Discovery and Tracking of a Novel Sulfolane-Degrading Bacterium through Laboratory and Field Studies

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Sulfolane is a constituent of concern at former oil and gas sites where is used in the Sulfinol process for sweetening gas. Sulfolane is an emerging contaminant with chemical properties that promote transport in groundwater, including high solubility, low sorption potential and low volatility. While biodegradation of sulfolane under aerobic conditions has been demonstrated in laboratory and field- scale studies, it is critical to establish the site-specific intrinsic capacity for sulfolane biodegradation and to evaluate remedial strategies to enhance sulfolane biodegradation, for full-scale remedial design.

A former gas plant was piloted for enhanced attenuation via biosparging to increase the aerobic biodegradation of sulfolane in groundwater. Biosparging amended the groundwater with oxygen to enhance the growth of sulfolane-degrading microorganisms and increase sulfolane degradation rates. Field-scale biosparging resulted in sulfolane biodegradation to below criteria. To confirm and understand the observed decreases in sulfolane, multiple lines of evidence were employed, including traditional site data, culture- based techniques, and molecular biological tools to identify and assess enhancement of sulfolane-degraders.

To propagate sulfolane degraders, a defined microbial growth media containing sulfolane was inoculated with site groundwater. Next generation sequencing (NGS) was used to characterize the culture's microbial community and to assess changes due to varying sulfolane concentration. To isolate putative sulfolane degraders, the culture was plated on a differential media and colonies of a putative *Rhodococcus* were identified. *Rhodococcus* has not previously been reported to degrade sulfolane but is a known degrader of phenolic compounds and polycyclic aromatic hydrocarbons. The 16S rRNA sequence of the isolate was used to develop a quantitative polymerase chain reaction (qPCR) test used to quantify this microbe in the culture and in site groundwater.

Assessment of the site groundwater microbial community by qPCR indicated increases in total microbial abundance by over 100-fold in response to biosparging, while NGS indicated a shift to a more aerobic microbial community. The putative *Rhodococcus* isolate was tracked via the developed qPCR test and its abundance was positively correlated of with the presence of sulfolane and biosparging.

In summary, we have developed an enrichment culture capable of degrading sulfolane, identified a novel putative sulfolane-degrader, developed and demonstrated a qPCR assay for in situ tracking of this microbe and corelated it's abundance with zones with high sulfolane degradation rates. Ongoing work to sequence the genome of the novel isolate as well as tests to identify genes involved in the degradation of sulfolane will be discussed.

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Linda Eastcott is a Senior Technical Advisor with Imperial Oil where she has worked for 20 years. Linda holds a Master of Applied Science in Chemical Engineering from the University of Toronto. She has over 30 years of experience in the field of contaminated site assessment and remediation across Canada. Major projects have included the evaluation of remediation options, design and implementation of in-situ remedial systems as well as several large excavation projects involving multiple stakeholders. Linda currently serves as a Technical Advisor to the Environmental & Property Solutions group, supports remedial system technology applications, leads a research focus area on upstream related issues, and co- chairs the Reclamation Remediation Research Committee of PTAC.

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