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Development of a Monooxygenase Gene-based assay to characterise 1,4-dioxane bioremediation potential

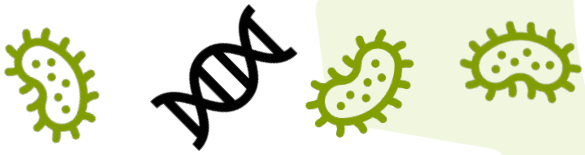
"This new method provides a way of characterising the diversity of SDIMO genes present in samples from contaminated sites. Although, the focus of this study was the biodegradation of 1,4-dioxane, SDIMO enzymes are known to be involved in the breakdown of a wide range of contaminants, so this could be a valuable tool in many other areas in the contaminated land sector".
Dr Simon Gregory, British Geological Survey

THE PROBLEM

1,4-Dioxane is an 'emerging contaminant', which is hazardous to the environment and human health. Its high solubility, and low degradability under some conditions, means it can be a challenge to remediate. It is known that it can be degraded by some types of monooxygenase enzymes. To better understand the potential for bioremediation at different sites, more information is required on which enzymes in this broad family of monooxygenases are most important in biodegradation, and under what conditions.

APPROACH

We used a qPCR and DNA sequencing approach to tackle this. Existing literature was reviewed to identify key monooxygenase enzymes thought to be important in bioremediation of 1,4-dioxane, and existing PCR primers sets were aligned to gene sequences in available databases to select those with optimal coverage and specificity. Once optimized, these were tested on samples from sites with 1,4-dioxane contamination. A DNA sequencing assay was also developed which provides information about the diversity of monooxygenase genes and identifies additional monooxygenase targets. These datasets were combined with physico-chemical data from the samples to evaluate the optimal conditions for bioremediation.



RESULTS

This project focussed on a tool to characterise the diversity of SDIMO genes present in samples from contaminated land sites. This gene family consists of six groups, of which Groups 5 and 6 are particularly associated with 1,4-dioxane degradation. A bespoke DNA sequencing assay was developed using low-cost Oxford Nanopore Flongle sequencing platform after PCR amplification. A small database was used with a bespoke bioinformatics pipeline to assign each sequence to a SDIMO group. This new assay and analysis pipeline were applied to groundwater samples from several contaminated sites.

The current assay is capable of identifying the proportion of SDIMO genes in each of the recognised families. Additional work on the analytical pipeline will further improve classification of the SDIMOs, potentially identifying known and previously undiscovered microbial genera/species that can degrade 1,4 dioxane and other emerging contaminants. Additional field validation will be required to raise the TRL before it is applied commercially. This could lead to more efficient remediation approaches and technologies.

REFERENCES:
Expanding the 1,4-dioxane remediation arsenal
By Caitlin Bell and Monica Heintz in Advances in Remediation, Volume 3. pp23-28. An Arcadis publication.