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Effects of a Nutrient-Surfactant Compound on Solubilization Rates of TCE

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ABSTRACT

BioTreat™, a commercially available nutrient-surfactant compound, was investigated for its ability to solubilize TCE. Potential mechanisms for enhancing biodegradation rates by the use of nutrient-surfactant mixtures are: increased solubilization of TCE into the aqueous phase, and increased nutrients for the bacteria and greater numbers of colony forming units (CFUs). In aqueous systems, no measured solubilization of 0.1 and 1.0 ppm TCE from the headspace into the liquid phase was observed with BioTreat added at concentrations <0.5%. However, at BioTreat concentrations in excess of the CMC (≥0.5%), increased solubilization of TCE was measured. A second question was the nutrient effect of BioTreat on the growth of the TCE-degrading bacterium, *Burkholderia cepacia* G4 $PR1_{301}$. The added nutrients provided by BioTreat was evident and lead to increased cell numbers. The effect of BioTreat on the expression of ortho-monooxgenase, the enzyme necessary for TCE degradation by *B. cepacia* was also investigated. Enzyme expression as detected by a colorimetric assay was inhibited for BioTreat concentrations >0.05%.

Index Entries: TCE; degradation; surfactants; enzyme; biodegradable.

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INTRODUCTION

Soils contaminated with volatile organic compounds (VOC) can pose a problem for remediation when the contaminant is sorbed to the soil, is present as a nonaqueous-phase liquid, or volatilized within the pore spaces. For bioremediation to occur, the contaminant must be bioavailable, i.e., solubilized, into the aqueous phase. Surfactants can facilitate this process of solubilization by reducing the interfacial tension between the soil–liquid interface. Surfactants can also facilitate solubilization from the pore spaces into the liquid phase by reducing the interfacial tension at the gas–liquid interface *(1)*.

Aqueous-phase surfactant concentrations can easily be quantified by measuring their ability to reduce the interfacial tension of the liquid. Typically, surfactants used for contaminant solubilization are used at or above the critical micelle concentration (CMC). The CMC is the concentration at which surfactant monomers self-aggregate into micelles with the hydrophobic tails oriented to the inside and the hydrophilic heads oriented outside. At concentrations above the CMC, monomers and micelles are present, but below the CMC, only surfactant monomers are in solution *(1)*.

Fertilizers have been found to increase remediation rates by stimulating bacterial growth. The oleophilic fertilizer, Inipol EAP22, was used in the remediation of the Exxon Valdez oil spill in Prince William Sound, AK to increase natural biodegradation rates of stranded oil by the indigenous microorganisms *(2)*. In contrast to this fertilizer, we examined the effects of an aqueous-based nutrient plus surfactant mixture and its application to hazardous waste remediation. This bioenhancing compound, Bio Treat™, along with two nonionic synthetic surfactants, Poly-Tergent 42® and Tween-80® were used in these experiments in order to investigate enhanced TCE solubilization for biodegradation by *Burkholderia cepacia* G4 $PR1_{301}$, a constitutive TCE degrading bacterium *(3)*. Potential mechanisms for enhanced biodegradation rates are: (1) increased solubilization of TCE into the aqueous phase, and (2) increased nutrients or alternate carbon source for the bacterium, thus increased cell numbers. In this study, we investigated the effects of this nutrient-surfactant product on TCE solubilization, *B. cepacia* growth, and expression of the enzyme necessary for TCE degradation.

EXPERIMENTAL PROCEDURES AND MATERIALS

Bacterial Strain, Culture Conditions, and Chemicals

The bacteria used throughout these experiments was *B. cepacia* G4 $PR1_{301}$, a nongenetically engineered constitutive TCE degrader (M. Shields, University of West Florida, Pensacola, FL; 3–4). The bacteria was grown in continuous culture in basal salts media (BSM) *(5)* with 20 m*M*

glucose as the sole carbon source. Liquid cultures were routinely started from nonselective agar plates of R2A growth media (Difco Laboratories, Detroit, MI) or a selective growth media of BSM + 1.7% noble agar, and either 20 m*M* glucose or 20 m*M* sodium lactate. The plates were scraped after 7 d, and the bacteria resuspended in 10 mL of BSM + 20 m*M* glucose in a 15-mL sterile centrifuge tube (Corning, Corning, NY). After a 2-d incubation on a rotary shaker (250 rpm) at ambient temperature, the optical density (OD) increased to 0.2–0.5 at 600 nm (Gilford Response UV-Visible spectrophotometer, Oberlin, OH). These cultures were transferred into 90 mL of fresh media in a 250-mL Erlenmeyer flask and returned to the shaker until OD ≥ 2.0.

Expression of the enzyme responsible for TCE degradation by *B. cepacia*, *ortho*-monooxygenase *(3)*, was measured using the triflouromethyl phenol or *m*-hydroxy benzotrifluoride (TFMP) oxidation assay. The rate of production of TFHA (7,7,7-trifluoro-2-hydroxy-6-oxo-2,4-heptadienoic acid), a yellow product, from TFMP correlates to the potential rate of TCE degradation by the enzyme *(4)*.

The synthetic surfactants used in these experiments were Poly-Tergent 42 (Olin, Stamford, CT), Tween 80 (Sigma Chemical, St. Louis, MO), and a commercially available nutrient–surfactant mixture, BioTreat (Rem-Tec, Clemmons, NC). All surfactant stock solutions were made in Milli-Q water and filter-sterilized through a 0.2-μm filter (Nalgene, Rochester, NY). The CMC values for these surfactants are: 0.01% BioTreat, 0.05% Poly-Tergent 42, and 0.03% Tween 80. The surfactants were tested at concentrations below, equal to, or above their aqueous CMC.

Growth of *B. cepacia* on Surfactants

B. cepacia G4 $PR1_{301}$ was assayed with 0.05% BioTreat and 0.05% Poly-Tergent 42 as the sole carbon sources. Poly-Tergent 42 was chosen as the nonionic surfactant, because it was reported to be biodegradable (Olin Corporation, personal communication). Bacteria were initially cultured on agar plates for 7 d, scraped, and resuspended in sterile minimal salts media *(6)* to OD = 1.5 at 600 nm. The bacterial suspension (300 μL) was inoculated into 100 mL of minimal salts media in a 250-mL Erlenmeyer flask. BioTreat and Poly-Tergent 42 that were filter-sterilized were added to a final concentration of 0.05%. The sterility of the surfactants was verified by streaking on nutrient agar plates (Becton Dickson Company, Cockeysville, MD) and observing for growth. The flasks were shaken at room temperature at 250 rpm on a rotary shaker and daily optical density measurements were recorded at 600 nm. The cultures were amended with an additional 0.05% surfactant until the OD = 2.

B. cepacia was also grown with BioTreat provided as a secondary carbon source. The bacteria were cultured as described previously with 20 m*M* glucose as the primary carbon source, and 0.01%, 0.05%, and 0.1%

BioTreat as an additional carbon/nutrient source. Optical density measurements at 600 nm were recorded at days 3, 4, and 5. The specific activity of the enzyme was assayed at day 5 using the TFMP oxidation assay.

Abiotic TCE Solubilization

TCE solubilization experiments utilized sterile 15-mL glass vials (EPA vials) containing 5 mL of sterile phosphate-buffered solution (PBS, 1.2 g Na_2HPO_4, 2.2 g NaH_2PO_4, 8.7 g NaCl/L water). Filter-sterilized BioTreat was added at concentrations of 0.001, 0.01, 0.05, 0.5, 1 and 5%. Tween 80 was tested at concentrations of 0.01, 0.03, 0.1, 0.5, 1 and 5% and was chosen based on its ability to solubilize low concentrations of carbon tetrachloride *(7)*. The vials were sealed with Teflon-coated septa prior to the addition of 0.1 or 1.0 ppm TCE, which was added through the septa via syringe. The vials were inverted and equilibrated overnight at ambient temperature on a rotary shaker at 250 rpm. Headspace measurements (30 µL) were performed on a gas chromatograph (GC).

Analytical Methods

A GC (Hewlett Packard 5890 Series II Plus, San Fernando, CA) equipped with an electron capture detector and a megabore capillary column DB624 (30 m 0.53 mm inner diameter) (Alltech, Deerfield, IL) was used to analyze headspace gas samples. Argon-methane was the carrier gas. The injector temperature was 100°C. The oven temperature started at 70°C and increased to 110°C at a rate of 10°C/min. The detector temperature was 300°C, and column carrier gas flow was 7.0 mL/min. A headspace gas volume of 30 µL was injected for each sample.

RESULTS AND DISCUSSION

Previous work has shown that surfactants can enhance solubilization of chlorinated solvents in soil systems *(8–10)*. The effect of surfactant-enhanced partitioning, i.e., solubilization, of TCE from the headspace into the liquid phase is important when determining the bioavailability of chlorinated solvents and other VOCs. This work examined abiotic TCE solubilization in aqueous systems. The amount of TCE partitioned into the headspace was followed throughout the surfactant treatments. All solubilization data presented here are expressed in GC area units of TCE in the gas phase.

Initial experiments examined low surfactant concentrations and their affect on TCE solubilization. Surfactants facilitate solubilization of VOCs from the headspace into the aqueous phase by reducing the interfacial tension at the gas–liquid interface. Relatively low surfactant concentrations (<0.1%) had no effect on the enhanced TCE solubilization for either 0.1 or 1.0 ppm in an aqueous system (Figs. 1 and 2). Data presented

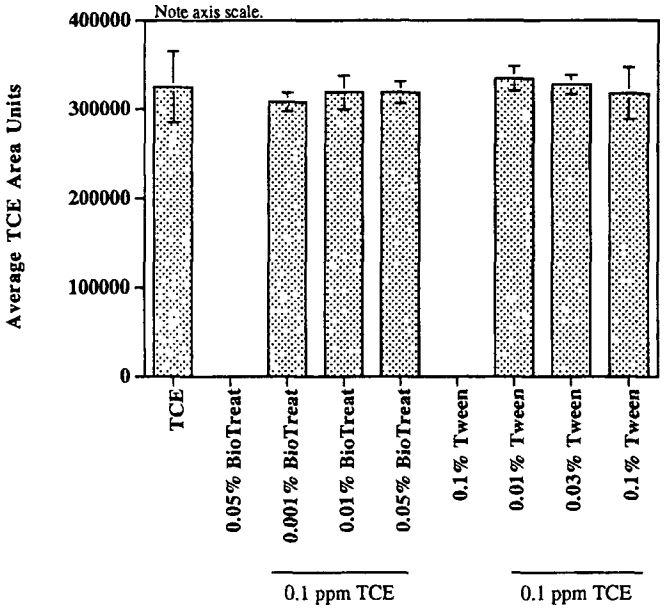

Fig. 1. Gas-phase measurements for the abiotic solubilization of 0.1 ppm TCE. Standard deviations are within bars, n = 3. BioTreat concentrations represent below (0.001%), at (0.01%), and above (0.05%) aqueous CMC. No enhanced partitioning of TCE from headspace into liquid phase visible. Tween concentrations represent below (0.01%), at (0.03%), and above (0.1%) aqueous CMC. No enhanced partitioning of TCE is detected.

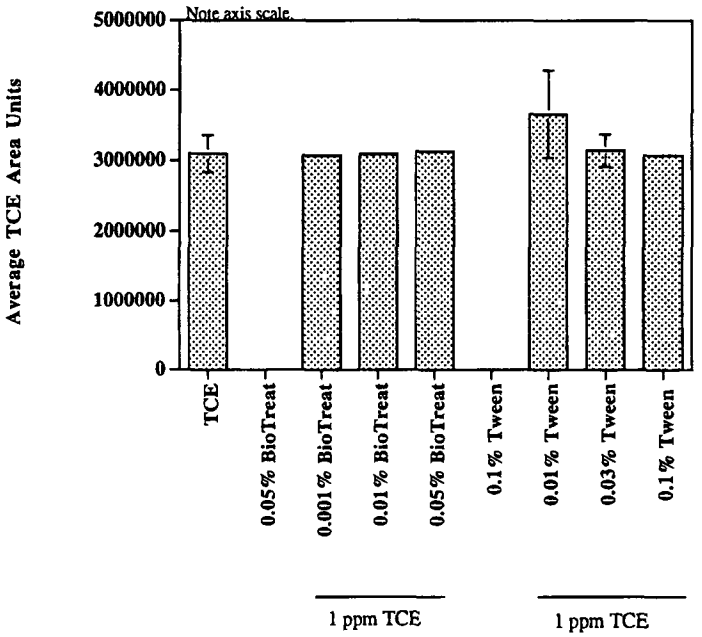

Fig. 2. Gas-phase measurements for the abiotic solubilization of 1.0 ppm TCE. Standard deviations are within bars, n = 3. No enhanced solubilization of TCE at 0.001, 0.01, or 0.05% BioTreat. No enhanced solubilization of TCE at 0.01, 0.03, and 0.1% Tween 80.

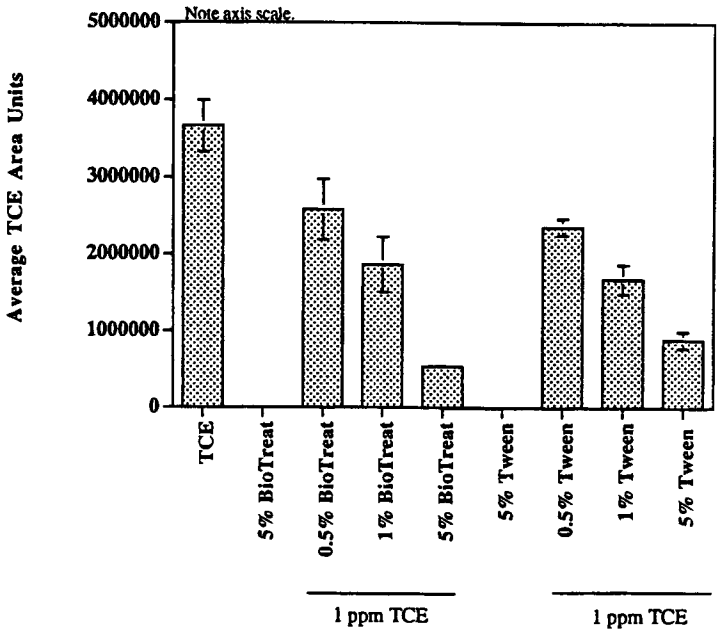

Fig. 3. Gas-phase measurements for the abiotic solubilization of 1.0 ppm TCE. Standard deviations are within bars, $n = 3$. Samples contain surfactant in excess of the CMC (0.5, 1, 5% BioTreat; 0.5, 1, 5% Tween 80). Increased partitioning of TCE from headspace into aqueous phase as surfactant concentration increased.

here showed that this was true for both Tween 80 and the nutrient–surfactant compound, BioTreat. This is expected based on previous work by other investigators, which showed that below the CMC, surfactants exist only as monomers, which do not enhance TCE solubilization *(15)*. However, as the surfactant concentrations increased in excess of the CMC (>0.5%), there was a decrease in TCE headspace concentration compared to controls. These data support an increased solubilization of TCE into the aqueous phase (Fig. 3). This enhanced solubilization of TCE from the headspace into the aqueous phase makes the contaminant ultimately more bioavailable to the microorganisms for degradation.

An important consideration in choosing a surfactant for remediation is surfactant persistence and toxicity *(11)*. The surfactant chosen for primary consideration in this study was BioTreat. Furthermore, the addition of nutrient solutions to contaminated soil has also been shown to increase remediation rates *(2,12,13)*. For example, Inipol EAP22, an oleophilic fertilizer used in the cleanup of the Exxon Valdez oil spill, also has surfactant properties *(14,15)*. BioTreat, although an aqueous-based fertilizer, is similar to Inipol EAP22 in that it demonstrates surfactant properties in addition to its nutrient-providing capabilities. Thus, the ability of *B. cepacia* to metabolize BioTreat as its sole carbon source (Fig. 4) was measured. An initial concentration of 0.05% BioTreat supported microbial growth to an OD = 0.32. All OD measurements were performed with a blank con-

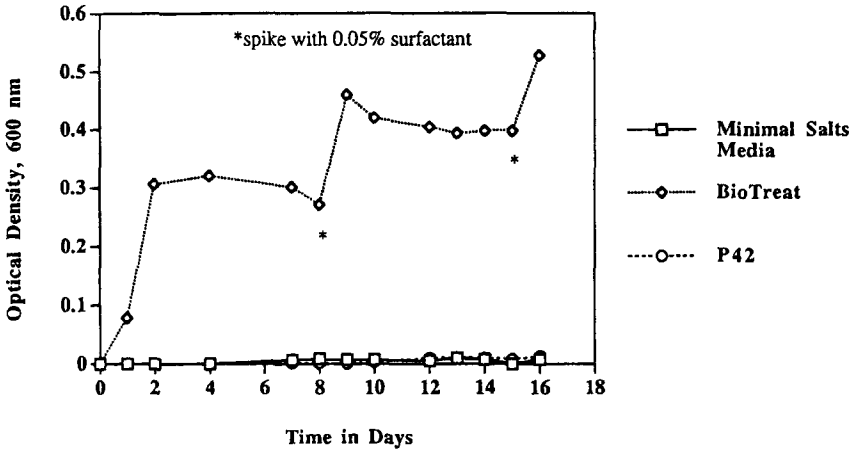

Fig. 4. Growth of *B. cepacia* G4 $PR1_{301}$ with surfactants as sole carbon source. Optical density measurements over time. Samples were spiked with additional 0.05% surfactant at days 8 and 15 in order to provide more carbon to the system. Bacteria can metabolize BioTreat, but not Poly-Tergent 42.

sisting of surfactant and water to account for the surfactant's possible effect on absorbance. Subsequent additions of BioTreat resulted in sharp increases in cell growth, followed by stationery growth periods. Stationery phases represent a depletion of the carbon source, inorganic nutrients, or a buildup of toxic cellular byproducts *(16)*. In TCE degradation experiments in our lab (data not shown), CFUs were increased by 34% over controls when BioTreat was added. Previous experiments indicated that Poly-Tergent 42 was not actually biodegradable by *B. cepacia* and therefore was used as a control. No growth of *B. cepacia* was observed after 16 d of exposure to 0.15% Poly-Tergent 42.

Although BioTreat at concentrations higher than the aqueous CMC can increase surfactant partitioning and act as a nutrient source for *B. cepacia*, the bacteria does not produce the enzyme necessary for TCE degradation when grown in the presence of high concentrations of BioTreat. *B. cepacia* was cultured with 20 m*M* glucose and varying concentrations of BioTreat as a secondary carbon source. Enzyme expression was inhibited at 0.05 and 0.1% BioTreat, but was not affected by BioTreat concentrations of 0.01% (Fig. 5). However, these low surfactant concentrations are not effective at increasing the TCE solubilization. BioTreat does appear to increase cell numbers, which would increase rates of TCE degradation by changing the TCE equilibrium concentrations between the aqueous and headspace phases. It is apparent that *B. cepacia* growth is not inhibited by the surfactant properties of BioTreat. However, the additional source of carbon does not correspond to an increase in OD over that of controls with glucose only. It is currently unclear if there may be other factors within the system that would slow cell growth, such as limited trace elements in the BSM media. Additional data from the TFMP

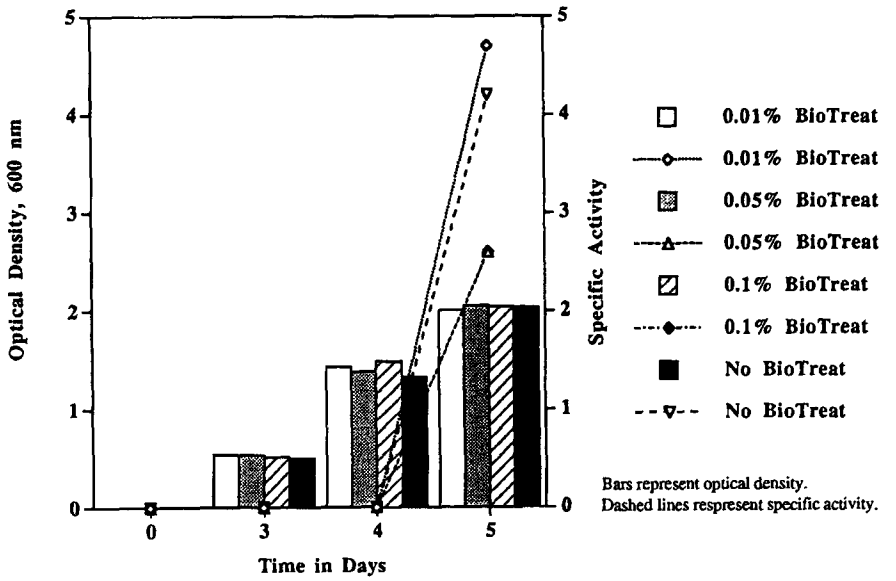

Fig. 5. Growth of *B. cepacia* G4 $PR1_{301}$ on glucose in the presence of BioTreat. Optical density and specific activity measurements over time. Glucose 20 m*M* present as carbon source. BioTreat (0.01, 0.05, 0.1%) did not inhibit growth of bacteria. Concentrations of 0.1% BioTreat inhibited enzyme production.

assays confirm that the bacteria does not produce the enzyme when grown on BioTreat only. An alternative explanation is the interference of BioTreat with the colorimetric TFMP enzyme assay. Work is currently being done to verify that no enzyme is produced vs the inability to detect enzyme production by the TFMP oxidation assay.

The BioTreat product used in this study acted as a nutrient to the bacteria, thus leading to higher cell numbers, but did not increase TCE bioavailability or solubilization at low concentrations. Although utilization of the compound at concentrations greater than the CMC increased TCE headspace partitioning, i.e., solubilization, and thus increased bioavailabilty, these same concentrations inhibited the expression of *ortho*-monooxygenase, which is essential for TCE degradation by *B. cepacia* G4 $PR1_{301}$. These results suggest no additional value in adding BioTreat to aqueous systems when increased solubilization of TCE for biodegradation by *B. cepacia* G4 $PR1_{301}$ is the only desired effect. Current work is examining the solubilization and nutrient effects of BioTreat in soil-slurry systems in preparation for use in a potential field demonstration.

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