THE FATE OF HYDROGEN PEROXIDE AS AN OXYGEN SOURCE FOR THE REMEDIATION OF BIOLOGICALLY ACTIVE SUBSURFACE TREATMENT ZONES

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INTRODUCTION

The natural ability of microorganisms to degrade contaminants *in situ* is a highly recognized phenomenon. In certain cases, this process of contaminant degradation by microorganisms, which may also be referred to as bioremediation or biodegradation, requires additional nutrients, carbon sources, and/or electron acceptors; therefore, the process becomes enhanced *in situ* bioremediation. *In situ* bioremediation is the use of indigenous biological agents to reclaim soils and waters polluted by substances hazardous to human health and/or the environment; it is an extension of biological treatment processes that have traditionally been used to treat waste in which microorganisms typically are used to biodegrade environmental pollutants (Atlas and Bartha 1993). In the environmental industry, the use of bioremediation technologies for soil and groundwater clean-up are gaining wide acceptance. In the most common practice of this technology, indigenous aerobic microbes are used to degrade hydrocarbon contaminants. Nutrients and oxygen are supplied to enhance this natural process (Fagan 1994). Recently, hydrogen peroxide has been used to facilitate biotreatment of subsurface contamination (Spain et al. 1989; Morgan and Watkinson 1992). These studies emphasize the fact that hydrogen peroxide might serve as an oxygen source for microorganisms.

In situ bioremediation is a promising and innovative technique to restore some of our polluted aquifers. This method exceeds conventional methods (i.e., pump and treat) by offering a potentially more effective and economical clean-up technique through partial or complete destruction of the contaminants (Atlas and Bartha 1993). Most currently used techniques rely principally on pumping from wells to recover contaminated groundwater followed by treatment and injection at delivery wells. These pump and treat techniques reduce concentrations to acceptable levels for some contaminants under certain saturated site conditions. But these technologies are generally not applied to tight formations where hydraulic conductivities are less than 10^{-4} cm/sec. Currently, the delivery of remediating materials is limited to injecting liquids through wells. Methods of delivering solid or vapor phases are for the most part unavailable, although such methods would facilitate some remedial actions.

Existing technologies are usually not effective at sites with contaminants of low water solubilities or those that are readily adsorbed to soil. Current delivery or recovery methods are also usually ineffective for contaminated unsaturated soils. Low hydraulic conductivities and local stagnant zones in unsaturated media inhibit the rates and ultimate effectiveness of most delivery or recovery technologies. Furthermore, some current recovery methods applied to the unsaturated zone require an underlying impermeable barrier that precludes their use at some sites. Another problem not addressed by current delivery and recovery technologies is the presence in some natural soils of preferred pathways of high conductivity separated by blocks of low conductivity. Delivery or recovery from such soils is limited by rates of diffusion through the soil matrix blocks to the preferred pathways (Murdoch et al. 1990).

Although they are not clearly defined, several environmental factors are known to influence the capacity of indigenous microbial populations to degrade contaminants. These factors include dissolved oxygen, pH, temperature, oxidation-reduction potential, availablility of mineral nutrients, salinity, soil moisture, the concentration of specific pollutants, and the nutritional quality of dissolved organic carbon in the ground water (Wilson et al. 1986). In field applications of enhanced *in situ* bioremediation, oxygen supply is usually the limiting factor (Hinchee 1991). In aerobic respiration, free molecular oxygen accepts electrons released by an

electron donor and is reduced to a lower oxidation state. Oxygen, if not present in adequate concentrations, will limit the ability of aerobic microorganisms to degrade contaminants. The rate of aerobic biotransformation and, thus, contaminant persistence has been reported to be controlled by the transport of oxygen into the contaminated ground water (Huling et al. 1990).

Dissolved oxygen is degraded by a number of consumption reactions such as reactions with metals to form oxides. In addition, dissolved oxygen has a very limited solubility in aqueous solutions: 9mg/l at 25°C and 11mg/l at 5°C in water, and 2-4mg/l at atmospheric pressure (Freeze and Cherry 1979). Alternatively, oxygen may be delivered to the subsurface in the form of hydrogen peroxide (H_2O_2). Hydrogen peroxide has several attributes which recommend its use as a supplemental oxygen source; it is reasonably inexpensive, is nonpersistent, and is not likely to represent a serious health hazard if used properly (Britton 1985). Hydrogen peroxide in the presence of Fe^{2+} and Fe^{3+} produces reactive hydroxyl (OH•) radicals and possibly other reactive species. The reagent components are easy to handle and environmentally benign, making this system attractive for treating aqueous or soil-bound contaminants (Pegnatello 1992). Hydrogen peroxide dissociates to produce one-half mole of dissolved oxygen per mole of hydrogen peroxide (Huling et al. 1990) $H_2O_2 \rightarrow H_2O + {}^1/_2\, O_2$ (1). The stoichiometry of Equation 1 indicates that 47.1% by weight of decomposed hydrogen peroxide will be pure oxygen.

When added to soil, hydrogen peroxide is unstable due to the presence of inorganic and organic catalyst such as: iron, manganese, and the hydroperoxidases, catalase, and peroxidase. The chemical environment in the subsurface is determined by the interactions between the immobile (soil) and mobile phases at a given location. Hydrogen peroxide introduced into the subsurface is subject to physical, chemical, and biochemical transformations. Hydrogen peroxide is very reactive, and transport of this material through an aquifer can be very difficult. Consideration of hydrogen peroxide sinks and appropriate hydrogen peroxide concentrations must be included in the design of an *in situ* biotreatment system. In addition to the factors mentioned already, hydrogen peroxide can mobilize metals such as lead and antimony; and, if the water is hard, magnesium and calcium phosphates can precipitate and plug the injection well or infiltration gallery (Wilson et al. 1986).

MATERIALS AND METHODS

Water used in all solutions was distilled, deionized, and filtered through a 0.45 μm membrane filter prior to being placed in solution. Hydrogen peroxide solutions were all made from a 50% (w/w) solution from Fisher Scientific. A stock solution of hydrogen peroxide was made by dissolving 1.0 ml of 50% H_2O_2 in a 500 ml measuring flask and filling with sterile H_2O to approximately 10,000 mg/l. This solution was labeled as stock A. Several serial dilutions of known concentrations of hydrogen peroxide were made to yield the appropriate concentration. A variety of analytical techniques for hydrogen peroxide were evaluated during these studies. The reasons for evaluating several methods was that the poor light transmittance, complex chemical matrix, and rapid reactions associated with soil constituents made analysis of hydrogen peroxide using traditional techniques difficult. Various soils were collected from their natural environments and include: (1) Wes reference soil from Vicksburg, MS, (2) Tellico loam from Pope County, Arkansas, (3) Gessie from Newton County, MS, (4) Crot from Custer County, Oklahoma, (5) Alligator Clay from Leflore County, MS, and (6) Ottawa Sand purchased from U.S. Silica, Ottawa, Illinois.

Soil Characterization Analysis

The bulk mineralogy and the clay minerals content in the five test soils (excluding the Ottawa sand) was determined by X-ray diffraction (XRD) analysis. This investigation was an attempt to obtain as much information as possible with an emphasis on linking the observed mineralogy to the chemical properties of the soils. Table 1 lists soils with the appropriate identification type, particle size distribution, and selected biological and chemical properties. In preparation for X-ray diffraction of the bulk sample, a portion of each sample was ground in a mortar and pestle to pass a 45 μm (No. 325) mesh sieve. For subsequent analysis of the clay-size fraction, a slurry of the powder with water was made and a suspension was placed on a substrate and allowed to air dry overnight. An X-ray diffraction pattern was collected on glycol atmosphere overnight at room temperature (23°C), and another X-ray diffraction pattern was collected for each sample. Bulk sample random powder mounts were analyzed using X-ray diffraction to determine the mineral constituents present in each soil. Microbial density was determined by acridine orange direct count (AODC) analysis.

The Fate of H_2O_2 in the Presence of Sterile H_2O (Control)

To a 250 ml nalgene bottle labeled control, 4 ml of a 1,000 mg/l H_2O_2 solution (prepared by diluting stock A by a ratio of 1:10) was mixed with 196 ml of sterile H_2O. The new solution contained a 20 mg/l H_2O_2 test solution. The test solution was analyzed for hydrogen peroxide concentration at regular intervals (i.e. 2 min., 4 min., 6 min...). Analyses were performed in triplicate using the reflectoquant analysis system (RQFlex) by merck.

The Fate of H_2O_2 in Presence of Soluble Elements (Aqueous solutions)

To six (6) 250 ml nalgene bottles labeled A1, A2, A3, A4, A5, and A6, 20 g (dry wt.) of each test soil (one soil type per bottle) were added to 80 ml of sterile water to yield a 20% slurry of each of the six soil types. The slurries were placed on an automatic shaker and shaken for 24 hours. After the 24 hour period, the samples were removed and centrifuged at 13,000 x g for 15 minutes and filtered through a 0.45 µm membrane filter. A second set of samples was prepared as the first. The first set of samples was autoclaved at 121°C for 25 minutes and allowed to cool, the second set were not autoclaved. After cooling, 196 mls of both sets of solutions were placed in a 250 ml nalgene bottle with 4 ml of a 1,000 mg/l H_2O_2 stock solution. The new solutions contained 20 mg/l H_2O_2 in two (2) solutions of each soil type with one autoclaved and the other not autoclaved. Samples were collected and hydrogen peroxide concentrations were measured at regular intervals using the RQFlex.

The Fate of H_2O_2 in Presence of Soil Surfaces and Soil Bound Particles (20% slurries)

To six (6) 250 ml nalgene bottles labeled B1, B2, B3, B4, B5, and B6, 20 g (dry wt.) of each test soil (one soil type per bottle) were added to 80 ml sterile water to yield a 20% slurry of each of the six soil types. The slurries were placed on an automatic shaker and shaken for 24 hours. A second set of soil slurries was prepared as the first. After the 24 hour period, the first set of samples was autoclaved and allowed to cool; the second set was not autoclaved. After cooling, 196 ml of both set of slurries were placed in a 250 ml nalgene bottle with 4 mls of a 1,000 mg/l H_2O_2 stock solution. The new slurry solutions contained 20 mg/l H_2O_2 in two (2) slurry solutions of each soil type, with one autoclaved and the other not autoclaved. Samples were then filtered continuously through a 0.45 µm membrane filter and analyzed for hydrogen peroxide concentration at regular intervals.

RESULTS AND DISCUSSION

Soil Characterization

These data are summarized in Table 1. The Quartz was the predominant mineral in all of the soils. Na and K-feldspar were also common constituents in each soil. Other phases that were present in minor or trace amounts in most soils include kaolinite, illite or mica, chlorite, a hydroxy-interlayered smectite, and/or smectite. Smectite and hydroxy-interlayered smectite are subdivided in this case based on their differing ability to expand to 1.7 nm upon exposure to ethylene glycol. Because these soils differ in the absence or presence of certain crystalline phases, each soil is described on the bases of its prevalent mineralogical characteristics and the effect of the mineral components on the chemical and physical property measured. Wes reference soil had a trace of hydroxy-interlayered smectite. Hematite was found in the Tellico soil. This iron oxide also provided the reddish hue of the sample. No expandable clays were detected in this sample. The Gessie soil had a minor amount of dolomite, a trace of hydroxy-interlayered smectite, and a trace of calcite. Although the Alligator clay had a minor amount of hydroxy-interlayered smectite, it had the most smectite of all the samples. This sample also had the greatest aggregate amount of silt and clay (that is, it was the most uniformly fine-grained). Calcite was a major phase in the Crot soil and accounted for the high Ca and Mg concentrations in the sample. K-feldspar was also a major phase and accounted for the high K in the sample. Analcime, a sodium-rich zeolite, was a minor constituent. The smectite in this sample appeared to have little hydroxy-interlayers and expanded to 1.7 µm after treatment with ethylene glycol. All the samples examined in this study contained clay minerals. The samples with the larger amounts of clay present (especially expandable smectite) also showed the highest total organic carbon (TOC) content. The concentrations of specific elements observed in certain samples correspond to increased proportions of minerals containing those elements. The chemical composition reflected the mineralogy. Tellico sample was iron-rich due to hematite. Crot sample had higher levels of cations such as Ca^{2+}, Na^{+}, and Mg^{2+}, and anions such as Cl^{-} and SO_4^{2-} due to the presence of calcite and a zeolite, analcime. The highest Na concentration was found in the Alligator sample and is attributed to the smectite fraction. Gessie had the highest TOC; this is attributed to the clay abundant fraction.

Hydrogen Peroxide's Degradation Kinetics in Various Solutions

Results of batch hydrogen peroxide degradation experiments in aqueous solutions (Figures 1-A and B) show hydrogen peroxide's decomposition rate to be significant when compared to sand control in all solutions with the exception of the unsterilized gessie, tellico, and alligator as well as the sterilized gessie soils, which are $(6.3 \pm 5.60) \times 10^{-4}$ 1/min ($R^2 = 0.173$), $(1.18 \pm 4.60) \times 10^{-4}$ 1/min ($R^2 = 0.009$), $(1.20 \pm 0.81) \times 10^{-3}$ 1/min ($R^2 = 0.265$), and $(3.50 \pm 3.92) \times 10^{-4}$ 1/min ($R^2 = 0.104$), respectively. Not only did these solutions show insignificant hydrogen peroxide degradation over time, but regression analyses show there to be very little, if any, correlation between decomposition rate and time. Results of batch experiments in soil slurry solutions (Figures 2-A and B) show the fastest rate of hydrogen peroxide decomposition to be in the unsterilized tellico soil, with the other unsterilized soil solutions following closely behind, with the exception of the sand control and the inclusion of the sterilized tellico soil solution. Sterilized soil solutions decomposed hydrogen peroxide at a rate which represents roughly an order of magnitude difference (slower) than that of the unsterilized solutions (Table 2). These findings suggest a strong contribution by biotic soil components to hydrogen peroxide's decomposition rate. Because the highest rates of hydrogen peroxide's decomposition were recorded in the unsterilized tellico and gessie soil solutions as well as in the unsterilized crot solution (Table 2), it is evident that both iron and calcium, as well as the extreme pH in the case of the crot solution, play a large role in hydrogen peroxide's degradation kinetics.

The rate of hydrogen peroxide decomposition in the unsterilized soil solutions appeared to be first order with respect to hydrogen peroxide and was calculated to have an average of $(3.03 \pm 0.50) \times 10^{-1}$ 1/min ($R^2 = 0.876$) for reactive soil types, with a rate of $(5.70 \pm 0.77) \times 10^{-3}$ 1/min ($R^2 = 0.932$) in the sand control, based on plots of the ln H_2O_2 concentration versus time (Figure 2-A). Sterilization by autoclaving reduced bacterial population and, except in the case of the tellico soil solution, hydrogen peroxide decomposition rates to very low levels (Figure 2-B). The rate of H_2O_2 decomposition in the unsterilized aqueous solutions appeared to be first order with respect to H_2O_2 concentration in the crot soil solution only (Figure 1-A), with the other solutions showing minimal, if any, H_2O_2 decomposition in 30 minutes. Sterilization by autoclaving reduced H_2O_2 decomposition rates to undetectable levels (Figure 1-B) in all aqueous solutions. The dramatic reduction in decomposition rates indicates that most of the decomposition was biologically mediated. Table 1 summarizes results of soil characterization analysis giving results of bacterial enumerations as microbial density per acridine orange direct count (AODC) method. No attempt was made to identify the bacteria but, on agar plates, virtually all of the bacteria from the slurry samples were catalase positive (released gas when the plate was flooded with 1 percent H_2O_2).

CONCLUSION

Understanding the fate of hydrogen peroxide in the subsurface environment is essential to its efficient use as an oxygen source in the *in situ* biodegradation process. A substantial amount of literature exists on hydrogen peroxide in *in situ* bioremediation of a variety of contaminants (Britton 1985; Huling et al. 1990). However, many of these sources express concerns about the rapid decomposition of hydrogen peroxide as well as its toxic effects on microorganisms when used in large concentrations (greater than 500 mg/l). The laboratory studies reported in this paper used batch experiments to monitor hydrogen peroxide degradation kinetics under a variety of subsurface environmental conditions. Much was learned about the fate of this compound and its reaction rates under these conditions. These studies should prove very instrumental in the design and implementation of an efficient *in situ* bioremediation system which uses hydrogen peroxide as its oxygen source.

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Table 1. Soil samples with Mineralogic Composition, Particle Size Distribution, and Selected Biological and Chemical Properties.

Soil	Major Mineral	Particle size distribution[1] %Sand	%Silt	%Clay	microbial density[2] (AODC)	Remarks	pH
Sand	Quartz	96	4	0	$(3.4 \pm 0.16) \times 10^6$		6.8
Tellico Loam	Quartz Hematite	38	40	22	$(7.0 \pm 0.04) \times 10^6$	51600 mg/kg Fe 3850 mg/kg Mn 671 mg/kg K 6.48 mg/kg OPO	6.6
Gessie	Quartz Dolomite Calcite	48	46	6	$(1.4 \pm 0.01) \times 10^7$	1090 mg/kg TKN 14296 mg/kg TOC	7.2
A. Clay	Quartz	13	65	22	$(2.0 \pm 0.01) \times 10^7$	7503 mg/kg Na 16.7 meq/100g CEC 15.1 mg/kg NH_2N	5.5
WES Ref	Quartz	8	76	16	$(2.1 \pm 0.01) \times 10^7$		5.3
Crot	Quartz Calcite	56	24	20	$(14.0 \pm 0.01) \times 10^7$	59500 mg/kg Ca 15000 mg/kg Mg 4470 mg/kg K 166 mg/kg SO_4 249 mg/kg Cl	10

[1]The particle sizes are defined as follows: sand = 2 to 0.05 mm, silt = 0.05 to 0.002 mm and clay = <0.002 mm. [2]Data given in colony forming units per milliliter solution (cfu/ml). AODC = Acridine Orange Direct Count of microorganisms.

Table 2. Summary of H_2O_2 Rate Constants in Various Solutions.				
Soil Type	Phase	Steriled	$k_{[H2O2]}$[d] 1/min	r^2
Tellico	20% Slurry	No	$(5.07 \pm 0.99) \times 10^{-1}$	0.898
Gessie	20% Slurry	No	$(3.77 \pm 0.16) \times 10^{-1}$	0.993
Crot	20% Slurry	No	$(3.40 \pm 0.78) \times 10^{-1}$	0.865
Tellico	20% Slurry	Yes	$(2.55 \pm 0.10) \times 10^{-1}$	0.994
Wes	20% Slurry	No	$(1.39 \pm 0.43) \times 10^{-1}$	0.774
A. Clay	20% Slurry	No	$(1.29 \pm 0.08) \times 10^{-1}$	0.986
Gessie	20% Slurry	Yes	$(7.50 \pm 0.44) \times 10^{-2}$	0.977
A. Clay	20% Slurry	Yes	$(4.27 \pm 0.40) \times 10^{-2}$	0.965
Crot	20% Slurry	Yes	$(4.26 \pm 0.56) \times 10^{-2}$	0.950
Crot	Aqueous	No	$(2.90 \pm 0.08) \times 10^{-2}$	0.994
Wes	20% Slurry	Yes	$(9.23 \pm 2.68) \times 10^{-3}$	0.628
Sand	20% Slurry	Yes	$(6.06 \pm 0.29) \times 10^{-3}$	0.985
Sand	20% Slurry	No	$(5.70 \pm 0.77) \times 10^{-3}$	0.932
Wes	Aqueous	No	$(2.71 \pm 1.34) \times 10^{-3}$	0.369
Wes	Aqueous	Yes	$(2.07 \pm 0.56) \times 10^{-3}$	0.669
Crot	Aqueous	Yes	$(1.41 \pm 0.30) \times 10^{-3}$	0.764
A. Clay	Aqueous	Yes	$(1.32 \pm 0.79) \times 10^{-3}$	0.315
A. Clay	Aqueous	No	$(1.20 \pm 0.81) \times 10^{-3}$	0.265
Gessie	Aqueous	No	$(6.26 \pm 5.60) \times 10^{-4}$	0.173
Tellico	Aqueous	Yes	$(5.80 \pm 2.27) \times 10^{-4}$	0.451
Sand	Aqueous	No	$(4.90 \pm 10.8) \times 10^{-4}$	0.048
Sand	Aqueous	Yes	$(4.10 \pm 1.73) \times 10^{-4}$	0.445
Gessie	Aqueous	Yes	$(3.50 \pm 3.92) \times 10^{-4}$	0.104
Tellico	Aqueous	No	$(1.18 \pm 4.60) \times 10^{-4}$	0.009

[d]Error limits are 95% confidence intervals on the slopes, all data at 25 ± 1°C.

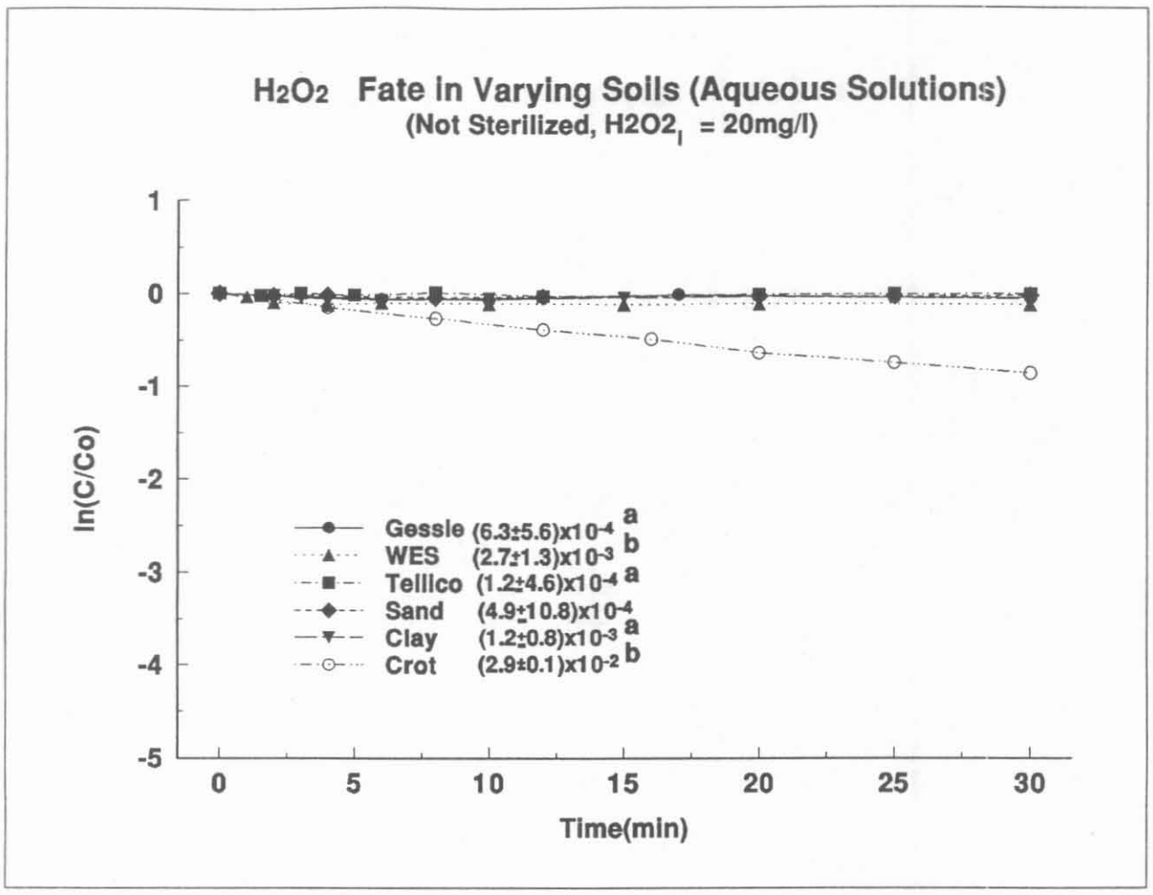

Figure 1-A. Hydrogen peroxide degradation in the presence of various soils; aqueous solutions (test for effects of soluble elements), not sterilized. Samples were prepared and analyzed at 25 ± 1°C and at relatively constant light intensity. Data presented are means of triplicate analysis. [a]Not Significantly different from sand control (t-test; $p>0.05$). [b]Significantly different from sand control (t-test; $p\leq0.05$).

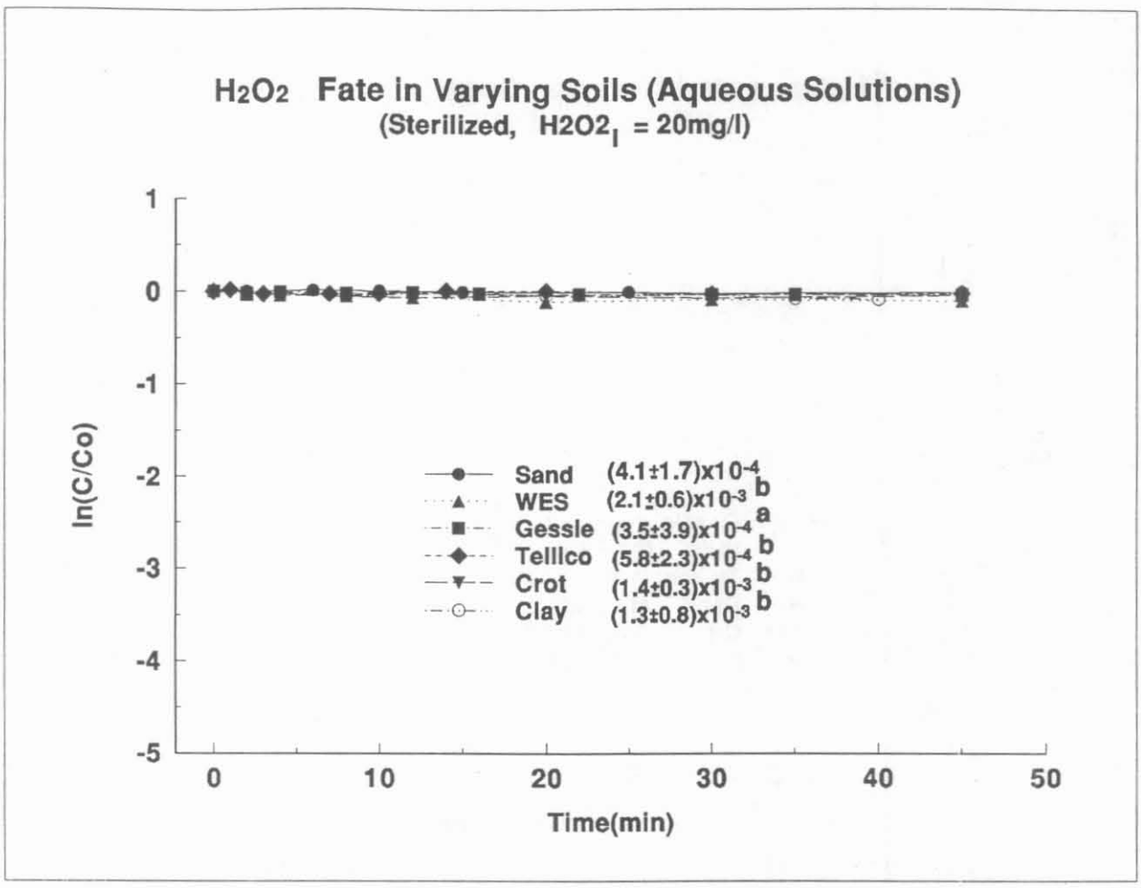

Figure 1-B. Hydrogen peroxide degradation in the presence of various soils; aqueous solutions (test for effects of soluble elements), sterilized. Samples were prepared and analyzed at 25 ± 1°C and at relatively constant light intensity. Data presented are means of triplicate analysis. [b]Significantly different from sand control (t-test; $p \leq 0.05$).

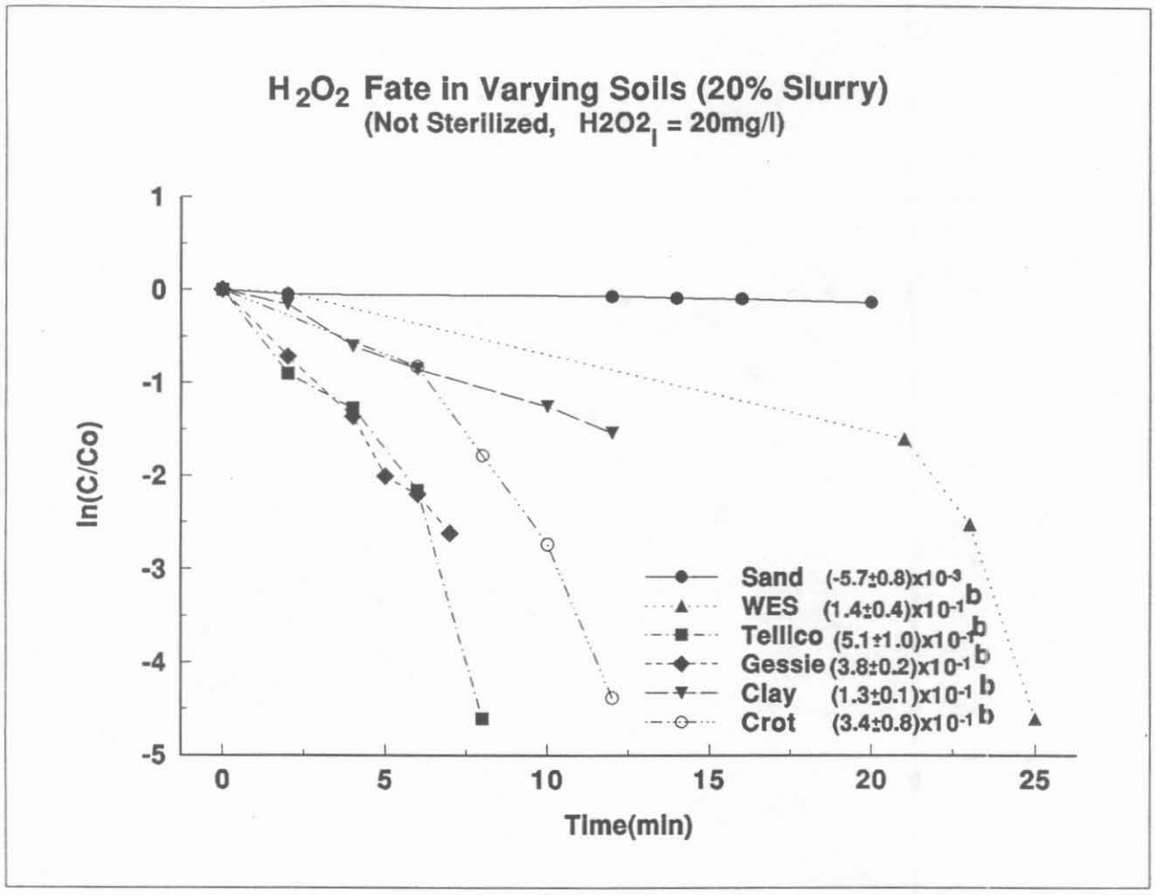

Figure 2-A. Hydrogen peroxide degradation in the presence of various soils; 20% slurries (test for effects of soluble elements), not sterilized. Samples were prepared and analyzed at 25 ± 1°C and at relatively constant light intensity. Data presented are means of triplicate analysis. [b]Significantly different from sand control (t-test; $p \leq 0.05$).

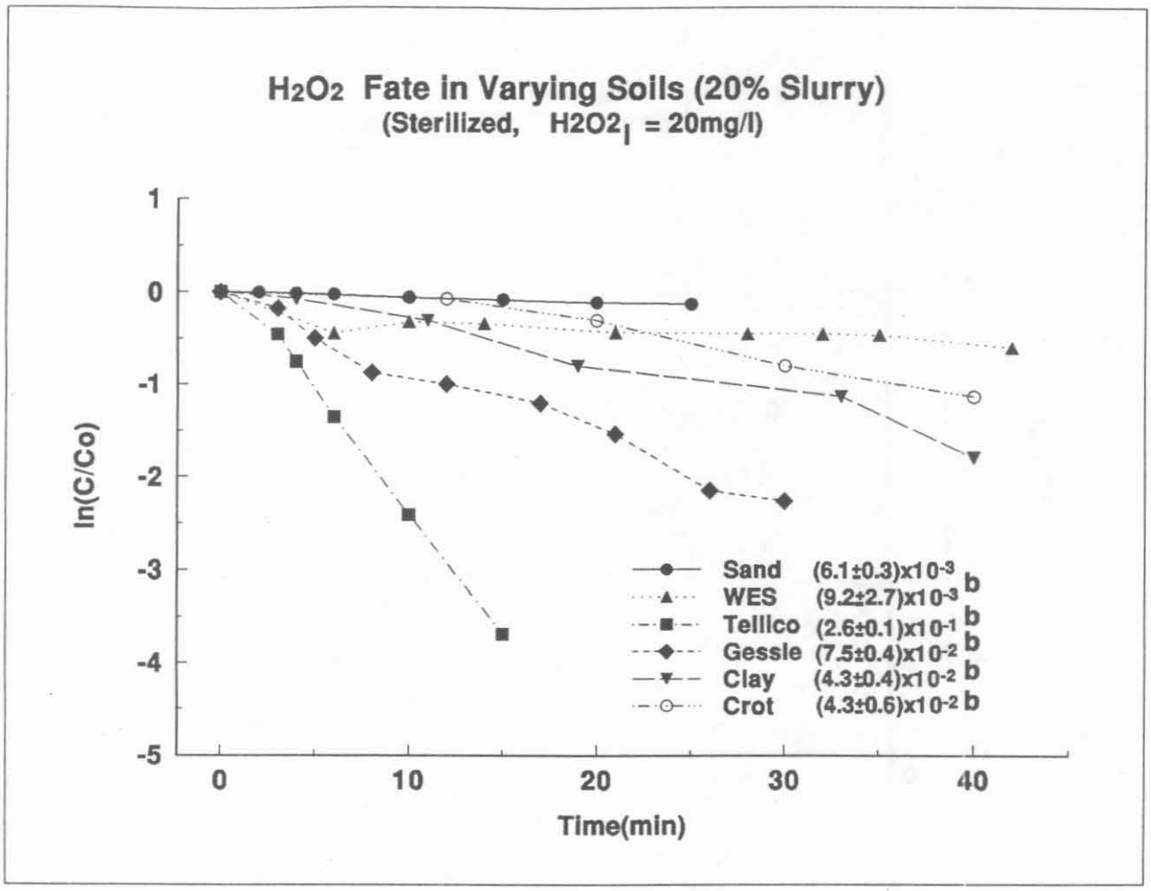

Figure 2-B. Hydrogen peroxide degradation in the presence of various soils; 20% slurries (test for effects of soluble elements), sterilized. Samples were prepared and analyzed at 25 ± 1°C and at relatively constant light intensity. Data presented are means of triplicate analysis. [b]Significantly different from sand control (t-test; p≤0.05).