# Mycoremediation of Naphthenic Acid Fraction Compounds Sourced from Oil Sands Process Affected Water

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Extraction of bitumen from Alberta's oil sands requires large volumes of water, leading to an abundance of oil sands process-affected water (OSPW) that must be remediated prior to discharge or reuse. OSPW contains a variety of toxic compounds, including polyaromatic hydrocarbons, benzene, toluene, ethylbenzene, xylenes (BTEX), and naphthenic acid fraction compounds (NAFCs). NAFCs are known to be one of the major recalcitrant contaminants in OSPW, and a significant contributor to toxicity, which poses a threat to the environment. With the ample presence of organic compounds, microorganisms are active in tailings ponds. It has been established that NAFCs can be removed from OSPW via microbial degradation under aerobic conditions thereby decreasing the toxicity of OSPW. Comprehensive genomic studies of the native microbial community have revealed substantial eukaryotic diversity present within OSPW, with abundant fungal presence. Ongoing efforts into a cost and energy efficient remediation strategy of NAFCs have focused on bioremediation, however, limited research has been conducted on the use of mycoremediation in the treatment of NAs. The objective is to characterize the capacity for NAFC degradation of the fungal isolate *Trichoderma Harzianum*, and to harness this process for surface OSPW mycoremediation.

Through selective enrichment of OSPW, a microbial community enriched in NAFC-degraders was established, and several microbial isolates were identified including for the first time a fungal isolate ***T. harzianum***. This study conducted microcosm experiments to elucidate and characterize the capacity of *T. harzianum* to degrade labile commercial NAFCs (Merichem) and OSPW sourced NAFCs. Additionally, two model NAFC compounds, a simple single ring cyclohexane carboxylic acid (CHCA) and complex diamondoid 1-adamantane carboxylic acid (ADA) were utilized to determine the influence of organic structure on degradation. ***T. harzianum*** degraded 14% of CHCA, 13% of ADA, and 23-47% of Merichem NAFCs.

An important finding of this study was that a fungi isolate was able to degrade a portion of ADA, which was previously thought to be recalcitrant to biodegradation, at a faster rate than the more labile CHCA. Orbitrap-MS revealed shifts in the chemical profiles of Merichem and OSPW sourced NAFCs especially compounds containing ≥17 carbons, that led to a 59% and 52% decrease in toxicity over the course of the experiment as per Microtox™ bioassay, respectively.

Overall, results indicate that *T. harzianum* may be an ideal addition to a microbial community, with synergistic relationships where metabolites are consumed, leading to more comprehensive NAFC removal. However, treatment technologies and applications of ***T. harzianum*** require further research, such as elucidating the degradation pathways for model compounds CHCA and ADA, to improve understanding of how fungi can support the remediation of OSPW.

## Sarah Miles

Sarah joined Geosyntec in 2021 as a senior staff scientist after earning her Doctorate in Environmental Engineering from the University of Alberta and B.Sc. in Earth Science at Memorial University of Newfoundland. During her PhD, she completed extensive work on oil sands tailings remediation projects, specifically on treatment of the naphthenic acid fractions within oil sands tailings water. With a focus on potential bioremediation strategies, her projects used microbial isolates, the native microbial community and chemical oxidation multistep treatments to degrade this target organic fraction. Sarah also completed an undergraduate research project that focused on potential carbon monoxide utilization pathways by microbial communities found at ultra-basic environments through stable isotope probing. Sarah now focuses at Geosyntec on remediation of hydrocarbons at contaminated sites, with a keen interest in R&D projects.