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Evaluation of The Efficiency of Various Commercial Products for The Bioremediation of Hydrocarbon Contaminated Soil

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Summary. Bioremediation has become an important method for the treatment of terrestrial oil spills and is often favoured over strictly physical-chemical methods. In this study, enzymatic analyses and signature lipid biomarkers were employed to evaluate the efficacy of selected bioremediation products on control and oil contaminated soil plots. It is envisioned that these biological indicators may be used as possible adjuncts to the strictly physical-chemical criteria most commonly employed. The application of the enzymatic and signature biomarker methods for product evaluation proved successful. The enzymatic assays provided a valuable insight into shifts in the functional diversity of the soil microbial communities resultant from the various treatments. Stimulation or inhibition of the microbial communities as a result of the various treatments was also demonstrated, particularly with regards to dehydrogenase activity. Phospholipid fatty acid profiles proved sufficiently sensitive to allow differentiation between products and resultant microbial communities that corresponded to satisfactory and unsatisfactory petroleum hydrocarbon removal.

Keywords: bioremediation, enzymatic activity, hydrocarbon, PLFA, soil

1. Introduction

The effect of oil spills and the remediation thereof is well publicised since the occurrence of accidental contamination of soil with hydrocarbons commonly occurs due to oil production and handling operations (Vasudevan and Rajaram, 2001). Current South African legislation concerning spill management dictates immediate response and containment of a spill and either chemical or biological remediation of the contaminated site (Minerals Act 50/1991). In the selection of an appropriate remediation strategy, the risk associated with the spill, as well as financial restraints and the respective advantages and/or disadvantages of the various strategies should be considered (Dorn *et al*., 1998). The use of indigenous microorganisms to degrade toxic compounds, such as hydrocarbons in soil holds several advantages (Sabaté *et al*., 2004). It is simple to maintain, applicable to large areas, cost-effective and usually leads to complete degradation of the contaminant (Bento *et al*., 2005). The increasing need for soil remediation has called for efficient and cost-effective technologies as well as alternative monitoring tools (Maila and Cloete, 2005). Physical and chemical analyses of the soil during the evaluation of bioremediation strategies only supply limited information on the effect of a particular treatment regime. Furthermore, chemical and physical characteristics are slow to change and the response of these parameters can only be measured effectively over an extensive period (Pascual *et al*., 2000). Conventional methods for the analysis of soil microbial communities are restrictively insensitive and provide limited insight into the environmental and nutritional status

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of the community *in situ* (Pascual *et al*., 2000; White *et al*., 1996). In order to overcome these limitations, alternative techniques for the investigation of microbial activities and community structure have been developed. These include the analysis of potential enzymatic activity (Aon *et al*., 2001; Bandick and Dick, 1999) and signature lipid biomarkers (White *et al*., 1996; Zelles, 1999).

In this study, the efficiency of five different bioremediation products were assessed in order to identify the most effective remediation strategy based on percentage total petroleum hydrocarbon (% TPH) removal.

2. Materials and methods

2.1. Experimental design

A suitable piece of land was identified and prepared by means of degrassing. The area was divided into 42 plots of equal size (4 m^2). The individual plots were separated from each other by a 1 m buffer zone that was not degrassed. Uncontaminated and contaminated treatment plots were designated based on a randomised plot design. Equal volumes (2 L/m^2) of used heavy machine oil were introduced to the contaminated plots while no oil was introduced to the uncontaminated plots.

Five different treatments for the remediation of hydrocarbon contamination were evaluated and compared with a control site. For each treatment, uncontaminated and contaminated plots were prepared. Each plot was prepared in triplicate to facilitate statistical analysis of the results obtained for each treatment. The five treatments evaluated included Products A, B, C, D, and E. A description of each product is included in Table 1. The financial implications of each treatment product were also considered during the evaluation. While some of the products are relatively inexpensive, more treatment is required after application, elevating costs. Product A was a fertiliser treatment based on stimulation of the indigenous soil microorganisms for the remediation of the hydrocarbons by the addition of appropriate nutrients. This product contained no microorganisms and was applied as an inexpensive, low-maintenance treatment product – the fertiliser was only applied on the soil surface and no further treatment of the plots was carried out. The equivalent of 120 kg KNO_3 and 250 kg super phosphate per hectare was applied. Products B, C, D, and E represent commercially available and trademarked formulations of sorbents, microorganisms, and/or nutrients. These products were added in the recommended dosage and the plots were treated as prescribed by the respective manufacturers. From the initial soil analysis, it was obvious that the soil pH was slightly acidic; NO_3 has a known alkalising effect on soil (Bergmann, 1992) and was therefore chosen as the form of nitrogen applied.

2.2. Sampling procedure

Representative soil samples from the various treatment plots were obtained using a random sampling design

Table 1. Description of bioremediation products applied during this study

Product name	Description
Product A	Fertiliser treatment regime based on augmentation of indigenous soil microbiota for the remediation of hydrocarbons by addition of appropriate nutrients and correction of C:N:P ratio.
Product B	Aqueous suspension of selected natural bacteria, nutrients, and cleaning products (0–5% anionic surfactant; 0–5% non-ionic surfactant). Formulated to remove hydrocarbon residues by bacterial oxidation to CO_2 and water, with no adverse environmental impact or harmful residues.
Product C	100% milled, screened natural solids. Solids are carefully dried to enhance absorption ability while preserving naturally occurring hydrocarbon degrading microorganisms within the solids mixture. After absorption of petroleum hydrocarbons to solids, microorganisms present in the formulation facilitate the degradation of the contaminants.
Product D	Dual-purpose oil/fuel absorbent and bioremediation agent containing naturally occurring microorganisms (high concentration of fungi)–for use on direct spills or hydrocarbon contaminated soil. When supplied with a hydrocarbon source and kept moist with water, microorganisms rapidly propagate to break down the contaminant into its non-harmful constituents. The absorbent component of the product will absorb and encapsulate the contaminant and render it available to the microorganisms within the formulation for oxidation.
Product E	A natural agricultural cotton cellulose product for the absorption, encapsulation, and bioremediation of petroleum hydrocarbon liquids in soils. Microorganisms contained within the formulation are considered indigenous to agriculturally produced materials. Serves as a dual-purpose oil/fuel absorbent and bioremediation agent for use on direct spills or hydrocarbon-contaminated soil.