

Title: Methanotrophic Bacteria in an Oilsands End-Pit Lake, and their Potential Contribution to Naphthenic Acid Degradation

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Summary

Surface mining operations of the Alberta oil sands produce fluid fine tailings (FFT) during the bitumen extraction process. The FFT are deposited into large engineered areas known as tailings ponds. Naphthenic acids (NA) are a major component of concern in the oil sands process-affected water (OSPW) in tailings ponds. They have been identified as primary source of acute aquatic toxicity of the waste. The persistence of NA in tailings thus represents a major challenge to the detoxification of OSPW and reclamation of mining impacted areas. To date, microbial attenuation has been proven to be a feasible and cost-effective NA bioremediation approach for OSPW. However, the diversity of NA complicates this effort as implicated microorganisms only target a specific or minor subset of NA. As a novel approach, the potential exists for aerobic methanotrophic bacteria to non-specifically biodegrade or biotransform NA. Methanotrophs utilize methane as sole source of carbon and energy, harboring the promiscuous methane monooxygenase enzyme (MMO) that has been shown to cometabolize a variety of hydrocarbon or organic pollutants. This they achieve by hydroxylating the terminal methyl or sub-terminal alkyl groups of their structure, as such oxidizing many organic compounds. If MMO could oxidize NA, this would thus help to mitigate OSPW toxicity.

Based on our 16S data from BML community analysis, the predominant methane oxidizing bacteria are *Methylobacter spp.* and *Methylocaldum spp.* With an abundance of methane efflux in tailings ponds, our hypothesis is that methanotrophic bacteria will play a role in the co-oxidation of NA and similar pollutants found in tailings ponds. We have investigated NA biodegradation in experimental microcosms using indigenous OSPW methanotrophs, we conducted both routine chemical analytics (GC, GC/MS) and molecular (16S rRNA gene sequencing) to profile the active microbial community and quantify NA loss over time. OSPW were obtained from Base Mine Lake (BML), the first full-scale end-pit lake for the Canadian oilsands industry and compared them with communities from an active tailings pond, Mildred Lake Setting Basin (MLSB), as well as with communities from a more natural freshwater body, Beaver Creek Reservoir (BCR). The microbial communities were assessed using high-throughput Illumina sequencing of 16S rRNA gene amplicons for bacterial communities. The species diversity of bacteria shows a mixture of some species typical for oilsands tailings ponds and some typical of natural waters.

In evaluated NA co-oxidation, microcosms with and without an active methane oxidation activity and a sterile control showed very different rates of surrogate NA biodegradation, despite similar microbial community structure. Of all three experimental set ups, systems with active methane oxidation were the most effective in the complete biodegradation of the tested NA. This preliminary observation supports the role of methanotrophs in NA degradation and hence can



play a role in the detoxification of OSPW. Efforts are ongoing to further elucidate the mechanism of methanotrophic NA bioconversion.