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The use of solid peroxides to stimulate growth of aerobic microbes in tundra

Daniel M. White [a,*], Robert L. Irvine [b], Craig R. Woolard [c]

[a] *University of Alaska Fairbanks, 248 Duckering, Box 755900, Fairbanks, AK 99775-5900, USA*
[b] *Department of Civil Engineering and Geological Sciences, University of Notre Dame, Notre Dame, IN 46556-0767, USA*
[c] *University of Akaska Anchorage, 3211 Providence, School of Engineering, Anchorage, AK 99508, USA*

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Abstract

Solid peroxides and peroxyhydrates degrade into a basic salt, water, and molecular oxygen when in contact with biologically active soils. Column reactors were used to quantify the extent to which three solid peroxides would stimulate growth of aerobic, heterotrophic bacteria and fungi in contaminated tundra soil. Soils in contact with a peroxide compound were incubated in column reactors at field moisture conditions at either 12 or 25°C with no mixing. After 1200-h incubations, localized concentrations of bacteria and fungi were at least 2 orders of magnitude greater in soil amended with sodium carbonate peroxyhydrate than in soil containing either calcium peroxide or magnesium peroxide. Only in soil containing sodium carbonate peroxyhydrate did microbes grow to an appreciably higher concentration than in control soil, which contained no peroxide. Stimulation of both bacterial and fungal growth occurred primarily at distances of less than 5 cm from the peroxide, suggesting that under static moisture conditions, only localized microbial growth can be expected in acidic tundra soils. © 1998 Elsevier Science B.V.

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1. Introduction

A critical factor in the aerobic bioremediation of contaminated soils is effective soil oxygenation. Although aqueous phase hydrogen peroxide can be used to oxygenate contaminated soils, hydrogen peroxide is toxic at high concentrations and frequently

* Corresponding author. Tel.: +1 907 474 6222; fax: +1 907 474 6087; e-mail: ffdmw@aurora.alaska.edu.

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promotes biofouling in the delivery system [1–3]. Solid compounds such as simple peroxides and peroxyhydrates are an alternative source of oxygen with application in bioremediation systems [4–7]. The simple peroxides (e.g., CaO_2) combine with water to form hydrogen peroxide, where M is a divalent

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

metal [8]. Peroxyhydrates (e.g., $Na_2CO_3 \cdot 1.5H_20_2$) also release hydrogen peroxide upon dissolution in water. Catalase, a microbial enzyme, and certain inorganic catalysts in soil convert two moles of hydrogen peroxide into one mole of O_2 and two moles of H_2O. Solid peroxides are normally used in laundry detergents and food products [9,10]. This research evaluated the effect of three peroxides on the growth of aerobic microorganisms in tundra soil contaminated with crude oil.

2. Experimental

2.1. Soil description

All soils were collected from Umiat, Alaska (69°21′00″N, 152°10′ 00″W). Prior to soil collection, the surface vegetation and rhizosphere were removed from a 30×200 cm section of tundra. Soil was excavated to permafrost, approximately 36 cm. The soil mineral fraction was divided evenly between silt and clay with only a minor fraction of sand [11]. The soil located 15–30 cm deep contained fibrous and partly decomposed plant material, while that located between 30–36 cm contained highly decomposed organic material. The C:N ratio of a representative sample of soil was 18.45 (9.6–10.5% C) as determined by carbon, hydrogen, nitrogen (CHN) analysis (Carlo Erba CHNS-O EA1108 Elemental Analyzer).

2.2. Soil preparation

After collection, all soils were shipped from Umiat to the University of Notre Dame where they were stored at 4°C. The concentration of aerobic, heterotrophic bacteria in the soil prior to reactor preparation was 7.9×10^4 CFU/g dry soil (4.9 log units), measured by Standard Method 9215 'Heterotrophic Plate Count' [12]. The concentration of fungal propagules in the soil was 3.2×10^3 CFU/g dry soil (3.5 log units), measured by Standard Method 9610 C [12]. The initial pH of the soil was 3.8, measured in a 1:10 dilution with distilled water. At field conditions, the soil contained 37% moisture (by drying overnight at 103°C) and 20% organic material (by loss on ignition at 550°C for 4 h). Prior to reactor preparation, all soil was forced through a #10 sieve (2 mm spacing) at the field moisture content to break up clay aggregates and fibrous mats. Sieved soil was homogenized by gently mixing without reaggregating the soil. The soil was not dried before sieving to preserve the field moisture condition. The homogenized soil was artificially contaminated in the laboratory with 15,000 mg/kg of Kuparuk River crude oil on a dry weight basis. The soil was gently mixed by hand until the oil was evenly distributed.

2.3. Reactor preparation

Soil column reactors were prepared by packing a total of 48 g of soil into Pyrex tubing, 18 cm long with an inside diameter of 1.6 cm. The soil was packed into the columns 3 cm at a time using a glass rod. The soil occupied all but roughly 2 cm^3 of the reactor. Three grams of SPO were added as a solid plug in the remaining reactor volume and held in place with a rubber stopper (see Fig. 1a). During experimentation, the reactor was inverted, so the SPO was at the bottom of the column. The open end of each reactor was covered with parafilm to reduce, but not eliminate, oxygen diffusion. Since oxygen diffuses through parafilm at 150 cc m^{-2} day^{-1} @ 50% relative humidity and 73°F [13], the parafilm allowed any oxygen released by the SPO to diffuse out of the reactor while preventing soil desiccation. Four replicate soil columns were prepared with each SPO along with four controls with no SPO. For each set of four replicates, two reactors were incubated at 12°C and two at 25°C. The incubation temperature of 12°C represents the maximum temperature expected at the surface of Arctic soils. All reactors were incubated for 1200 h.

All solid peroxides were kept in sealed containers at 4°C to prevent natural degradation before reactor preparation. Each of the peroxides was used within 3 months of delivery from the suppliers. Although oxygen is released from each of the SPO compounds through a hydrogen peroxide intermediate, the purity of each compound and the percent active oxygen are critical for bioremediation applications (see Table 1). Magnesium peroxide, for example, has a high mass percent oxygen, but since it is only prepared in 24% purity, the active oxygen content is the lowest of the three compounds tested.

2.4. Monitoring

At the end of the 1200-h incubation, each soil column was aseptically extruded (see Fig. 1b) and split into 3 units of equal length (see Fig. 1c). One centimeter of soil at each end of the column was discarded to reduce end-effects. Soil units one, two, and

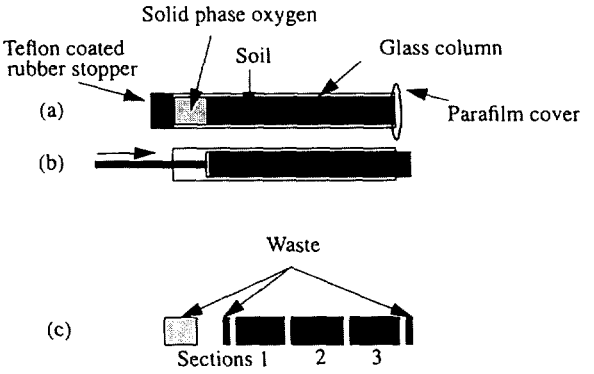

Fig. 1. Illustration of the soil column extrusion (a, b) and sampling (c) procedure.

Table 1
Properties of solid phase oxygen compounds

	Manufacturer	Active ingredient	Purity (wt.%)	Active O_2 (wt.%)[a]
SPO1	FMC	CaO_2	80	16.9
SPO2	Fluka	MgO_2	24	6.9
SPO3	Solvay/Interox	$Na_2CO_3 \cdot 1.5H_2O_2$	85	15.0

[a]Weight percent active oxygen takes into account the purity of the compound shown.

three were made up of soil originally positioned 1–5 cm, 5–10 cm and 10–15 cm from the SPO, respectively. Each soil unit was homogenized and subsampled to quantify the concentration of aerobic, heterotrophic bacteria using Standard Methods 9215 'Heterotrophic Plate Count', fungal propagules using Standard Method 9610 C [12], and soil pH. Soil pH was quantified in a 1:10 (w/w) dilution with distilled water.

3. Results and discussion

3.1. Soil pH

The pH in all three soil units of the controls and the reactors containing SPO1 and SPO2 were roughly equal (see Figs. 2 and 3). Since calcium peroxide (SPO1) and magnesium peroxide (SPO2) degrade into basic salts, both were expected to neutralize the soil pH. Neither peroxide, however, had any neutralizing effect on the soil. The absence of a basic reaction is probably the result of slow diffusion of the basic ions in the moist, but unsaturated soil. Previous studies showed that slurries of the same soil reached pH > 9 when either calcium and magnesium peroxide were added [11].

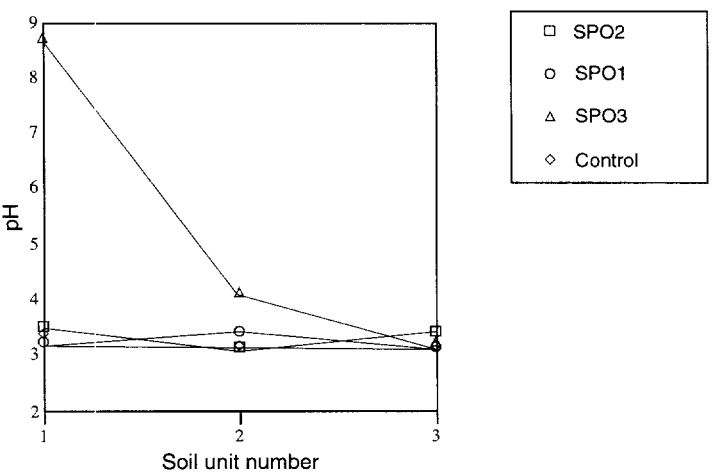

Fig. 2. Distribution of pH in soil columns (25°C).

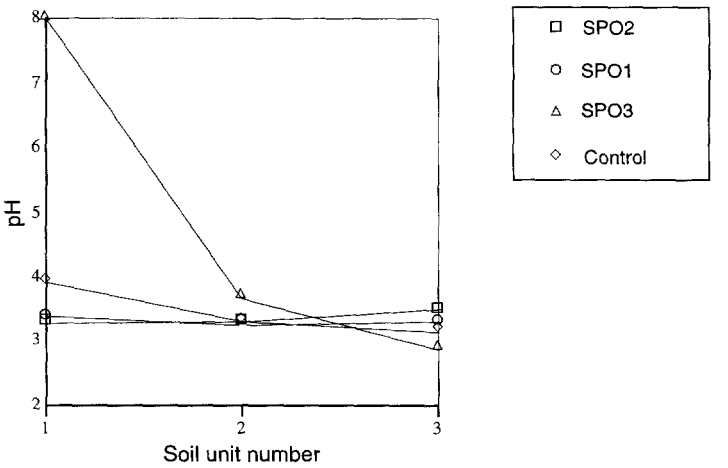

Fig. 3. Distribution of pH in soil columns (12°C).

The pH in reactors containing SPO3 reached 8 and 8.7 in the first soil unit at incubation temperatures of 12 (see Fig. 3) and 25°C (see Fig. 2), respectively. The pH in soil units two and three did not increase appreciably at either incubation temperature.

3.2. Microbial enumerations

3.2.1. Control reactors

As shown in Figs. 4 and 5, the concentration of bacteria in the control reactors incubated at 12 and 25°C was almost an order of magnitude greater in soil unit three (i.e., the end covered by parafilm) than soil unit one. Since diffusion of air through the

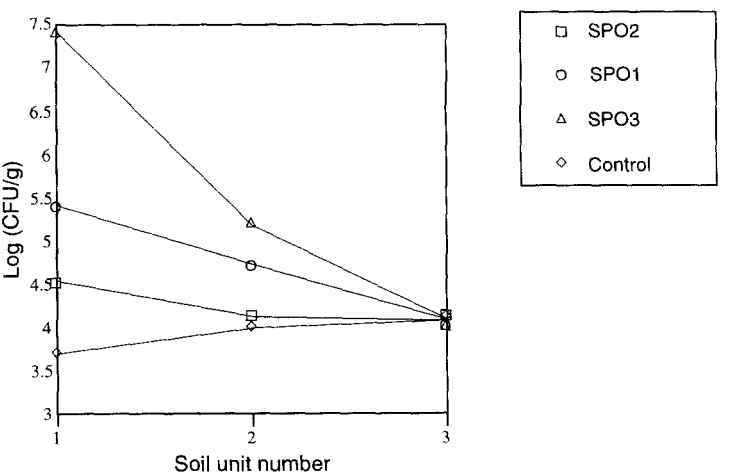

Fig. 4. Distribution of bacteria in soil columns (25°C).

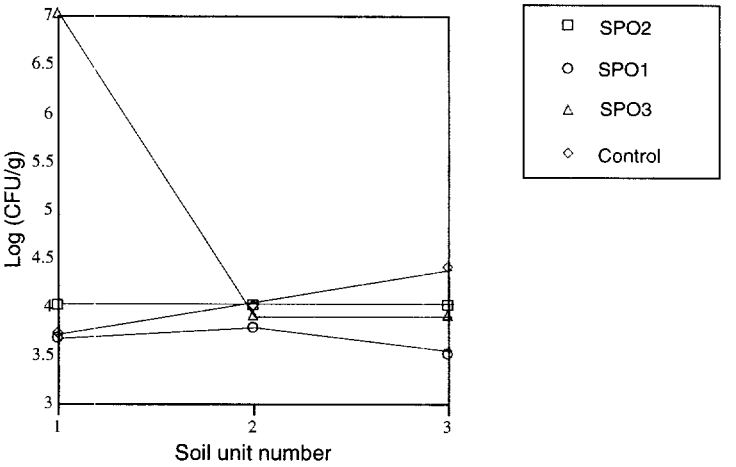

Fig. 5. Distribution of bacteria in soil columns (12°C).

parafilm cover was the only source of oxygen in the control reactors, higher concentrations of organisms in soil unit three were expected.

3.2.2. Reactors containing SPO

In all reactors incubated at 25°C and containing an SPO, the concentration of bacteria in soil unit three was similar to the concentration measured in the control reactors. These data suggest that oxygen released by an SPO did not reach the furthest soil unit located 10–15 cm from the SPO.

In the first soil unit of reactors containing SPO2, the concentration of bacteria was roughly one order of magnitude greater than in the control reactor. While the result suggests that some oxygen was released from SPO2, it did not drastically increase the number of bacteria in the soil. A somewhat larger increase in bacterial numbers was

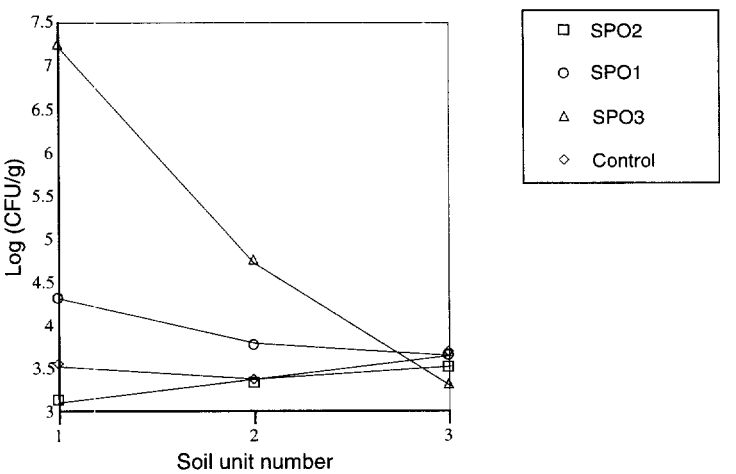

Fig. 6. Distribution of fungi in soil columns (25°C).

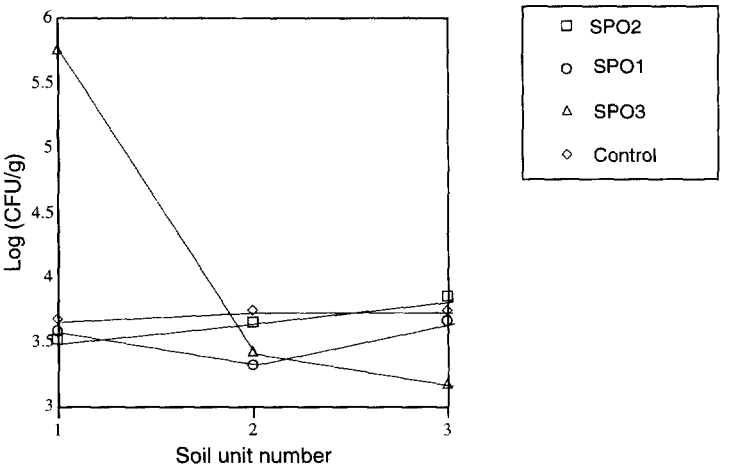

Fig. 7. Distribution of fungi in soil columns (12°C).

observed in the first soil unit of the reactor containing SPO1. The largest increase in bacterial concentration (4 log units greater than the control) was observed in soil unit one of the reactor containing SPO3. Similar results were obtained for the SPO3 reactor at 12 and 25°C. The plate counts observed in soil unit one with SPO1 and SPO2 indicate that these oxygen sources were not as effective at 12°C as they were at 25°C.

Fungal plating was included in the monitoring program since fungi may be important in bioremediation [14], and are not adversely affected or prefer low pH. Despite their pH preference, however, the distribution of fungi in all reactors were proportional to the concentrations of bacteria at both temperatures (see Figs. 6 and 7).

4. Conclusions

The research results demonstrated that the growth of bacteria and fungi in tundra soil can be enhanced by the addition of solid peroxides. In systems where SPO will increase the soil biomass concentration, it can be an effective tool to improve bioremediation system performance.

Of the three solid peroxides evaluated in this research, SPO3 had the greatest stimulatory effect on heterotrophic bacterial and fungal counts observable at the end of a 1200-h incubation period. Data indicated that soil in contact with SPO3 supported a population of bacteria two orders of magnitude larger than soil in contact with either SPO1 or SPO2. The observed increase in organism growth occurred primarily at a distance of less than 5 cm from the SPO, suggesting that only localized microbial growth should be expected in acidic tundra soil.

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