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Anaerobic Remediation of Dinoseb from Contaminated Soil

An On-Site Demonstration

Scientific Note

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INTRODUCTION

Dinoseb is a teratogenic, nitrophenolic herbicide that was used extensively in the Pacific Northwest of the United States. Because of its toxic properties, the use of dinoseb was banned in 1986 by the US Environmental Protection Agency (EPA), but still persists as a soil contaminant at many sites where it has accumulated as a result of crop-dusting activities, leaking storage containers, and/or poor disposal practices. There are very few treatment options open to the parties responsible for these contaminated sites. Incineration and the haul-and-store options are the only currently acceptable methods for treatment of soils contaminated with nitroaromatic compounds. Future "land ban" regulations may soon limit the types of dinoseb waste that can be accepted by hazardous waste storage facilities. These disposal methods are expensive and are one cause for the bankruptcy of responsible parties.

In order to provide an alternative treatment procedure, the degradation of dinoseb in soils by use of biological procedures has been investigated. Investigations have shown that dinoseb biodegradation apparently does not occur under well-aerated conditions in liquid culture (*1,2*) or under microaerophilic or denitrifying conditions. Under these conditions, microorganisms transform dinoseb to its amino and acetamido forms (*2–5*),

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which apparently retain significant toxicity (*3*). These reactive amino derivatives can undergo oxidation to form polymeric materials (*6–8*).

It has been shown that dinoseb can be degraded anaerobically in aqueous culture to CO_2 and acetate (*2*). The application of anaerobic culture techniques to large-scale soil treatments requires some modification of anaerobic technique. An inexpensive scheme for the bioremediation of dinoseb-contaminated soil has been developed and tested at the bench-scale (*9*) and in small-scale field demonstrations (*10*). The process involves the addition of an external carbon source (starch), and inoculation with either a dinoseb-acclimated aqueous culture or soil containing organisms acclimated to the degradation of dinoseb. This procedure has been used to establish anaerobic conditions efficiently in open containers of soil and to demonstrate the bioremediation of dinoseb-contaminated soil without specialized bioreactors or other sophisticated equipment (*9,10*).

Here a large-scale application of this technology to an on-site treatment of a soil acutely contaminated with dinoseb is described. The problems faced in conducting scientific studies at remote sites are discussed, as well as the response of this treatment system to climactic changes and overwintering.

METHODS

Treatment Procedure

The demonstration was carried out in a polypropylene-lined pit on-site. All personnel wore protective suits, full-face respirators, gloves, and protective footwear during all procedures done in the pit. The demonstration consisted of three stages.

Stage 1 consisted of three 2600-L fiberglass static reactors, which were loaded with approx 350 L of irrigation water, 2.3 kg of K_2HPO_4, and 686 g of KH_2PO_4. Ingredients were mixed until the salts dissolved. Approximately 315 kg of contaminated soil were then added to each reactor using a backhoe. Then 6.3 kg of starch substrate (described below) were added, as well as 35 kg of dried treated soil (described below). The surface area of the aqueous phase was approx 1130 cu in. The pH of the aqueous phase of each reactor was adjusted to 7 with 35*M* sodium hydroxide or concentrated (14.7*M*) phosphoric acid as required. pH, temperature, and redox electrodes were suspended from the top of each reactor and immersed into the aqueous phases to a depth of 10–30 cm below the surface. The data were collected and stored in an A/D data-logging unit connected to a 12-V deep-cycle battery. The reactors were covered with 6-mil polypropylene, which was secured under the lip of each tank with an elastic cord. Duplicate aqueous (one deep and one shallow each) and soil (from below the surface) samples were taken from random locations in each reactor at

time 0 and then every 2 or 3 d thereafter. Reactors were mixed once after 15 d of incubation.

Stage 2 consisted of five reactors set up identically to stage 1 reactors, except that the soil contents from stage 1 were split evenly between the five reactors as inoculum. The contents of the reactors were allowed to incubate for 13 d prior to being transferred to the stage-3 reactors. This stage was designed to generate inocula for the third stage. Quintuplicate soil and aqueous samples were taken initially and after 13 d of incubation.

The contents of the five stage-2 reactors were used as inoculum for the stage-3 static reactors. Each of the three reactors had a 6000-L capacity and was loaded with approx 2000 L of irrigation water, 12.4 kg of K_2HPO_4, 3.7 kg of KH_2PO_4, 32 kg of starch substrate, and approx 2000 kg of contaminated soil. The surface area exposed to the air in stage-3 reactors was approx 6230 sq in. Temperature, redox potential, and pH were monitored as described for stage 1. Triplicate soil and aqueous-phase samples were taken from random locations in the reactors at time zero and then once a week.

Initial Inoculum

Bench-scale experiments performed to determine if this soil contained organisms capable of dinoseb degradation showed that the use of an inoculum of soil containing dinoseb-acclimated organisms improved the degradation of dinoseb in this soil (*9*). It was determined that a 10% inoculum by weight provided the appropriate number of organisms to metabolize the starch supplied, thus consuming oxygen (*9*). The inoculum necessary was obtained from successful pilot-scale experiments performed on another dinoseb-contaminated soil (*10*). HPLC analyses confirmed that dinoseb and all detectable aromatic intermediates were removed from the soil, which was air-dried at room temperature. The dried soil cakes were then crushed, weighed into plastic bags, and stored at 4°C until needed. The treated soil was stored for approx 2 wk prior to beginning the demonstration.

Starch Substrate

A byproduct "centrifuge cake" from a potato processing plant was provided by the J. R. Simplot Co., Caldwell, ID. The composition of the starch substrate was analyzed as described previously (*9*), and found to contain 42% solids, 215 mg/g available starch, 6.7 mg/g total nitrogen, 2.6×10^4 culturable heterotrophic bacteria/g, and 8×10^3 culturable amylolytic bacteria/g. The centrifuge cake was not sterilized prior to use in the demonstration. Previously published results showed that the starch source contained no dinoseb-degrading microorganisms (*9*).

Sampling Procedures

In all experiments, pH and redox potential were measured potentiometrically by placing the electrodes 10–30 cm below the aqueous surface in the reactors. Two aqueous-phase samples were taken from randomly selected depths in the reactors. Results of HPLC, nitrate, and ammonium analyses were averaged to provide approximations of the concentration of the analytes in the total aqueous phase or analyzed by depth to determine the effect of depth on the dinoseb concentration in the aqueous phase of the reactors.

Quintuplicate soil samples of approx 10 g were taken using a long-handled sampling scoop. The excess aqueous phase was allowed to drain from the soil samples before 5 g were weighed and placed on dry ice for transport to the laboratory for analysis as described below. Extractions were performed on these saturated soils to prevent irreversible adsorption of dinoseb to the soils that could occur during soil-drying procedures. The results from these extractions were used for comparative purposes and as an indication of the completeness of the treatment procedure.

Analytical Methods

Dinoseb concentrations were determined by high-performance liquid chromatography (HPLC) using a 250×2 mm Phenomonex (Torrance, CA) Spherex 5 μm C_{18} reverse-phase column as described previously (*9*). Detection of dinoseb and possible transformation products was accomplished using the diode-array UV detector, recording adsorption at 210 nm with continuous scanning of the adsorption spectrum of each peak from 190–600 nm. The detection limit for dinoseb was determined to be 1 mg/L.

Dinoseb was extracted from 5 g of soil by sonication for 5 min in 5 mL acetonitrile at 5°C. Prior to extraction, each sample was amended with 100 μL of a 5000 mg/L stock solution of 4,6-dinitro-*o*-cresol (DNOC) in methanol (which acted as an extraction standard for monitoring intermediate production), and the methanol was allowed to evaporate. Aliquots of 1 mL of the extracts were then passed through a precolumn filter containing C_{18} packing material, and 10 μL of the filtrate were injected onto the HPLC column. Extraction efficiencies were determined to be at least 98% for DNOC in all of the extractions. Dinoseb was recovered at an efficiency of 100% from dinoseb-spiked samples. Aqueous samples were analyzed directly.

Redox potentials of the cultures were measured using a platinum electrode (Orion model 96-78). Nitrate and ammonium concentrations were determined using an ion-sensitive electrode (Orion model 95-12).

RESULTS

Site Description

The site is located in southern Idaho approx 30 miles west of Twin Falls, ID. The geological description identifies the exposed rock unit as the Tuana Gravels of Pleistocene Age, consisting of coarse gravels interbedded with sands and silts. The immediate area consists of the Taunton soil series, which is moderately deep and well drained. The permeability is moderate (0.6–2.0 in/h), and the soils are moderately alkaline (pH 7.4–8.4). The soils are thought to contain low organic matter and to have a low clay content. There is a silica-calcium-cemented layer at 70–100 cm that is restrictive to the downward movement of solutions. Samples of the soil collected from the site were determined to have a pH of 7.5. These samples contained 288 μg/g total K, 294 μg/g NO_3^- as N, 217 μg/g NH_3 as N, and trace amounts of the extractable cations Ca, Mg, Na, K, Zn, Mn, Cu, and Fe.

Demonstration

Stages 1 and 2 were carried out during the months of August and September. The temperature of the aqueous phase in the tanks cycled daily between 25 and 32°C; the average temperature was 28°C. Visual observation indicated that there was no obvious evaporative loss of the aqueous phases during the incubations. The results of these incubations have been published previously (*10*). The major parameters can be described as follows. The pH of the aqueous phases remained within 0.2 pH units of 7. The redox potential in the aqueous phase of the reactors dropped rapidly and was below 0 mV by 2 d of incubation. Nitrate was removed from the aqueous phase of the reactors within 5 d of incubation. Ammonium concentrations were initially averaged at about 40 ppm and were reduced slowly to approx 10 ppm as N by 30 d, a level that was maintained throughout the remainder of the incubation. Dinoseb was undetectable in both the aqueous and soil phases by 15 d. A transient unidentified intermediate was detected in the aqueous-phase samples and in soil extracts. The concentration of intermediate was reduced to near the detection limits of the analyses within 45 d of incubation (stage 1).

The stage-3 incubation was carried out throughout the months of October to May. Initial average temperatures of the aqueous phase were in the mid 20s, but as the climate reflected the change of season, the temperatures in the reactors decreased. The temperature of the reactor aqueous phase averaged below 20°C by midNovember. Figure 1 shows the daily minimum and maximum air temperatures for the course of the incubations. The stage-3 reactors were allowed to overwinter with no attempts

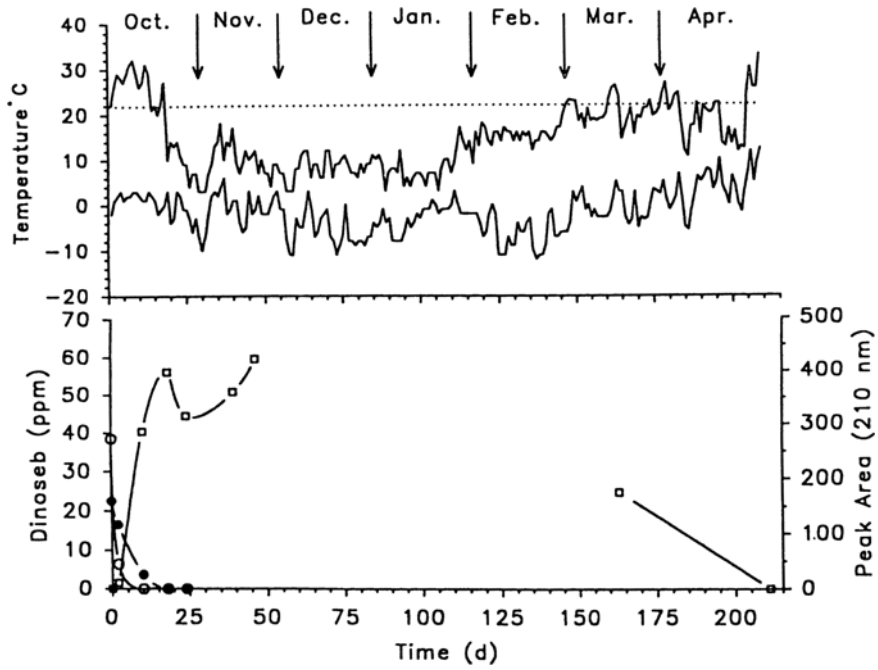

Fig. 1. Air temperature and results of HPLC analyses of the contents of the large-scale dinoseb treatment reactors. ○ Aqueous dinoseb, ● soil dinoseb, □ intermediate.

to insulate them from environmental conditions other than the tarp covering the top of the reactors. The reactor contents were frozen from early December to early March. During this period, the electrode monitors were removed from the reactors in order to prevent damage. Samples were not taken after day 45, since there was a 2-in layer of ice on top of the reactor contents.

The pH was initially maintained within 0.2 pH units of 7, and the redox dropped to below 0 mV within 2–3 d. The results of HPLC analyses of the samples taken before winter are summarized in Fig. 1. Samples collected in March (after the reactor contents thawed) showed that intermediate 1 (as yet unidentified) was still present in the reactors. The temperature of the reactor contents was 14°C, and the redox potentials were all < -290 mV at this time. The reactor contents were allowed to incubate further with periodic sampling. The intermediate was removed from the reactor aqueous and soil phases by 211 d of incubation (total). The clean aqueous phase was then pumped onto an uncontaminated drainage site nearby, and the soil was backfilled and tilled into the pit.

DISCUSSION

The excavated soil contained large chunks of material that were very compact and difficult to break. This material appeared to be heavily contaminated with dinoseb and resisted wetting during the treatment procedure. Extra precautions were taken to crush these chunks in order to achieve a more homogeneous soil layer in the reactors. Because of the amount of soil to be treated, it was not feasible to sieve the soil to obtain a truly uniform distribution of the soil into the reactors. The demonstration of the procedure was done in such a manner as to mimic an actual treatment at a level of technology as low as possible, so that any problems in the procedure would be encountered as early in the development of the industrial method as possible. The problems in distribution of soil size and contamination will be overcome in future treatments by the use of a hopper to crush the soil and mix it with buffer as it is being added to the reactor vessels. The second problem encountered was the remote location of the site (approx 400 miles from the laboratory). This limited the frequency at which samples could be taken. Laboratory studies and earlier small-scale demonstrations (*9,10*) had shown that differences in the scale of the treatment did not greatly affect the time-course of metabolic events, so the authors were reasonably certain of the sequence of events that would be followed in this treatment. The monitoring of the reactor contents was done as often as possible to allow the results to be compared to those obtained during earlier studies.

The results of earlier experiments showed a rapid drop in redox potential and dinoseb concentration when a 10% (by weight) inoculum of soil containing dinoseb-acclimated organisms was used (*9*). This also occurred in all of the reactors in all of the stages of this demonstration. This rapid drop in the redox potential suggested that the microorganisms in the dinoseb-accliminated inoculum used for stage 1 and in the inoculum scaled up from stage 1 to 2 and from stage 2 to 3 were able to remove the oxygen and nitrate rapidly. This was confirmed by nitrate analyses. These activities allowed the establishment of anaerobic conditions quickly, and thus avoided polymerization reactions, which at this scale would tie up large amounts of the target compound, prolonging or halting the treatment period. The presence of ammonium in the aqueous phase throughout the incubation period suggested that nitrogen was not limiting during the course of the incubation. The rapid removal of dinoseb (within 10–15 d in all stages) confirmed that the use of an acclimated inoculum from previous treatments or from one stage to another was an efficient way to obtain rapid large-scale removal of dinoseb from contaminated soil. The incubation times for dinoseb removal from the soil/buffer mixture at these scales were not significantly different from the incubation times observed

in smaller-scale experiments (*9,10*), suggesting that even larger-scale treatments will occur in similar time frames. This is encouraging, since these were static tanks. Anaerobic, stirred tanks would presumably require even less time for the bioremediation process to occur, as indicated by our experiments with another dinoseb-contaminated soil in the state of Washington (*10*).

The accumulation of an intermediate of dinoseb degradation in the aqueous phase of the reactors demonstrated again the importance of monitoring the intermediates of the degradation of a compound, rather than only the parent compound. Dinoseb was completely removed from the soil and aqueous phases well before disappearance of the intermediate in stage 1. The intermediate was eventually removed from the reactor contents in this stage. The intermediate, however, was not removed from the aqueous phase of the stage 3 reactors before the reactor contents were frozen and sampling was stopped. The removal of the intermediate was accomplished, however, once the tank contents warmed up again in the spring. The authors have observed that at temperatures of 22°C and lower, the degradation of dinoseb and its metabolic intermediates is significantly reduced as compared to degradation at 25°C and up (*11*). The cessation of activity observed in the reactors after 25 d of incubation correlates with the drop in air temperature that occurred at this time. Although it is difficult to determine the time when activity in the reactors resumed after overwintering, it most likely coincided with the warming trend observed in the air temperature in early March. The only comparison that could be found in the literature to the problem of overwintering biological treatment systems was that of land farming (*12*). Land farming often takes two or more growing seasons for the complete removal of contaminant compounds, so the overwintering of degradative microbial populations is a known phenomenon. There is little doubt that in our bioreactors the population of active microorganisms was reduced because of freezing. As in most natural systems, the population quickly reestablished itself. The soil environment also provided some microenvironments in which the physical effects of freezing temperatures did not prove hazardous to the microorganisms.

In this demonstration, it has been shown that the removal of the nitroaromatic compound dinoseb could be accomplished at large scales and that the remediation procedure could withstand overwintering. This process is quite rapid as compared to other biological remediation methods and is more economical than physical methods, such as incineration (*10*). The process may also be applied to the treatment of other nitroaromatic compounds. This demonstration is the first instance in which the Idaho Department of Environmental Quality has authorized the reapplication of a once-contaminated soil to a site as a noncontaminated backfill and waste water.

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