A bioremediation of crude oil contaminated soils study was carried out in engineered laboratory biopile systems. In soil bioremediation, biopiles are ‘ex situ’ treatment systems that consist of excavated and aerated contaminated soils amended by addition of biological, mineral or organic material depending upon specific needs. When bacteria are added to the contaminated soil undergoing biological treatment, the latter is referred to as a bioaugmented treatment system. Such soils are arranged above ground in a pile fashion and hence the term biopile. The study has shown virtually identical trends in respiration rates when indigenous and commercial added bacteria where employed. However, the bioaugmented experiments yielded much greater respiration rates and lead to a reduction of 75% of the initial oil in just 118 days, compared to 1 year in similar studies in the literature. The benefits of engineered biopile systems include safe operations, facilitated material balance and process controllability. The benefits of bioaugmentation were clearly demonstrated and it was found that when no nutrients are added to the soil, bacteria tend to metabolize hydrocarbons into carbon dioxide and water rather than assimilate carbon in cell growth. Polyaromatics analysis of treated soil in this investigation pointed out that simpler and more bioavailable crude oil components were degraded first. A simple sigmoid model for the carbon dioxide generation was developed from the respiration data.

Keywords: bioremediation; biopile system; contaminated soil; crude oil.

INTRODUCTION

Concern over soil contamination by crude oil or hydrocarbon products in general, is gathering momentum after a similar feeling has been around for a while on marine oil spills, which enjoy more media coverage because of the often spectacular visual effects images conveyed to people. There are similarities and differences between inland and offshore crude oil spills. Similarities include hazards to life in all its forms, contamination of valuable fresh water resources from aquifers or desalination plants and an uncertain long term environmental impact despite unsubstantiated claims that nature fully recovers in ‘a few years’. On the other hand, the differences concern mainly the behaviour of spilled oil, its interaction with the surrounding environment and the corresponding approach to remediation.

In the case of soil contamination by hydrocarbon products, there has been a great deal of work on biologically based treatment processes from several disciplines of the scientific community. This is not an odd phenomenon since environmental research concerns just as many disciplines and more importantly attracts funding support from government and private sources. However, the diversity of backgrounds of the researchers created a collection of schools of thought as well as, sometimes convenient, basis for agreement or disagreement in interpretation of laboratory or field data on bioremediation. For people seriously engaged in large scale bioremediation of oil contaminated soils, this situation leads to the legitimate question: is bioremediation of crude oil contaminated soils a black art or an engineering challenge? The following notes are extracts from relevant literature findings that cannot be considered as a comprehensive survey, for the sake of conciseness.

Al-Daher et al. (1998) carried out what seemed to be a series of intermediate size (20 m³) bioremediation tests on crude oil contaminated Kuwaiti soil by means of windrow piles. Treated soils were initially referred to as lightly and heavily contaminated without providing a measure of hydrocarbon content that could easily be measured as TPH or equivalent TOC. The windrow piles employed were inoculated by sprinkling with solutions containing sewage sludge, mature compost and non-identified heterotrophic bacteria isolated from contaminated soil and grown in the laboratory on hexadecane as the sole source of carbon. Whilst the study was found to be interesting,
justified and timely, the approach and interpretation of results were subject to flaws and errors. For example, their measurement of respiration rate (CO₂) is based on a method that is known to us to be unreliable and yields incorrect results. They used the sodium carbonate precipitation method that produces colloidal barium carbonate, a very difficult solid to filter, that yields residual carbonate which can be titrated alongside the sodium hydroxide, thus producing erroneous and misleading titration results. In addition, the reduction of total extractable matter (TEM) was allocated to bioremediation when it was clear from their windrow biopile systems that loss by evaporation can also reduce TEM. Indeed, their aeration was crude and consisted of ploughing, a procedure that promotes partial evaporation of hydrocarbons. Al-Daher et al.'s study also found no significant influence of different additives (nutrients, biomass) on the biodegradation, a fact contradicted by most reliable investigations. Their study lasted 10 months and only a brief test was conducted with a covered windrow pile, aimed primarily at moisture retention. No further details were provided on this single odd test and therefore their conclusion on its apparent enhanced performance is difficult to ascertain.

D’el Arco and Franca (1999) investigated the bioremediation of soil sediment contaminated by light Arabian crude oil and found a clear evidence of improved bioremediation performance between soil with indigenous bacteria and soil with added landfarming farming bacteria. The latter were accelerated during the landfarming process and improved considerably the oil degradation in the contaminated sediment. Micro-organism acclimation is a technical term often employed to refer to adaptation to certain conditions that include source of carbon, pH and other nutrients.

Straube et al. (1999) carried out a bench scale study to optimize bioaugmentation strategies for treatment of soils contaminated with high molecular weight polyaromatics. The latter compounds are extremely toxic and often considered as ‘recalcitrant’. Their study shed some light on aspects of bioaugmentation, namely how it improved biodegradation of insoluble polyaromatics. Their study suggests that addition of light oil and bacteria that can produce surfactants, stimulated by added light oil, had the same effect as adding small amounts of surfactants that enhanced bioavailability of poly-aromatic hydrocarbons (PAH), and hence their degradation.

Trindade et al. (2004) carried out a comparative bioremediation study between a weathered and recently oil contaminated soil samples from Brazil. The weathered contaminated soil was about 4-years old. They concluded that bacteria from the weathered soil performed better than the ones in the freshly contaminated soil and explained this in terms of acclimation of bacteria in the former case. They also found that bioaugmentation (addition of previously hydrocarbon acclimated bacteria) and biostimulation (addition of nutrients) doubled the bioremediation efficiency (percent total petroleum hydrocarbon, TPH, removal). Although their study appeared to be superficially interesting and raised important issues, their rudimentary experimental approach however, coupled with unjustified assumptions on the fraction of carbon mineralized or metabolized as biomass, cast serious doubts on the reliability of their results and thus conclusions. The greatest weakness is associated with the assumption made in the relationship between total organic carbon (TOC) and TPH. The relation is not a simple percentage, but depends on the nature of crude oil (fraction of heavy components). This is a typical study that is scientifically sound in principle but seriously flawed in technical and engineering terms.

The study of Becker (1999) on the order in aerobic microbial communities from hydrocarbon contaminated soils suggested that such biomass communities were essentially self-regulating and only depended on the bioavailability of the source of carbon. In other words, this implied most heterotrophic bacterial communities displayed similar hydrocarbon degrading patterns, perhaps due to complementary contributions of individual members. This is indeed an interesting topic that will undoubtedly shed light on the issue of bioaugmentation and acclimation.

**EXPERIMENTAL**

The experimental part of this soil bioremediation work consisted of several stages, starting with the preparation of four soil samples collected from the Sahel oil field (Abu Dhabi, UAE): clean soil, soil artificially contaminated by crude oil to saturation level (17% w/w), crude oil contaminated soil (17% w/w crude oil) with added commercial bacterial product and crude oil contaminated soil with added HgCl₂. The latter sample served for a control test in which most of the indigenous bacteria were killed by the added biocide. The biological product employed in the bioaugmented biopile system was Anmite P300 (Cleveland Biotech, UK) and consisted of immobilized Pseudomonas Putida (gram negative) and Bacilli Subtilis (gram positive) bacteria on a cereal (bran). The bacteria were conditioned to degrade petrochemicals by the vendor. The concentration of biomass in Anmite P300 was approximately 9 × 10⁷ CFU g of bran.

The soil texture was characterized gravimetrically according to the ASTM method using soil sieve fractions and classified as ‘sandy loam’ using a standard soil characterization triangle chart. The average particle size was 150 μm (constituting 46% w/w of the size range). Its bulk density was measured as 1.6 g ml⁻¹. The soil field capacity (water absorption to saturation level) and oil absorption to saturation were determined as 23% w/w and 17% w/w, respectively. The pH and conductivity of the soil were 8.85 and 634 μS cm⁻¹ respectively. The soil chemical composition was also determined by X-ray fluorescence to identify elements present that may be of help in the explanation of results. The alkaline nature of the soil was consistent with the carbonate content.

The crude oil involved had the composition shown in Figure 1. Bu Hasa oil field was near Sahel oil field where soil samples were collected from.

The total petroleum hydrocarbon (TPH), polyaromatic hydrocarbons (PAH), total organic carbon (TOC), polychlorinated biphenyls (PCB), elemental analysis (H, N, S, P) of the soil samples before and after bioremediation treatment were determined in the Central Laboratory Unit of the UAE University and may be invoked in the interpretation of bioremediation results. The TPH analysis is based on a gravimetry and Fourier Transform Infra Red (FTIR)
method according to the US Environmental Protection Agency methods 418.1 and 9071.

The basic soil related tests described above were followed by the design and commissioning of a biopile treatment system shown in Figure 2. The carefully engineered biopile system was self-contained, reliable and safe to use in a conventional chemical engineering laboratory since no harmful emissions were allowed, owing to the activated charcoal trap installed to capture all VOC’s released during the treatment period which extended to 118 continuous days. The biopile system was designed to remove completely CO₂ from atmospheric air supplied to the biopile cell and capture completely CO₂ generated by the bioremediation process within the biopile cell. The humidifying unit had a dual function: to provide moisture to the aerating stream of air entering the biopile system and remove traces of NaOH that may be picked up in the CO₂ traps prior to the biopile cell. The arrangement shown in Figure 2 ensures that CO₂ analysed originates solely from the bioremediation process. The biopile outlet was designed to enable insertion and removal of a thin thermocouple to measure the temperature of the soil when desired. The main biopile cylindrical cell has a diameter of 16 cm i.d. and a height of 7.4 cm. The hollow conical bottom has a height of 6 cm. The upper compartment has identical dimensions.

The total biopile charge was 1.875 kg and included, where appropriate in the various biopile systems employed, crude oil (17% w/w soil), Amnite P300 (10% w/w soil) and biocide HgCl₂.

The three biopile systems employed in this investigation and referred to as systems 4, 5 and 6 have the following characteristics:

- System 4 consisted of soil, crude oil and Amnite P300.
- System 5 consisted of soil and crude oil.
- System 6 consisted of soil, crude oil and HgCl₂.

No nutrient supplements were added. The soil, oil and added bacterial products were well mixed by means of blending blades. The biopile charge was also accurately weighed after treatment in order to assist in establishing a material balance exploiting the TPH, PAH, TOC, PCB, elemental analysis (H, N, S, P) data.

The air flowrate was maintained constant in all cases at 500 cm³ min⁻¹ using a flow regulator. Such level of aeration ensured supply of a large excess of oxygen.
The contaminated soil charge was loaded into the biopile cell made of ceramic material and fitted with perforated base and top ceiling. The base was lined with a geotextile cloth lining to prevent loss of soil particles through the holes. There was no need to fit geotextile cloth on the top ceiling since no fluidization of soil took place. In this arrangement aeration was uniform throughout the cross section of biopile.

The bioremediation analysis system consisted of an ‘in-house’ developed titration method based on the standard titrimetric determination of CO₂ trapped in a concentrated NaOH solution (as shown in Figure 2). Our method made use of two titration colour indicators: methyl orange and phenolphthalein employing hydrochloric acid at two concentrations for each indicator. This method, though tedious, was very reliable and cost effective. The titration error was estimated as 4% or less from a series of triplicate runs. CO₂ from the atmosphere that is trapped in the pre-treatment NaOH traps was also measured once by off-line gas chromatography and the agreement was found to be good. Daily samples of NaOH trap solutions were analysed. This method also has the advantage of storing the reaction history in solution as well as being recorded in a spreadsheet. The NaOH solutions were replaced whenever they were considered to be weak and also when the liquid level dropped below a pre-determined height that was considered important for maximum CO₂ absorption. The bioremediation processes in systems 4, 5 and 6 were conducted at laboratory temperature which did not vary appreciably and the measured biopiles temperatures were constant at 23°C.

RESULTS AND DISCUSSION

The crude oil composition shown in Figure 1 clearly demonstrates the complex nature of crude oil and therefore the fate of the various petroleum fractions present will follow a complex path according to prevailing conditions present during the treatment process. Since an oil saturated soil sample was considered in this work, one might ask the following question: how is the crude oil combined with soil? To answer such question, a series of optical microscopy images of clean, contaminated and contaminated-bioaugmented soil samples were taken (at 400× magnification). Figure 3(a) depicts the appearance of oil saturated soil with added bacterial product. It depicts oil present as a film on the tiny soil particles. The combination of oil and bacterial product created ‘aggregates’ with channels between them, enabling relatively easy flow of air during aeration. There is no visible presence of ‘free’ oil in the soil. This environment also makes it easy for water moisture to be added (determined experimentally as 10–20% of the field capacity) during aeration to deposit on the surface of such soil ‘aggregates’, thus facilitating bioavailability of nutrients to bacteria. The appearance of the treated soil sample is depicted in Figure 3(b). One can see that the soil looks cleaner than contaminated soil and has a layer of fungus on the surface. This is due to the presence of bran in the bacterial product.

The daily measurements of CO₂ generated by the bioremediation process enabled the calculation of cumulative CO₂ production over the treatment period (shown in Figure 4 for system 4), daily respiration rates as well as periodically averaged respiration rates.

The pattern of daily rates depicted in Figure 5 shows an overall trend with random irregularities, typical of soil respiration rates. The averaging of periodic rates serves to ‘smoothen’ the trend and extract very useful information such as lag phase, maximum activity and activity decline. Figures 6 and 7 show such periodically averaged respiration rates for systems 4 and 5. The averaging procedure has to be done with some care in order to retain data representativity whilst highlighting clearly the phases described above. System 4 was the bioaugmented biopile and system 5 was the biopile with indigenous bacteria. One can see clearly similarities in trends but with widely differing magnitudes of respiration rates.

Both systems appear to show a trend of ‘adaptation’ period, ‘maximum oil degradation’ period and a ‘decaying’
The rate of oil degradation period. The striking feature is that the maximum oil degradation period is almost identical at around 50–70 days from the start of the treatment process. However, the magnitude of the respiration rates are manifolds higher in the case of bioaugmented biopile. This result clearly demonstrates the benefit of bioaugmentation.

An enhanced comparison between the three systems investigated in this work can be seen in Figure 8. System 6 is a control system where most of the indigenous bacteria were killed with a biocide (HgCl₂). The mild increase in CO₂ in system 6 is probably due to residual bacteria that resisted the biocide action of HgCl₂. The cumulative CO₂ generation curves for systems 4 and 5 needed different Y axis scales such was the difference in magnitudes even though the shape of the curves were very close. Note that the units in Figure 6 have been conveniently expressed as mg CO₂ kg soil. The adaptation period is identical for all systems and lasted approximately 500 h (20 days). This suggests that indigenous and added bacteria could be of the same genus. During this period, the bacteria acclimatize to their new source of carbon.

In system 4 where biodegradation rates were significantly higher, a carbon balance facilitated by CO₂ generation rates, TOC and TPH data before and after biological treatment, was performed. 74% of the initial crude oil was degraded over a treatment period of 118 days. This was equivalent to a reduction of TPH from 197 g to 50 g in the soil load. This is a remarkable result considering no additional nutrients were provided to the commercial bacteria employed (Amnite P300). In future runs, additional nutrients can be added to extend the treatment process beyond the 74% TPH reduction achieved thus far. The leveling-off of the CO₂ generation curve in Figure 4 is probably caused by available nutrients exhaustion (other than carbon sources). A similar field study carried out by Gogoi et al. (2003) yielded a 75% degradation of the initial petroleum in the contaminated soil which was bioaugmented and biostimulated with nutrients. Their runs lasted 1 year. They aerated and humidified their contaminated soils intermittently and their initial hydrocarbon content was much lower (13% w/w maximum) and the soil texture was not reported.

The reduction in PAH in our treated soil was slight and suggests that bacteria start degrading simple components first, followed by other ‘bioavailable’ complex components. Those components that are for example, strongly
adsorbed onto soil particles will probably persist until they remain the sole carbon source and be made available to bacteria by suitable transport.

Our biopile treatment investigation had shown that only properly engineered systems can help investigate the oil degradation process under controllable conditions and where material balances can be carried out on key elements such as carbon, nitrogen, phosphorus and potassium. Furthermore, daily estimation of respiration rates can be of great help in identifying key stages in the biological treatment process that can be improved and therefore improve the overall process.

In terms of modeling CO₂ generation, a simple sigmoid-type function represented by equation (1) was found to fit adequately the data collected for system 4 (bioaugmented):

\[ C_{\text{CO}_2} = \frac{0.0617}{0.0124 + \exp(-0.0728t)} \]  

where \( C_{\text{CO}_2} \) is the cumulative amount of carbon dioxide (in mol) produced and \( t \) is time (in days). The model parameters were easily obtained using Excel Solver by minimizing the sum of square of errors (objective function). The correlation coefficient \( R^2 \) was greater than 0.99. The major benefit of such simple models is the quick estimation of asymptotic CO₂ generation (i.e., large time values) when they are available for various systems, enabling a reliable comparison of performance between them.

CONCLUSION

The bioremediation of crude oil contaminated soils by means of engineered biopile systems with indigenous and added bacteria has clearly shown that such processes can be amenable to process analysis. The contaminated soil preparation coupled with measurable process variables such as aeration rates, CO₂ generation rates and humification rates can lead to very efficient bioremediation operations. Whether the biological treatment relies on indigenous bacteria or added bacteria, the treatment always follows a three step process: adaptation (14–21 days), maximum degradation (50–70 days) and declining degradation (past 100 days). However, the addition of bacteria (commercial or otherwise) had a dramatic increase in the respiration rate, clearly highlighting the benefits of bioaugmentation. When macro and micro nutrients are not added, bacteria tends to metabolize hydrocarbons into CO₂ and water to a greater extent. This is of great importance for urgent soil bioremediation.

The engineered biopile system adopted in this work can easily incorporate a biological filter to degrade volatile components otherwise trapped in activated carbon in this investigation. This is indeed highly desirable and should displace the landfarming practice that merely shifts part of the problem from the soil to the atmosphere. In terms of treatment cost, biopile systems developed by the US Navy (technical memorandum TM-2189-ENV) were found to be in the range 40–100 US$/yd³. The higher end of the cost range was associated with shorter treatment periods (4 months). Despite the positive potential highlighted in this work, a great deal of engineering work is still required to optimize the biological treatment of contaminated soils.

REFERENCES


Becker, P.M., 1999, About the order in aerobic heterotrophic microbial communities from hydrocarbon contaminated soils, International Biodegradation and Biodegradation, 43: 135–146.


ACKNOWLEDGEMENT

The authors would like to acknowledge the financial support of the UAE University to this work, under grant 03-7-12/02.

*This paper was presented at the 7th World Congress of Chemical Engineering held in Glasgow, UK, 10–14 July 2005. The manuscript was received 15 December 2004 and accepted for publication after revision 11 April 2005.*