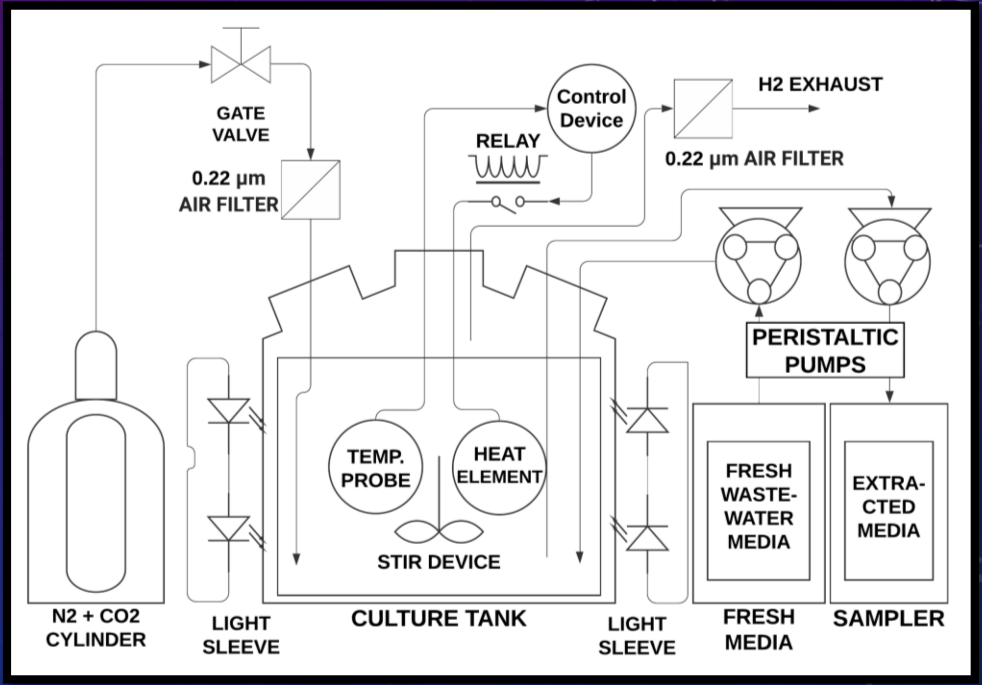

Figure 4: An early design for an upscaled system later used in this work

METHODS – PRELIMINARY RESEARCH

- **Preliminary Research: Determining Inhibition**
  - Two test groups were compared with one control
    - **Control**: Nitrogen-Fixing Growth Media with 0.03 M Sodium acetate (Acetate is an excellent substrate)
    - **Acetate-Containing Wastewater**: Wastewater Media with 0.03 M Sodium acetate (To compare for inhibition)
    - **Acetate-Lacking Wastewater**: Wastewater Media without Acetate (To compare for organic viability)
  - All samples were provided with **N2 gas, vitamins, and concentrated base solution** prior to inoculation
  - Simulated wastewater compositions of an early planetary base were derived from research conducted at the Johnson Space Center[1]

Figure 5: Inoculated simulated wastewater samples

METHODS – MAINLINE RESEARCH

- **Mainline Research: Upscaling**
  - Photobioreactor Design
    - Temperature and Lighting-Controlled Photobioreactor used to grow up to 3L media with *R. palustris*
    - Aerobic conditions established by 45 minutes of N2 gas sparging at 250 mL / min, then at 15 minute intervals every 60 minutes
    - Sampling of 2 L media every 10 days, followed by addition of 2L fresh wastewater media to replenish carbon content and restart cycle

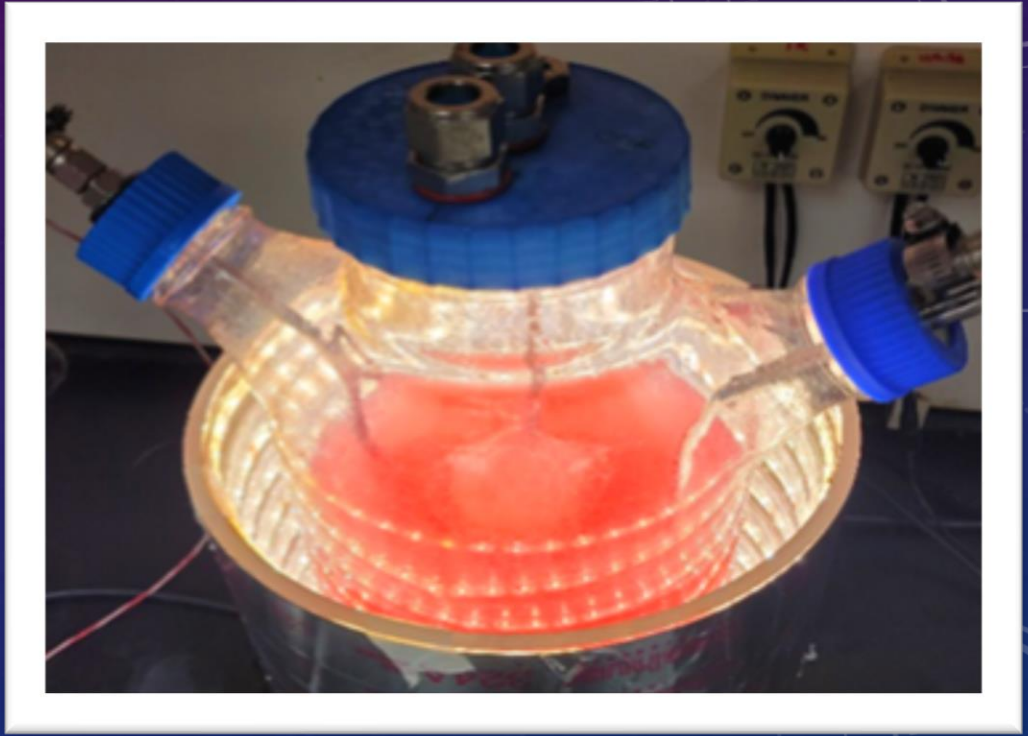

Figure 6: Successful execution of upscaled, anaerobic system in wastewater culturing

RESULTS

- **Preliminary Research:**
  - Ammonium bicarbonate, soap determined to be inhibitory components of wastewater
  - Removal of these components resulted in accelerated growth in **acetate-containing test trial** compared to the control
  - Acetate-lacking successfully grew, though not beyond optical densities < 1
  - Acetate-lacking growth justified upscaling to determine harvestability and upscaling improvements

Figure 7: Visual growth results of wastewater samples. (Left: Acetate-Lacking. Center: Control. Right: Acetate-Containing)

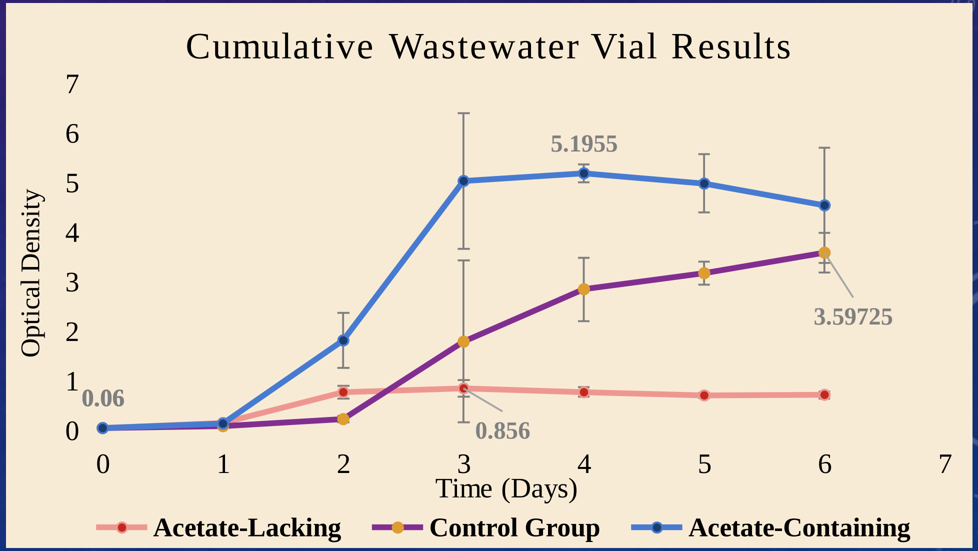

Figure 8: Average growth results of R. palustris nif A* in wastewater and control group vials.

RESULTS

- **Mainline Research:**
  - *R. palustris* was successfully cultivated in upscaled conditions
  - Growth was improved beyond that of preliminary research (Determined using optical density values)
  - Replenishing wastewater apparently accelerated biomass production, yielding more biomass on second harvest

Figure 9: Wastewater Biomass Harvest Day 20

Table 1: Current results of biomass harvests after 10-day growth intervals

Day	Mass Harvested		Mass Harvested/Volume Ratio	
10	1.67	g	0.835	g/L
20	4.67	g	2.335	g/L

CONCLUSIONS AND FUTURE WORK

- **Conclusions:**
  - *R. palustris* demonstrates an ability to utilize wastewater carbon in photoheterotrophic growth
  - Wastewater culturing can be upscaled to increase biomass output
  - Replenishing wastewater apparently increases growth rate
    - This is promising when considering a steady-state, steady-flow system

- **Future Work:**
  - Longer-term culturing in upscaled system is required to optimize biomass output
  - Integration in simulated agriculture systems is a next-step to test the efficacy of wastewater biomass output in agriculture
  - Developing a second bioreactor system with the same features as current, but including a temperature control will be important in accelerating research and determining best biomass production methods

ACKNOWLEDGEMENTS

Faculty, Advisors, and Mentors

- Dr. Lance Seefeldt, Department Head and Professor – USU Department of Biochemistry
- Dr. Anna Doloman
- Seefeldt Lab – USU Department of Chemistry and Biochemistry

Funding

- The Peak Summer Research Fellowship
- NASA Center for the Utilization of Biological Engineering in Space (NASA CUBES)

REFERENCES

1. Harwood, Caroline. “Harwood Lab: Rhodopseudomonas Palustris.” Harwood Lab - Rhodoseudomonas, depts.washington.edu/cshlab/html/organisms/rhodopseudomonas.html.

2. Verostko, C. E., Carrier, C., and Finger, B. W. (2004). Ersatz Wastewater Formulations for Testing Water Recovery Systems. 2004-01-2448. doi:10.4271/2004-01-2448

This material is based upon work supported by NASA under grant or cooperative agreement award number NNX17AJ31G/NNH16ZOA001N-16STRII_B3. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Aeronautics and Space Administration (NASA).