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Inhibitory Effects on Degradation of Diesel Oil in Soil-Microcosms by a Commercial Bioaugmentation Product

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The effectiveness of bioaugmentation, i.e. inoculation using specific microbial degraders to enhance bioremediation of oil polluted soil, has been investigated by various authors: Jobson et al. (1974) and Lehtomäki & Niemelä (1975) reported only marginal effects on degradation upon inoculation. Two commercial bioaugmentation products were recently tested by Venosa et al. (1992), who found no positive effects on oil degradation. Dott et al. (1989) reported that the oil degrading capacity (in liquid culture) of 9 commercial bioaugmentation products were inferior to that of activated sludge. In spite of these results, commercial products for bioaugmentation are readily available. The present study describes the effects of inoculating diesel oil polluted soil with a commercial bioaugmentation product. The investigation was carried out in laboratory scale using soil-microcosms. The results indicated that addition of small amounts of bioaugmentation product was without effects on diesel oil degradation. Addition of larger amounts of the product directly inhibited degradation during the entire experimental period of 61 days.

MATERIALS AND METHODS

Bioaugmentation product: The acquired product consisted of a powder of freeze dried material. According to the supplier the powder contained micro-organisms (bacteria and yeast) and small amounts of surfactants on a starch-based carrier material. The micro-organisms were claimed to be specially adapted to grow on various petroleum products, paraffins, naphthalenes and lubricant oils. Prior to use, the product was assessed for viability and oil degrading capacity: The number of heterotrophic bacteria was determined by suspending the product in a buffer solution and plating appropriate dilution's on plate count agar. The number of oil degrading bacteria was determined by plating the same dilution's on basic mineral medium agar. The plates were sprayed with a solution of octadecane in ether leaving an opaque layer on the surface. Colonies capable of plaques formation in the octadecane layer were regarded as oil degraders. The plates were incubated at 25 °C for three days or for three weeks before enumeration of heterotrophic bacteria and oil degrading bacteria, respectively.

Soil: The soil used in this study was a diesel oil polluted sandy soil excavated from the vadose zone at a storing facility near Copenhagen. The average diesel oil content was 8.8 ± 0.5 mg/g dry soil. The pH of the soil was 6.8. The soil was passed through a 4 mm sieve and carefully mixed by hand prior to use.

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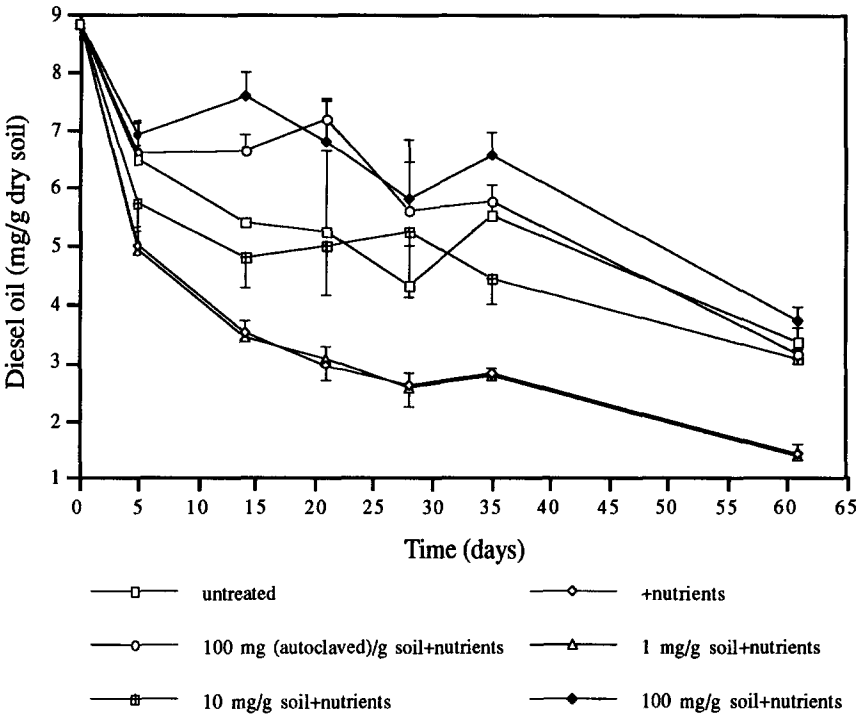

Figure 1. Effects of different amounts of bioaugmentation product on diesel oil degradation in soil-microcosms. Each data point represents the average of 3 microcosms, apart from day 0 where the initial diesel oil content of the soil was used. Bars (one sided) represent 1 standard deviation or are covered by the data points.

Soil-microcosms: 100 g (dry weight) of soil was placed in 1 L infusion flasks with airtight rubber stoppers. The following flasks were set up in triplicate: untreated controls receiving distilled water, flasks receiving only nutrients, flasks receiving both nutrients and different amounts of bioaugmentation product (1, 10 and 100 mg/g soil) and flasks receiving 100 mg/g autoclaved product. Nutrients were supplemented in aqueous solution in the form of K_2HPO_4 and KNO_3 according to the specifications of the supplier to give a final C:N:P-ratio of 100:7:1 (the carbon content of the pollutant was calculated by assuming that the formula of diesel oil was $(CH_2)_n$). The bioaugmentation product was soaked with tepid water to activate the micro-organisms before it was mixed up with the soil. The water content of the soil was adjusted to 12 % (60 % of the water holding capacity) by addition of distilled water or the nutrient solution. The incubation temperature was 25°C.

Quantification of diesel oil in soil microcosms: subsamples of 10 g of soil (dry weight) were placed in glass flasks and mixed up with 20 mL of distilled water. The flasks were placed for 5 min on an orbital shaker (250 rpm). 10 mL of pentane was added, the flasks were vigorously shaken by hand after which they were placed for 1 hr on an orbital shaker (250 rpm) The diesel oil content was determined by injecting 1 µL samples of the pentane phase into a Hewlett Packard 5890 gas-chromatograph connected to a Shimadzu C-R5A integrator. A 30 m x 0.532 mm DB-1 (J & W Scientific) fused silica capillary column was used. N_2 at a

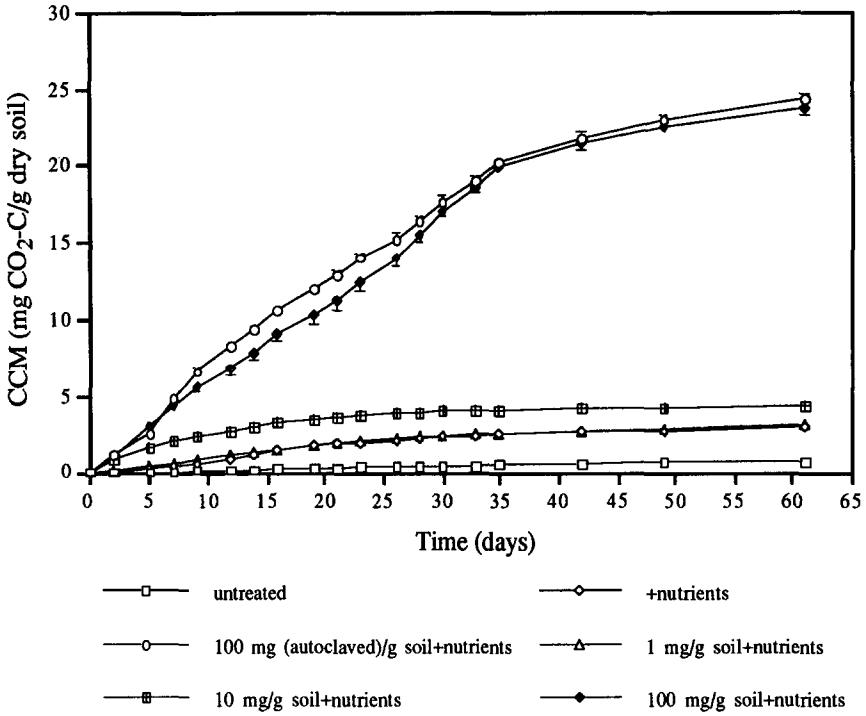

Figure 2. Effects of different amounts of bioaugmentation product on cumulative carbon mineralised (CCM) in soil-microcosms. Each data point represents the average of 3 microcosms. Bars (one sided) represent 1 standard deviation or are covered by the data points.

rate of 24.0 mL/min acted as carrier gas. The injector- and detector temperatures were 275°C and 300°C, respectively. The following temperature program was used: 70°C for 5 min, then a rise of temperature to 275°C at a rate of 12°C/min. This temperature was held for 12 min. The diesel oil content was calculated by comparing with a standard curve of known amounts of diesel oil in pentane.

Quantification of CO_2 - evolution: 1 mL air-samples from the microcosms' head spaces were injected into a gas-chromatograph (Mikrolab CC 82-12, Århus, Denmark) equipped with a glass column (4.51 x 3 mm ID Porapak Q 80/100 mesh). The injector and detector temperatures were 60°C, and the column temperature was 30°C. Hydrogen (40 mL/min) was used as carrier gas. The microcosms were flushed with water-saturated air after each measurement.

RESULTS AND DISCUSSION

The number of heterotrophic and oil degrading bacteria in the bioaugmentation product was $5.5 \cdot 10^{10}$ and $6.8 \cdot 10^{9}$ colony forming units/g, respectively.

In figure 1, diesel oil degradation in the soil-microcosms during the incubation period is shown. The coefficients of variation averaged 6.7%. At the highest concentration of bioaugmentation product - both living and autoclaved - degradation of diesel oil was retarded during the entire experimental period. Degradation at the 10 mg/g level was more rapid during the first 30 d but almost the same diesel oil con-

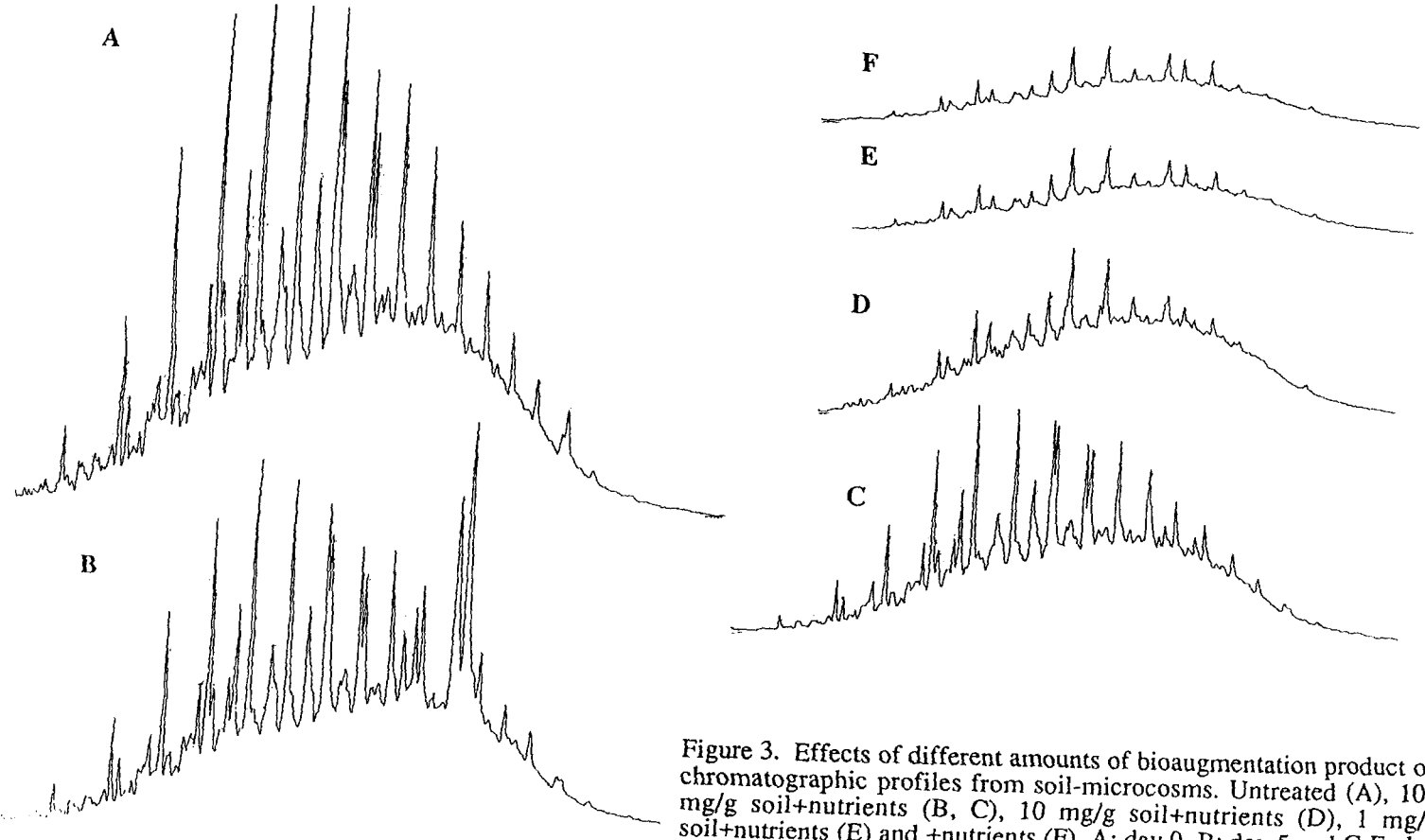

Figure 3. Effects of different amounts of bioaugmentation product on chromatographic profiles from soil-microcosms. Untreated (A), 100 mg/g soil+nutrients (B, C), 10 mg/g soil+nutrients (D), 1 mg/g soil+nutrients (E) and +nutrients (F). A: day 0, B: day 5 and C-F: day 61.

centration was reached after 61 d. Diesel oil degradation in the untreated soil followed the same pattern as the 10 mg/g level. In contrast, diesel oil degradation commenced rapidly and ended at a considerably lower concentration after 61 d in the microcosms only receiving nutrients; an almost identical pattern was seen in the microcosms receiving 1 mg/g of bioaugmentation product.

The cumulative carbon mineralised (CCM) in the microcosms - calculated from CO_2-evolution - is shown in figure 2. The opposite pattern of the degradation data was reflected: The CCM at the highest concentration of bioaugmentation product markedly exceeded the CCM measured at the other treatments, followed by the CCM of the intermediate concentration. The CCM´s in the microcosms that received only nutrients and the lowest concentration of bioaugmentation product, respectively, were less than 1/8 of the CCM reached at the highest concentration of bioaugmentation product. The CCM of the untreated microcosms remained low during the experiment.

Comparison of the degradation data and the CCM leads to the conclusion that the excessive mineralisation rate at the highest concentration of bioaugmentation product did not originate from degradation of diesel oil, but from degradation of the product itself. Possibly, the starch-based carrier material constituted a source of readily available carbon. Bossert et al. (1984) reported a similar effect of adding a alternative carbon source to oil polluted soil.

The chromatograms of figure 3 shows quantitative and qualitative effects of adding the bioaugmentation product to diesel oil polluted soil. Figure 3 (A) shows the chromatographic profile of untreated soil at day 0; at this time straight-chained alcanes constituted a prominent part of the oil. In figure 3 (B) various peaks not encountered in the untreated soil were seen originating from the bioaugmentation product. Figure 3 (C-F) represents chromatograms from the end of the experimental period of soil only supplemented with nutrients and supplemented with the tree different concentrations of bioaugmentation product, respectively. It is evident, that the high and intermediate concentration level inhibited diesel oil degradation; at the highest concentration a substantial amount of straight-chained alcanes were still left undegraded after 61 d. In contrast, the chromatographic peaks from the bioaugmentation product had disappeared, probably because the components were degraded. The chromatograms of the soil supplemented only with nutrients and the soil supplemented with the lowest concentration of bioaugmentation product were indistinguishable and showed the residual diesel oil almost depleted of straight-chained alcanes leaving the unresolved complex mixture typical of biological oil degradation (Gough & Rowland, 1990).

The present study shows, that diesel oil degradation in soil microcosms was inhibited by a bioaugmentation product, when added in the amount of 100 mg/g soil and 10 mg/g soil (the lowest addition level of 1 mg/g soil did not have any detectable effects on diesel oil degradation compared to addition of nutrients alone). The reason for the inhibition was probably the presence of a readily degradable carbon source (starch) in the formulation of the bioaugmentation product. Probably, the selection pressure shifted the populations towards micro-organisms capable of rapid growth on starch instead of oil degradation. Alternatively, no shift in the composition of the population took place, but the oil degrading micro-organisms - both of indigenous origin and from the bioaugmentation product - preferred starch degradation to diesel oil. In any case, the introduced micro-organisms were not able to express their oil degrading capacity. The conversion of large amounts of starch to biomass and CO_2 could also have had a negative effect on oil degradation by depleting the soil of the

nutrients necessary for oil degradation. In the microcosms enriched with 100 mg product/g soil a marked CO_2 production occurred. This was probably coupled to a stochiometric O_2 consumption leading to partial anaerobiosis. In large scale bioremediation efforts, the soil aeration rate would have to be very high to prevent the soil turning anaerobic and thereby inhibiting degradation, since the presence of molecular oxygen is necessary for substantial oil degradation to take place (Leahy & Colwell, 1990).

The results of the present study corroborates the statement of Pritchard (1992), that inoculant formulation is a important factor determining the success of inoculation in bioremediation efforts. We, therefore, suggest that use of bioaugmentation products containing readily available carbon may be inappropriate in connection with bioremediation of oil polluted soil.

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