GRO Summer Internship Final Report

Microbiological Treatment of Drinking Water and Arsenic Removal

Matthew Tancos
Ball State University, Muncie, IN

This summer I had the privilege to conduct research at the National Risk Management Research Laboratory's Treatment Technology Evaluation Branch (TTEB) of the Environmental Protection Agency under the guidance of Branch Chief, Dr. Darren Lytle, in Cincinnati, OH. My research focused on the quantitative detection of arsenite oxidase genes in microbial communities that inhabit water treatment plant filters. The overall goal of the research was to determine the significance of bacteria in the removal of arsenic from water distribution systems, and their future applications in the microbiological treatment of drinking water. Currently, microbial treatment of drinking water is not employed in the United States due to the stigma associated with bacterial contamination and the paradox it presents with the removal of bacteria from the same distribution systems. However, biologically active filters would dramatically lower costs, reduce chemical dependence, and attenuate the toxic compounds that permeate our water supply, such as arsenic.

Arsenic enters the hydrologic cycle via natural leaching from sediments, mining, industry, and agriculture and speciates into two toxic forms, arsenite [As (III)] and arsenate [As (V)]. Strong chemical oxidants (permanganate and chlorine) need to be added, which transform arsenite to the less mobile arsenate. As a result, the arsenate coalesces with iron oxyhydroxides and clay minerals to form this iron/arsenic matrix that can be removed with conventional iron-removing techniques.

This research design started with the Greene County water treatment plant in Dayton, OH, which had unusually low amounts of arsenic in their treated water. This proved to be enigmatic because the pre-treated water had normal amounts of arsenic; however, no strong oxidizing chemicals were added. Research concluded that microbial processes were primarily responsible for the oxidation of arsenite in the drinking water.

My objective was to develop a Real-Time PCR (polymerase chain reaction) protocol that would quantify the number of bacteria in the filters. Therefore, the technique would allow arsenic-oxidizing bacteria to act as indicators by demonstrating what a healthy population is and its sensitivity to water chemistry. In the future, these applications will be vital when trying to determine what could be hindering microbial growth and if arsenic oxidizers are present in the filters.

New degenerate oligonucleotide primers were designed to hybridize with the arsenite gene. This proved to be the most tedious aspect of the research because the arsenite-oxidase gene sequence is not highly conserved between different species of bacteria and archaea (a specific group of microorganisms). As a result, the most homologous (put simply, similar) protein sequences had to be aligned in order to find conserved regions. Primers were then designed to amplify a product between those two conserved regions, producing a 133 base pair amplicon (DNA fragment).

Time constraints prevented the project from being completely finished; however, only a few experiments remain. Hopefully, one of the Master's degree students with whom I was working will be able to complete the remaining experiments, and a paper will be published on our work.

My past research with PCR and designing primers was used greatly, and significantly helped the project get off to a quick start. Having past lab experience, computer skills, and the ability to

work with others proved to be invaluable. However, through the research, I developed new skills such as troubleshooting, designing projects, and utilizing new technology. For instance, I gained experience in molecular techniques such as cloning and DGGE (denaturing gradient gel electrophoresis). Working with such a multi-disciplinary group of people granted me knowledge in water chemistry, engineering, and microbiology. Therefore, I was not submerged in only my work, but worked with others and participated in their research. This open mindset actually allowed me to work on another project that involved characterizing a microbial bioflim in a corroded lead drinking water pipe. This experiment was even more exciting because this type of research was never performed before. I could have easily overlooked this opportunity, which allowed me to expand my knowledge on a new subject and become a co-author on a publication.

The summer experience strengthened my desire to pursue my degree in genetics and biology and reaffirmed my career choice as a biologist. Internships are fantastic for that reason, because you quickly realize whether or not you could see yourself doing this as a vocation. This experience will certainly increase my credentials for prospective graduate schools since it demonstrated my commitment to the field of biology. Working in a Federal research facility and helping to fulfill the EPA mission made the GRO Fellowship even more rewarding. Not only could the research help millions of Americans and make drinking water safer, but we also pioneered a new green technology that could reduce dependence on certain chemicals.

Working with the EPA over the summer was an excellent experience that allowed me to work with a group of talented individuals and broaden my horizons. The atmosphere of the EPA lab was always welcoming, which allowed an easy transition. The experience was invaluable because I developed contacts, increased my laboratory skills, and strengthened my career choice. For future GRO Fellows, I would recommend stepping outside of your comfort zones and trying new things. The internship experience is meant to reaffirm your field of study or allow you to realize its shortcomings. Either way, getting involved in more activities besides your own only makes the experience more worthwhile.