REQUEST FOR PROPOSALS (RFP)

Next-Generation Analytical Methods for Understanding Biological Nutrient Removal Processes (5291)

Date Posted
Friday, September 20, 2024

Due Date
Proposals must be received by 3:00 pm Mountain Time on Thursday, November 21, 2024.

WRF Project Contact
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Project Sponsors
This project is funded by The Water Research Foundation (WRF) as part of WRF's Research Priority Program.

Project Objectives
- Using a combination of literature review, utility surveys/case studies, and lab study, investigate how wastewater monitoring using next-generation analytical methods (i.e., amplicon sequencing, genomics, transcriptomics, proteomics, and metabolomics) can be applied for understanding, optimization, troubleshooting, and control of biological nutrient removal (BNR) processes.
- Identify case studies of successful application of microbial population monitoring for wastewater treatment processes using next-generation methods, with remediation strategies and lessons learned.
- Develop an up-to-date guidance document for use by treatment plant process control and operations staff to implement next-generation analytical methods.
- Identify high-priority research gaps that impede robust and consistent application of next-generation analytical methods for understanding and monitoring BNR processes.

Budget
Applicants may request up to $250,000 in WRF funds for this project.

Background and Project Rationale
Technological developments over the last two decades have led to increasing availability coupled with dramatic decreases in cost of next-generation analytical tools, such as high-throughput nucleic acid sequencing (which can include amplicon sequencing, metagenomics and metatranscriptomics), proteomics, and metabolomics. These next-generation tools can be

leveraged to understand microbial community structure, function, and activity, and they are even more powerful when used to complement one another (sometimes referred to as 'multi-omics'). Next-generation methods have been used to characterize the species, pathways, and enzymes responsible for individual BNR processes. This has greatly increased our understanding of the dominant microbial communities underlying performance of BNR systems, changes in community structure and composition over time or in response to different operating conditions, and important functional genes and metabolites at each stage in nutrient removal.

Unfortunately, understanding of how to leverage next-generation analytical approaches for actionable process guidance (e.g., process design, modeling, troubleshooting, and ultimately control) has lagged in the wastewater sector. For example, metagenomic analytical tools, which allow for more specific identification of microorganisms, have become increasingly available and less expensive but are still not widely used in wastewater treatment monitoring contexts. Traditional microscopic evaluation and characterization of organisms by morphology and staining remain the standard troubleshooting tools, as most utilities do not have the institutional capacity for conducting next-generation microbial analyses in-house. Utilization of third-party services for this type of analysis is an option, but may also add significant costs or turnaround time; thus, there is a need to develop robust guidance on current state of 'omics' tools and other next-generation analytical methods for BNR processes.

In the context of this RFP, "next-generation analytical tools/methods" refers to genomics (ranging from amplicon sequencing to metagenomics), transcriptomics, proteomics, and metabolomics. Microscopy methods are not within the scope of this project; however, proposers are encouraged to incorporate other emerging high-throughput technologies for evaluating microbial communities and processes if applicable.

Research Approach

This RFP is intentionally flexible in the research approach to encourage creativity and originality from proposers. Proposers should describe how they will conduct the research to meet the objectives listed above. A successful proposal will evaluate **all** the well-established next-generation analytical methods (genomics, transcriptomics, proteomics, metabolomics) and describe nascent technologies if applicable. It will examine the use of these methods in various BNR processes, such as nitrification processes, biological phosphorous removal processes, mainstream or sidestream annamox processes and partial denitrification-annamox (PdNA), densification, and bulking and foaming.

The following approach is intended as a suggested starting point:

1. Conduct a literature review (building on findings from WRF 4961, 4878, and 4827a [Garner et al. 2023, Park and Chandran 2018, Hoar et al. 2019]) that synthesizes currently available methods and findings relevant to BNR processes at wastewater treatment facilities, including their technology readiness level.
2. Conduct an industry/treatment plant survey. The information collected from the survey should be useful for identifying challenges related to BNR process control and meeting nutrient discharge limits that could be addressed with next-generation tools, and for

understanding the degree of readiness/willingness of utilities to adopt next-generation tools. The survey should include both facilities that have attempted to utilize next-generation methodologies to inform process operation or troubleshooting and those that have not. The survey should focus on treatment plants with BNR processes and should include plants with a variety of operating conditions such as:

- Size: from small (< 1 MGD) to large (> 10 MGD) plants
- Operating temperature: cold (40 - 60^{o} F) to hot weather (80 - 100^{o} F)
- Various types of BNR processes and configurations (e.g., activated sludge, membrane bioreactor, granular activated sludge, etc.)

3. Develop criteria to evaluate currently available tools. Identify tools with high potential for of full-scale application and widespread adoption.
4. Based on findings from the above project components, design and perform experiments (bench scale and/or in-situ) to evaluate the selected tools using wastewater samples from treatment plants with various operating conditions. The experimental timeline would ideally extend over 1+ years and over multiple treatment plants and results should inform the research questions listed below.
5. Perform data analysis and integration. This effort would include the potential to integrate data with process modifications/control and/or with mathematical process modeling.
6. Provide a cost matrix for adoption of a routine monitoring program that uses next-generation tools (both for building and operating an in-house lab or consistent use of third parties), including comparisons to alternative (conventional) methods. This will help utilities with their business case approval to establish regular monitoring with next-generation technologies.
7. Develop a guidance document and identify key remaining research gaps/needs. This document should help utilities assess whether these tools would be helpful for their needs, understand the limitations of the tools, determine what should be considered when setting up a sampling and analytical plan, and understand how to interpret the data. This document should provide recommendations for:

    - Simple process control parameters like sludge volume index (SVI), dissolved oxygen (DO), solids retention time (SRT), mixed liquor suspended solids (MLSS), oxidation-reduction potential (ORP), oxygen uptake rate (OUR), and influent food to microorganism ratio (F/M)
    - Optimal sampling locations, sample frequency and sample storage requirements
    - Data analysis
    - Control charting
    - Monitoring microbial community changes and succession during seeding/inoculation, routine operation, and as an early warning system for process inhibition or foaming and bulking

A successful proposal will address **all** the following research questions:

- How can next-generation tools most effectively be used to monitor:
  - Seeding or inoculation success in bioreactors?
  - The health of a wastewater system?
  - Changes at pump stations or headworks that may impact process performance?
- What is the link between microbial community composition and function in selector zones (if present) and zones of secondary treatment processes? How does composition and function relate to process performance efficiency?
- What are the minimum quality assurance/quality control (QA/QC) requirements for results to be robust enough to inform operations?
- What are the time, equipment, and financial investments required of a utility to implement various types of next-generation methods on a consistent basis?

Proposers are also encouraged to specify additional research questions. Some **optional** suggested questions include:

- What is the value and function of various biodiversity indices (e.g., Simpson Reciprocal Index, Shannon Index) for evaluating results from these methods?
- How can next-generation sequencing (NGS) help in better understanding and predicting changes in microbial communities as a result of using different external carbon sources? What operational strategies can then be used to mitigate risks to final effluent quality, settling characteristics, and sludge dewatering?
- Can information gained from next-generation analytical tools/methods be used to design rapid, accessible/low-cost molecular tools as a surrogate for routine monitoring, early-warning, or process troubleshooting? This can include focused monitoring and troubleshooting for nutrient removal or filaments/settleability, etc.
- How can 'omics result be used to improve wastewater amplicon sequencing databases, the interpretation of amplicon sequencing data, and the connection between microbial composition/relative abundance and BNR "health"?
- How can results from next-generation analytical methods inform biological inputs (e.g., kinetic and stoichiometric parameters) within process model applications (e.g., BioWin, Sumo, GPSx, etc.)?
- How can next-generation results be leveraged for out-selection of certain microbial groups where beneficial to promoting a BNR process?

Expected Deliverables

- Final Research Report and presentation
- Guidance document for utilities seeking to adopt next-generation methods for monitoring BNR processes, including a cost matrix
- A fact sheet for utilities considering the use of next-generation methods outlining options and associated resource needs in terms of sampling type and frequency, equipment, staffing, software, communication to operations, and limitations.

- Case studies of successful applications of microbial population monitoring or other innovative, emerging analytical methods for BNR process understanding, design, modeling, monitoring, control, or optimization/troubleshooting

We encourage proposers to suggest other types of deliverables if appropriate.

Communication Plan
Please review WRF's *Project Deliverable Guidelines* for information on preparing a communication plan. Conference presentations, webcasts, peer-reviewed publication submissions, and other forms of project information dissemination are typically encouraged.

Project Duration
The anticipated period of performance for this project is 24-30 months from the contract start date.

References and Resources
The following list includes examples of research reports, tools, and other resources that may be helpful to proposers. It is not intended to be comprehensive, nor is it a required list for consideration.

WRF Projects:

- Garner, E., M. F. Blair, C. Brown, A. Cullom, B. C. Davis, S. Ghosh, M. Gnegy, S. Gupta, I. Keenum, K. Liguori, A. Maile-Moskowitz, E. Milligan, J. Pan, A. J. Prussin II, K. Scott, L. S. Heath, L. C. Marr, P. J. Vikesland, L. Zhang, and A. Pruden. 2023. The Use of Next-generation Sequencing (NGS) Technologies and Metagenomics Approaches to Evaluate Water and Wastewater Quality Monitoring and Treatment Technologies. Project 4961. Denver, CO: The Water Research Foundation.
- Park, M -R., and K. Chandran. 2018. Nationwide Meta-omics Survey of Denitrifying Microbial Communities in Wastewater Treatment Systems. Project 4878. Denver, CO: The Water Research Foundation.
- Hoar, C., J. H. Ahn, and K. Chandran. 2019. Assessment of Nitrification Inhibition. Project 4827A. Denver, CO: The Water Research Foundation.

Peer Reviewed Literature:

- Diaz, R., B. Mackey, S. Chadalavada, P. Heck, and R. Goel. 2022. Enhanced Bio-P removal: Past, present, and future–A comprehensive review. *Chemosphere*, 309, 136518.
- Knisz, J., P. Shetty, R. Wirth, G. Maróti, T. Karches, I. Dalkó, M. Balint, E. Vadkerti, and T. Bíró. 2021. Genome-level insights into the operation of an on-site biological wastewater treatment unit reveal the importance of storage time. *Science of the Total Environment*, 766, 144425.
- Liu, S., Q. Liu, H. Wu, W. Jiang, A. Kahaer, Q. Tang, Z. Hu, C. Hong, and D. Liu. 2022. Integrative chemical and omics analysis of the ammonia nitrogen removal characteristics and mechanism of a novel oligotrophic heterotrophic nitrification-aerobic denitrification bacterium. *Science of The Total Environment*, 852, 158519.

- Ngara, T. R., P. Zeng, and H. Zhang. 2022. Biological nitrogen removal database: a manually curated data resource. *Microorganisms*, 10(2), 431.
- Rosas-Echeverría, K., C. Fall, E. Gutiérrez-Segura, M. P. Romero-Camacho, and K. M. Ba. 2023. Mechanisms of persistence and impact of ordinary heterotrophic organisms in aerobic granular sludge. *Bioresource Technology*, 384, 129346.
- Stewart, R. D., K. S. Myers, C. Amstadt, M. Seib, K. D. McMahon, and D. R. Noguera. 2024. Refinement of the "Candidatus Accumulibacter" genus based on metagenomic analysis of biological nutrient removal (BNR) pilot-scale plants operated with reduced aeration. *Msystems*, 9(3), e01188-23.
- Wang, X., G. Zhang, A. Ding, L. Zheng, E. Xie, D. Yuan, T. Qiuyang, Y. Xing, and H. Wu. 2023. Nitrite-resistance mechanisms on wastewater treatment in denitrifying phosphorus removal process revealed by machine learning, co-occurrence, and metagenomics analysis. *Environmental Pollution*, 327, 121549.

Proposal Evaluation Criteria

The following criteria will be used to evaluate proposals:

- Understanding the Problem and Responsiveness to RFP (maximum 20 points)
- Technical and Scientific Merit (maximum 30 points)
- Qualifications, Capabilities, and Management (maximum 15 points)
- Communication Plan, Deliverables, and Applicability (maximum 20 points)
- Budget and Schedule (maximum 15 points)

PROPOSAL PREPARATION INSTRUCTIONS

Proposals submitted in response to this RFP must be prepared in accordance with WRF's *Guidelines for Research Priority Program Proposals* and *Instructions for Budget Preparation*. These guidelines contain instructions for the technical aspects, financial statements, indirect costs, and administrative requirements that the applicant must follow when preparing a proposal.

Proposals that include the production of web- or software-based tools, such as websites, Excel spreadsheets, Access databases, etc., must follow the criteria outlined for web tools presented in the *Technology Deliverables Guidance*.

Eligibility to Submit Proposals
Proposals will be accepted from both U.S.-based and non-U.S.-based entities, including educational institutions, research organizations, governmental agencies, and consultants or other for-profit entities.

WRF's Board of Directors has established a *Timeliness Policy* that addresses researcher adherence to the project schedule. Researchers who are late on any ongoing WRF-sponsored studies without approved no-cost extensions are not eligible to be named participants in any proposals. Direct any questions about eligibility to the WRF project contact listed at the top of this RFP.

Administrative, Cost, and Audit Standards
WRF's research program standards for administrative, cost, and audit compliance are based upon, and comply with, Office of Management and Budget (OMB) Uniform Grants Guidance (UGG), 2 CFR Part 200 Uniform Administrative Requirements, Cost Principles, and Audit Requirements for Federal Awards, and 48 CFR 31.2 Contracts with Commercial Organizations. These standards are referenced in WRF's *Guidelines for Research Priority Program Proposals* and include specific guidelines outlining the requirements for indirect cost negotiation agreements, financial statements, and the Statement of Direct Labor, Fringe Benefits, and General Overhead. Inclusion of indirect costs must be substantiated by a negotiated agreement or appropriate Statement of Direct Labor, Fringe Benefits, and General Overhead. Well in advance of preparing the proposal, your research and financial staff should review the detailed instructions included in WRF's *Guidelines for Research Priority Program Proposals* and consult the *Instructions for Budget Preparation.*

Budget and Funding Information
The maximum funding available from WRF for this project is $250,000. The applicant must contribute additional resources equivalent to at least 33% of the project award. For example, if an applicant requests $100,000 from WRF, an additional $33,000 or more must be contributed by the applicant. Acceptable forms of applicant contribution include cost share, applicant in-kind, or third-party in-kind that comply with 2 CFR Part 200.306 cost sharing or matching. The applicant may elect to contribute more than 33% to the project, but the maximum WRF funding

available remains fixed at $250,000. Proposals that do not meet the minimum 33% of the project award will not be accepted. Consult the *Instructions for Budget Preparation* for more information and definitions of terms.

Period of Performance
It is WRF's policy to negotiate a reasonable schedule for each research project. Once this schedule is established, WRF and its sub-recipients have a contractual obligation to adhere to the agreed-upon schedule. Under WRF's *No-Cost Extension Policy*, a project schedule cannot be extended more than nine months beyond the original contracted schedule, regardless of the number of extensions granted.

Utility and Organization Participation
WRF encourages participation from water utilities and other organizations in WRF research. Participation can occur in a variety of ways, including direct participation, in-kind contributions, or in-kind services. To facilitate their participation, WRF has provided contact information, on the last page of this RFP, of utilities and other organizations that have indicated an interest in this research. Proposers are responsible for negotiating utility and organization participation in their particular proposals. The listed utilities and organizations are under no obligation to participate, and the proposer is not obligated to include them in their particular proposal.

Application Procedure and Deadline
Proposals are accepted exclusively online in PDF format, and they must be fully submitted before 3:00 pm Mountain Time on Thursday, November 21, 2024.

The online proposal system allows submission of your documents until the date and time stated in this RFP. To avoid the risk of the system closing before you press the submit button, do not wait until the last minute to complete your submission. Submit your proposal at https://forms.waterrf.org/cbruck/rfp-5291.

Questions to clarify the intent of this RFP and WRF's administrative, cost, and financial requirements may be addressed to the WRF project contact, Maitreyi Nagarkar at 571.384.2117 or mnagarkar@waterrf.org. Questions related to proposal submittal through the online system may be addressed to Caroline Bruck at 303.347.6118 or cbruck@waterrf.org.

Utility and Organization Participants

The following utilities have indicated interest in possible participation in this research. This information is updated within 24 business hours after a utility or an interested organization submits a volunteer form, and this RFP will be re-posted with the new information. **(Depending on your settings, you may need to click refresh on your browser to load the latest file.)**

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