

Discovery, and Characterization of a Novel Sulfolane-Degrading Microbe at a Former Gas Plant Site

Philip Dennis, SiREM

Trent Key, ExxonMobil Biomedical Sciences, Inc

Courtney Toth and Elizabeth Edwards, University of Toronto

Matthew Schoeppic, Imperial Oil

Sulfolane is a constituent of concern at former oil and gas sites where it is used in the Sulfinol process for sweetening gas. 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.

At a former gas plant, field-scale biosparging resulted in sulfolane biodegradation to below criteria. To confirm and understand the observed decreases in sulfolane concentrations, multiple lines of evidence were employed, including traditional site data combined with culture-based techniques, and molecular biological tools to enrich, identify and assess microbial sulfolane-degraders.

A multi-year study, with the goal of characterizing sulfolane degraders in site groundwater, included propagating sulfolane degraders from groundwater on a microbial growth media containing sulfolane, and 16S rRNA amplicon sequencing to characterize the culture's microbial community, and to assess changes due to varying sulfolane concentrations. Culture based methods were used to isolate putative sulfolane degraders, and genomics and proteomics were used in biomarker discovery, specifically identifying genes and proteins integral to sulfolane degradation. An enrichment culture (SC25) capable of degrading sulfolane with half-lives of 3.5 hours was developed from site groundwater. 16SrRNA gene amplicon sequencing and quantitative polymerase chain reaction (qPCR) indicated a novel *Rhodococcus*, designated as *Rhodococcus* sp. strain SC25, as the dominant microbe in the enrichment culture and its abundance was correlated with sulfolane biodegradation. Multiple lines of evidence suggest that *Rhodococcus* sp. strain SC25 represents a novel aerobic sulfolane degrader belonging to the phylum Actinomycetota, differentiating it from other reported sulfolane degraders which belong to the Proteobacteria

A putative novel sulfolane monooxygenase (SMO) gene associated with the novel *Rhodococcus*, was identified using proteomics and genomics. The novel SMO was used to develop a quantitative PCR (qPCR) test that was applied to groundwater samples at the former gas plant site. The qPCR test results indicated that the SMO was most prevalent in the source area and the biosparge zone, which had higher sulfolane concentrations, and was less prevalent in areas near the plume fringe where sulfolane concentrations were lower. These data suggest that the novel *Rhodococcus* harbours the SMO and may be involved in sulfolane degradation at the site.

In summary, we have developed a media- based culture capable of degrading sulfolane, identified a novel putative sulfolane-degrader and identified putative genes and enzymes involved in the sulfolane degradation process which are promising biomarkers to track sulfolane biodegradation potential in situ.

Phil Dennis

Phil Dennis is a Senior Principal Scientist at SiREM. Phil holds a M.A.Sc., in Civil Engineering from the University of Toronto and an Honours B.Sc., Molecular Biology and Genetics from the University of Guelph. Phil has over 30 years of experience in research and development and management of molecular biology, microbiology, and environmental remediation laboratories. Phil is a founding member of SiREM, and currently directs molecular genetic testing services and is innovation lead for SiREM's research and development program contributing to multiple ongoing research and development projects.

