**PROJECT TITLE:** *Cold Temperature Ammonia Consuming Bacteria during Wastewater Treatment*

**I. PROJECT STATEMENT**

This project will investigate the presence and activity of ammonia-consuming bacteria during the winter months in Minnesota’s wastewater treatment facilities. These organisms are critically important for protecting Minnesota’s surface water quality, by preventing the release of ammonia (which is especially toxic to fish) and estrogenic hormones. Ammonia-consuming bacteria, however, are exceptionally sensitive to cold temperatures, such that ammonia discharges from wastewater treatment facilities are typically not regulated from November through April. Because these organisms are slow-growing in the laboratory and ammonia is not regulated in the winter, our knowledge and understanding of these critically important organisms during the cold weather months is sorely lacking. Research is needed to better understand these organisms to better protect Minnesota’s surface water quality, to optimize energy consumption for wastewater treatment, and to better remove hormones and other contaminants of emerging concern from Minnesota’s wastewaters.

Ammonia is a critically important environmental pollutant because of its toxicity to fish, its contribution to eutrophication (i.e., excessive growth of weeds and algae in lakes and streams), and its cost of treatment. Ammonia is not typically a primary pollutant (i.e., directly released to the environment), but instead forms as proteins decompose. Because municipal wastewater contains a substantial quantity of protein, ammonia is a particularly important pollutant to wastewater treatment plant operators. Ammonia is one of the most difficult pollutants to remove from wastewater because it requires a lot of aeration to fully treat (which is expensive) and the organisms that consume ammonia are very slow-growing.

Ammonia-consuming organisms are historically thought to be inactive at low temperatures, which is why most wastewater treatment facilities in Minnesota are not regulated for ammonia discharges in the winter. Our recent research, however, has demonstrated that these organisms are retained during the winter months at similar quantities as the summer months. This suggests that these organisms remain active during the winter, even if they are not responsible for measurable ammonia removal. If ammonia-consuming bacteria remain active during the winter, this has several beneficial impacts on the State of Minnesota. First, wastewater treatment facilities need to be less concerned about the resumption of ammonia removal each Spring. This would allow wastewater treatment operators to better control aeration rates, potentially saving Minnesota taxpayers an unnecessary expense. Second, ammonia-consuming bacteria have been linked to the degradation of numerous contaminants of emerging concern, such as the hormone estrone. If ammonia-consuming organisms remain active in the winter months, Minnesota’s wastewater treatment facilities can discharge lower quantities of estrogenic substances, reducing the feminization of fish and help sustain Minnesota’s fish populations.

**II. PROJECT ACTIVITIES AND OUTCOMES**

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| **Activity 1 Title:** *Identify and characterize the ammonia consuming organisms in Minnesota’s wastewater treatment facilities* **Description:**Samples will be collected from up to 10 municipal wastewater treatment facilities in Minnesota in February (cold) and in August (warm). We will then characterize the ammonia-consuming bacteria by using DNA sequencing technology on a gene common to all bacteria (16S rRNA gene) and a gene that is specifically involved in ammonia consumption (*amoA*). We will then sequence all of the DNA in these samples and then use this data to assemble the majority of the genome of the ammonia-consuming bacteria in Minnesota’s wastewater treatment facilities (this technique is known as “shotgun metagenomics”). This work will allow us to determine if the ammonia-consuming bacteria vary from summer to winter and from treatment plant to treatment plant. The assembled genome sequences will then provide us the fundamental information to allow us to track the activity of ammonia consuming organisms in Activity 2.**ENRTF BUDGET: $227,426** |  |
| **Outcome** | **Completion Date** |
| *1. Sample collection and Genomic DNA extractions* | March 31, 2021 |
| *2. DNA sequence analysis* | August 31, 2021 |
| *3. Analysis of DNA sequence data* | March 31, 2022 |

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| **Activity 2 Title:** *Quantify the activity of ammonia consuming bacteria during wastewater treatment in the winter***Description:**This activity will determine the activity of ammonia consuming bacteria during the winter months. We will quantify the expression of different genes during the winter months by targeting their RNA (a direct measure of activity) rather than their DNA (linked to the potential to do an activity). Because ammonia consuming bacteria appear to be active in the winter but yet do not consume ammonia to a great extent, we will also track their ability to remove estrone, the most prominent estrogenic compound in municipal wastewater. This research is important because ammonia consuming bacteria have been previously linked to the removal of estrone from wastewater, such that the reduction in ammonia consumption could also be linked to the removal of estrogenicity in the winter months.**ENRTF BUDGET: $227,425**

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| **Outcome** | **Completion Date** |
| *1. Sample collection, RNA extraction, and cDNA Synthesis* | May 31, 2022 |
| *2. DNA sequence analysis* | December 31, 2021 |
| *3. Analysis of DNA sequence data* | December 31, 2022 |
| *4. Quantification of estrone degradation activity* | December 31, 2022 |

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| **Activity 3 Title:** *Disseminate research results to stakeholders.***Description:**The first two activities will significantly improve our knowledge of the ammonia consuming organisms in the Minnesota. The final activity will be to disseminate these results at local conferences (e.g., the Annual Innovative Conference by the Central States Water Environment Association). In addition, we will publish our research results in open-access, peer-reviewed journals, which will allow us and LCCMR staff to disseminate our results to the fullest extent possible.**ENRTF BUDGET: $7,500**

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| **Outcome** | **Completion Date** |
| *1. Presentations at in-state scientific conferences (on-going/continuous)* | June 30, 2023 |
| *2. Publication in open access scientific journals* | June 30, 2023 |

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**III. PROJECT PARTNERS AND COLLABORATORS:**

The project team will be led by Drs. Timothy LaPara and Sebastian Behrens (University of Minnesota, Department of Civil, Environmental, & Geo-Engineering) who are experts in wastewater treatment and in microbiology. The team also will include one post-doctoral research associate, a graduate student, and numerous participating wastewater treatment facilities.

**IV. LONG-TERM IMPLEMENTATION AND FUNDING:**

The long-term goal of the proposed research is to protect the Minnesota’s surface water through better wastewater treatment. This research will help optimize removal of ammonia from Minnesota’s municipal wastewater throughout the year and hopefully reduce the estrogenicity of Minnesota’s wastewater discharges.