NM WRRI Student Water Research Grant Final Report

Progress Report due Tuesday, April 1, 2025
Draft Final Report due Friday, August 29, 2025
Final Report due Tuesday, September 30, 2025

1. Student Researcher: Derek Belka
   Faculty Advisor: Dr. Andrew Schuler

2. Project title:

Metagenomic and metabolic characterization of previously unidentified polyphosphate-accumulating organisms in a laboratory culture removing phosphorus from wastewater

3. Description of research problem and research objectives.

We cultivated a biomass that performed biological phosphorus removal in a laboratory batch reactor. However, our preliminary analysis indicated an extremely low population of known phosphorus-removing bacteria and a large population of other bacteria with unknown physiology. The objective of this research was to identify the most likely bacteria responsible for the observed phosphorus removal using advanced genetic identification and profiling, enabling us to better understand their potential role in phosphorus removal at full-scale water resource recovery facilities.

4. Description of methodology employed.

We used an advanced technique known as hybrid assembly of metagenomes from metagenomic DNA. This method combines short-read Illumina and long-read Pacific Biosciences DNA sequencing technologies to reconstruct the genomes of the bacteria in our bioreactor. The long-read sequences act as a backbone, capturing long segments of DNA that short-read technology cannot, while the short-read sequences provide error correction and gap filling of the longer sequences. Collectively, the hybrid method produces more complete genomes with reduced error rates. The sequencing data was processed through computer algorithms that construct the genomes and identify the genes of the bacteria in our samples so that we can assess their potential for phosphorus removal metabolism.

5. Description of results; include findings, conclusions, and recommendations for further research.

The main results from this research are called metagenome-assembled genomes (MAGs). We assembled 67 MAGs, 24 of which are considered High-Quality Draft MAGs and will be submitted to the National Center for Biotechnology Information database. From these MAGs, we identified at least three new candidate polyphosphate-accumulating organisms based on their ability to process organic carbon anaerobically, scavenge phosphate at low concentrations, and similarity in polyphosphate accumulation.

Future research would confirm the metabolic potential of these organisms through the reconstruction and analysis of genome-scale metabolic models, along with experimental

validation. Utilizing our cultivation methods, future researchers could also cultivate these organisms and subject them to various batch tests that further elucidate their metabolism under different conditions, allowing for comparisons to computer simulations. This methodology would enable others to develop specific parameters for integration into existing water resource recovery process models, leading to more accurate model-based evaluations of treatment.

6. Provide a paragraph on who will benefit from your research results. Include any water agency that could use your results.

Any water resource recovery facility and, by extension, the entirety of New Mexico, benefits from the knowledge gained through this research since it enhances our understanding of phosphorus removal mechanisms at water resource recovery facilities and our ability to protect sensitive aquatic ecosystems. State water agencies are not responsible for wastewater treatment in New Mexico and do not receive any direct benefit.

7. Describe how you have spent your grant funds. Also provide your budget balance and how you will use any remaining funds. If you anticipate any funds remaining after September 30, 2025, please contact Carolina Mijares immediately (575-646-7991; mijares@nmsu.edu).

The grant funds were spent on commercial metagenomic DNA sequencing services and travel to the 2024 New Mexico Water Conference. The remaining $97.39 was budgeted for New Mexico Water Conference travel costs that were not utilized.

8. List presentations you have made related to the project.

Predictive Biology Tools for Identification of Unidentified Polyphosphate Accumulating Organisms in Wastewater Treatment. New Mexico Water Conference, November 5, 2024, Pojoaque, NM.

9. List publications or reports, if any, that you are preparing. For all publications/reports and posters resulting from this award, please attribute the funding to NM WRRI and the New Mexico State Legislature by including the account number: NMWRRI-SG-FALL2024.

Belka, D., Winston, A., Nelson, W., McDermott, J, and Schuler, A.J. (2026, In Prep). Members of the genera *Austwickia* and *Herpetosiphon* may contribute to enhanced biological phosphorus removal using amino acids.

The publication above will also be incorporated into my Ph.D. dissertation.

10. List any other students or faculty members who have assisted you with your project.

No other students or faculty members assisted. However, scientists at the Pacific Northwest National Laboratory helped with the sequencing analysis and will be listed as co-authors on any publications.

11. Provide special recognition awards or notable achievements as a result of the research including any publicity such as newspaper articles, or similar.

None.

12. Provide information on degree completion and future career plans. Funding for student grants comes from the New Mexico Legislature and legislators are interested in whether recipients of these grants go on to complete academic degrees and work in a water-related field in New Mexico or elsewhere.

I will be completing my Ph.D. in the fall of 2025. I will be working as a wastewater treatment subject matter expert for a consulting firm that services clients across the United States and relocating to the Denver, CO area.

You are encouraged to include graphics and/or photos in your draft and final report.

Final reports will be posted on the NM WRRI website and should be verified by the student's advisor.