

Statement of Research for Ananda Shankar Bhattacharjee

February 2023

Summary: I am an environmental engineer specializing in microbial genomics. My research is inherently interdisciplinary because it demands a working knowledge of cutting-edge molecular biology, microfluidics, advanced microscopy, bioprocess engineering, computational biology, and classical microbiology. My research focuses on the microbiome of built environments (microbiology of wastewater and water) and wastewater treatment processes (engineered ecosystems for nutrient recovery and removal). I have always been intrigued by developing and implementing novel aerobic and anaerobic-engineered bioreactors for resource recovery (chemical and/or microbiological) to protect human health and the environment. I have received extensive laboratory and field training throughout my research career. I intend to use my training to continue research avenues related to microbiology and engineering, to develop the best water resource management practices for the benefit of future generations.

The 14 Grand Engineering Challenges developed by The National Academy of Engineers raise public awareness of our times' critical global issues and the roles engineers must play in addressing them. My research goals focus on four grand engineering challenges:

1. Manage the nitrogen cycle by involving ecosystem functions mediated by microbes and viruses. Develop innovative wastewater treatment technologies such as nitrite/nitrate-dependent anaerobic methane oxidation (nDAMO), anaerobic ammonium oxidation (anammox), and granular activate sludge bioprocess engineering.
2. Provide access to clean water by applying innovative treatment processes to safeguard the water quality. For example, a few treatment processes are anammox, nDAMO, granular activated sludge (nutrient removal and recovery), and virus-based biocontrol of antibiotic-resistant (BSL-2 microorganisms) bacteria (microbial pathogen control) in treated wastewater.
3. Engineer the tools of scientific discovery, utilizing computational tools for water microbiome engineering.
4. Restore and improve urban water and wastewater infrastructure with innovative treatment processes.

Scientific Focus: My research seeks to create novel aerobic and anaerobic biotechnologies that recover nutrients (e.g., methane, nitrogen, phosphorus, and potassium) from municipal and agricultural waste streams using microbiome and environmental engineering (bioprocess) research. These novel biotechnologies' microbiomes (microbial and viral communities) will provide a fundamental understanding of the assembly and functions. The research entails two interconnected themes; (1) the microbiome of the complex-built environment and (2) engineered microbiomes for sustainable water systems. My diverse educational background (engineering, life science-microbiology, and environmental studies) and research experiences have facilitated my convergent research skills. It is reflected in my diverse publication record in leading journals like *Nature Communication*, *ISME Communication*, *Environmental Science & Technology*, *Water Research*, *Frontiers in Microbiology*, *AAPG Bulletin*, *Environmental Microbiology*, *Biotechnology*, and *Bioengineering*.

The microbiome of the complex-built environment:

All the structures built by humans are a part of the built environment. Microorganisms are found in every part of the built environment. The microbiome (microbial communities) and their metabolites are beneficial (e.g., treatment of water and wastewater, food and beverage production) and detrimental (e.g., human diseases, food spoilage). My research focuses on understanding the water microbiome and its metabolisms that remove and recover nutrients from wastewater. The water microbiome of bioreactors (complex-built environment) is a metropolis of viruses and microorganisms (bacteria, archaea, protozoa, and unicellular eukaryotes). The microorganisms and viruses share and compete for resources (e.g., nutrients and microbes for infection by viruses). However, the engineering of water microbiomes to achieve specified functions remains challenging. These challenges are due to microorganism, viruses, and their interactions (e.g., syntrophy) being poorly characterized, and the tools to predict and manage their (virus and microorganism) functions are lacking. A digital twin platform of water microbiome, with metabolisms and bioreactor operation, will assist wastewater operators in efficiently operating the nutrient removal and recovery process. These modules for the digital twin platform are discussed in engineered microbiomes for sustainable water systems.

Recent DNA sequencing and microbiome analysis advancements provide insight into the community structure, metabolisms, and interaction among microorganisms and viruses. I study the metabolic networks and microbial interactions underpinning microbiome function at a molecular level. My research involves integrating bioprocess engineering (e.g., substrate uptake, metabolite production, nutrient removal), biological information (e.g., biomass growth), and multi-omics approaches. The multi-omics approaches include what might be happening (metagenome) to what is happening (e.g., metatranscriptome and metaphenome) during nutrient removal and recovery in bioreactors. The microbiome analysis uses metagenomic data sets to recover draft and complete genomes of microorganisms and viruses. These genomes are starting points for reconstructing the metabolism of microorganisms in the water microbiome community. For example, metabolic reconstruction with genomes and metatranscriptome (RNA expression sequences) identified the functional role of poorly understood heterotrophs in anaerobic ammonia-oxidizing (anammox) bioreactors (*Nat Commun*, 2017, 8, 15416). The putative metabolic exchanges between heterotrophs and anammox bacteria improve nitrogen removal from wastewater. The microbiome analysis of the bioreactors for nutrient removal and recovery provides us with metabolism and interactions. How microbiome analysis assists environmental engineers in designing sustainable water and wastewater treatment facilities is discussed in ‘engineered microbiome for sustainable water system’.

Metabolism of microorganisms drives the nutrient removal and recovery in a bioreactor under constraints imposed by viruses. However, the diversity and role of viruses in bioreactors' water microbiomes are not yet understood. The viruses infect bacteria, archaea, and protozoans. These infections are a significant source of mortality among the host, leading to microbial composition changes, the flow of nutrients (N, C, P, and S), and energy in the bioreactor (*Water Res*, 2015, Vol. 81 Pg. 1-14). To elucidate the diversity of viruses in the microbiome of the bioreactor, I used a partial nitrification bioreactor (PN/A: nitrification and anammox) that removes ammonia. Our preliminary results from the metagenome and transmission electron microscopy of the viral fraction extraction indicate a diverse viral population (unpublished work). For the study, a combination of viral, microbial analysis (omics), microbiological techniques (microscopy), and convolutional neural networks (CNN, machine learning) were used to elucidate viral diversity in the engineered water microbiome of the PN/A bioreactor. The results provide proof of the concept that diverse viral communities exist in engineered bioreactors. Research is needed to

delineate the diversity, ecology, and role viruses play in engineered microbiomes of the bioreactors.

Based on the preliminary finding that viruses are a part of the water microbiome of bioreactors, I hypothesize that *'viruses can 'break or make' these engineered bioreactors by; (a) killing the host cells or (b) providing fitness genes to host microorganisms through lateral gene transfer.'* Viral lyses of host microorganisms (lytic infection) release organic carbon and nutrients. Viruses can also introduce novel genetic information (lateral genes transfer) in the host genome during infections. To test the hypothesis, the ecology of the viruses of the water microbiome of the bioreactor will be studied by elucidating the viral diversity and their putative microbial hosts. The study will include the type of transmission/viral infection cycle (lytic or lysogenic) for the viruses, including their hosts. Viruses of cultivable bacteria of the bioreactor microbiome will be isolated. These viral isolates will be classified based on morphology (microscopy). The type of infectious life cycle will be determined for the viruses. Viruses of uncultivable microorganisms in a bioreactor will be investigated from the metagenome. Briefly, for the research, I will use a combination of bioprocess engineering, classical microbiological culturing techniques, microfluidics, single (cell and viral particle), genomics (*Environmental Microbiology*, 2020), and computational biology tools (machine learning). My research will infer interactions between viral and microbial populations with the dynamic model of a virus-microbe community (*Systems*, Vol. 3, Pg. 1-14).

In wastewater treatment plants, microbial biofilm growth leads to the biofouling of membranes. Biofouling is the primary cause of reduced flow/flux across the membrane and loss of nutrient removal and recovery from the wastewater treatment plants (WWTPs). Biofouling necessitates frequent treatment or replacement of membranes, increasing the cost of operation and maintenance of WWTPs. Physical and chemical treatment of biofouled membrane is ineffective as the biofilm formation revives after a period. The biofilm-forming microbes have often been resistant to antimicrobials (e.g., antibiotics). The antimicrobial-resistant microbes pose a threat to human and environmental health. Virus-based biocontrol of biofilm-forming microorganisms is an efficient and cost-effective treatment. Viruses lyse the biofilm-forming microbe and disrupt the biofilm-formation process. Biofilm disruption maintains the flow/flux across a membrane (*Biotechnol Bioeng*, Vol. 8, Pg. 1644-1654). The virus-based biocontrol of biofilms forming microorganisms in a built environment, especially water and wastewater treatment infrastructure, has industrial-scale and public health applications. Based on published research findings, I hypothesize that *'viruses and biofilm-forming antimicrobial-resistant microbes reside in the same environment.'* My research goal to test the hypothesis will be to investigate the microbiome of the WWTP. Identify the antimicrobial-resistant microorganism that forms biofilm and develop enrichment cultures of them. Test these enrichment cultures with a viral fraction from the WWTPs and several other environments. Isolate viruses that infect and lyse each microbe (lytic infection cycle). Then develop bioprocess engineering parameters (e.g., # of the virus to microbe) to control the biofilm outbreaks with the viral isolates.

The short-term plan to test my hypotheses will be to train students (undergraduates and graduates) to isolate and identify antibiotic-resistant (BSL-2 microorganisms) bacteria (ARBs) from local WWTPs. Identify potential pathways the ARBs may escape into the natural environment from built environments. Isolate viruses from built environments (local WWTPs) that prey upon the ARBs and stop them from forming biofilms and escaping into the natural environment. Long term goal for the hypotheses will be to establish the virome and microbiome

of local, state, and national WWTPs. The virome and microbiome will assist in understanding the interaction between viruses and microbes in built environments.

To test both my hypotheses for the short and long term, I plan to support the work with funding from the state division of water quality, National Science Foundation (NSF) under the Ecosystem Studies Program (ES), Systematics and Biodiversity Science Cluster (SBS), and Environmental Sustainability program (CBET 7643), DOE Joint Genome Institute's Community Science Program, and Gordon and Betty Moore Foundation under the symbiosis in aquatic systems initiative.

Engineered microbiomes for sustainable water system:

Water resource management is vital for safeguarding human and environmental health. The water and wastewater treatment utilities are essential in preserving the food, energy, and water (FEW) nexus. The wastewater treatment sector is amidst a renaissance to embed energy and resource efficiency to pursue clean water. The wastewater treatment is becoming energy positively by producing energy from the treatment processes (e.g., methane from anaerobic digesters). My research efforts are to improve traditional wastewater treatment processes. To achieve improved WWTPs, I integrate microbiome research with environmental engineering. Microbial communities (microbiomes) drive the Earth's biogeochemical cycles through their metabolisms. The microbiomes have been harnessed as biotechnologies to clean up environmental pollutants, e.g., groundwater remediation and nutrient removal from wastewater. Although microbiomes perform valuable functions, a vast majority of microbe's metabolic capabilities are yet to be understood, unlocked, and harnessed for human and environmental health benefits. The high-throughput DNA sequencing-driven insights shed light on the community structure, genetic diversity, putative metabolisms of uncultured microorganisms, and their crucial roles in diverse environments. These insights into the potential metabolic capabilities of microorganisms provide us with novel biotechnology applications. For example, nitrite-dependent anaerobic methane oxidation by bacteria (nDAMO) (*Water Res*, 2016, Vol. 99 Pg. 244-252). The nDAMO bacteria reduce nitrite and oxidize methane simultaneously.

The traditional wastewater treatment processes (bioprocess engineering) have relied on regulating environmental variables (e.g., nutrients, temperature, etc.) to steer biological processes toward a desired steady state of nutrient recovery. This approach results in a complex microbiome that offers little control over the byproducts (e.g., N₂O, a potent greenhouse gas) during nutrient removal and recovery. Understanding the bioreactor microbiome will assist us in mitigating the issue of harmful byproduct release while improving nutrient removal and recovery. Elucidating the microbiome community structure and putative metabolisms of microorganisms will pinpoint the bacteria-producing harmful byproducts. A digital twin platform for water microbiomes that incorporates microbial community structure, metabolisms, and metabolite exchange interactions with 'omics' data will provide a framework for understanding the metabolic potential of the bioreactors. With the knowledge of the metabolism of harmful bacteria, environmental variables (e.g., nutrients, temperature, level of oxygen, etc.) can be regulated to outcompete other microbes in the bioreactors treating wastewater. My research will develop novel high-throughput methods that integrate systems biology, water microbiome digital twin platforms, and machine learning to create optimized engineered microbiomes. These microbiomes will remove and recover nutrients at commercially viable rates from agricultural and municipal wastewater streams. The objectives are; (1) systematic assembly and analysis of engineered aerobic and anaerobic microbiomes in digital twin and laboratory scale biological

reactors; and (2) metabolism optimization through microbiome metabolic networks of non-model aerobic (e.g., granular activated sludge microorganisms) and anaerobic (e.g., anammox bacteria) microorganisms.

Microbiome research, with a systems biology approach, allows harnessing the metabolic potential of microorganisms for complete nutrient removal and recovery such as anammox (*Environ Sci Technol* 2017, Vol. 51, Pg. 4317-4327; *Nat Commun*, 2017, 8, 15416), nDAMO (*Water Res*, 2016, Vol. 99 Pg. 244-252), and granular activated sludge (*Manuscript in preparation*). The microorganisms (bacteria, archaea, and viruses) involved in aerobic (granular activated sludge) and anaerobic (anammox, nDAMO) nutrient removal processes primarily grow as suspended (granules) and attached (membrane or carrier) multi-species biofilms. The microorganisms and viruses in the biofilms share metabolites. For example, the association between the heterotrophic bacteria receiving organic carbon needed for growth from the anammox bacteria. In return, the heterotrophs convert nitrate to nitrite, a form of nitrogen that anammox bacteria require for growth. The exchange of metabolites between anammox and heterotrophic bacteria improves nitrogen removal (*Environ Sci Technol* 2017, Vol. 51, Pg. 4317-4327; *Nat Commun*, 2017, 8, 15416). Such metabolite exchange interactions among the microorganisms take place in the biofilm. Several microorganisms (multi-microbial species) form the biofilms in the bioreactor microbiomes. For example, the anammox bacteria reside in a multi-species biofilm. The interactions (e.g., syntrophic) and metabolic exchanges between anammox bacteria and other bacterial species in the biofilm are yet to be understood. To shed light on these microbial interactions, I will follow (goal 1) a systematic assembly and analysis of engineered microbes critical for efficient nutrient removal and recovery. With multi-omics (metagenomics, metatranscriptomics, and metabolomics), I will investigate the metabolism of the water microbiome of the bioreactors treating wastewater (e.g., anammox). Metabolisms of the water microbiome will provide putative microbial interactions via metabolite exchanges. These microbial interactions and putative metabolic genes of microorganisms will be an input for incremental kinetic learning (supervised machine learning). The microbial interactions (e.g., syntrophy) will be 'learned' from predicted proteins (metagenome/meta-transcript assembled genome), metabolites (metabolomics), mass spectrometry (proteomics), nutrients (nitrogen species), and organics in bioreactors. A water microbiome digital twin platform that incorporates the microbial community structure and metabolisms will be developed. The platform will be used for simulations (e.g., changes in environmental parameters, pH, and temperature) to increase biological reactor efficiency and novel metabolisms.

I will use bioprocess engineering and microbiome research for my objective 2, metabolism optimization of the bioreactor treating wastewater. Environmental variables (e.g., nutrients, temperature, dissolved oxygen, organic carbon, etc.) will be regulated while applying a suite of omics approaches, metagenomics (putative metabolic genes), metatranscriptomics (expression of metabolic genes), metaproteomics (metabolism), and metabolomics (metabolites produced). The multi-omics approach will provide metabolisms of the microbiome for changes in environmental variables. These will provide novel parameters for the water microbiome, a digital twin platform. The metabolisms and environmental variables with supervised learning will help predict the biokinetics of the bioreactor microbiome in the platform. The biokinetics of the microbiome will form the metabolic networks. Metabolism-optimized bioreactors for enhanced nutrient recovery and removal will be designed based on our understanding of biokinetics. External perturbations, temperature changes (ambient), introducing chemical species (nitrate, sulfide, etc.) in higher concentrations, or mechanical failures lead to temporary or permanent bioreactor failures. The

bioreactor failures can be directly linked to the microbiome. Bioreactor biokinetics and understanding the metabolic networks with machine learning (kinetic learning) will assist in simulations and train models to modules for the water microbiome digital twin platform. These modules will predict scenarios of bioreactor failure and strategies for a road to recovery. Ahead of time, the modules (machine learning) will equip microbiologists and environmental (bioprocess) engineers with strategies for precession recovery of the reactor operations. These modules will be developed for several processes employed across a conventional wastewater treatment plant. The water microbiome digital twin platform, with novel modules, can be incorporated with the commercially available platforms for wastewater treatment plant operation (e.g., MyNereda).

The short-term goals are to develop strategies to enrich microorganisms involved in novel biologically mediated transformation (particularly nitrogen and carbon) pathways that can be harnessed for wastewater treatment applications by engaging cutting-edge omics, molecular biology, and classical microbiological culturing techniques. I will work with WWTPs to develop aerobic and anaerobic biotechnologies for nutrient removal.

Briefly, long-term goals are to develop a) engineered microbiomes for sustainable and safe water systems and b) a research, teaching, and mentorship program that is nationally recognized for solving environmental challenges at local, state, national, and global levels.

I plan to support my research (short and long-term research goals) with funding from the state division of water quality, NSF under the Environmental Engineering and Sustainability cluster, Ecosystem Studies Program (ES), Systematics and Biodiversity Science Cluster (SBS), the Department of Energy, and the Water Research Foundation.

I look forward to developing a leading research program in each area mentioned above.



Figure 1: My research themes directly advance six of the National Science Foundation's top 10 ideas: (1) Understanding the Rule of Life- The environmental factors shape the microbial metropolis in natural and engineered ecosystems (image: suspended and attached anaerobic ammonia-oxidizing (Anammox) bioreactors; Possible interaction between microbes in Anammox Reactors); (2) Growing Convergence Research- The microbiome of engineered ecosystem play vital role in nutrient removal. (Image: Wastewater Treatment plant (source: Google) and anaerobic ammonia oxidation metabolism pathways of Anammox organism enriched in Utah bioreactors); (3) Harnessing the Data Revolution- Large microbiome datasets, multimodal imaging, operation data of engineered ecosystem, and environmental datasets are used for network analysis by computational network biology analysis (Image: Relatedness among viral genomes from the Great Salt Lake, and phylogeny of microbes); (4) NSF INCLUDES- Diverse STEM education for students, professionals and lifelong learners of all ages (Image: Diversity in STEM fields (source Google)); (5) Navigating the New Arctic- The increasing temperature in the Arctic, has opened up

the environmentally fragile region for oil and natural gas exploration. Ancient microbes and viruses frozen in the ice can cause infection outbreaks; therefore, surveillance of virome and microbiome using single viral and cells genomics is vital (Image: High resolution three emission channel (red, green, and blue) wide-field auto-fluorescence image overlaid on a black and white bright field image showing broad size range of fine quartz grains (light green to periwinkle blue) and pyrite (dark green) in New Albany shale rock; Image: Oil platform (source google)). (6) Astrobiology*- Virome and microbiome unearthed from the ocean crust of the Juan *de* Fuca Ridge in the Pacific Northwest give scientists a window into ancient organisms that survived when the earth was without oxygen. (Images: Andrew Fisher).

Reference: **Coenen, A. R., & Weitz, J. S. (2018). Limitations of correlation-based inference in complex virus-microbe communities. *Msystems*, 3(4).

All references from my list of publications.