Transactions on Ecology and the Environment vol 20, © 1998 WIT Press, www.witpress.com, ISSN 1743-3541

Solvent enhanced bioremediation of weathered oil contamination

G. Gray, K. Chappell & V. K. Broscomb-Smith
School of Environmental and Applied Sciences, University of Derby, Derby, UK.
K.R.Chappell@derby.ac.uk

Abstract

This paper describes a novel bioremediation process for the treatment of oil spills on land. The method was developed specifically to deal with long term oil contamination where the volatile fractions have evaporated leaving the more recalcitrant fractions. A model system of sand and Kuwaiti crude oil was used to test the system. A combined treatment which introduced an additional solvent component was found to enhance mobility and availability of oil, enhancing bioremediation.

1 Introduction

The Gulf War in Kuwait has resulted in one of the largest man made environmental disasters, and almost certainly the greatest acute contamination of land and water with oil, in history. A total of 788 wells were sabotaged by the Iraqi army on their retreat, of which 613 were set alight and 76 left to pour oil onto the land [1]. In addition to the wells, oil distribution centres, oil loading terminals, storage tanks and refineries were also severely damaged. For 300 days in 1991, between 2 and 4 million barrels of oil per day were spilled into the Kuwaiti desert. The Gulf was also heavily contaminated with estimates being between 160 and 340 million gallons of crude oil entering the sea.

A legacy of this contamination has been the Kuwaiti lakes of oil which formed from a mixture of crude oil and large quantities of sea water used to extinguish the well fires. Oil density in these lakes[2] is approximately 1 g ml^{-1} with oil penetration varying from surface contamination to a depth of 1 m, average penetration is calculated to be 0.2 – 0.3 m. These heavily contaminated sites along with soils contaminated by oily mists and soot pose a serious environmental problem in the region.

Penetration of oils into the soil provides a significant threat to valuable groundwater supplies. The main risk is from the monoaromatic fractions, especially benzene, toluene, ethylbenzene and xylene. Risks are also posed to wildlife with large kills of birds, mammals and insects being reported.

In the seven years since the withdrawal of Iraqi troops there has been a massive clean-up operation utilising a wide range of physical, chemical and biological techniques. However, the oil lakes and large areas of contaminated sands still pose a significant problem. Economic considerations mean that, potentially, the most favourable approach for decontamination of the area is bioremediation.

There are several problems with the use of bioremediation in Kuwait, these are as a result of environmental conditions and the current condition of the oil in the lakes and sand. Environmental conditions which can prove problematic are high desert temperatures and low precipitation. Temperatures which can exceed 40 ^{o}C in July and August, and low precipitation rates, annual average of 115 mm, place water at a premium[2]. Soils in Kuwait are mainly sandy calcareous aridisol soil with an organic matter content of under 0.02%, nutrients are lacking and high calcium and magnesium content further decrease the availability of nutrients. Typically, soils contain 10^5 – 10^6 bacteria, and 10 – 10^2 fungi [6]. Due to the use of sea water in the fire fighting operations, and subsequent evaporation, salinity levels are high in the lakes and surrounding soils[1].

Attempts at the application of bioremediation in Kuwait, including landfarming, windrow composting and static soil piles, have met with varying degrees of success [7]. Up to 74.1% of alkanes were degraded within six months using a landfarming technique whilst the use of static piles achieved 53.8% degradation over the same time period. Static soil piles, however, provide a relatively cheap option with lower labour requirements. Work at the Bioremediation Unit at the University of Derby has previously identified enhanced bioremediation in such systems with the addition of solvents to water/nutrient mixes. Low concentrations of solvents appear to increase oil mobilisation and availability to bacteria. A recirculating system avoids risks posed to groundwater by increased mobility of the oil.

The following paper describes a laboratory scale application of a solvent enhanced system to sand contaminated with weathered Kuwaiti crude oil.

Transactions on Ecology and the Environment vol 20, © 1998 WIT Press, www.witpress.com, ISSN 1743-3541

2 Experimental Method

Schematic Diagram of Model System

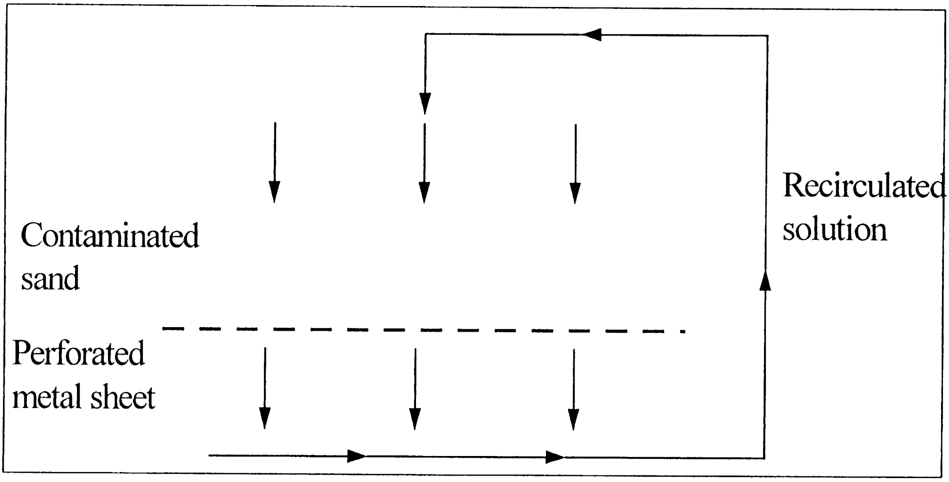

Figure 1.

2.1 Preparation of weathered crude on substrate

Washed sharp sand was coated with crude oil at a ratio of 47:1 sand and oil by weight. The resulting substrate was mixed and then spread thinly in enamelled trays. The trays where heated at a constant 60 °C in a fume cupboard for 7 days. The resulting weathered crude was extracted from the sand (see 2.3) and subsequent analysis by GC/MS confirmed that in excess of 80% of the original mass of the crude oil had been lost. Whilst evaporation greatly reduced the mass of all major constituents, concentrations of all aliphatics smaller than C_{16} were reduced to trace levels.

2.2 Trials

Six simultaneous systems where constructed for the treatment of 320g of weather crude oil on sand (Fig. 1). Each system was activated by the addition of 80 ml of aqueous solution to the system. The composition of the solution for each solution is detailed in Table 1. This solution was collected from the base of apparatus after passing through the sand. The volume of the solution was adjusted to 80 ml by the addition of water

(Deionised or saline as appropriate), prior to being feed back onto the sand. This process was repeated for all six trials for 14 days under ambient conditions. Chemical composition and bacterial abundance were assessed for each system at day 0, day 2 and day 14.

System Solution Composition

System	Water	Nutrient	Limonene
F	Fresh	None	None
FN	Fresh	Yes	None
FSN	Fresh	Yes	Yes
S	Saline	None	None
SN	Saline	Yes	None
SSN	Saline	Yes	Yes

Table 1.

2.3 Extraction and Analysis of Oil Components

1 g samples were transferred to paper thimbles (Whatman) and under went an 8 hr soxhlet extraction into Dichloromentane (HPLC grade). Sample volumes were adjusted by rotary evaporation at 20 °C and the volume samples were then made up to 10.00 ml samples. Determination of aliphatic constituents was performed GC FID/MS using a Hewlett Packard 5890 Series II GC with 50 m C_{18} capillary column and 5971 Series Mass Selective Detector.

2.4 Bacterial Counts

Water samples (2 ml) were removed from each system on days 2 and 12 for total bacterial counts. Samples were preserved with 2% final concentration neutral filtered formaldehyde and refrigerated for up to 48 hours prior to counting. Two replicate subsamples were stained with acridine orange, concentrated on black, 0.2μm pore size polycarbonate membrane filters and counted by epifluorescence microscopy [3 4] using a Leica DMLB microscope. More than 600 cells were counted per preparation; 95% confidence intervals were < ±8% of the count [5].

Transactions on Ecology and the Environment vol 20, © 1998 WIT Press, www.witpress.com, ISSN 1743-3541

3 Experimental Results

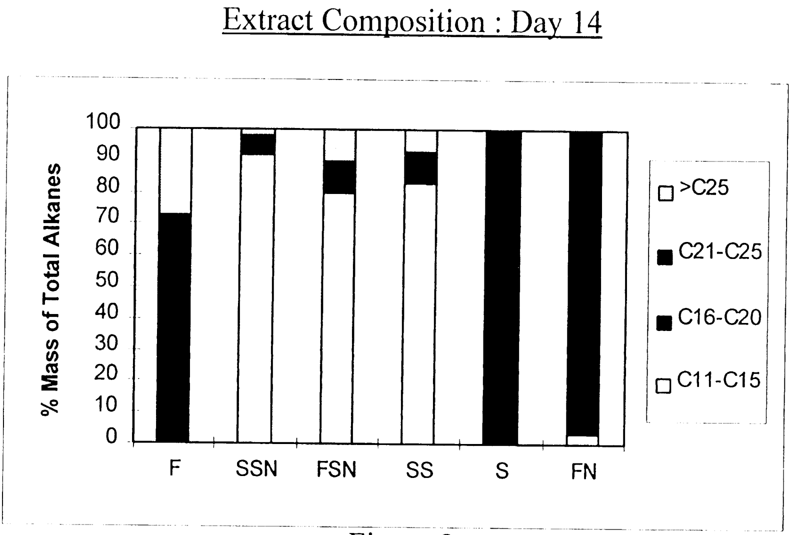

Figure 2.

The use of deionised water without additional nutrient resulted in no detectable change in the composition of the weathered crude oil. The addition of a nutrient mix (System FN) resulted in a marked reduction in the mass of total hydrocarbon. Concentrations of larger aliphatics (> C_{20}) were found to be greatly reduced and a corresponding increase in the relative abundance of aliphatic fraction ($C_{16\text{-}20}$) was observed. The major species remaining in the oil remained alkanes and their branched homologues. The addition of limonene solvent (system FSN) resulted in enhanced breakdown of all fractions from C_{16} and above compared to the non solvent systems. Despite the presence of a substantial carbon source, growth within system F, with the addition of just deionised water, was limited. This is possibly due to the absence of macro and /or micro nutrients. Nutrients for other systems were provided in the form of sea weed nutrient mix, however system S (sea water alone) gave similar results to those of system FN (deionised water with added nutrient) indicating that perhaps the use of could reduce the need for additional nutrient addition. Further support for this is provided by the absence of any enhancement of breakdown on the addition of nutrient to the sea water and solvent system.

Most systems exhibited significant bacterial growth between days 2 and 14, indicating the development of established microbial populations.

Bacterial numbers on both days 2 and 14 indicate that the FSN system was the most favourable for microbial growth. Within the sea water systems the addition of solvent did not promote increased microbial growth but did promote breakdown. It is possible that mobilisation of oil within the solvent increased the available surface area for breakdown, this would also indicate that microbial growth rates were limited by factors other than availability of the carbon source. It is likely that different rates of breakdown between populations of similar sizes are due to differences in population composition, this is clearly an area requiring further work.

Bacterial Numbers

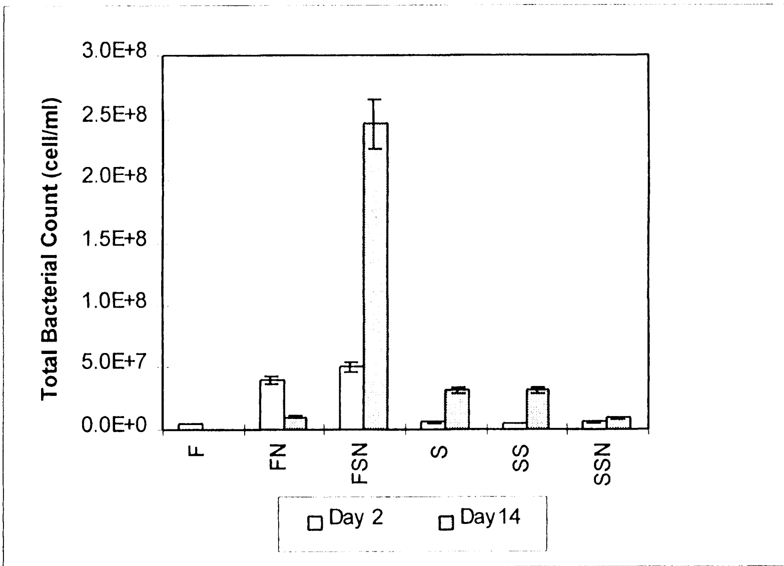

Figure 3.

4 Conclusions

The results of this preliminary trial indicate marked enhancement of bioremediation processes with the addition of a solvent component. It appears that this is due to increased mobilisation of oils, thus providing additional surface area for bacterial action. Use of either sea water or freshwater had little effect on the rate of oil degradation. This may have significant implications for the use of seawater in bioremediation in Kuwait, provided problems with soil salination can be overcome. This

Transactions on Ecology and the Environment vol 20, © 1998 WIT Press, www.witpress.com, ISSN 1743-3541

trial presents only preliminary work and it is clear that more work is required in order to develop a working system for large scale treatment of weathered oil.

5 References

1. Bader, M.S.H. and Clarkson, W.W. 1995. An Overview of the Oil Lakes in Kwait: The Problem and Remedial Methods, *Journal of Environmental Science and Health*, A30 (5).

2. Al-Houty, W., Abdal, M. and Zaman, S. 1993. Preliminary Assessment of the Gulf War on the Kuwait Desert Ecosystem, *Journal of Environmental Science and Health,* A28 (8).

3. Carr O.J. and Goulder R., 1993. Directly counted bacteria in a trout farm and its effluent, *Aquacult. Fish. Managem.*, 24, 19-27.

4. Daley R.J., 1979. Direct episluorescence enumeration of native aquatic bacteria: uses, limittations, and comparative accuracy. in Costerton, J.W. and Colwell, R.R. (Eds), *Native Aquatic Bacteria: Enummeration, Activity, and Ecology. Am. Soc. Testing Materials, Spec. Tech. Publ. No. 695.* pp 29-45.

5. Jones J.G., 1979. *A Guide to Methods for Estimating Microbial Numbers and Biomass in Fresh Water. Freshwater Biol. Asssoc. Sci. Publ. No.39.* 112pp.

6. Al-Awadhi N.; Al-Daher R.; El-Nawawny A.; Balba M.T., 1996, Bioremediation of oil contaminated soil in Kuwait: Land farming to remediate oil contaminated soil, *Journal of Soil Contamniation*, Vol. 5, (3).

7. Balba M.T.; Al-Awadhi N.; Al-Daher R., 1995, Bioremediation: An overview based on international project experience, Journal of Arid Land Studies.