Statement of Research for Ananda Shankar Bhattacharjee

February 2021

***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 (Figure 1). The specific focus of my research encompasses natural and engineered ecosystems for water and urban wastewaters. I have always been intrigued by how to best develop and implement strategies for the biological treatment of contaminants (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 that training to continue exploring various environmental engineering research avenues to find the best practices for future generations' societal and environmental needs.

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, and 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 such as anammox, nDAMO, granular activated sludge (nutrient removal and recovery), and phage-based biocontrol antibiotic-resistant bacteria (microbial pathogen control) in treated wastewaters.
3. Engineer the tools of scientific discovery, utilizing computational tools for microbiome engineering.
4. Restore and improve urban water and wastewater treatment infrastructures with innovative treatment facilitate.

Scientific Focus: My research is interdisciplinary and directly addresses the assurance of high-quality water supplies now and in the future. The research entails two interconnected themes; (1) engaging ecosystem functions to secure sustainable supply and protection of water, and (2) engineering of the built environment. 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*, *Environmental Science & Technology*, *Water Research*, *Frontiers in Microbiology, AAPG Bulletin, Environmental Microbiology, and Biotechnology, and Bioengineering*.

***Engaging Ecosystem Functions to Secure Sustainable Supply and Protection of Water*:** Engineering solutions have played a significant role in addressing water resource management's challenges, mainly focusing on areas of access to clean water, sanitation, water for irrigation, protection from floods, and energy generation. Despite their proven benefits, these conventional engineering solutions are increasingly criticized because of their negative environmental, societal, and economic impacts. Higher reliance on ecosystem functions combined with environmental engineering as an alternative to conventional engineering solutions is being advocated to achieve optimal present-day water management solutions. Microorganisms are vital in mediating these functions through biogeochemical cycles such as Nitrogen (N), Carbon (C), Phosphorous (P), and Sulfur (S). However, the relationship between microbial and viral community structure and the ecosystem processes mentioned above is not adequately understood.

My research involves understanding the role of microbes and virus-mediated biogeochemical cycling in nutrient impaired riverine (Jordan River) (*Water Res*, 2016, Vol. 99, Pg. 244-252), impounded wetland (*Unpublished*), and hypersaline lake (Great Salt Lake) ecosystems (*Front Microbiol*, 2017, Vol. 8, Pg. 1-15). My interest influences my research choices in novel microbe mediated biogeochemical pathways of N transformation processes that can enhance water quality (such as nDAMO, anammox, COMplete AMMonia OXidiser, Comammox). My research program will understand microbes' role and their viruses in the biogeochemical cycling of N, C, P, and S nutrients in natural habitats and habitats impacted by treated wastewater discharge and excess agricultural nutrient runoffs.

The nDAMO microbe metabolism connects both the nitrogen (denitrification) and carbon (methane oxidation) cycle. These microbes play a significant role in N and C (as methane) transformation in anoxic sediment habitats (*Water Res*, 2016, Vol. 99, Pg. 244-252). The nDAMO microbe contribution at local and global denitrification and methane oxidation budget is not yet understood. That is due to the difficulty in molecular detection and slow growth rate (duplication time of 20-30 days) of nDAMO microbes. My research aims to estimate the fraction of anaerobic oxidation of methane (AOM) that can be attributed to denitrification in the nutrient impaired riverine, wetlands and freshwater lakes (e.g., Flathead). Ecologists will use the AOM estimates for denitrification and methane flux models. I will use a combination of molecular detection of novel functional genes (*Water Res*, 2016, Vol. 99, Pg. 244-252) culturing enrichment consortiums, biogeochemical, genomics, and computational network biology tools (artificial neural network in machine learning).

Microbial metabolism mediates biogeochemical pathways in nature under the constraints imposed by viruses. It is critical to delineate how viruses that infect microbes affect the nitrogen, carbon, and sulfur cycling in the environment. My primary objective will be to investigate viruses that infect microbes of AOM and denitrification (Anammox, DNRA, and COMAMMOX) pathways in natural and enrichment cultures.

The fundamental understanding of microbe and virus-mediated biogeochemistry in natural ecosystems leads to novel technologies (anammox, nDAMO, Commamox) to improve wastewater streams' water quality. I plan to support this work with funding from the state division of water quality, National Science Foundation (NSF) under the 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.

***Engineering of the Built Environment*:** Water resource management is vital for safeguarding human and environmental health. The wastewater treatment utilities play an essential role in preserving the food, energy, and water (FEW) nexus. The wastewater treatment sectors are in the midst of a renaissance to embed energy and resource efficiency to pursue clean water. The treatment of wastewaters processes is even becoming energy positive and producing energy from the treatment process. My research efforts will improve traditional wastewater treatment processes by integrating viral and microbial ecology with environmental engineering for energy recovery. The two research themes are (a) "manage nitrogen cycle, reducing nitrogenous pollution in an environmentally sustainable approach"; (b) "safeguard of water and wastewater treatment infrastructures by biocontrol of multi-species biofilms using viruses and enzymes produced by viruses."

Microbial ecology allows harnessing the metabolic potential of microorganisms for complete nutrient removal 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), 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 microbes and viruses in the biofilms share metabolites among each other. One such association is between the heterotrophic bacteria receiving organic carbon that they need for growth from the anammox bacteria. In return, the heterotrophs convert nitrate to nitrite, a form of nitrogen that anammox bacteria require for growth (*Environ Sci Technol* 2017, Vol. 51, Pg. 4317-4327; *Nat Commun*, 2017, 8, 15416). This interaction and exchange of nutrients take place in the biofilm. The anammox bacteria reside in a multi-species biofilm. The interactions between anammox bacteria and other bacterial species in the biofilm are yet to be understood. Using incremental kinetic learning (supervised machine learning), the anammox and other bacteria interactions will be 'learned' from predicted proteins (metagenome/meta-transcript assembled genome), metabolites (metabolomics), nutrients (Nitrogen species), and organics in bioreactors. Supervised learning will facilitate in predicting the kinetics of bioreactor metabolic networks. Bioreactors that have enhanced nutrient recovery and removal can be designed with biokinetic knowledge. External perturbations, temperature changes (ambient), the introduction of chemical species (nitrite, sulfide, etc.) in higher concentration, mechanical failures will lead to temporary or permanent bioreactor failures. The bioreactor failures can directly be linked with the microbiota of the reactor. Bioreactor kinetics knowledge gained with machine learning (kinetic learning) will assist in simulations and train models to modules. These modules will be able to predict scenarios of bioreactor failure and strategies for a road to recovery. Ahead of time, the modules (Machine learning) will equip bioprocess engineers with strategies for precession recovery of the reactor operations. Similar modules will be developed for denitrification processes that are employed in conventional wastewater treatment plants.

The primary goal will be to understand the metabolisms, metabolic flux, and biokinetics of the anammox and nDAMO pathways (Innovative nitrogen transformation process (INTP)). The side stream wastewater from anaerobic digestors has a high nitrogen load. A combination of anammox and nDAMO bioreactors can reduce nitrogen load while trapping dissolved methane in a wastewater treatment plant's side-stream water. The research focus will cater to INTP's enhanced biological reactors for agricultural and municipal wastewater treatment. I will use a combination of culturing enrichment consortiums, biogeochemistry, genomics (single-cell and meta omics), and machine learning for INTP's.

Bioreactors treating wastewaters are virus and microbe metropolis. The established hypotheses, (a) *kill the winner*, and (b) *piggyback the winner* are models of population growth of bacteria, archaea, protozoans, and viruses. The viruses infect bacteria, archaea, protozoans (host). 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). The diversity and ecology of viruses in the biofilm of anammox bacteria, methanotrophs, methanogens, and DNRA (multi-species anammox globule biofilm) are not understood. Preliminary, viral, and microbial analysis, combining omics, classical microbiological techniques, and convolutional neural networks (CNN), show viruses in anammox bioreactors (*unpublished*). However, research is needed to delineate the diversity of viruses in anammox bioreactor. The ecology of the bioreactors' viruses will be studied by examining their interactions with their hosting species. The type of transmission/infection cycle that exists for these viruses, including their hosts. Introduction of novel genetic information (lateral genes transfer) in the host, lyses of microbial cells releasing organic carbon and nutrients are a few interactions between viruses and microbes. My research will infer interactions from viral and microbial population time series with the dynamic model of virus-microbe community (*Msystems*, Vol. 3, Pg. 1-14). For my research, I will use a combination of classical microbiological culturing techniques, microfluidics, single (cell and viral particle), genomics (*Environmental Microbiology*, 2020), and computational biology tools (machine learning).

The second research theme is the biocontrol of biofilms formed in the built environment. In water and wastewater treatment plants, microbial biofilm growth leads to the biofouling of membranes involved in the nutrient removal process and pipes' corrosion. The biofouling leads to a drop in flux across the membrane and loss in nutrient removal and recovery from the wastewater treatment plant's treated discharge waters. The biofouling necessitates frequent replacement of membranes, increasing the cost of operation and maintenance. The addition of a virus that infects the microbe that forms biofilms on the membrane's surface disrupts the biofilm. The disruption of biofilm increases the flux across (*Biotechnol Bioeng*, Vol. 8, Pg. 1644-1654). The virus-based biocontrol of biofilms on the built environment, especially water and wastewater treatment infrastructure, have industrial-scale applications. My research goal will be to investigate the microbial ecology of the multi-species biofilm and develop enrichment cultures. Test the enrichment cultures with viral lysate from several environments. Isolate viruses that infect each species. Develop bioprocess engineering parameters to control the biofilm outbreaks with the viral isolates. For my research, I will use a combination of classical microbiological culturing techniques, microfluidics, single (cell and viral particle), genomics (*Environmental Microbiology, 2020*), and computational biology tools (machine learning).

I plan to support my research works 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 of the areas mentioned above.

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

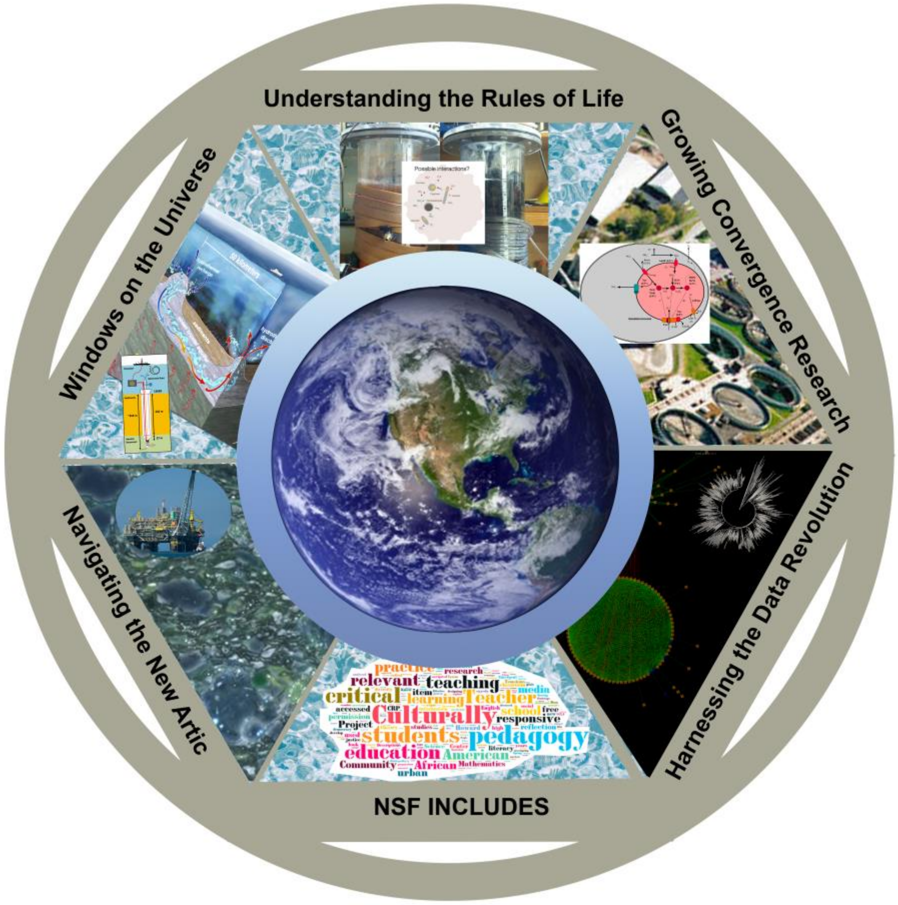

Figure 1: My research themes directly advance six of the National Science Foundation's top 10 ideas: (1) Understanding the Rule of Life- The environmental factors shape the microbial metropolis in natural and engineered ecosystems (image: suspended and attached anaerobic ammonia oxidizing (Anammox) bioreactors; 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 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 Artic- Increasing temperature in the Artic, 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 overlain on a black and white bright field image showing broad size range of fine quartz grains (light green to periwinkel blue) and pyrite (dark green) in New Albany shale rock; (6) Windows on the Universe- Virome and microbiome unearthed from ocean crust off the Juan *de* Fuca Ridge in the Pacific Northwest give scientists a window into ancient organisms that survived when the earth was without oxygen.