Impact of bioremediation treatments on the biodegredation of buried oil and predominant bacterial populations

R.P.J. Swannell[1], D.J. Mitchell[1], J.C. Waterhouse[2], I.P. Miskin[2], I.M. Head[2], S. Petch[2], D.M. Jones[2], A. Willis[3], K. Lee[4], J.E. Lepo[5]

[1] AEA Technology plc, National Environmental Technology Centre, Abingdon, Oxfordshire, OX14 3DB, UK
[2] University of Newcastle upon Tyne, Newcastle upon Tyne, NE1 7RU, UK
[3] University of Sunderland, Sunderland, UK
[4] Fisheries and Oceans, IML, Québec, Canada
[5] University of West Florida, Pensacola, USA

ABSTRACT

A field trial was conducted to assess the application of mineral fertilizers as a bioremediation treatment for oil buried in fine sediments. Biodegradation of the crude oil was studied by monitoring changes in residual hydrocarbons. Changes in predominant bacterial populations were determined using Denaturing Gradient Gel Electrophoresis (DGGE) analysis of bacterial 16S rRNA gene fragments amplified from nucleic acids extracted from the sediments. The chemical analysis showed that the application of mineral fertilizer to the oil-contaminated sediments significantly ($p = 0.0001$) increased the rate of biodegradation of hydrocarbons. The predominant bacterial populations in untreated plots and plots treated with fertilizer alone were stable throughout the experiment. Changes in the bacterial populations occurred in response to oil and oil and fertilizer addition, though different populations were stimulated by each treatment. This study has shown the potential of bioremediation to treat oil buried in fine sediment and also indicated that bacterial populations change rapidly and reproducibly in response to inputs of crude oil and bioremediation agents.

Introduction

Bioremediation has now been shown to be effective on a range of shoreline types [9, 11]. Field studies have demonstrated that it can be used successfully to clean rocky, cobble [1] and coarse sand [14] shorelines. These studies have led to the formulation of some operational guidelines on the use of bioremediation after a marine oil spill [6, 11]. Much less attention, however, has been given to fine sediments such as those found in the upper parts of mudflats around the UK coast. These areas often have poor access and are difficult to clean using conventional methods. Moreover, the field experiments carried out to date have also concentrated on the ability of bioremediation to treat surface contamination of shoreline sediments [10], and less consideration has been given to the potential of bioremediation to treat buried oil. Oil stranded on shorelines can become buried by clean sediment deposited by tidal action, or inadvertently as a result of beach cleaning operations [7]. In order to fill these important gaps in our current understanding of the potential of

Microbial Biosystems: New Frontiers
Proceedings of the 8th International Symposium on Microbial Ecology
Bell CR, Brylinsky M, Johnson-Green P (eds)
Atlantic Canada Society for Microbial Ecology, Halifax, Canada, 1999.

bioremediation, our field experiment was designed to ascertain the feasibility of using bioremediation on the upper part of a mudflat to treat oil contamination buried at a depth of 15 cm. The biodegradation of the oil was monitored by following compositional changes in the oil over time. The response of the bacterial populations in the beach sediments were determined using MPN counts of hydrocarbon-degrading bacteria and denaturing gradient gel electrophoresis (DGGE) of ribosomal RNA (rRNA) gene fragments, PCR-amplified from DNA extracted from the beach sediments.

Methods and Materials

Specification of Field Site and Placement of Plots

A field site in the south-west of England (Stert Flats: 51º 12.3' North, 03º 03.9' West) was chosen for the experimental work. This site has been previously used for bioremediation field experiments and tidal and sediment movements on this site have been well characterised. A total of 12 plot areas were marked on an 80 m stretch of sand (mud content of 3.2%; 80% of particles in 125-180 µm range) using stainless steel poles, which also served to anchor the mesh enclosures in the beach. The plots are all located at the same point in the tidal cycle. Twelve mesh enclosures were manufactured using Nitex (pore size = 200 µm) material [4], measuring 0.4 m (L) x 0.4 m (W) x 0.05 m (D). These were filled with beach material from the field site and buried at each of the delimited plot locations at a depth of 0.15 m, 6 m apart.

Experimental Design

The plots were divided into three Blocks of four randomly assigned treatments (Fig. 1). For the oiled plots, oil was applied at a rate of 4 l m^{-2} bag area. Inorganic fertilizer (sodium nitrate and potassium dihydrogen orthophosphate) was applied at fortnightly intervals. This application rate was determined in laboratory studies using Stert sediment [12] at 100:2:0.2 (oil:nitrate:phosphate w/w). Fertilizer was applied in seawater to the mesh containers after partial excavation. Average background of total oxidised nitrate concentration was 13 µM (SD=16 µM, n= 23), average NH_4^+ was 50µM (SD= 22, n= 23), and average inorganic phosphate was 7 µM (SD= 2, n= 23).

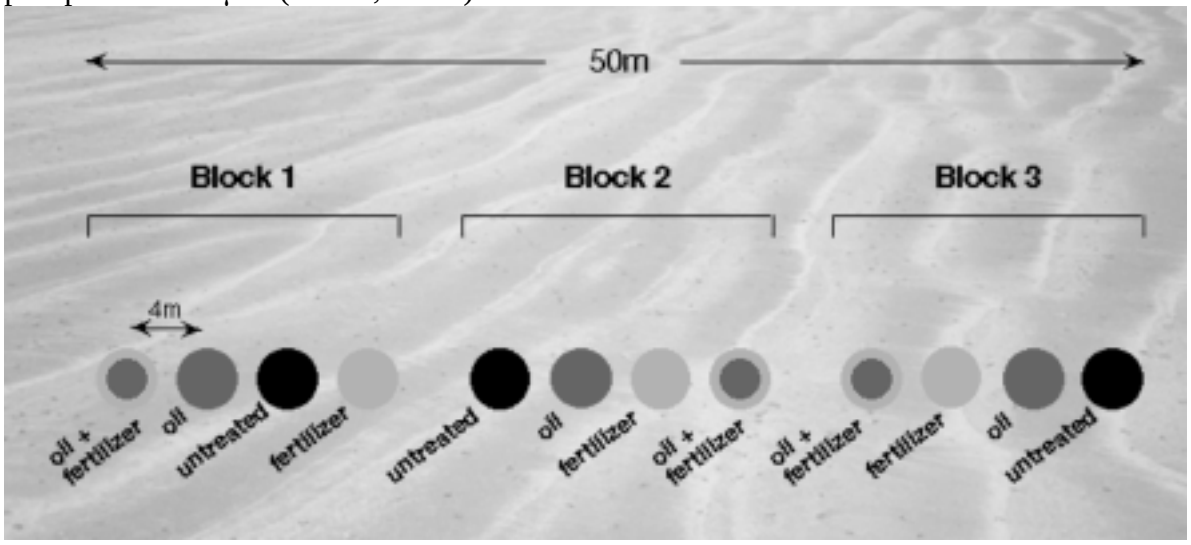

Fig. 1. Design of field experiment.

Preparation of Oil

Arabian Light Crude Oil was chosen as the test oil for the experiment as it is known to contain a high proportion of biodegradable components [12]. The oil was weathered by agitation with air at room temperature until a constant weight was achieved. This process removed 20% of the oil by volume. The oil was then emulsified with artificial seawater (Instant Ocean), using a mechanical mixer (Silverson Ltd., Buckinghamshire, UK) to form a 25% water-in-oil emulsion. The weathering and emulsification were used to simulate oil spilled at sea and washed ashore [5].

Monitoring

The success of the bioremediation treatment was monitored using a variety of methods. Samples were extracted at random points within the enclosures at Day 0, 42 and 101 and analysed for residual hydrocarbons using a method described elsewhere [12]. In addition to a range of aliphatic and aromatic hydrocarbons, the analysis process identified a range of geochemical biomarkers (e.g., pristane and phytane) including 17α(H), 21β(H)-hopane that were used to determine the extent of biodegradation independent of any physical removal of oil that may have occurred [1]. The analysis was completed using GC/FID and GC/MS with a method described elsewhere [13]. Culturable hydrocarbon-degrading bacteria were determined by MPN counts using a procedure [12] modified from the sheen screen method of Brown and Braddock [2]. MPN counts were conducted in 24-well microtitre plates using weathered Arabian light crude oil as the carbon source [12]. The diversity of the predominant bacterial populations present in the beach sediments was analysed using DGGE analysis of PCR-amplified rRNA gene fragments. The PCR amplification and DGGE were conducted according to Muyzer et al. [8].

Results and Discussion

Residual Hydrocarbon Analysis

At the end of the trial, residual hydrocarbon analysis suggested that the oil was more degraded in the fertilized plots than in the oiled, unfertilized control plots (Fig. 2). The data from the oiled fertilized and oiled unfertilized plots were analysed using three way, factorial analysis of variance (for effect of treatment, block and date). The results for the effect of block and treatment are reported here (more details of the results of the statistical analysis are given in [13]). This demonstrated that differences in the ratio of resolvable and detectable aliphatic hydrocarbons relative to hopane between the oil-only plots and the plots treated with oil and fertilizer were highly significant ($p < 0.0001$; Table 1), and that the effect varied with block. There appeared to be no significant effect of treatment on the degradation of aromatic hydrocarbons. A further effect was seen in that there was a significant difference in the rates of degradation in Block 2 when compared to Blocks 1 and 3. A much lower degradation rate was seen in the plots positioned in the middle of the experimental area (Block 2).

Table 1. Results of the statistical analysis of the oil residue analyses.

Oil Component*	p - Value for Comparison	
	Treatment	**Block**
n-C18/Phytane	0.0001	0.0001
TGCD (AL)	0.0001	0.062
TGCR (AL)	0.0001	0.0001
TGCR (AR)	0.715	0.051

* n-C18/Phy = n-octadecane / phytane; TGCD (AL) = Total GC Detectable Aliphatics / 17α, 21β hopane; TGCR (AL) = Total GC Resolvable Aliphatics / 17α, 21β hopane; TGCR (AR) = Total GC Resolvable Aromatics / 17α, 21β hopane

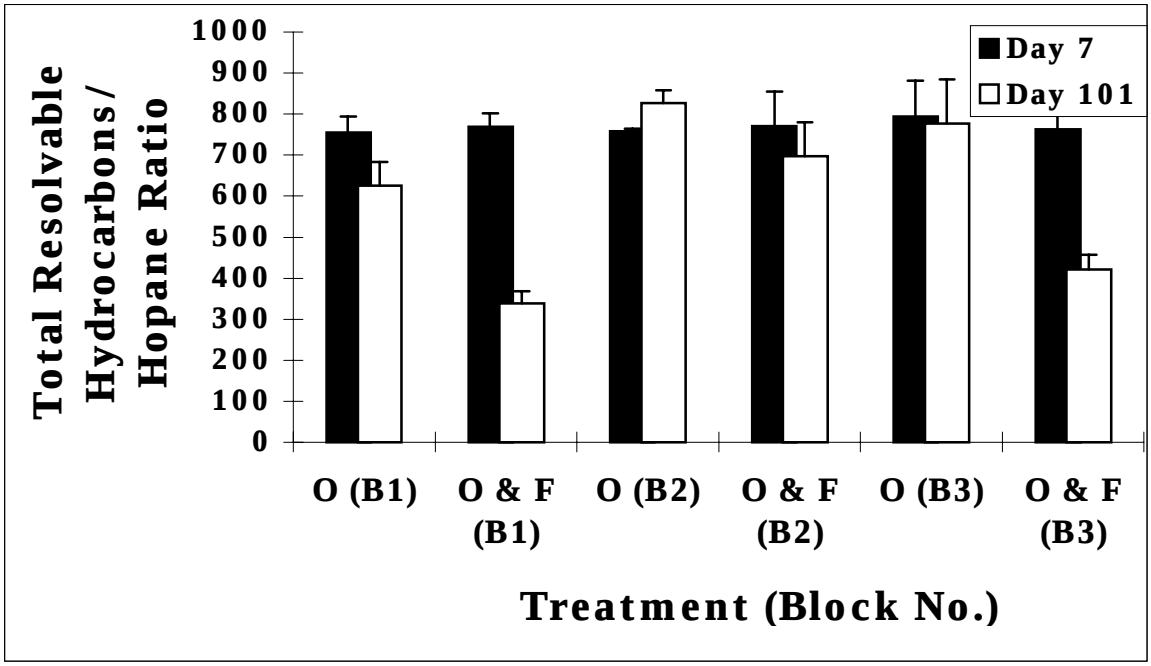

Fig 2. Effect of bioremediation on oil composition. The bars reflect the unbiased standard deviation of the mean (n = 3). Oil was applied on Day 0 after sampling for CO_2 evolution and MPNs. Fertilizer was first applied on Day 6. (O = Oil; F = Fertilizer)

Bacterial populations

MPN counts indicated that oil treatment and treatment with oil and fertilizer increased the abundance of hydrocarbon-degrading bacterial populations in the beach sediments. In plots treated with fertilizer and oil the population increase was greater than in plots treated with oil alone (Fig. 3). DGGE analysis demonstrated that the bacterial populations in the native beach sediment were dominated by relatively few predominant community members (Fig 4a) and the plots treated with fertilizer alone showed the same predominant bacterial populations as untreated plots. The predominant bacterial populations in oiled sediments were considerably different from those in untreated sediments (Fig 4b and 4c) and the bacterial populations responded rapidly to the addition of fertilizer. Within 24 hr of fertilizer treatment the predominant populations in plots treated with oil and fertilizer were

clearly different from those treated with oil alone (Fig. 4b and 4c). This population shift was reproducible within plots but not between blocks. Also by day 42 and day 101 of the experiments the reproducibility of the DGGE analysis both within and between plots was far lower (data not shown). It is an important observation that different bacterial populations were stimulated in response to oil alone and a bioremediation treatment (fertilizer addition to oiled plots). From our analyses we cannot unequivocally demonstrate that these represent different populations of hydrocarbon-degrading bacteria. However since oil treatment increased the organic carbon content of the sediment by at least 10 fold a reasonable working hypothesis is that the populations stimulated are likely to be hydrocarbon-degraders. It is thus clear that the effect of the bioremediation treatment was not simply a stimulation of the same bacterial populations that predominated in the oiled beach sediments, but in fact selected for different sub-populations present in the beach sediments.

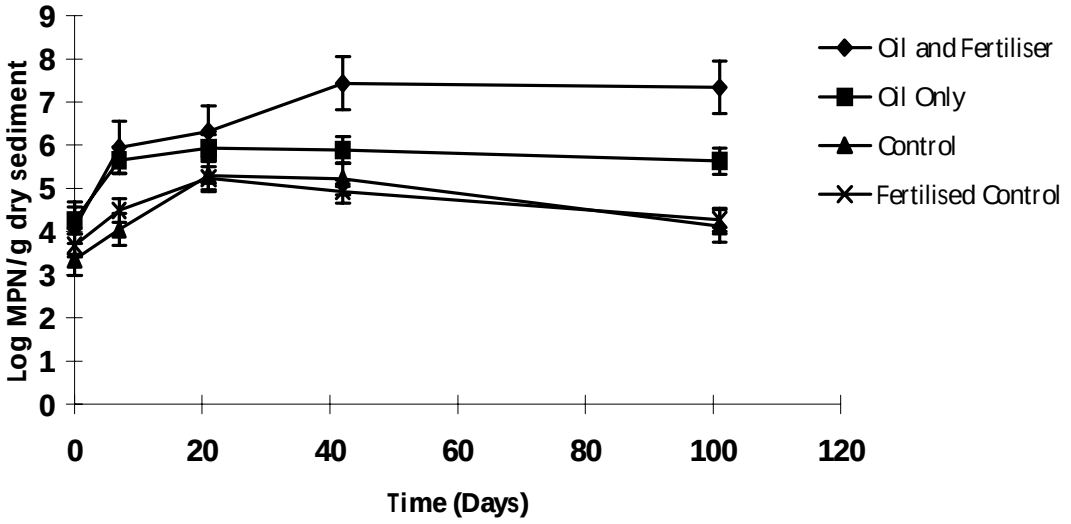

Fig. 3. Changes in the MPN counts of the hydrocarbon-degrading population. Oil was applied directly after sampling on Day 0. Fertilizer was first applied on Day 6. Error bars are the standard deviation of the mean (n = 3).

Conclusions

The residual hydrocarbon analysis clearly showed that the addition of inorganic fertilizers to an oiled oxic fine sediment significantly enhanced the level of biodegradation in comparison to untreated oiled sediment. However, the degree of stimulation varied between the experimental blocks, with a much smaller effect being recorded on Block 2. These findings indicate that bioremediation may be considered for the treatment of buried oil in oxic fine sediments. Oil can become buried on sandy shorelines following tidal and sediment movements, or even as a result of conventional physical methods used to clean oiled fine sediment substrates. Both these phenomena were noted during the response to the recent

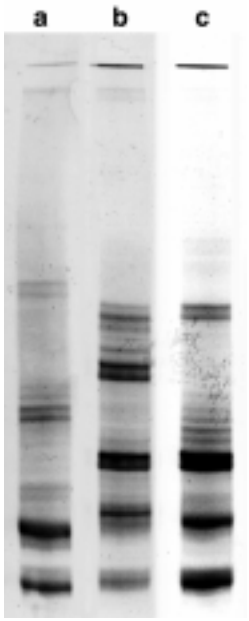

Fig. 4. DGGE analysis of rRNA gene fragments amplified from beach sediment DNA 24 hr after the first addition of fertilizer. DGGE profile of the predominant bacterial populations in: (a) untreated beach sediments; (b) sediments treated with oil alone; and (c) sediments treated with oil and fertilizer.

Sea Empress incident [7, 3], and hence our findings expand the range of conditions under which bioremediation may be considered as a response to an oil spill incident. Moreover, bioremediation of buried oil is less intrusive and may be less damaging to the environment than traditional physical techniques. Our molecular biological analysis has suggested that the predominant bacterial population structure changes rapidly in response to spilled oil, and that a bioremediation treatment may stimulate different predominant populations from those that occur in sediments challenged with oil alone.

Acknowledgements

This work has been supported by USEPA through the EPA-UWF Co-Operative Agreement No. CR-822236-0 and by the Marine Pollution Control Unit of the UK Coastguard Agency.

References

1. Bragg JR, Prince RC, Harner EJ, Atlas RM (1994) Effectiveness of Bioremediation for *Exxon Valdez* Oil Spill. Nature 368:413-418
2. Brown EJ, Braddock JF (1990) Sheen screen: a miniaturised most-probable-number method for the enumeration of oil-degrading micro-organisms. Appl Environ Microbiol 56:3895-3896
3. Colcomb K, Bedborough D, Shimwell S, Lunel T, Lee K, Swannell R, Wood P, Bailey N, Halliwell C, Davies L, Sommerville M, Dobie A, Mitchell D, McDonagh M (1997) Shoreline Clean-Up and Waste Disposal Issues During the Sea Empress Incident. In: Proceedings of 1997 Oil Spill Conference, API, Washington DC, USA, pp 195-203.
4. Lee K, Levy EM (1992) Microbial Degradation of Petroleum in an Intertidal Beach Environment - in situ Sediment Enclosure Studies 1987. In: Marine Ecosystem

Enclosed Experiments, Proceedings of a Symposium held in Beijing, People's Republic of China. Published by International Development Centre, Ottawa, Canada, ISBN 0-88936-543-1, pp 140-155

5. Lee K, Swannell RPJ, Sveum P, Guillerme M, Merlin F, Reilly T, Oudot J, Ducreux J (1995) Protocol for the Assessment of Bioremediation Strategies on Shorelines. In: Proceedings of the 1995 Oil Spill Conference", API, Washington, DC, pp 901-902
6. MPCU. (1995) Scientific, Technical and Operational Advice (STOP 2/95). "Operational Guidelines for the Application of Bioremediation Agents. Marine Pollution Control Unit, Coastguard Agency, Southampton, UK
7. MPCU. (1996) The Sea Empress incident. Report by The Marine Pollution Control Unit, published by The Coastguard Agency, Southampton, UK, ISBN-1-901518-00-0
8. Muyzer G, de Waal EC, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl Environ Microbiol 59:695-700
9. Prince RC (1993) Petroleum Spill Bioremediation in Marine Environments Crit Rev Microbiol 19:217-242
10. Rosenberg ER, Legmann R, Kushmaro A, Taube R, Adler E, Ron EZ (1992) Petroleum bioremediation - a multiphase problem. Biodegradation 3:337-350
11. Swannell RPJ, Lee K, McDonagh M (1996) Field Evaluations of Oil Spill Bioremediation. Microbiol Rev 60 (2):342-365
12. Swannell RPJ, Croft BC, Grant AL, Lee K (1995) Evaluation of Bioremediation Agents in Beach Microcosms. Spill Science and Technology Bulletin 2:151-159
13. Swannell RPJ, Mitchell DJ, Jones DM, Petch S, Head IM, Willis A, Lee K, Lepo JE (In press) Bioremediation of oil contaminated fine sediments. In: Proceedings of 1999 Oil Spill Conference", API, Washington DC, USA
14. Venosa AD, Suidan MT, Wrenn BA, Strohmeier KL, Haines JR, Eberhardt BL, King D, Holder E (1996) Bioremediation of an Experimental Spill on the Shoreline of Delaware Bay. Environ Sci Technol 30:1764-1775