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Solid oxygen source for bioremediation in subsurface soils

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Abstract

Sodium percarbonate was encapsulated in poly(vinylidene chloride) to determine its potential as a slow-release oxygen source for biodegradation of contaminants in subsurface soils. In laboratory studies under aqueous conditions, the encapsulated sodium percarbonate was estimated to release oxygen over about a two-month period. Microbial survival in the presence of encapsulated sodium percarbonate was markedly increased compared to the unencapsulated compound. In laboratory studies, the encapsulated sodium percarbonate was used to provide oxygen as an electron acceptor for microorganisms during the biodegradation of propylene glycol. In 30 days at 12 °C (similar to subsurface soil temperatures), the concentration of propylene glycol was reduced tenfold and the number of propylene glycol degrading organisms increased tenfold compared to live controls without the encapsulated sodium percarbonate. Killed controls did not show significantly reduced concentrations of propylene glycol compared to the live controls. Acidic soil conditions (pH 4.7) were neutralized to a pH of about 8.3 by the encapsulated sodium percarbonate.

1. Introduction

There is a strong interest in the use of bioremediation to eliminate organic contaminants in subsurface (below 1.5 m) soils, saturated or unsaturated. In soils with low hydraulic conductivities ($< 10^{-5}$ cm/s), bioremediation may be limited by oxygen. In many cases where subsurface soil bioremediation has been attempted, oxygen was determined to be the limiting factor [1, 2]. This is especially true in overconsolidated silts and clays. This paper will describe a solid source of oxygen for use in subsurface bioremediation.

Sodium percarbonate was identified as a potential source of oxygen. Sodium percarbonate ($Na_2CO_3 \cdot 1.5H_2O_2$) releases hydrogen peroxide which decomposes

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to oxygen. Poly(vinylidene chloride) (PVDC) was chosen as an encapsulating agent because it is nonreactive with sodium percarbonate and inert in the soil. This paper describes the results of the experiments designed to evaluate the effectiveness of encapsulated sodium percarbonate as a source of oxygen to support biodegradation under laboratory test conditions.

2. Material and methods

2.1. Microencapsulation method

Sodium percarbonate was obtained from Fluka Chemicals (Ronkonkoma, NY). Microencapsulation was performed by the R.T. Dodge Co. (Dayton, OH). Poly(vinylidene chloride) (PVDC, as Serfene 121) was used as the wall material and was obtained from Morton International (Danvers, MA) as a preparation of 50% solids in water. It was further diluted to 30% solids in water. Sodium percarbonate was loaded into the 23-cm bed of a Wurster coater (Coating Place, Verona, WI) and the air inflow rate adjusted to the point that the compound was fluidized. The PVDC was sprayed in at a rate of 50 to 60 g/min. The final encapsulated product was 50% PVDC (as wall) and 50% (w/w) sodium percarbonate. Thus, 6.5% of the final product is hydrogen peroxide. The coated product was left in the Wurster until dry and then sieved through a 2.36-mm sieve to remove any coarse material. The grain size analysis of the final product was determined using ASTM Method D1140-54.

2.2. Oxygen formation studies

Studies of oxygen formation from sodium percarbonate were performed using the vessel shown in Fig. 1. The dissolved oxygen monitoring probes used were Wheaton 227840 (Wheaton, Millville, NJ). Three identical test systems

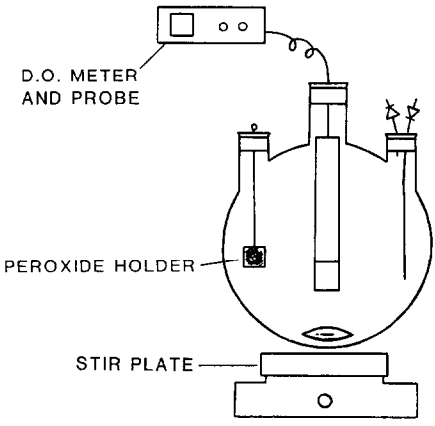

Fig. 1. Vessel configuration used to measure oxygen release.

were set up in a controlled environment room held at 12 °C (typical subsurface soil temperature). The vessels were standard five-liter, three-neck distillation flasks (Fig. 1). The volume as configured was 5.25 l. A large amount of water and the small amount of sodium percarbonate were used so that the level of dissolved oxygen would not reach saturation (below 8 ppm). The vessels were sealed with rubber stoppers secured with wire. Each vessel was nearly filled with deionized water and then the vessel was sparged with oxygen-free nitrogen gas. The oxygen in the vessels was reduced to about 0.5 ppm. The sodium percarbonate was held in a screen basket made of stainless steel. The amounts introduced per vessel were 3.6 g of the encapsulated sodium percarbonate (SPE) and 1.8 g of unencapsulated sodium percarbonate (SPU). These amounts provide equivalent oxygen potential. The vessels were then sealed and the remaining gas displaced by quickly pumping deionized water from another deoxygenated vessel. Once full, the vessels were sealed. The water in the vessels was mixed with a magnetic stirrer set at low speed (about 30 rpm). The output from the dissolved oxygen probes was monitored with Dianachart data acquisition software (Dianachart, Rockaway, NJ).

2.3. *Microbial survival*

Two bacterial isolates were used to test the toxicity of sodium percarbonate. Isolate F199 was obtained from Pacific Northwest Laboratory, Richland, WA, and has been described by Fredrickson et al. [3]. This Gram-positive bacterium, which has yet to be identified, was obtained from deep subsurface soil samples. A fluorescent *Pseudomonas* sp. isolate, designated B1, was obtained from a local hydrocarbon-contaminated soil sample by making selections on S1 medium [4]. Both isolates were grown in R2A broth at room temperature (about 22 °C). After 48 h, the cultures were centrifuged in the Sorval RC5B centrifuge with GSA rotor at 5,850 × g for 5 min. The resulting pellets were suspended in 100 mL of sterile deionized water and the centrifugation repeated. The final bacterial pellets were resuspended in 10 mL of sterile deionized water. The suspensions were placed in sterile serum bottles and sealed. Aliquots of these suspensions were used in the tests.

The toxicity of the sodium percarbonate was determined in 100 mL salts medium (SM), pH 6.8 [5]. The following solutions were prepared in triplicate: 0.0425 g SPE/100 mL and 0.0213 g SPU/100 mL. These concentrations provided equivalent concentrations of hydrogen peroxide. A suspension of about 1×10^5 cells/mL was made by diluting the prepared cell suspensions in the solutions described above. The suspensions were incubated with the SPE or SPU in 125 mL dilution bottles at 12 °C, on a reciprocating shaker (60 shakes per minute), for 2–4 days. The surviving cells were monitored by plating 0.2-ml aliquots on R2A plates using a Spiral Platter (Spiral System Inc., Cincinnati, OH). The control bottles, without SPE or SPU, were similarly plated. The plates were incubated at 29 °C for 24–48 h and then the number of colony-forming units (CFU) was determined as described above.

2.4. Degradation studies

Eighteen, 250-ml rounded flasks (Pyrex 5160) were used to test degradation of a surrogate organic contaminant in soil when exposed to SPE. Six flasks were live controls, six were killed controls and six were treatment flasks. All flasks received 0.1 g of IBDU fertilizer (PAR-EX 24-4-12, Vigoro Industries, Fairview, IL) added to the bottom of each flask. The six killed flasks and the six treatment flasks received 3 g of SPE and the six live control flasks received 3 g of sterile sand. One hundred grams of silty clay soil, obtained locally from a depth of 1.5 m, was added to cover these additions. For the six killed flasks, the soil was first autoclaved four times for 1 h with 24 h between each autoclaving. Next, 20 mL of water, which had been deoxygenated by sparging with oxygen-free nitrogen, was added to each flask. For the killed flasks, the 20 mL of water contained 5% sodium azide. One hundred μL (0.103 g) of propylene glycol (PPG) (Ashland Chemical, Cincinnati, OH) was added to each of the 18 flasks. The PPG acted as a surrogate contaminant. The flasks were sealed with rubber stoppers and retained with wire. Each flask was then purged with oxygen-free nitrogen delivered through a no. 22 hypodermic needle for 20 min. The flasks were incubated statically for 30 days at 12 °C.

After incubation, the flasks were analyzed. The amount of PPG in the soil was determined by taking five 5-g random grab samples from the soil in each of the test flasks. Extractions of the soil were performed as described in Method 3580 from EPA SW-846 [6]. The sample was extracted with 30 mL of Milli-Q water (Millipore Inc., Bedford, MA). The gas chromatograph used was a HP 5890 (Hewlett-Packard, Avondale, PA) with a Nukol capillary column (15 m, 0.53 mm i.d.). The operating conditions were: injection port 200 °C, detector 275 °C, initial oven temperature 75 °C for 2 min, ramping to 190 °C at 10 °C/min, final holding time 10 min. Column flow was with ultra-high purity (UHP) nitrogen set for flow rate of 15 mL/min through the column, with auxiliary make-up gas of UHP nitrogen set at the detector to make a total flow of 30 to 35 mL/min. The detector was flame ionization. A 1–3 μL aliquot of extract was injected into the gas chromatograph using the solvent flush technique.

To determine the number of microorganisms present, six random grab samples, of 1 g each, were removed from each flask and added to 5 mL of sterile, deionized water in each of six sterile 15 mL test tubes (Falcon model 2025). The soil and water were then sonicated at 90% power for 3 min using a Heat Systems sonicator model W-375 (Heat Systems, Plainview, NY) equipped with a cup horn, model H1. After sonication, the suspensions were diluted by adding 1 mL to 100 mL of sterile water and then plated on R2A medium using the Spiral Platter. The suspensions were also plated using the Spiral Plater on SM containing 1% PPG and solidified with 2% agar. The R2A and SM plates were incubated at 29 °C for 24–48 h then the number of CFUs were determined as described above. The population on R2A was described as the heterotrophic population and those growing on the SM plates as the PPG-degrading population. No additional microbes were added in any of these treatments.

The pH of the soil was determined both before treatment and after treatment by adding 5 g of soil to each of three 15-mL test tubes (Falcon 2025) and 5 mL of deionized water. The tubes were mixed on a New Brunswick shaker model G2 (New Brunswick, NJ) at room temperature (about 22 °C) for 30 min then allowed to sit for 1 h. The pH was determined using a Cole Parmer (L-05992-62) (Chicago, IL) soil pH probe and Orion Ion Analyzer EA920 (Boston, MA).

3. Results

3.1. Microencapsulation

A 1:1 ratio (wall to core) was selected as the final product because formulations with thinner walls released oxygen too quickly (data not given). The grain size of the microcapsules was 93% between 0.85 and 0.425 mm in diameter and all of the microcapsules were between 2.0 and 0.25 mm.

3.2. Oxygen formation

The rate of oxygen formation from the unencapsulated sodium percarbonate was about 40% faster than from the encapsulated sodium percarbonate (Fig. 2).

3.3. Microbial survival

Exposure of the bacterial isolates to SPU eliminated the cultivable bacterial populations in one day (Fig. 3). However, the bacteria in the control vessels

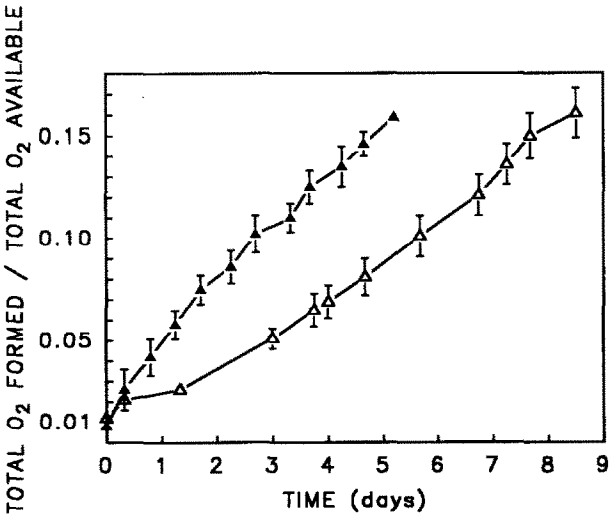

Fig. 2. Dissolved oxygen concentrations as a function of time from unencapsulated and encapsulated sodium percarbonate at 12 °C. To three vessels of 5.25 l of deionized water was added either (△) 1.8 g of SPU or (△) 3.6 g of SPE. Mean and standard deviations from the three test vessels are shown.

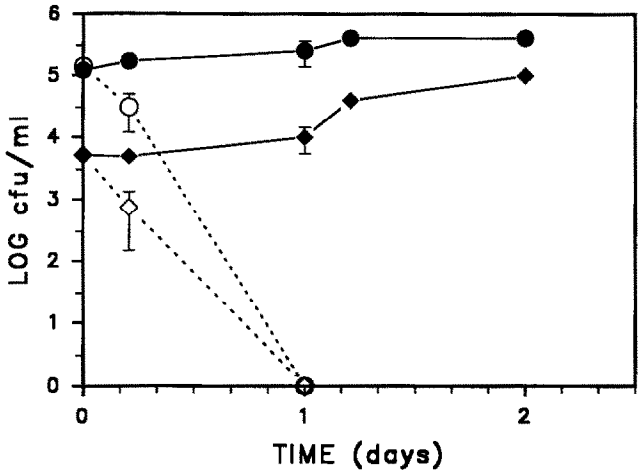

Fig. 3. Toxicity of SPU to bacteria. (◇) Isolates B1 and (o) F199 exposed to SPU in salts medium. Controls for each (◇, ●) were without added SPU. Values are the mean and standard deviations of three replicates.

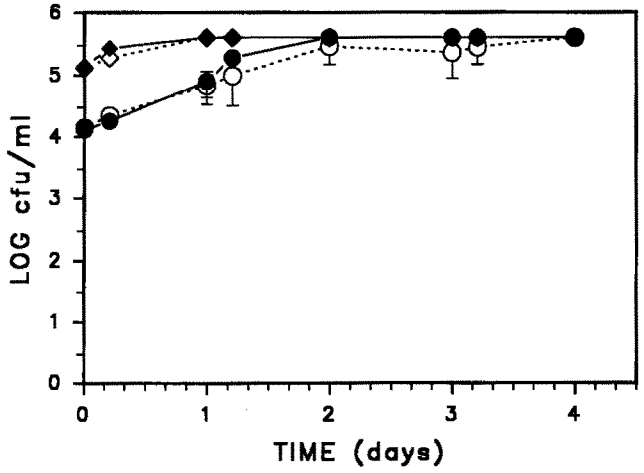

Fig. 4. Toxicity of SPE to bacteria. (◇) Isolates B1 and (o) F199 were exposed to SPE in salts medium. Controls for each (◇, ●) were without added SPE. Values are the mean and standard deviations of three replicates.

(without sodium percarbonate) were unaffected throughout the duration of the experiment (Fig. 3). In contrast, when the bacterial isolates were exposed to SPE their populations were the same as the unexposed control populations (Fig. 4).

TABLE 1

Biodegradation of propylene glycol (PPG) in soil with encapsulated sodium percarbonate as the oxygen source

Treatment	Heterotrophic population (cfu/g soil)	PPG degraders (cfu/g soil)	pH	[PPG] (μg/g soil)
Treatment (with SPE)	$(5.4 \pm 1.6)^a \times 10^7$	$(4.7 \pm 2.2) \times 10^7$	8.3±0.08	<70
Killed control (with SPE)	0	0	8.3±0.15	512±68
Live control (no SPE)	$(5.9 \pm 2.1) \times 10^6$	$(3.6 \pm 1.3) \times 10^6$	4.7±0.03	638±56

[a] Mean plus or minus the standard deviation.

3.4. Biodegradation studies with encapsulated sodium percarbonate

Table 1 shows the results of the tests of biodegradation of propylene glycol using encapsulated sodium percarbonate as the oxygen source. A tenfold increase in the population of heterotrophic organisms and PPG degraders was observed in the vessels containing the SPE oxygen source (Table 1). However, no microbial cells were cultured from the killed flasks. In addition, the soil pH was increased from 4.7 in the control vessels, to 8.3 in the flasks with SPE added (Table 1).

The concentration of the surrogate contaminant decreased about one order of magnitude from 638 to less than 70 μg/g (Table 1) in the SPE treated compared to the live control flasks. In the killed flasks, the PPG concentration was reduced to 512 μg/g but this mean was not significantly different from the mean of 638 μg/g in the treatment flasks at the 0.05 confidence level as determined using the Student-*t* test.

4. Discussion

This laboratory has conducted experiments to determine the feasibility of using encapsulated sodium percarbonate as the oxygen source for aerobic biodegradation. It has been shown that sodium percarbonate produces oxygen when placed in water. The specific chemical reaction involved in this reaction was not determined in this study but has been considered in detail by others [7,8]. There are many catalysts including catalase, iron, etc., potentially available in the oxygen monitoring arrangement since the system is neither sterile nor free of metals.

Although the rates of oxygen formation changed over time, the average rate of oxygen formation was about 1.8% per day of the total available oxygen for

encapsulated sodium percarbonate. By extrapolating from this data, the oxygen should be produced for up to 55 days from the SPE. In this study, encapsulated sodium percarbonate was tested in saturated, well-mixed conditions. In soil, the degree of water saturation and water (with catalysts) movement around the capsules would probably be markedly less than in our laboratory experiments. Thus, the rate of oxygen formation is expected to be slower in the field than in the laboratory. However, this remains to be determined.

Sodium percarbonate was toxic to the two microorganisms tested at the concentration tested. Encapsulation reduced this toxicity. Further tests will be required to see if this is true for other microbial species. However, we have shown that even at a ratio of 33 : 1 (ww soil to SPE), as used in the degradation experiment, the SPE was not toxic to the soil microbial population. It is possible that the encapsulated sodium percarbonate was toxic to some components of the microbial population. This could have been masked by the general increase in microbial numbers observed. One to two orders of magnitude increase in microbial populations have been reported after oxygen and nutrients were added to soils [9]. Thus, the tenfold increase in microbial population in one month is within the reported range.

The observed disappearance of the PPG in the treatment flasks, we have associated with biodegradation. Although hydrogen peroxide has been used as a chemical oxidizing agent, Fenton's Reagent [10], we did not see evidence of statistically significant losses of PPG in the killed flasks. However, the apparent loss of PPG in the killed flasks may be due to this chemical oxidation.

Sodium percarbonate is a strong alkali and may increase the pH of soil. In acidic subsurface soils this may actually be beneficial. Generally, near neutral pH is most conducive to microbial biodegradation. Verstraete et al. [11] reported a near doubling of the rates of biodegradation of gasoline in soil after adjusting the pH from 4.7 to 7.4. Dibble and Bartha [12] observed that the optimal pH for biodegradation of oily sludge in soil was 7.8. The soil used in our experiments was acidic and responded to SPE treatment with an increase in pH. These more neutral conditions apparently were favorable for microbial activity. Not all soils are acidic. If treatment with SPE will result in highly alkaline conditions, then buffering might be achieved with acidic nutrients, such as phosphoric acid, nitric acid, or sulfur compounds. The potential use of these may be limited by site or regulatory interests.

Another set of possible problems arising from the use of SPE may be related to the specific geochemistry at a particular site and how that is affected by the introduced SPE. For example, it remains to be seen whether the sodium will be precipitated and possibly cause plugging of the soil pores. This and other possibilities will need to be studied further.

How would this material be delivered to the site of subsurface contamination? Researchers in our laboratory have developed a technique, termed hydraulic fracturing (HF), to deliver solids in large volumes into subsurface soils [13]. The HF technique creates horizontal pancake-shaped fractures that are 5 m or more in diameter and 1–2 cm in maximum thickness. The fractures are

stacked vertically with spacings as close as 20 cm. Typically, 300 to 700 kg of granular material is injected into each fracture. The granular material can be coarse-grained sand, which results in permeable fractures that increase drainage, or it can be a chemically active compound, such as SPE.

The advantage of an encapsulated solid oxygen source, when coupled with a delivery system like hydraulic fracturing, is that the oxygen can be delivered rapidly, deep into the contaminated soil and then made available slowly. Instead of having to move many meters through the soil, the oxygen can be made available in the contaminated soil directly. Also, we expect that requirements for pumping and related equipment at the ground surface will be reduced compared to other methods of bioremediation. The next stage of this research will be the field testing of the SPE product at a contaminated site.

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