Natural attenuation of petroleum hydrocarbons in anaerobic granitic groundwater

- The significance of biodegradation

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Abstract

Sweden is one of several countries where refined petroleum products are stored in unlined vaults within granitic rock. When these vaults are emptied and decommissioned, it is important to investigate the removal of the remaining petroleum in the vicinity of the vaults. The transport and biodegradation processes of hydrocarbon contaminants in granite have been shown to be restricted to water-bearing fractures.

In this thesis, the significance of anaerobic biodegradation of the remaining petroleum in these fractures was investigated. Reduced and anaerobic conditions in petroleum-contaminated groundwaters from the fractures were reported. The A/H quota, an indicator of biological degradation of organic carbon established in this thesis, was significantly higher in the contaminated groundwaters than in pristine groundwater. Many oxidized hydrocarbons, putative metabolites in the hydrocarbon degradation could be detected with gas chromatography – mass spectrometry (GC – MS). Furthermore, it was shown with the most probable number method (MPN) that bacteria utilizing nitrate, ferric iron, and sulfate as electron acceptors were abundant in contaminated groundwaters. Cloning and sequencing, and denaturant gradient gel electrophoresis (DGGE) revealed that many of the dominating bacteria *in situ* were closely related to petroleum hydrocarbon-degrading ferric iron- and sulfate-reducing bacteria. A bacterium closely related to the ferric iron-reducing bacterium *Rhodoferax ferrireducens* was particularly abundant. This bacterium was shown to be involved in a previously not reported anaerobic degradation initiation of the monoaromatic hydrocarbon propylbenzene via a hydroxylation of the aromatic ring. The produced metabolites, propylphenols, were found to be enriched intracellulary. This showed the importance of including intracellular metabolites when estimating biodegradation in a contaminated area. The importance of fracture surfaces in degradation processes was proved by findings that ferric iron-reducing bacteria, residing on fracture surfaces were involved in anaerobic alkane degradation. This is the first investigation to show alkane degradation under iron-reducing conditions. We also performed a field scale study of transport and natural attenuation of propylbenzene in fractured rock. Such a study of a petroleum hydrocarbon has never previously been reported in this specific environment. This field scale study confirmed the importance of biodegradation of propylbenzene *in situ*.

Keywords: 16S rDNA, A/H quota, alkanes, Iron-reducing bacteria, monoaromatic hydrocarbons, propylbenzene, *Rhodoferax*, Sulfate-reducing bacteria

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