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Use of calcium peroxide to provide oxygen for contaminant biodegradation in a saturated soil

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Abstract

Laboratory studies were conducted in solid-phase reactors on a silty loam contaminated with bis-(2-ethylhexyl) phthalate (BEHP) to determine the conditions under which calcium peroxide (CaO_2) would promote the aerobic bioremediation of water-saturated soil. Closed 500 ml solid-phase reactors were operated to determine whether CaO_2 stimulated the biodegradation of BEHP in saturated soil. Ex situ bioremediation conditions were then simulated by mixing water-saturated soil for 6 h before placing the soil in three vented, 2 l solid-phase reactors for 50 days. Biodegradation of BEHP was quantified using four different measurements of microbial activity: (1) oxygen concentrations in the reactor gas; (2) bacterial colony-forming units (CFU); (3) fungal CFU; and (4) 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride dehydrogenase activity (INT-DHA). CaO_2 released molecular O_2, which retarded dewatering but substantially enhanced BEHP biodegradation. After 20 days, BEHP in the amended reactor was reduced from 20.3 to roughly 5 g kg^{-1} vs. 15 g kg^{-1} in the reactor without CaO_2. Bacterial growth was favored over fungal growth at elevated moisture and BEHP levels.

Keywords: BEHP; Dehydrogenase activity; Oxygenation; Peroxides; Solid-phase

1. Introduction

Water-saturated soils are often encountered during in situ bioremediation, but are also problematic for ex situ operations. Examples include dredged sediments, fine-grained

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residuals from soil washing operations, and slurried soil requiring further treatment. Such soil must be drained of its pore water before oxygen can be provided by venting. Weeks or months are required to dewater fine-grained soils and sediments, thus increasing the total time needed for remediation. While bioslurping and soil fracture techniques can increase the contact area at the gas/liquid/solid interfaces, the interior points of water-saturated soils will remain anaerobic for extended periods of time in contaminated soils that support aggressive oxygen consumption rates. This paper describes laboratory investigations of the ability of calcium peroxide (CaO_2) to serve as an oxygen-releasing compound to promote contaminant biodegradation in water-saturated soil. The results will help develop further the application of solid oxygen sources to bioremediation.

Investigators have recognized the potential for adding a homogenization step to solid-phase bioremediation to enhance a remediation process that is otherwise characterized by slow biodegradation rates and non-uniform treatment [1,2]. Simpkin and Giesbrecht [3] bioslurried soil to remove readily biodegradable polycyclic aromatic hydrocarbons (PAHs) followed by land treatment to remove the recalcitrant residuals. Harmsen et al. [4] used a mixed solid-phase bioreactor to treat readily bioavailable pentachlorophenol and PAHs, followed by land application to achieve cost-effective removal of the less bioavailable contaminant fraction. Cassidy and Irvine [5] slurried soil before solid-phase treatment to bioremediate two contaminated soils. Slurrying markedly enhanced the performance of the solid-phase reactors, but weeks were required to dewater the soils sufficiently to allow venting in the solid-phase reactors. These findings underscore the need to investigate ways of oxygenating saturated soils.

Hydrogen peroxide is the oxygen source most commonly used to oxygenate saturated soils for bioremediation applications. Hydrogen peroxide decomposes to form molecular oxygen and water in the presence of catalase (an enzyme found in aerobic microorganisms) and certain abiotic catalysts [6]. Virtually all aerobic microorganisms produce catalase [7]. Hydrogen peroxide can be effective in promoting contaminant biodegradation in soil [8,9], but is readily scavenged by metals and humic substances. This results in rapid exhaustion of the oxygen source [10]. Oxygen released at a rate greater than by which it can be consumed by microorganisms escapes unused via volatilization or dissolved in groundwater. Furthermore, hydrogen peroxide can be toxic at concentrations required to achieve biological treatment [8,10].

Solid oxygen sources decompose in the presence of water to produce hydrogen peroxide, and have been investigated as a means of releasing oxygen in soil more slowly than the direct addition of hydrogen peroxide. Sodium carbonate peroxyhydrate ($Na_2CO_3 \cdot 1.5H_2O_2$), commonly referred to as sodium percarbonate, has been used to oxygenate saturated soils [11,12], but encapsulation is required because its high water solubility results in rapid release of hydrogen peroxide. Magnesium peroxide (MgO_2) and CaO_2 are orders of magnitude less water-soluble than sodium percarbonate [13], which allows them to release oxygen over prolonged periods without encapsulation. Several studies have reported that addition of MgO_2 in saturated soil resulted in increased concentrations of dissolved oxygen and enhanced biodegradation of dissolved contaminants [14–17]. However, these studies did not determine whether microbial growth was stimulated by adding MgO_2.

MgO_2 and CaO_2 combine with water to form hydrogen peroxide according to the following equation, where M represents the divalent metal [18]:

$$MO_2 + 2H_2O \rightarrow M(OH)_2 + H_2O_2. \quad (1)$$

Production of $M(OH)_2$ can drastically increase in pH in poorly buffered soils [19]. While CaO_2 has been less studied than MgO_2 as a solid oxygen source, White et al. [20] reported that CaO_2 resulted in greater stimulation of microbial growth on crude oil in an arctic soil than did MgO_2. A major advantage of CaO_2 over MgO_2 is that commercial CaO_2 can deliver three to four times more molecular oxygen than MgO_2 on a mass basis. MgO_2 is prepared at a mass purity of only 15–25%, as compared with 60–80% for CaO_2 [20,21]. $M(OH)_2$ is the major impurity in the production of commercial peroxides. Another advantage of CaO_2 is that it is considerably less expensive than MgO_2, and is easily produced in the field by heating lime with hydrogen peroxide [18].

Bis-(2-ethylhexyl) phthalate (BEHP) is a priority pollutant frequently found in soils [22]. Estimated annual production of BEHP in 1980 was 350 million lb [23]. A suspected carcinogen and teratogen [22], BEHP is used as a plasticizer for polyvinyl products. BEHP is readily biodegradable [24]. BEHP has a low water solubility (0.3 mg l^{-1}, at 25°C) and a very low vapor pressure (6.5×10^{-6} mmHg, at 25°C) [22].

2. Materials and methods

2.1. Contaminated silty loam

The silty loam used in these studies was originally contaminated with 40 g BEHP/kg soil, but was treated biologically in previous studies to BEHP concentrations less than 150 mg kg^{-1} [1]. The soil was re-contaminated with BEHP for these studies. Table 1 lists some of the properties of the silty loam after being passed through a 0.5-mm sieve. The treated soil had a volumetric water content of 55% saturation. Active BEHP-degrading microbes were present in the soil from the original contamination.

2.2. Chemicals

CaO_2 was obtained from Solvay/Interox (Holzreuth, Germany), consisting of 22% (by weight) molecular O_2. Carbon disulfide (CS_2) and BEHP were purchased from Aldrich Chemical. O_2 and CO_2 standards were from Linde (Munich, Germany).

Table 1
Properties of the silty loam after sieving [5]

Parameter	Measured value
Clay (wt.% < 2 μm)	15.2
Silt (wt.% 2 μm–0.2 mm)	62.3
Fine sand (wt.% 0.2–0.5 mm)	23.3
Carbonates (wt.%)	7.8
Cation exchange capacity (mEq/100 g)	13.8
Humic substances (wt.%)	5.1

2.3. Contamination with BEHP and slurry homogenization

The soil was re-contaminated by adding 400 g of BEHP to 20 kg of soil in an 80-l cement mixer with 1.75 l of distilled water (enough water to provide 100% saturation). N and P were provided by adding 140 g of powdered ammonium phosphate, resulting in a C:N:P ratio of 10:1.5:1. The soil was then slurried in the cement mixer for 1.5 h to ensure a homogeneous water content, nutrient distribution, and BEHP concentration.

2.4. Closed shakeflasks

Closed shakeflasks were used to simulate unvented solid-phase bioreactors. The shakeflasks consisted of 500 ml Pyrex respirometer bottles with a septum-filled monitoring port to allow headspace sampling. A pneumatic seal was maintained by a ground-glass connection between the tops and the flasks. The total volume of the closed slurry reactors was 540 ml. Fifty grams (dry weight) (30 ml) of soil (i.e. pre-slurried, saturated) from the cement mixer were placed in the shakeflasks. Four different reactor types were established: (1) biologically active soil with CaO_2; (2) biologically active soil without CaO_2; (3) autoclaved soil with CaO_2; (4) autoclaved soil without CaO_2. Five grams of CaO_2 were mixed into the soil in the slurry reactors with a stainless steel spatula. This amount of CaO_2 would result in a maximum release of 1.1 g of molecular oxygen. Ten-milliliter gas samples were drawn from the septum-filled ports to monitor O_2 concentrations. After 16 days, the shakeflasks were opened, and the soil was mixed thoroughly with a stainless steel spatula before sampling. The laboratory temperature was 22°–24°C.

2.5. Vented solid-phase bioreactors

The vented solid-phase bioreactors (Fig. 1) consisted of glass Imhoff cones with a total volume of 2 l [1]. Each reactor received 1.7 l (approximately 2.3 kg) of slurried soil from the cement mixer. A fitted polyvinyl chloride (PVC) lid equipped with a rubber O-ring and vacuum grease was used to seal the reactors. Glass wool was packed into the bottom 50 ml of the reactors to prevent the passage of soil. The 1.7 l of contaminated soil in the reactors extended approximately 30 cm from the top of the glass wool. The diameters of the soil/air interface (top of the soil) and the soil/glass wool interface (bottom of the soil) were approximately 15 and 5 cm, respectively. The exterior of the reactors was covered with aluminum foil. Two grams of granular activated carbon (GAC) were used to collect volatile organics in the effluent air. The reactors were equipped with a flask to collect leachate. The laboratory temperature was between 22° and 24°C during the study period.

Three bioreactors were established: (1) biologically active with CaO_2; (2) biologically active without CaO_2; and (3) autoclaved control without CaO_2. The soil was added to the reactors in three 567-ml increments, which were packed into the vessels by a Teflon stopper attached to a steel rod. One hundred grams of CaO_2 (which would release a maximum of 22 g of molecular oxygen) were added by mixing 33.3 g of CaO_2 into each 567 ml of soil with a stainless steel spoon. Immediately upon closing, air was

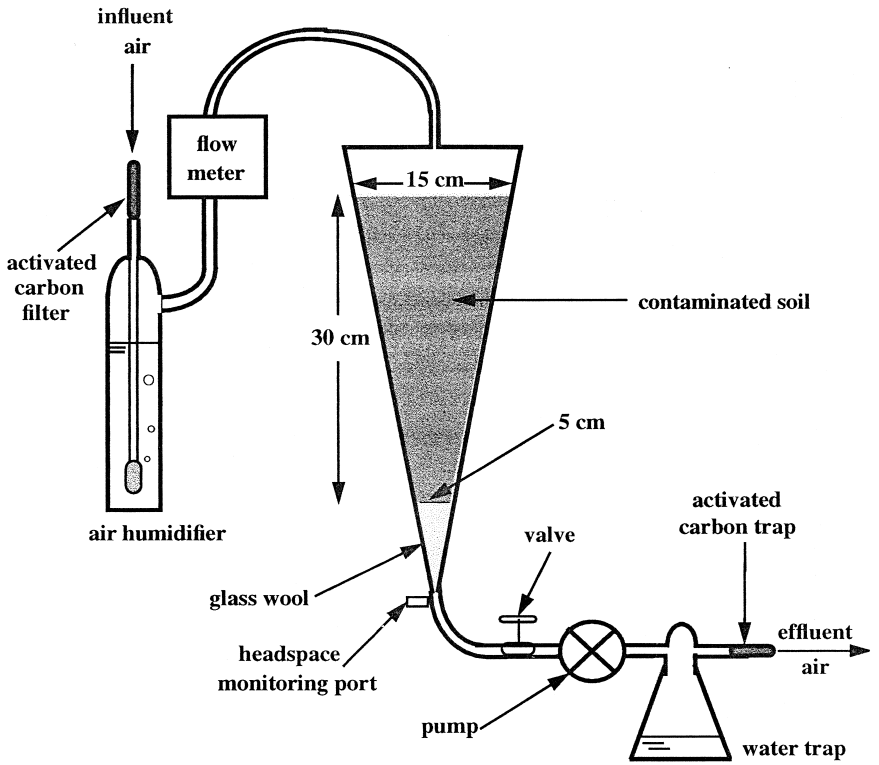

Fig. 1. Solid-phase bioreactors used in the experiments.

pulled through the reactors to encourage dewatering and, ultimately, to vent the soil. Only water was removed from the soil immediately after turning on the pumps. However, within the first 2 days, sufficient dewatering had occurred to allow the passage of air.

Air was pulled through the soil using a membrane vacuum pump. The influent air was purified with an activated carbon filter and humidified to minimize drying of the soil during venting. Tygon tubing was used to make all connections for air flow. The rate of air flow was measured with a gas flow meter, and controlled with the valve. Air was vented at 5 ml min^{-1}, resulting in a gas exchange rate of 15 pore volumes per day.

The concentration of oxygen was measured in 10 ml samples of the effluent air taken from the bottom of the solid-phase reactors (i.e. in air that had already passed through the soil). Each reactor was opened by removing the lid every 5 days to collect a composite soil sample from the entire 15 cm depth of soil using a hollow stainless steel tube with an inner diameter of 1.5 cm. Each soil core was emptied into a beaker, mixed thoroughly with a sterilized stainless steel spatula, and analyzed for BEHP and quantification of microbial activity. While both the initial slurrying and the continuous passage of air through the reactors minimized vertical variations in oxygen penetration and soil

heterogeneity, the composite sample allowed a depth-weighted average concentration of constituents to be monitored over the time-course of the study. Oxygen, released from CaO_2 lost via venting and/or opening the reactors for sampling, was not quantified.

2.6. Soil analyses

Extraction of BEHP was done according to Irvine et al. [24]. Quadruplicate 5 g soil samples were placed in 100 ml screw-cap test tubes with 25 ml of distilled water, 10 ml of carbon disulfide (CS_2), and 500 μl of diethyl phthalate (DEP) (internal standard). The test tubes were vortexed for 30 s, shaken for 60 min on a wrist-action shaker at the maximum setting, and centrifuged at 6000 rpm for 15 min. The CS_2 was removed with an air-tight syringe and placed in 1.5 ml vials for analysis with a gas chromatograph (GC). DEP recovery in excess of 92% throughout the study period indicated that the extraction procedure was effective. Except that only 5 ml of CS was used, extraction of the GAC traps was identical to that for the soils. One-microliter CS_2 samples were injected into a Carlo Erba GC equipped with flame ionization detection and a 15-m capillary Supelco DB-5 column. Oven, injector, and detector temperatures were 240°, 315°, and 330°, respectively. Standards were in CS_2. Soil pH was measured using duplicate 5 g soil samples according to the electrical method [25]. Water content was measured using the gravimetric method [26].

2.7. Quantification of biological activity

Respiring bacteria and fungi reduce 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride (INT) to the red precipitate INT-formazan [27]. INT-DHA (dehydrogenase activity) was quantified with duplicate 5 g soil samples using a modification of the method provided by Alef [27]. The modifications consisted of using 5 g soil in 100 ml vials, 20 ml of INT solution, no buffer solution, and incubation for 6 h at 25°C. The 100 ml of extraction solution (*N*,*N*-dimethylformamide and ethanol) was filtered (0.45 μm) and measured spectrophotometrically at 464 nm against a blank. Results are expressed as micrograms of INT-formazan produced per gram of oven dried soil per day ($\mu g\ g^{-1}\ day^{-1}$). Spread plate methods in Standard Methods for the Examination of Water and Waste Water [28] were used to quantify heterotrophic bacteria (Method 9215C) and fungi (Method 9610C) on quadruplicate soil samples. Results are reported as colony-forming units (CFU).

2.8. Gas analyses

The concentration of oxygen in the 10-ml gas samples taken from the bioreactors was measured by injecting into an Illinois Instruments Model 3600 Oxygen and Carbon Dioxide Gas Analyzer. The analyzer was calibrated weekly using ambient air (assuming 20.9% O_2) and a gas standard consisting of 10.0% CO_2 and 90% N_2 (a zero-point for O_2).

3. Results and discussion

3.1. Closed shakeflasks

Fig. 2 shows the time profile of the mass of oxygen measured in the headspace of the closed shakeflasks containing saturated soil during the 16-day study period. Approximately 150 mg of oxygen was initially present in the 510 ml of air in all the shakeflasks. A maximum of 1.1 g of oxygen was available through the added CaO_2, for a total of 1.25 g oxygen in the shakeflasks containing CaO_2. The mass of oxygen in the reactors containing killed soil without CaO_2 remained constant at 150 mg, indicating that autoclaving effectively killed the soil microbes. In contrast, the mass of oxygen in the headspace of reactors containing biologically active soil without CaO_2 decreased at a rate of about 27 mg day^{-1} for the first 4 days, and the remaining 40 mg of oxygen was reduced to nearly zero during the next 4 days. The reactors containing killed soil with CaO_2 showed an initial rate of increase in oxygen of 20 mg day^{-1} over the first 6 days. By day 16, just over 300 mg of oxygen had accumulated in the shakeflask containing killed soil with CaO_2.

Oxygen was released and consumed simultaneously in the biologically active reactor with CaO_2. As can be estimated from Fig. 2, the rate of oxygen consumption was about 105 mg day^{-1} from the time that the decomposition of CaO_2 was complete (apparently sometime between days 3 and 5) and until the time that the partial pressure of oxygen in the headspace approached that of air (shortly after day 10). After day 10, oxygen was depleted at a rate of about 40 mg day^{-1} for the next 4 + days. Using an oxygen consumption rate of 105 mg day^{-1} for the time that the partial pressure of oxygen was greater than air (i.e. for the first 10 + days) and a rate of 40 mg day^{-1} for the next 4 days, the total mass of oxygen consumed over the first 14 days can be estimated to be

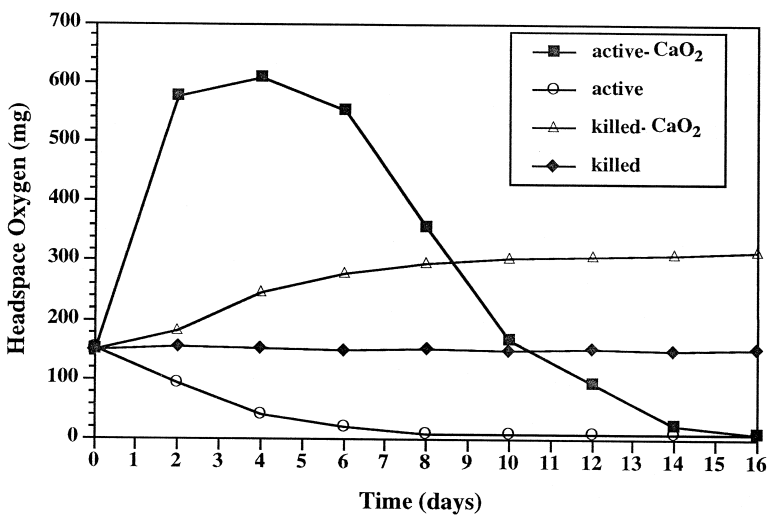

Fig. 2. Mass of oxygen in the headspace of the closed shakeflasks with time.

1.2 g, or very near the initial mass of oxygen present in the flask. The release rate of oxygen of 320 mg day^{-1} can be estimated simply by adding the oxygen consumption rate of 105 mg day^{-1} to the 215 mg day^{-1} rate of oxygen increase observed for the first 2 days. At this oxygen release rate, the CaO_2 added would be completely dissociated in 3.5 days. A biologically mediated oxygen release rate of 300 mg day^{-1} can be determined as the difference between 320 mg day^{-1} and the 20 mg day^{-1} estimated for the reactors containing killed soil and CaO_2.

The results from soil analyses in the shakeflask reactors are presented in Table 2. Each shakeflask contained approximately 1 g of BEHP (20.3 g $kg^{-1} \times 0.05$ kg). Neither of the reactors with killed soil had appreciable BEHP consumption or microbial activity. In contrast, the BEHP concentration decreased from approximately 20 to 9 g kg^{-1} (i.e. 0.55 g BEHP removed) in the biologically active soil containing CaO_2. Since 2.58 g oxygen are needed to oxidize 1 g of BEHP completely to CO_2 and H_2O (2.58 is the chemical oxygen demand (COD) equivalent of BEHP), 1.42 g of oxygen would be needed to mineralize the 0.55 g of BEHP consumed in the reactor containing CaO_2. Accordingly, the difference between the 1.2 g of oxygen actually consumed and the 1.42 g of oxygen needed for complete mineralization represents an unexerted oxygen demand that will be found in both the biomass produced and other by-products formed. Using an empirical formula for biomass of $C_5H_7NO_2$, 1.41 g oxygen is needed to mineralize 1 g of biomass. As a result, roughly 0.16 g of biomass was produced in the CaO_2 amended reactor for each 1.42 g of BEHP-COD consumed, assuming the presence of no other by-products. The resulting yield of 0.11 g biomass produced per gram of BEHP-COD consumed is less than the 0.21 reported by Cassidy [1] for BEHP-COD and the 0.3 by Pitter and Chudoba [29] for other wastes. Note also that 2.18 g ($= 1.2$ g $O_2/0.55$ g BEHP) of oxygen is utilized for each 1 g of BEHP consumed.

Fungi, as well as bacteria, were monitored using plating and spectrophotometric methods because several species of fungi are capable of degrading and/or mineralizing BEHP [30,31]. As can be seen from Table 2, however, the number of fungal CFU in the biologically active reactors did not increase above background values by more than the

Table 2
Results from the soil analyses after 16 days in the closed shakeflasks[a]

Reactor contents	BEHP[b]	Bacteria[c] (CFU$\times 10^6$ g^{-1})	Fungi[d] (CFU$\times 10^4$ g^{-1})	INT-DHA[e] (μg g^{-1} d^{-1})	pH[f]
Active CaO_2	9.3 ± 1.8	23.1 ± 13.1	2.7 ± 2.1	443 ± 31	8.1 ± 0.1
Active	18.3 ± 1.7	6.4 ± 3.2	2.4 ± 1.5	86 ± 13	8.3 ± 0.3
Killed CaO_2	20.4 ± 2.1	0	0	6 ± 4	8.2 ± 0.2
Killed	19.8 ± 1.6	0	0	16 ± 7	8.3 ± 0.1

[a] Data were averaged for the quadruplicates of each reactor type.
[b] Initial BEHP concentration in the soil was 20.3 g $kg^{-1} \pm 1.2$.
[c] Initial bacterial CFU in the soil was 3.5×10^6 CFU $g^{-1} \pm 2.3 \times 10^6$ (6.54 log units).
[d] Initial fungal CFU in the soil was 2.7×10^4 CFU $g^{-1} \pm 1.9 \times 10^4$ (4.43 log units).
[e] Initial INT-DHA in the soil was 8 μg g^{-1} $d^{-1} \pm 4$.
[f] Initial pH in the soil was 8.2 ± 0.1.

standard deviation during the 16-day incubation while the bacterial CFU increases in the same reactor were 6.6-fold (with CaO_2) and 1.8-fold (without CaO_2).

Because of the low fungal CFU, INT-DHA readings were used to correlate bacterial activity with bacterial CFU measurements. Both methods have difficulties. Spread plate techniques notoriously underestimate biological activity because only cells that grow on the medium selected and in the environment of the spread plate are detected [7]. In contrast, INT-DHA activity typically overestimates biological activity because abiotic reduction of INT to INT-formazan can occur, and all aerobically respiring cells (viable and non-viable) contribute to the measurement [7,32–34]. This pattern can be seen in the results of the shakeflask studies. For example, INT-DHA readings were not zero in the autoclaved controls as were bacterial and fungal CFU and the increases in INT-DHA in active soil with and without CaO_2 were 55-fold and 11-fold, respectively. Likewise, INT-DHA data indicate that biological activity was enhanced 5-fold by the presence of CaO_2, while CFU data indicate enhancement by a factor of only 3.6. Similar observations were made from studies in slurry and solid-phase bioreactors treating diesel-fuel-contaminated soil [1]. The pH was not affected by adding CaO_2 to this carbonate-rich, silty loam, but adding peroxides can increase the pH drastically in poorly buffered soils [12,20].

3.2. Vented solid-phase bioreactors

The data from the shakeflask studies showed that oxygen was released from CaO_2 added to the soil, and that the released oxygen stimulated microbial activity and promoted the biodegradation of BEHP without increasing soil pH. However, the saturated soil in the shakeflasks was not allowed the opportunity to drain. Vented, solid-phase bioreactors were operated to determine how amending soil with CaO_2 affects solid-phase bioremediation in reactors that allow drainage. The dose of CaO_2 in the vented solid-phase bioreactors was 40% of that used in the shakeflasks (0.04 g g^{-1} soil vs. 0.1 g g^{-1} soil), in part because less oxygen would be needed in reactors supplied oxygen through venting.

Dewatering of the soil in the solid-phase bioreactors over the 50-day experiment is shown by plotting the leachate collected from the reactors with time (Fig. 3). The soil was saturated when placed in the reactors. All three of the reactors produced approximately 270 ml of leachate. The two soils without CaO_2 were completely dewatered after day 15, whereas more than 20 days were required for the soil with CaO_2 to dewater. The observed hindrance in dewatering of the saturated soil caused by CaO_2 was likely the result of gas binding associated with the evolution of oxygen. Gas bubbles, presumably of oxygen, were observed in the soil containing CaO_2 during reactor sampling. The presence of a gas phase decreases the hydraulic conductivity in a porous medium and retards drainage. The water content in all three reactors after all the leachates were collected was approximately 55% saturation, but the soil continued to lose water due to evaporation. The final moisture content after 50 days of venting was 46%. The soil pH in all of the reactors remained at 8.2, showing that CaO_2 did not change the soil pH.

The BEHP concentrations in the silty loam during the study period are shown in Fig. 4. BEHP removal was observed in active soil with and without CaO_2 until a concentra-

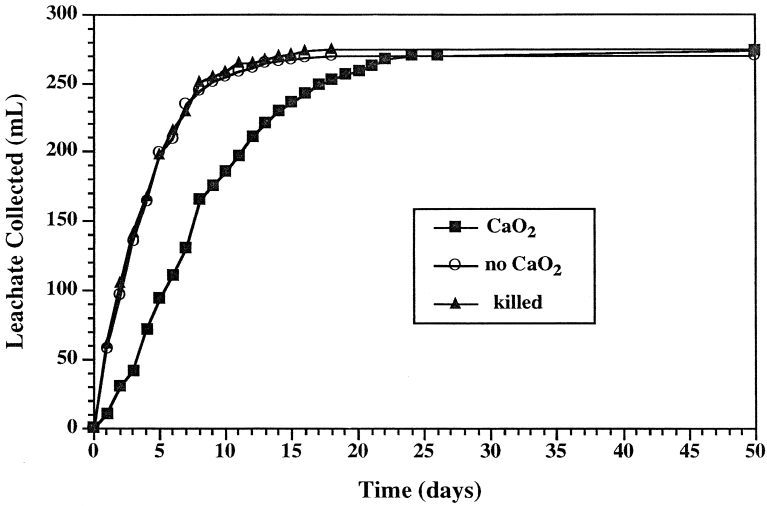

Fig. 3. Dewatering of soil in the solid-phase bioreactors with time.

tion of approximately 3 g kg^{-1} was reached. Slow BEHP removal below a concentration of 3 g kg^{-1} was not due to nutrient limitations, but rather to reduced bioavailability in the silty loam caused by the high humic and clay content (Table 1) [5]. No BEHP was lost in the leachate or the exhaust gas from the bioreactors, which is consistent with the results of previous solid-phase studies with BEHP-contaminated soil [1]. No BEHP removal was observed in the autoclaved soil. Throughout the first 20 days, the rate of BEHP degradation for the soil with CaO_2 was roughly 830 mg day^{-1} kg^{-1} soil, or 1900

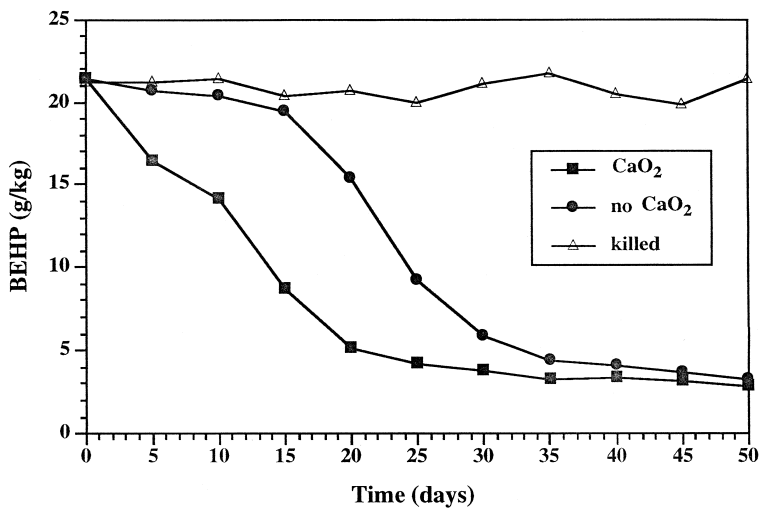

Fig. 4. BEHP concentrations in the solid-phase bioreactors with time.

mg day^{-1} for the 2.3 kg of soil present in the reactor (see Fig. 4). The soil without CaO_2 showed notable BEHP removal after the soil was dewatered on day 15. Only then was this reactor oxygenated sufficiently to allow aggressive biological degradation of the BEHP. Between days 15 to 25, the maximum rate of BEHP degradation in the soil without CaO_2 was roughly 1000 mg kg^{-1} day^{-1}, or 2300 mg day^{-1} for the 2.3 kg of soil present in the reactor.

The concentration of oxygen in the gas at the bottom of the solid-phase bioreactors is shown as a function of time in Fig. 5. Note that these measurements were made in gas that had already passed through the soil. The oxygen concentration in the killed control remained at 21% (atmospheric concentration) throughout the experiment, demonstrating no microbial activity in the soil. In contrast, the oxygen concentration in the reactor without CaO_2 decreased immediately to values less than 2% and then began to increase somewhat on day 10. Oxygen consumed by respiring microorganisms could not be replaced during the first 15 days because the soil was not sufficiently dewatered to allow adequate aeration (Fig. 3). The concentration of oxygen in the soil containing CaO_2 was greater than atmospheric during the first 4 days, due to the release of O_2 from the CaO_2. After day 4, microbial respiration consumed oxygen at a greater rate than it was released by the CaO_2 until approximately day 15 (Fig. 5) when the rate of BEHP consumption began to slow (Fig. 4). The increase in oxygen concentrations in the reactor containing CaO_2 after day 15 was due to oxygen provided by vented air. Oxygen concentrations approached atmospheric by day 50 because the bioavailable BEHP had already been consumed (Fig. 4). The oxygen concentration in both biologically active reactors neared atmospheric by day 50 because the BEHP had been consumed (Fig. 4).

Results from these solid-phase reactors show that CaO_2 released oxygen in the soil during dewatering, even as it retarded the dewatering required for soil venting. Because

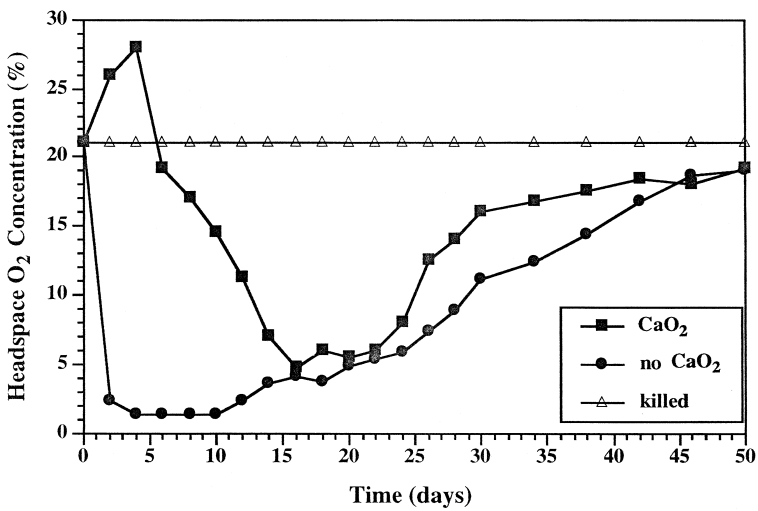

Fig. 5. O_2 concentrations in the gas of the solid-phase bioreactors with time.

venting and oxygen release from CaO_2 occurred simultaneously, the total mass of oxygen consumed in the solid-phase reactors cannot be quantified from the data in Fig. 5. Nevertheless, it can be concluded that no venting should have been attempted for the reactor with CaO_2 until the residual oxygen from the CaO_2 dropped below atmospheric levels in order to minimize the loss of oxygen generated from the decomposition of CaO_2.

Measurements of CFU and INT-DHA with time in the solid-phase bioreactors are shown in Fig. 6a and b, respectively. INT-DHA values in both biologically active soils increased from approximately 50 $\mu g\ g^{-1}\ day^{-1}$ to a maximum value of 450 $\mu g\ g^{-1}\ day^{-1}$. This represents a 9-fold increase in INT-DHA, which is greater than the 7-fold increase in total microorganism numbers observed in both biologically active soils (from 4×10^6 CFU g^{-1} to 28×10^6 CFU g^{-1}). INT-DHA measurements in the killed soil varied from 5 to 15 $\mu g\ g^{-1}\ day^{-1}$. No bacterial and fungal CFU were observed in the

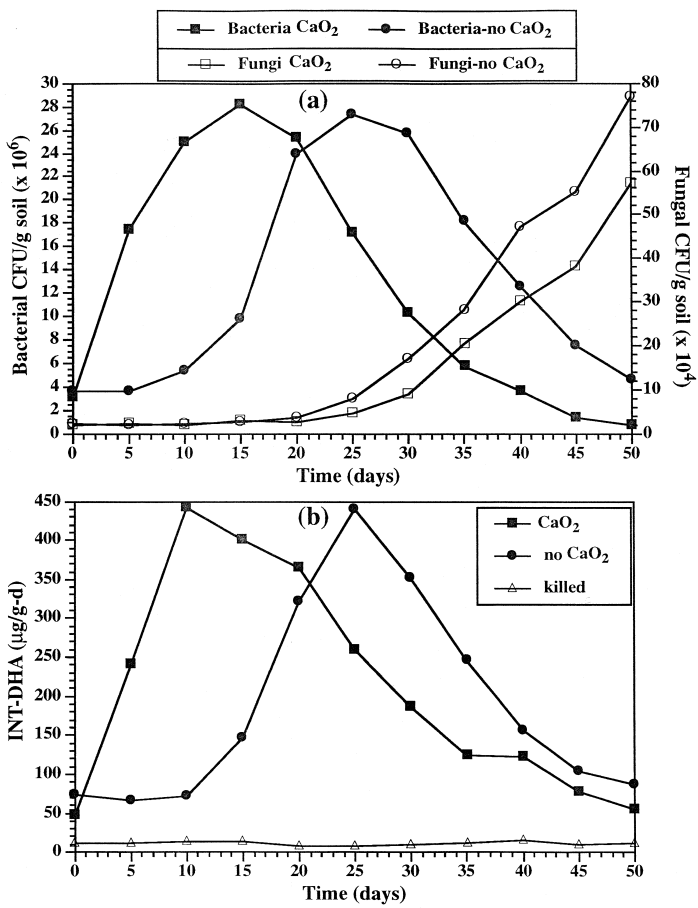

Fig. 6. (a) Bacterial and fungal CFU and (b) INT-DHA in the solid-phase bioreactors with time.

killed soil (data not shown). This is similar to the results observed in the shakeflasks. The soil amended with CaO_2 showed microbial growth within the first 5 days of operation (Fig. 6). Meanwhile, microbial growth in the soil without CaO_2 was delayed by approximately 10 days and remained relatively low until day 15 when the soil was fully dewatered and oxygen was provided through venting (Figs. 3 and 5). Maximum values of total CFU and INT-DHA were observed between days 10 and 15 in the soil with CaO_2, and between days 25 and 30 in the soil without CaO_2. The decrease in bacterial CFU and INT-DHA from maximum values coincided with the depletion of bioavailable BEHP in both biologically active reactors (Fig. 4). The bacterial CFU and INT-DHA results obtained in the solid-phase bioreactors were similar to measurements in the shakeflasks (Table 2). The results in Fig. 6 strongly support the observation that the oxygen released from CaO_2 stimulated microbial activity.

Fungal numbers remained at background values of approximately 2×10^4 CFU g^{-1} during the first 20 days in both biologically active reactors. However, fungal numbers increased dramatically after day 20 to values of approximately 6×10^5 CFU g^{-1} in the soil without CaO_2, and 8×10^5 CFU g^{-1} in the soil with CaO_2. This represents an increase in fungal numbers of more than an order of magnitude. Soil pH did not change during the study period, but the soil continued to desiccate through venting to a moisture content of 46%, which is less than the field capacity of the soil (55%). It has been observed in composting operations and in soils that fungal growth is often favored over bacterial growth as the moisture content decreases [35]. The moisture content decrease may have contributed to the observed increase in fungal numbers in the solid-phase reactors. Other factors are also possible. These include: (1) the release of available growth factors from 'dying' bacteria; and (2) the detection of fungal propagules or spores during unfavorable conditions established after most of the bioavailable BEHP were depleted [7]. The increase in fungal numbers had little or no impact on the INT-DHA levels (Fig. 6b) because the final levels, even though an order of magnitude above initial levels, constituted a relatively low respiratory level for the fungi (as compared to that of bacteria at earlier times).

4. Summary and conclusions

The objective of this study was to investigate the ability of CaO_2 to oxygenate water-saturated soil in order to promote contaminant biodegradation. The conclusions from these laboratory investigations can be summarized as follows:

- Adding CaO_2 to water-saturated soil releases oxygen over a period of days to weeks and promotes accelerated contaminant biodegradation;
- Amending the soil with CaO_2 under conditions of water saturation slowed the drainage of water from the soil due to the formation of gas (probably O_2) bubbles;
- The INT-DHA assay overestimated the biological activity in the soil relative to microbial enumeration using CFU measurements;
- Results from the INT-DHA assay are much less variable and are obtained in a much shorter time frame than those from plating techniques.

Recent studies in ex situ bioremediation indicate that solid-phase treatment of slurried soil has the potential to realize cost savings relative to using solid-phase bioremediation or bioslurry treatment alone. Possible treatment scenarios include a short slurry homogenization step followed by active aeration in solid-phase bioreactors [36], and extensive bioslurry treatment followed by a polishing step in a passively aerated system (e.g., land application) [1,3]. In both cases, the solid-phase treatment step relies on aeration to provide oxygen for contaminant biodegradation, which can be severely limited in water-saturated soils produced from slurrying. Results from these studies show that adding CaO_2 to water-saturated soil can release oxygen over a period of days to weeks to promote contaminant biodegradation in a solid-phase bioreactor. This suggests that CaO_2 and other solid oxygen sources have potential application to ex situ bioremediation, especially for soils requiring a long time for gravity drainage.

INT-DHA is a well-developed tool in uncontaminated soils [37,38], but application to contaminated soil has been limited. Dehydrogenase activity typically overestimates biological activity in soil. However, this study showed that the results from the INT-DHA assay showed much less variability than CFU results. Furthermore, the INT-DHA assay can provide results within a day, whereas up to a week or more are often required for plating techniques. This suggests that INT-DHA can be used alone to measure microbial activity in soils when qualitative results are sufficient, but should be correlated with other methods (e.g., CFU and respirometry) when more exact measurements are required.

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