Water Matters:
Wastewater Treatment and Personnel Exposure to Aerosolized Pathogens

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Learning Objectives/Internship Objectives

Environmental Resource Technician:

- Familiarity with wastewater treatment processes, biodigestion, and capture of compressed natural gas (CNG)
- Familiarity with local, state, and federal environmental laws, plant permit parameters, and American Public Health Association (APHA) Standard Methods for the analyses performed
- General understanding of WRRF Laboratory operations, procedures, processes, and workflow
- Analysis of water samples from the treatment process as well as municipal and industrial samples from various locations in the GRWRRF service area for different factors to determine water content and treatment efficacy, to ensure environmental standards are met
- Analyses include total and volatile solids; total and volatile suspended solids; phosphorous, ammonia, and hexavalent chromium content; and chemical oxygen demand
- Complete training; pass Initial Documentation of Capability to perform work independently
- Calibrate, operate, and maintain equipment
- Follow daily testing agenda and record results in electronic laboratory information management system, XLIMS
- Collect samples and prepare reagents, standards, and test solutions as needed
- Track samples throughout testing process; note any unusual samples or results
- Perform mathematical calculations

Bioaerosols Project:

- Detailed current literature review: understanding the treatment process, methods, and best practices for aerosol capture and analysis, and common pathogens of concern in aerosols (specifically COVID)
- Devise and validate aerosol collection method, including equipment, materials, media, and volumes
- Determine sample collection dates/locations times and pathogens of interest
- Collect samples, concentrate (if needed), nucleic acid extraction, qPCR analysis
- Analyze data and report results

Introduction

Michigan enjoys a unique geographic situation, surrounded by four of the five freshwater Great Lakes, and is home to more than 11,000 inland lakes[1] and 51,000 miles of rivers and streams.[2,3] Rich in natural resources, Michigan is a regional and national treasure for outdoor recreation, drinking water, agriculture, energy, and industry.[4]

With great resources come great responsibilities. Population growth, human encroachment on natural environments, and global warming are among the threats to Michigan's ecology and, by extension, human health. It is critical that we protect and preserve these resources.

Central to that responsibility is water management, including the effective treatment of wastewater. The Grand Rapids Water Resource Recovery Facility (GRWRRF) serves ten communities covering 125 square miles and treats, on average, 40 million gallons of wastewater per day.[5] To ensure the plant is operating optimally, water is treated effectively, and final effluent meets or exceeds the facility's National Pollutant Discharge Elimination System (NPDES) permit standards, the laboratory at the GRWRRF conducts daily testing on plant influent, process samples, and final effluent. The laboratory also monitors influent content by testing municipal and industrial discharge samples. In the lab, Environmental Resource Technicians (ERTs) and Chemists carry out the daily testing at one of four workstations, under the direction of Laboratory Superintendent [redacted]. This internship involved learning and executing the work of an ERT, specifically workstations 1 and 2, described in more detail below.

In addition, research has shown that wastewater treatment plant workers face higher than average exposure to biological pathogens.[6–11] Some of this exposure is likely due to the aerosolization of pathogens during the wastewater treatment process, but further testing is needed to understand and mitigate risk. Therefore, inspired by the work of the Grand Valley State University (GVSU) Molecular Monitoring (MoM) and Beaches laboratories and my experiences there, and with the support of the GRWRRF, this internship also included an additional project designing an experiment to capture and analyze plant bioaerosols to understand WRRF personnel exposure. This project is also described in more detail below.

Description of Work

Environmental Resource Technician

The standards for water treatment facilities are set by the Environmental Protection Agency (EPA) and, in Michigan, are enforced by the Michigan Department of Environment, Great Lakes and Energy (EGLE), as set forth in the plant's National Pollutant Discharge Elimination (NPDES) permit.[12]

To meet and exceed permit requirements, ERT work includes analysis of samples from the treatment process as well as river, municipal, and industrial samples from numerous locations in the city and surrounding areas. More than 30 plant samples are tested each day. Other testing

categories, such as Industrial Pretreatment Program (IPP) samples, can add 8-10 additional samples to the daily testing agenda. Multiple tests are performed on each sample to determine water content and treatment efficacy.

Work began with training on plant organization and communication systems, lab operations and safety, the laboratory's electronic Laboratory Information Management System, XLIMS, data integrity, inventory, instruments, and quality assurance. A plant tour and written documentation introduced the details of the treatment process. I became familiar with local, state, and federal environmental laws, plant permit parameters, and Job Hazard Assessments (JHAs) for the analyses performed. I also gained an understanding of WRRF Laboratory organization, operations, procedures, processes, and workflow. Twenty-one additional training modules covering topics from personal protective equipment (PPE) and ergonomics to fire, heat stress, pathogens, and active shooter scenarios were completed via the city's NeoGov training platform. Finally, for my own and others' benefit, I compiled a list of the many acronyms needed to understand the "code language" of the GRWRRF lab.

Training for individual tests included shadowing several experienced ERTs and reviewing documentation including detailed Job Breakdown Sheets (JBSs) (standard operating procedures); Laboratory Testing Procedures (LTPs), which include thorough training and troubleshooting documentation; and American Public Health Association (APHA) Standard Methods. Test-specific training included the use and maintenance of desiccators, vacuum filtration units, drying ovens, 500°C furnaces, water baths, IDEXX Colilert-18 and Quanti-Trays, and the Hach Test-in-Tube (TNT) system. Standard laboratory equipment such as balances, pipettes, glassware, and QA/QC for each test was also covered.

As an ERT, my responsibilities included following the JBSs to execute the daily testing agenda. Results were recorded in XLIMS, lab management software from EthoSoft specific to the wastewater treatment industry and customized for GRWRRF. Within XLIMS, test results were recorded along with inventory tracking numbers for all consumables, instruments, and reagents used, and technician name, date, and analysis time. Any unusual samples or results were flagged for further review. Additional responsibilities included tracking samples throughout the testing process, calibrating, operating, and maintaining equipment, collecting samples, preparing standards, performing mathematical calculations, checking and entering new items into inventory, and cleaning the sample room. Glassware, pipettes, and sample containers are washed and reused to minimize waste, which requires unloading and reloading as many as four dishwashers, up to four times per day.

Laboratory tests performed include total and volatile solids; total and volatile suspended solids; phosphorous, ammonia, and hexavalent chromium content; dissolved oxygen levels; chemical oxygen demand; and fecal coliforms. After completing supervised training for each procedure, I passed an Initial Documentation of Capability (IDOC) test to perform the work independently. In addition to these procedures, I was able to observe additional tests for pH and biological oxygen demand (BOD), procedures for sample login and distribution to the correct testing areas, and the plant's water treatment and biodigestion processes and facilities.

Through this experience I became familiar with the wastewater treatment process and better able to appreciate the necessity and complexity of the work. I became an active and contributing member of the lab, able to perform and report the test results, troubleshoot, identify and correct errors, and relieve some of the workload for permanent staff members.

Bioaerosols

The bioaerosols project was inspired by the work of the GVSU Molecular Monitoring and Beaches labs and the WRRF, as well as published research into the potentially heightened exposure of wastewater treatment plant (WWTP) workers to pathogens. In this case we sought to understand the exposure of WRRF personnel to pathogens that may become airborne during the treatment process – specifically SARS-CoV-2, but with the possibility of expanding to other pathogens as well. The GRWRRF is an ideal site to undertake such a project, as it is one of Michigan's largest single-site treatment plants.

The final project plan included:

1. Detailed current literature review: understanding the wastewater treatment process, review methods and best practices for bioaerosol capture and analysis
2. Project plan/experimental design
    a. Devise bioaerosol collection method, including equipment, materials, media and volumes
    b. Design workflow and develop procedure SOPs
    c. Determine pathogen(s) of interest
    d. Develop budget
    e. Determine sample collection dates/locations/times
3. Materials and method validation
    a. Order and assemble materials and equipment
    b. Test equipment and procedures for bioaerosol capture
4. Execute experiment
    a. Collect samples, concentrate (if possible), nucleic acid extraction, qPCR
5. Analyze data and report results

A review of more than 40 scientific papers, industry publications and technical documents revealed intriguing possibilities and numerous potential complications. Most notably, no accepted standard method or best practice for bioaerosol capture or processing exists.[13,14] Numerous methods have been employed, depending on the testing environment and the collected pathogens.[7–9,14–18] However, efficiencies are unknown, and the data may lack accuracy and precision.[13,14] For the capture of bacteria and viruses, specific challenges exist with sampling adequate volumes of air, maintaining sample viability, and sufficiently concentrating the sample for PCR analysis.[13] Therefore, the first and primary challenge was to determine which collection method could be employed in the plant environment to capture bacterial and viral particles for a modest budget.

Early in project development, we were hopeful of a project partner in the Antrum company, which offers indoor air monitoring technology initially developed at GVSU.[19] Unfortunately, it became clear that Antrum's technology is for continuous-flow monitoring and would be

unsuitable for bioaerosol sampling. However, Antrum remains a potential partner for future collaborations.

A review of the literature revealed numerous methods for bioaerosol capture, including both passive and active methods (Fig. 1). The literature also indicated the necessity of sampling a large volume of air to collect detectable levels of microorganisms from a relatively low-concentration environment, such as outdoor air.[9,14,20] Specifically, several cubic meters of sampled air are recommended.[16,20] Additionally, in active sampling methods, the airflow rate needs to be high enough to sample a large volume of air efficiently, but low enough to avoid the harmful effects of shear forces, particle bounce/re-aerosolization, damage to particles as they impact the collection surface, and evaporation or desiccation of collection media.[14–16] An airflow rate between 50 – 200L/min. appears optimal.[15,16,20]

Passive sampling methods, such as swabbing or those that rely on particles settling onto a surface such as an agar plate, are simple to use, readily available, and inexpensive.[15,16] However, these methods can sample only a limited volume of air, are subject to desiccation affecting pathogen viability and nucleic acid integrity, and appear unsuitable for viral capture and therefore this project.[14–16]

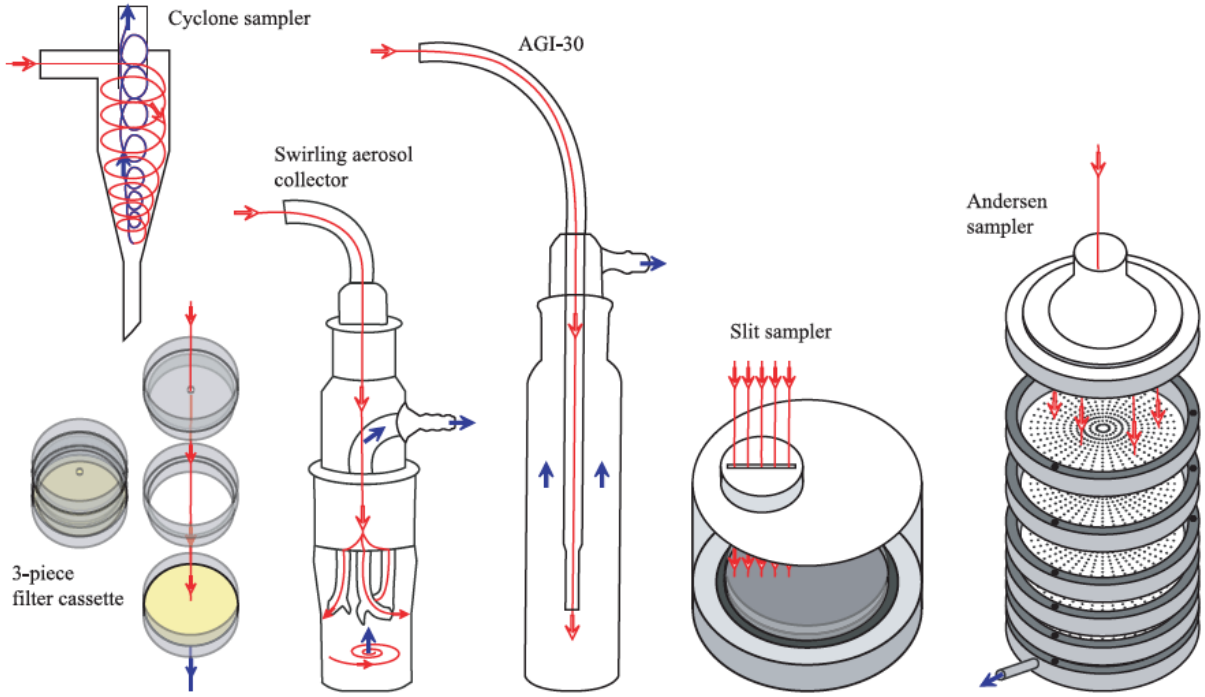

Figure 1. Passive and active bioaerosol sampling devices. Red lines: airflow in. Blue lines: airflow out. Verreault et al. 2008.

Active sampling methods include solid impactors, liquid impingers (sometimes called liquid impactors), liquid cyclones (similar to liquid impingers), forced-air filters, electrostatic precipitators, and condensation techniques.[9,13–16] These methods can sample larger volumes of air, may be able to separate particles by size and/or charge, and, as in the case of liquid methods, help maintain particle viability.[15,16] A liquid cyclone or impinger appeared to be the best method

for our purpose due to its ability to sample a large volume of air and comparatively gently deposit particles onto the surface of a relatively small volume of liquid such as phosphate-buffered saline (PBS), which is the recommended media for sample preservation.[9,15,20] The Coriolis Micro cyclone system from Bertin Technologies[21] stood out as a simple, effective, and complete system, but proved to be cost-prohibitive. That led us to the SKC biosampler, a glass device ("swirling aerosol collector" in Fig. 1) which accelerates the air as it moves through successively smaller channels before pushing it through three outlets angled toward a liquid surface. The air movement causes the liquid to swirl, collecting and capturing particles from the forced air while limiting particle deposits on the sides of the collection vessel.[15,16] Excess air is then released through the side of the device.[16,22] This device would be a cost-effective solution as it would allow us to use the Tygon® tubing and vacuum pump, a Welch 2567B-50 WOB-L, already in the lab's possession. This setup should enable us to sample three cubic meters of air by running the pump for 30 minutes at 100L/min. and collect the particles in 20mL PBS.

Lab Superintendent [redacted] was extremely helpful in devising a method to test the assembly by aerosolizing a water sample and collecting particles in the controlled environment of a biosafety hood (Fig. 2).

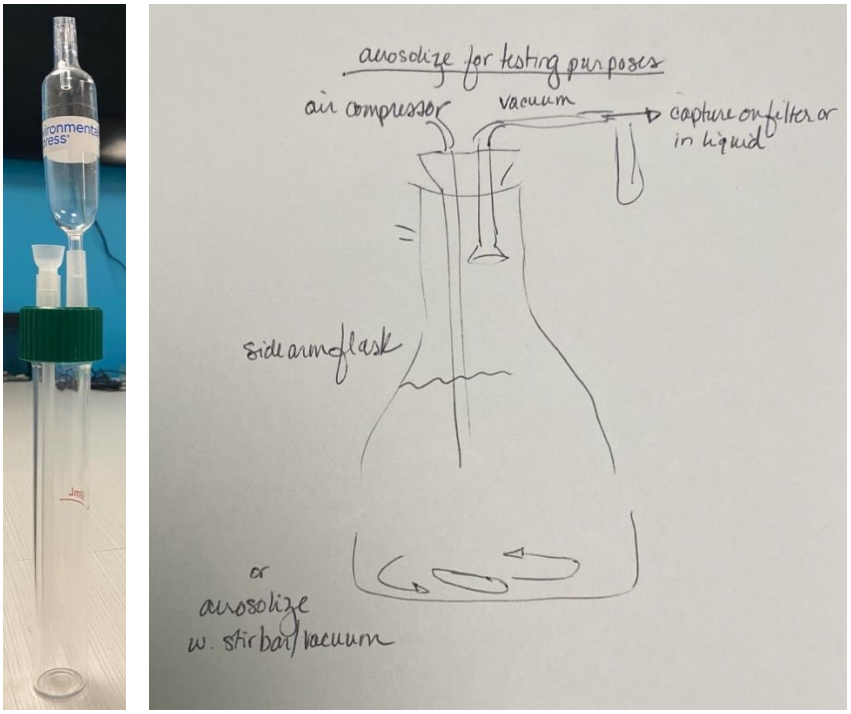

Figure 2. Bioaerosol collection method validation. Air forced into a flask containing a wastewater sample on a stir plate aerosolizes particles. The collection mechanism captures aerosols.

Next, sample collection sites were selected during a plant walk-through with [redacted]. A detail of the sample site map can be seen in Fig. 3. Eight sampling sites were chosen based on the likelihood of aerosolization due to mechanical agitation, swift flow/falling water, and/or active spraying. The presence of a nearby power supply was also a practical consideration.

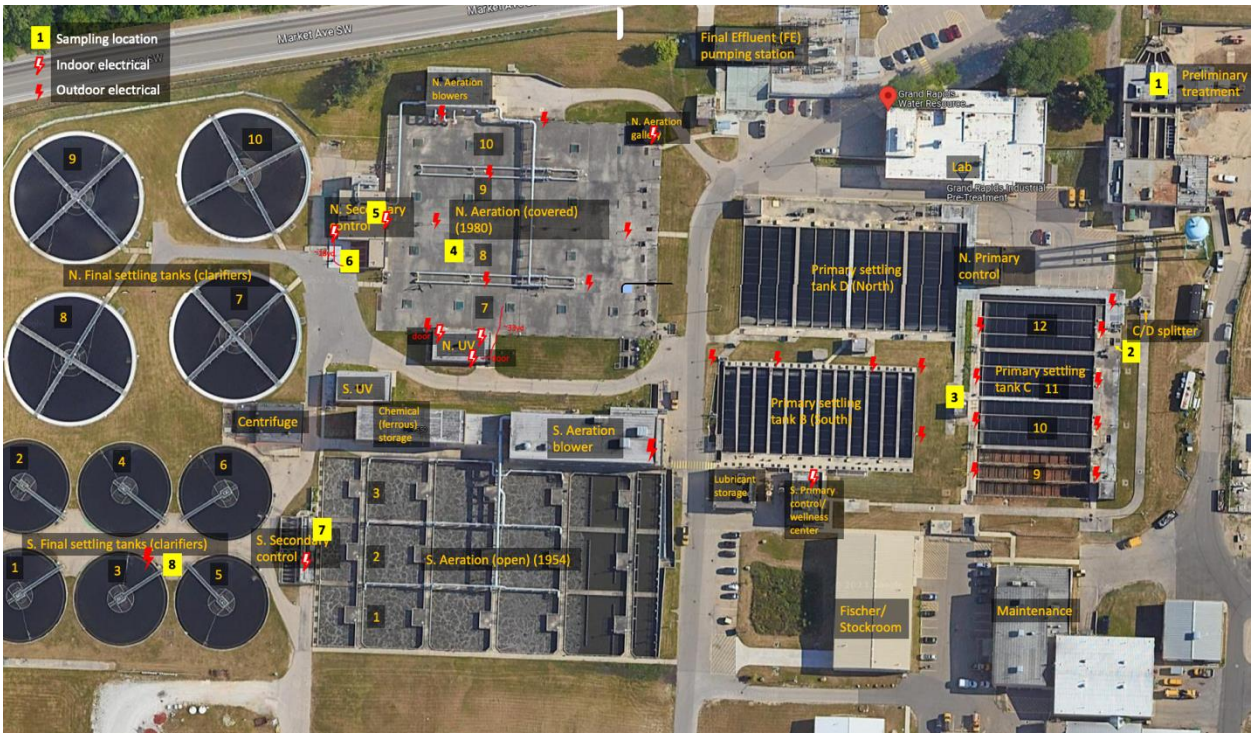

Figure 3. GRWRRF bioaerosol sample locations. 1. Preliminary treatment/bar screen (indoor, mechanical agitation) 2. Channel from preliminary treatment (outdoor, area of rapid flow) 3. Waterfall area from primary settling tank to channel that leads to aeration (turbulence, rapid flow) 4. Covered aeration tank – area of possible aerosolization due to strong current and aeration process, may concentrate particles to come out of grate 5. Waterfall area from aeration tank (turbulence, rapid flow) 6. Return activated sludge via open-air Archimedes screws (mechanical agitation) 7. Waterfall area from aeration tank (turbulence, rapid flow) 8. Spray onto clarifier surface

A workflow was laid out (Fig. 4) with assistance from [redacted] and Dr. [redacted], Primary Investigators in the MoM and Beaches laboratories. An early plan to use a Colilert-18 test for coliforms and *E. coli* as method validation was abandoned in favor of detection via qPCR. After sample collection, separate DNA and RNA extractions would be performed. For bacterial pathogens, DNA extraction would be done by filtering the sample, extracting material from the filter via the Qiagen PowerSoil kit, and concentrating via alcohol precipitation. RNA would be extracted via the Qiagen Viral RNA mini kit. Two different qPCR reactions would also be performed: the extracted DNA would be used to detect *E. coli* (EC23S) and *Bacteriodes* human markers (HF183/BacR287) as in the Beaches lab. EPA Method C and MST procedures would be modified and combined for this project. The extracted RNA would be used to detect PMMoV (Pepper Mild Mottle Virus) as in the MoM lab. These targets were selected for their known presence in wastewater, the ability to adapt familiar detection processes for this project, and the availability of the necessary materials and reagents. While these markers are not specifically pathogenic to humans, they represent the ability of bacteria and viruses to be aerosolized, and give insight into WRRF personnel exposure and the need for further testing. SARS-CoV-2 was not selected as a target due to its currently low levels in wastewater samples, making it unlikely to be detected in collected aerosols. Development of SOPs and qPCR plate maps also began at this time.

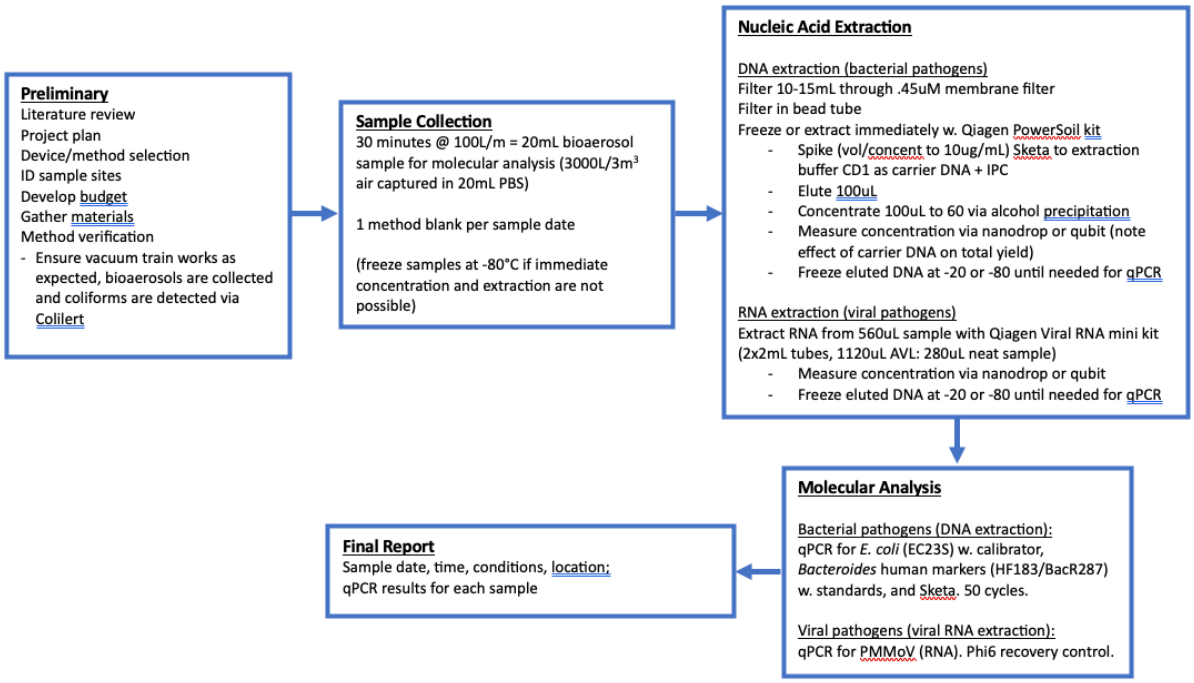

Figure 4. Bioaerosols project workflow.

Concurrently, a budget was developed to include one SKC Biosampler and materials and reagents needed for processing 24 samples: eight sample locations, two samples per location taken on different dates, at a rate of two samples and one blank per sample date, and two extractions per sample. Two samples and one blank appeared to be the daily limit based on sampling duration, the time needed to sterilize equipment between samples, and same-day nucleic acid extraction.

At this point (mid-October), the project was paused due to the late date. Bioaerosols are less likely to be captured in cool weather,[10] and it would take several more weeks to process purchases and receive supplies. Specifically, the SKC Biosampler would take three or more weeks to arrive. Given the calendar date and semester schedule, it appeared imprudent to expend finances and begin method validation and sample collection without assurance of completing the molecular analysis. While somewhat disappointing, in the overall course of the project we worked through unexpected challenges and put a solid plan together, which proved to be a tremendous learning experience.

Internship Discussion

During this internship, most, but not all, of the original objectives were achieved. As the project progressed, plans were adjusted, and additional needs were addressed as they arose. Specifically, as an ERT I learned the testing procedures associated with workstations 1 and 2, as intended. I was also able to train on additional tests for other workstations and observe additional tests for

pH and biological oxygen demand (BOD), procedures for sample login and distribution to the correct testing areas, and the plant's water treatment and biodigestion processes and facilities.

For the bioaerosols project, I quickly learned that what I imagined would be a simple matter of capturing aerosols was quite complex. The first two parts of the project, literature review and method selection, took considerably longer than anticipated, as there was much to learn and no accepted method to follow. The benefits and drawbacks of different devices and methods had to be reviewed and understood to select one that might produce the best result in this situation. Workflow and SOP development were needed because existing processes developed for water sampling had to be adapted to samples with a lower concentration of microorganisms. Sample concentration methods such as filtering, tangential flow, micro concentrators, and alcohol precipitation were reviewed and considered. The workflow and sampling program had to be well defined in order to develop a detailed, accurate budget. Budget development required diving into scientific pricing and sourcing, which was new to me but ultimately a helpful exercise. A budget was then developed after determining what items/quantities were needed for each step of the process. While I initially assumed the sampling, testing, and data analysis would be the majority of the project, it now seems this would be the “easy” part as they are much more familiar to me. The research, organization, and planning became the central part of the project. Having worked through it, I now have a much better appreciation for what goes into setting up a project.

I have benefitted greatly from this internship in many ways. In addition to the technical learning covered above, seeing the internal operations of an extremely well-organized and well-run lab will prove valuable long into the future. Specifically, seeing the format for very well-written and organized SOPs and other documentation, learning inventory management, seeing the value of clearly defined task lists and expectations, and a lab organized so that technicians have all the materials and equipment they need – and nothing else – at their fingertips to perform work effectively and efficiently, are things that I will be able to carry forward. Protocols have been developed for managing workload when personnel are unavailable or there is a holiday, ensuring critical functions are always covered. Additionally, the GRWRRF laboratory XLIMS system introduced me to electronic lab management. Finally, as I have always worked on a Mac platform, my city-issued PC allowed me to become more comfortable with an unfamiliar operating environment.

The PSM CMB coursework was also excellent preparation for much of the professional content of the internship. However, additional training in project planning, scheduling, budgeting, purchasing, and lab management would also be beneficial.

The challenges encountered are those typical of working in a new environment and learning or designing new processes and procedures. For example, as an ERT, simply learning my way around the lab, where things belong, and how to restock when things run out took some time. As a technician, it is frustrating when tests fail, especially when the cause is my own error, and naturally that did happen. The solution is to keep practicing, ask questions, take good notes – and always follow the SOP.

The most significant challenge with bioaerosols was figuring out how to collect them, which took longer than anticipated. Project planning, budgeting, quoting and sourcing challenges have

also been noted. However, from my previous work experience, I am well aware that things rarely go as planned and are never as straightforward as they seem, so these things were not particularly bothersome. Reading, studying, and seeking the advice of more experienced scientists enabled me to work through these issues.

Overall, I had a truly excellent experience with this internship. I was pleased to find a position where I could expand on my water quality work and increase my knowledge of water quality issues and solutions. I thoroughly enjoyed working with a very welcoming and helpful group, learning the treatment and testing processes, and seeing the systems in place in a well-run and efficient lab. The degree of planning for every contingency, from testing issues to days when fewer staff members are available, left a strong impression. The camaraderie and cooperation among the lab staff and throughout the plant were wonderful to see. A strong sense of mutual respect, shared responsibility, and an attitude of "I've got your back and you've got mine" is refreshing and encouraging. All of the plant staff I encountered recognize the importance of their work, leaving me with great confidence in the quality of Grand Rapids' water systems.

I would like to close with some thank yous and acknowledgments. First and foremost, I thank [redacted] for responding to my initial inquiry and for her support and encouragement in establishing and throughout this project. I also appreciate the support of additional Grand Rapids Environmental Services leadership, specifically [redacted]. Special recognition must be given to the excellent, patient, and accommodating ERT team and Chemists at the GRWWRF: [redacted]. Who knew that working with sewage could be fun? For their ideas, insight, encouragement, and responses to my many questions, [redacted]. Without them, this project would never have been conceived. Finally, to [redacted] for their willing and cheerful assistance with many "small" things.

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