EPA Research Grant Annual Report Summary

Period Covered by the Report: September 2021 – September 2022
Date of Report: November 2022
EPA Agreement Number: 84008601
Title: When a Detour Becomes a Shortcut: Going Full-Scale with Partial Denitrification/Anammox as an Alternative Strategy for Mainstream Deammonification and Incorporating Biological Phosphorus Removal
Investigators: Fevig, Stephanie; Chandran, Kartik; De Clippeleir, Haydee; Klaus, Stephanie; Riffat, Rumana; Wells, George
Institution: The Water Research Foundation (WRF), Columbia University (CU), District of Columbia Water and Sewer Authority (DC Water), Hampton Roads Sanitation District (HRSD), George Washington University (GWU), Northwestern University (NU)
Research Category: Harmful Algal Blooms, Water, Clean Water, Water Quality
Project Period: Year 2 (September 2021 – September 2022)

Objective of Research:

The goal of this project is to develop proof of principle for different partial denitrification/anammox (PdNA) configurations, with the integration of biological phosphorus (bio-P), and move PdNA into full-scale application. PdNA with bio-P has the potential to achieve low nutrient effluent concentrations with reductions in energy, chemicals, and treatment footprint. Ultimately, implementation of this process can provide significant cost savings to utilities while reducing nutrient discharges to the watershed and mitigating the formation of harmful algal blooms (HABs).

There are four key objectives of this research, each supported by a series of tasks with task leads and supporting team members:

- Objective 1: Develop new PdNA concepts using denitrifying phosphate accumulating organisms (dPAO)
- Objective 2: Develop proof of principle for different PdNA configurations
- Objective 3: Move PdNA into full-scale application
- Objective 4: Conduct outreach and technology transfer, in particular to utilities in regions with HABs

Progress Summary/Accomplishments (Outputs/Outcomes):

With the opportunity for innovative full-scale process deployment over the timeframe of this three-year project, outreach and technology transfer to water resource recovery facilities in HAB hotspots and other regions nationally will be important components of the project. Below are the accomplishments to date for the key outputs/outcomes of this project:

Development of New PdNA Concepts Using Denitrifying PAO

- NO_3^--N concentration (or VFA/NO_3^--N ratio) was shown to have a positive correlation with estimated % anoxic P uptake via NO_3^--N.
- dGAO may be preferentially selected at longer SRT, while Accumulibacter dPAO may be preferentially selected at shorter SRT. This is in line with our observation that decreased SRT in this study was associated with reduced dGAO, based on 16S rRNA amplicon sequencing results before carrier inoculation.
- The significantly higher NO_3^--N reduction and NO_2^--N accumulation rates in suspended solids resulted in a higher NTR ratio than in attached biofilm. This implies that microorganisms in suspended biomass may have a higher PdN efficiency than in biofilm biomass. Meanwhile, the suspended solids had a higher anoxic P uptake contribution, suggesting that dPAO enriched in suspended solids may prefer to use NO_3^--N over NO_2^--N as an electron acceptor to promote orthoP conversion to polyP.
- Inoculation with anammox-enriched biofilm carriers enabled proof-of-feasibility of integrated activity of dPAO-PdN with anammox-driven N removal. Ongoing efforts seek to increase anammox activity.

Development of Proof of Principle for Different PdNA Configurations

- DC Water Pre-anoxic IFAS
    - Overall, this study demonstrated that methanol can be used as a carbon source for both partial denitrification/anammox (PdNA) and full denitrification.

Movement of PdNA into Full-Scale Application

- James River
    - Startup of mainstream anammox IFAS is possible within 2-3 months without anammox biomass seeding, with low ammonia and nitrite concentrations.
    - Maximum anammox activity is continuing to increase beyond the in situ removal rates.
    - Anammox was detected in the full-scale aerobic IFAS zone**.**
    - Anammox was detected in the moving media full-scale PdNA demonstration tank.
- York River Filter Pilot
    - The results showed that PdNA filter startup without biomass seeding is feasible. With the right carbon dose, PdN was established even without the presence of AnAOB, and the nitrite availability concomitantly with the ammonium presence favored AnAOB activity. The performance, maximum activity tests, and molecular analysis results showed that glycerol has a higher potential to speed up AnAOB growth and enrichment when using virgin media.

Publications/Presentations:

Publications

Fofana, R., Parsons, M., Long, C., Chandran, K., Jones, K., Klaus, S., Trovato, B., Wilson, C., De Clippeleir, H., and Bott, C. (2022). Full-scale transition from denitrification to partial denitrification–anammox (PdNA) in deep-bed filters: Operational strategies for and benefits of

PdNA implementation. Water Environment Research, 94(5), e10727. https://doi.org/10.1002/wer.10727.

Macmanus, J., Long, C., Klaus, S., Parsons, M., Chandran, K., De Clippeleir, H., and Bott, C. (2022). Nitrogen removal capacity and carbon demand requirements of partial denitrification/anammox MBBR and IFAS processes. Water Environment Research, 94(8), e10766. https://doi.org/10.1002/wer.10766.

Schoepflin, S., Macmanus, J., Long, C., McCullough, K., Klaus, S., De Clippeleir, H., Wilson, C., Parsons, M., Chandran, K., and Bott, C. (2022). Startup strategies for mainstream anammox polishing in moving bed biofilm reactors. Water Environment Research, 94(6), e10723. https://doi.org/10.1002/wer.10723.

Presentations

Investigation of Raw Fermentate for Mainstream Partial Denitrification-Anammox Application. Paper was presented at WEFTEC 2022 in October 2022 (New Orleans, LA).

Advancing Full-Scale Partial Denitrification-Anammox (PdNA) Filters: Design and Operation Guidelines. Paper was presented at WEFTEC 2022 in October 2022 (New Orleans, LA).

Mainstream Anammox in Action! Implementation of Full-Scale Partial Denitrification-Anammox, IWA LET 2022 Reno, Stephanie Klaus, Megan Bachmann, Rahil Fofana, Mike Parsons, Haydee De Clippeleir, Charles Bott. Presented poster and poster pitch presentation in April 2022.

Current State of Knowledge on Operation and Implementation of Partial Denitrification – Anammox (PdNA) Filters. Rahil Fofana, DC Water; Megan Bachmann, HRSD; Kimberly Jones; Jeseth Delgado Vela, Howard University; Benay Akyon; Wenjun Liu, Xylem; Stephanie Klaus, HRSD. Paper was presented at WEF IPE 2022 in June 2022 (Miami, FL).

Success at Pilot-Scale Leads to the Full-Scale Application of PdNA in MBBR and IFAS and the Inadvertent Development of Mainstream PNA Along the Way. Megan Bachmann, Stephanie Klaus, Justin Macmanus, Michael Parsons, HRSD; Haydee De Clippeleir, DC Water; Charles Bott, HRSD. Paper was presented at WEF IPE 2022 in June 2022 (Miami, FL).

Developing Application Guidelines for Mainstream Partial Denitrification-Anammox Application with Fermentate. Paper was presented at WEF IPE 2022 in June 2022 (Miami, FL).

Robustness and Stability of Partial Denitrification – Anammox (PdNA) in Deep-Bed Polishing Filters: Low Temperature and Backwashing Shear. Coauthors: 1) R. Fofana, 2) M. Bachmann, 3) K. Jones, 4) J. Delgado-Vela, 5) B. Akyon, 6) W. Liu, 7) M. Parsons, 8) S. Klaus, 9) C. Bott, 10) C. deBarbadillo, 11) H. De Clippeleir. Presented virtually at IWA Wastewater, Water and Resource Recovery Conference Poznan in April 2022.

CWEA/CSAWWA Spring Meeting's Fresh Ideas Poster Competition: Title: Achieving Mainstream Partial-Denitrification Anammox (PdNA) Selection with Methanol. GW Students' poster won first place!

Future Activities:

Objective 1: Develop New PdNA Concepts Using Denitrifying PAO

- Continue reactor performance monitoring via in-cycle nutrient analysis.
- Expand suite of ex situ tests to understand segregation of biological P and N removal activities in suspended solids and on biofilm carriers separately.
- Elucidate Accumulibacter dPAO clade microdiversity and relative abundance enriched in the reactor via qPCR and shotgun metagenomic analyses.
- Determine reason behind the decline in anammox activity, and identify means to increase activity.
- Send out the first PCR amplification products of second batch samples for 16S rRNA sequencing, and complete data analysis upon receiving the raw results from sequencing facility.

Objective 2: Develop Proof of Principle for Different PdNA Configurations

- DC Water
    - The experimental runs for Task 2.5, pre-anoxic concept using IFAS anammox, with fermentate will commence. With this run, we hope to assess and develop startup strategies with virgin media for Blue Plains AWTP.
    - Prepare a journal paper that focuses on anammox bioaugmentation. Experimental runs conducted with acetate as the carbon source will be presented in the paper.
- HRSD
    - Continue operating the IFAS pilot glycerol vs methanol at steady state (Task 2.3).

Objective 3: Move PdNA into Full-Scale Application

- HRSD
    - Finish construction and begin startup of the full-scale post-anoxic PdNA fixed media IFAS demonstration at James River Plant (Tasks 2.3 and 3.3). Continue with startup of the moving media IFAS reactor.

Objective 4: Conduct Outreach and Technology Transfer

- Host State of the Science Workshop on the Status of Shortcut Nitrogen Removal: Revealing the Outcome of 10 Years of R&D Workshop and project meeting on December 14–15, 2022.
- Prepare and submit final project deliverable: PdNA blueprint/guidance document.
- Host final workshop and webinar in 2023.

Supplemental Keywords:

Biological Nutrient Removal (BNR), Nutrients, Treatment, Nitrogen, Phosphorus

Relevant Websites:

https://www.waterrf.org/harmful-algal-blooms-habs-when-detour-becomes-shortcut, https://www.waterrf.org/research/projects/mainstream-deammonification-biological-phosphorus-removal-1