TEAM SPEAR

SOLDIERS POLYFLUOROALKYL SUBSTANCES ENZYMATIC ACTION AND REMEDIATION

CDT Amber Matlach[1], CDT Isabelle Stoldt[1], and CDT Kinady Pierce[1]
Advisors: Dr. Beth Bachert[1], LTC Samuel Cowart[1], Dr. Simuck Yuk[1], Dr. Enoch Nagelli[1], and Dr. Charlie Sweet[2]
[1]Department of Chemistry and Life Science, United States Military Academy, West Point, New York 10996, USA
[2]Department of Chemistry, United States Naval Academy, Annapolis, MD 21402

BACKGROUND & COMPETITION RELEVANCY

Polyfluoroalkyl Substances (PFAS) are "forever" chemicals found in many Army products known to contaminate water sources and present a significant challenge to remove from the environment. Certain psychrophiles, cold-adapted microorganisms, have shown potential in the bioremediation of PFAS, though little is known about the mechanisms of the enzymes involved. Our research aims to isolate, characterize, and use these microorganisms as production factories for PFAS-degrading enzymes. To ensure we maintain a lethal fighting force overseas, we must first protect the warfighter at home. Through December 31, 2024, the DoD has determined that 723 active military installations require an assessment of per- and polyfluoroalkyl substances (PFAS) use or potential release (1). The military uses PFAS for several different training functions, and prolonged exposure to PFAS leads to serious health problems and disabilities in soldiers. As of recent, the U.S. Department of Veterans Affairs granted disability to soldiers who were exposed to per- and polyfluoroalkyl substances on military installations (2). This work will uncover novel capabilities in these microbes that the DoD may harness for bioremediation and biomanufacturing purposes. Our ultimate goal is to develop a solution that eliminates the threat of PFAS exposure to soldiers, enabling our military to maintain the most effective fighting force possible. The project is relevant because it focuses on an innovative STEM solution to a problem relevant to warfighters. The work aligns with addressing environmental and health challenges faced by the military, with the potential for significant operational impact.

PROJECT DESIGN

PHASE I: Sample collection and culture-based isolation of candidate microorganisms for bioremediation

Sample collection from sites across the Chesapeake Bay watershed in the winter and summer

Culture-based isolation followed by 16S rRNA sequencing for microbe identification

Rationale: The Chesapeake Bay watershed has been shown to contain high levels of various PFAS substances, suggesting microbes that thrive in these environments have likely adapted mechanisms to withstand and/ or degrade PFAS.

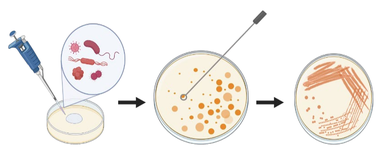


Image Source: Biorender

PHASE II: Evaluation of candidate psychrophiles for PFAS bioremediation

PFAS toxicity screening

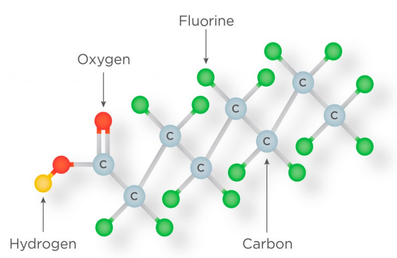
Positive growth

No growth/ weak growth

PFAS degradation assay

≥50% degradation

<50% degradation



PFAS Molecule: Science for Georgia

Whole genome sequencing

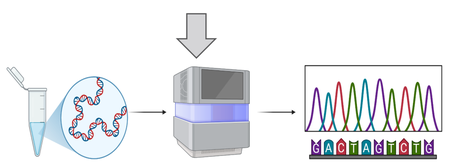
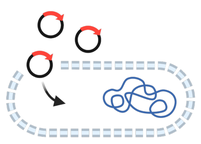


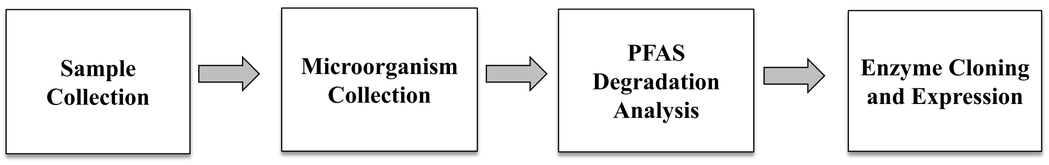
Image Source: Biorender

Cloning and expression of candidate enzymes



- *Image Source: Biorender*

INNOVATION



Conventional PFAS removal methods (such as incineration, UV lasers, and filtration) are costly and energy-intensive. They often generate harmful byproducts without fully destroying PFAS. Our approach utilizes microbes to enzymatically break down PFAS, offering a cleaner, low-energy solution that eliminates the compounds at the source.

RESULTS

Figure 1: Proposed sampling locations in Susquehanna River watershed. An initial exploration of the Susquehanna watershed (yellow highlight in the image) will use nine sampling sites SQ1-SQ9 covering the length of the river, including its multiple headwaters, riverine main stem, and tidal endmember/mouth.

Image Source: USDA

Proposed sampling sites:

SQ1 – North Branch headwater
SQ2 – North Branch main stem 1
SQ3 – North Branch main stem 2
SQ4 – West Branch headwater
SQ5 – West Branch main stem
SQ6 – Susquehanna PA main stem
SQ7 – Maryland feeder stream
SQ8 – Susquehanna MD tidal endmember
SQ9 – Mouth (Havre de Grace, MD)

Table 1. Stocked Bacterial Samples from Susquehanna River Water Samples

	Growth media and conditions‡								
	37°C (~48 hrs growth)			25°C (~72 hrs growth)			4°C (~4 weeks growth)		
Sample†	MG	MA	RA	MG	MA	RA	MG	MA	RA
SQ1-S	4	3	4	6	13	13	22	13	1
SQ1-W	---	22	32	---	16	39	---	22	32
SQ2-S	2	6	5	13	17	12	26	14	25
SQ2-W	---	29	29	---	24	31	---	29	29
SQ3-S	3	3	6	8	8	13	18	21	18
SQ3-W	---	19	36	---	19	29	---	19	36

†For each sample, 10 uL (25°C, 37°C) or 100 uL (4°C) of water was plated on each type of media and incubated at each temperature for the indicated times. After initial plate growth, single colonies were isolated and restreaked at least three successive times and stored long-term in a glycerol stock. S and W represent summer (2024) and winter (2025) samples.
‡Growth media tested were MG: Marine 2216 saltwater medium supplemented with gellan gum in place of standard agar, MA: Marine 2216 agar, RA: Reasoner's 2 Agar. MG was not used for the winter samples

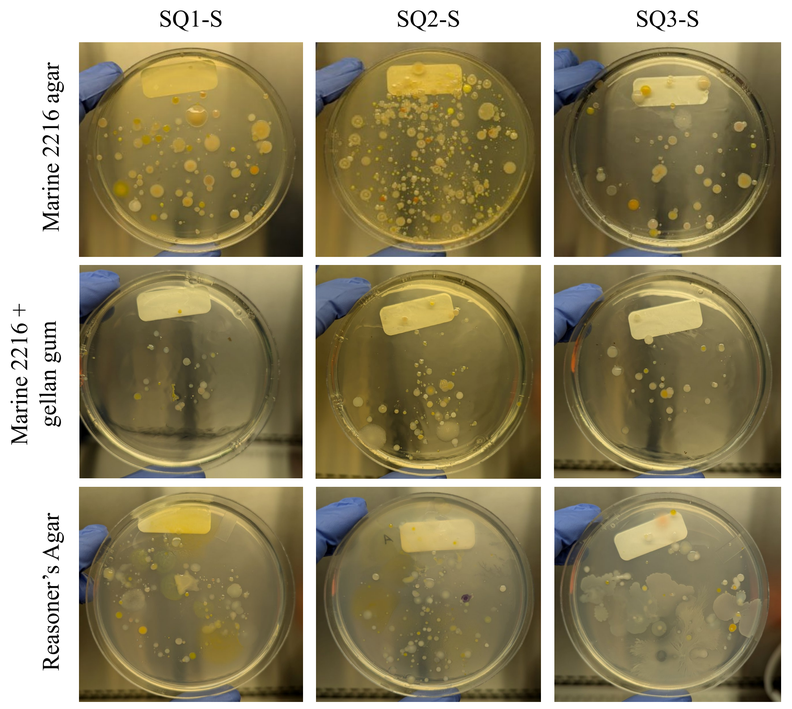


Figure 2. Representative photographs of plates from Summer SQ sites 1-3. 100 uL of water was spread across each plate and incubated at 4°C for approximately 4 weeks. Replicate plates (not shown) had similar colony counts and colony morphologies. Single colonies were picked from each plate to capture the overall phenotypic diversity of colonies, and restreaked for isolation 3 additional times. Glycerol stocks for each isolate were made and frozen at -80C for further characterization and sequencing.

IMPACT & CONCLUSION

IMPACT: Our Research on PFAS levels directly impacts the warfighter by addressing potential health risks that compromise the optimal ability of soldiers in the field. PFAS exposure has been linked to a list of long-term health effects, which reduce soldier readiness and effectiveness. This project aligns with the Army's mission by defining the gap in environmental and health safety, ensuring soldiers remain fit for duty. The broader impact of this work extends beyond environmental cleanup. It's about improving the health and safety of personnel on military bases, while also contributing to innovations in decontamination, detection technologies, and medical intervention strategies. The goal is clear: enhance soldier readiness and operational sustainability through cutting-edge biotechnological solutions.

CONCLUSION: Perfluoroalkyl substances pose a significant threat to those in contaminated locations. Treatment for this PFAS is expensive and can result in harmful byproducts. The potential use of enzymatic degradation for this material would provide a cost-effective and sustainable solution to this problem. To maximize the likelihood of identifying highly effective PFAS-degrading bacteria, we have collected over 700 bacterial isolates in the last nine months, which we will begin to move through our selection process when we gain access to additional resources. Beyond this project, the application of successful biodegradation processes will have enormous impacts on how the Army addresses all future chemical and biological challenges.

FOR FURTHER INFORMATION:

U.S. ARMY COMBAT CAPABILITIES DEVELOPMENT COMMAND ARMY RESEARCH LABORATORY:
WWW.ARL.DEVCOM.ARMY.MIL

POINT OF CONTACT:
Dr. Beth Bachert
beth.bachert@westpoint.edu
(845) 938 5814

REFERENCES:
(1) *ASD(EI&E) - Per- and Polyfluoroalkyl Substances (PFAS)*. www.acq.osd.mil. https://www.acq.osd.mil/eie/eer/ecc/pfas/data/cleanup-pfas.html.
(2) US Department of Veterans Affairs, Veterans Health Administration. *PFAS - Perfluoroalkyl and polyfluoroalkyl substances - Public Health*. Va.gov. https://www.publichealth.va.gov/exposures/pfas.asp.

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