

Effect of Nutrient Concentration on the Selection of Bacterial Communities and Oil Composition During Bio-Remediation of Contaminated Beach Sediments

Michael G Milner¹, D. Martin Jones¹, Richard P. J. Swannell², Fabien Daniel², David Mitchell² & Ian M. Head (i.m.head@ncl.ac.uk)¹

¹ Fossil Fuels and Environmental Geochemistry, Drummond Building, University of Newcastle, Newcastle upon Tyne, UK

² National Environmental Technology Centre, AEA Technology, Culham, Abingdon, Oxfordshire, UK

In situ bio-remediation techniques can provide a simple and cost effective solution to hydrocarbon contamination. Naturally occurring microbial populations may be stimulated by addition of nutrients lacking in a particular environment. However, the effects of different nutrient amendments upon the microbial community and oil degradation are poorly understood. An ecological theory (resource ratio theory) has been developed which relates community structure to partitioning of growth limiting nutrients between different members of the community (Smith, 1993). Preliminary evidence has indicated that resource ratio theory may be applied to oil spill bio-remediation where limiting resources (nitrogen and phosphorous) are added to stimulate microbial hydrocarbon degradation (Smith et al., 1998). Resource ratio theory predicts that provision of nutrients at different concentrations will select organisms with different kinetic properties and that these may be phylogenetically different. Therefore it has been suggested that addition of specific concentrations of nutrients will select specific assemblages of organisms with different catabolic and kinetic properties. Consequently, it may be possible to optimise hydrocarbon degradation efficacy by controlling the levels of limiting nutrients. The effects of nutrient concentration on bacterial community structure in oil-contaminated sediments were investigated using beach microcosms. Bacterial community structure was determined by denaturing gradient gel electrophoresis (DGGE) analysis of PCR-amplified of ¹⁶S rRNA gene fragments. Quantitative differences in the composition of crude oil at the beginning and end of the experiment were determined

using Gas Chromatography and Gas Chromatography-Mass Spectrometry. Frequent monitoring of CO₂ evolution throughout the experiment was used as a proxy for crude oil degradation. DGGE analyses showed that different nutrient amendments selected for different bacterial community composition as predicted by resource ratio theory. Oil chemistry data showed that nutrient addition significantly stimulated degradation of total petroleum hydrocarbons, alkanes and aromatic compounds and CO₂ evolution rates also increased with increasing nutrient concentration. In many instances differences in nutrient addition correlated with changes in population structure and the extent of hydrocarbon degradation. However, at nutrient concentrations around the value that elicited optimum hydrocarbon removal, differences in population structure were not related to alterations in the efficacy of hydrocarbon removal. These observations indicated that subtle differences in nutrient concentrations had little effect on the outcome of hydrocarbon bio-remediation despite the selection of different bacterial assemblages. The different bacterial populations therefore degraded hydrocarbons with equal efficiency. Alternatively, bacterial community composition does significantly affect hydrocarbon degradation, but the measures of hydrocarbon degradation used here were too crude to detect this.

Smith VH, Graham DW & Cleland DD, *Environ, Sci, Technol.*, **32**, 3386-3395, (1998).

Smith VH, *Adv. Microb. Ecol.*, **13**, 1-37, (1993).