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# The Fate of Hydrogen Peroxide as an Oxygen Source for Bioremediation Activities within Saturated Aquifer Systems

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## ABSTRACT

In situ bioremediation is an innovative technique for the remediation of contaminated aquifers that involves the use of microorganisms to remediate soils and groundwaters polluted by hazardous substances. During its application, this process may require the addition of nutrients and/or electron acceptors to stimulate appropriate biological activity. Hydrogen peroxide has been commonly used as an oxygen source because of the limited concentrations of oxygen that can be transferred into the groundwater using above-ground aeration followed by reinjection of the oxygenated groundwater into the aquifer or subsurface air sparging of the aquifer. Because of several potential interactions of $H_2O_2$ with various aquifer material constituents, its decomposition may be too rapid, making effective introduction of the $H_2O_2$ into targeted treatment zones extremely difficult and costly. Therefore, a bench-scale study was conducted to determine the fate of $H_2O_2$ within subsurface aquifer environments. The purpose of this investigation was to identify those aquifer constituents, both biotic and abiotic, that are most active in controlling the fate of $H_2O_2$. The decomposition rates of $H_2O_2$ were determined using both equilibrated water samples and soil slurries. Results showed $H_2O_2$ decomposition to be effected by several commonly found inorganic soil components; however, biologically mediated catalytic reactions were determined to be the most substantial.

### IMPLICATIONS

In situ bioremediation is an innovative treatment process that results in the cleanup of both groundwater and aquifer solids. The process typically involves the addition of oxygen and nutrients for simulation of the natural bacterial populations within the aquifer that ultimately use the pollutant(s) as a food source. The benefits of this process are reduced costs, treatment of contaminated aquifers under existing structures, and low site worker exposure to the pollutants.

This study focused on the fate of injected $H_2O_2$ within various aquifer matrices. Hydrogen peroxide was utilized as an oxygen source for in situ bioremediation. The results indicate that the bacterial populations themselves had the greatest impact of $H_2O_2$ degradation. Naturally occurring Fe had the next greatest impact, albeit at a much lower rate than the biomass-related reactions. The primary conclusion is that prior to injection of $H_2O_2$ into polluted subsurface environments, careful evaluation of potential scavengers needs to be addressed, especially oriented toward biotic reactions.

## INTRODUCTION

Many aquifer systems in the United States have become contaminated due to past military and industrial activities.[1,2] Many of these contamination problems are composed of highly biodegradable chemicals such as

petroleum hydrocarbons and wood-preserving products.[3] In general, remediation techniques may be categorized into two applications-based techniques: invasive and noninvasive technologies. Invasive techniques are ex situ treatment processes that require the excavation of soil prior to application. Examples include incineration, solidification/stabilization, composting, and bioslurry treatment.[4,5,6] Invasive technologies utilize highly engineered reactor units that offer elevated levels of process control and relatively rapid soil throughputs. The use of engineered reactors with such high levels of process control results in a treatment process that is kinetically more rapid and complete in terms of contaminant destruction than is typically afforded with noninvasive techniques. However, soil excavation and handling along with high capital and operational costs make invasive techniques more expensive than noninvasive approaches. Also, the potential for worker and community exposure to the contaminants is greatly increased with the use of invasive techniques.

Noninvasive techniques are inclusive of both in situ and pump-and-treat systems. They are usually more cost-effective than invasive techniques because of reduced capital and operational costs. Noninvasive techniques also offer an additional benefit in that they can be applied at facilities with existing structures, such as buildings or runways, that cannot be demolished to facilitate soil excavation. Pump-and-treat systems involve groundwater collection with subsequent treatment using water treatment processes, such as carbon adsorption, chemical oxidation, and activated sludge,[4,7] followed by reinjection of the groundwater effluents back into the aquifer to facilitate aquifer flushing or disposal of the treated water off-site. Pump-and-treatment systems are sometimes costly and time-intensive because of prohibitively slow removal of the contaminants due to sorptive limitations associated with many organic contaminants, resulting in potentially extended remediation times.[5,8] In situ systems essentially convert portions of the aquifers into subsurface reactors. The most common form of in situ treatment is biotreatment;[6] however, soil flushing and vapor extraction have also been successfully used.[9,10,11]

In situ biotreatment appears to offer the most promise of all of the in situ techniques under development for remediation of saturated aquifers because of the ease of application and the state of technology maturity. In situ bioremediation utilizes microorganisms to remediate contaminated aquifers in biologically active zones established within the contaminated soil structure.[12,13,14,15] In most cases, the stimulation of microorganisms within the subsurface requires the addition of electron acceptors and/or nutrients.[13,15,16] The most developed and commonly practiced form of in situ biotreatment involves the use of indigenous aerobic microbes.[5,6,17] Typically, aerobic biotreatment is used to degrade organic contaminants within polluted aquifers because of rapid removal rates and the achievable extent of contaminant degradation.[6,13]

During aerobic degradation, free molecular oxygen accepts electrons released by an electron donor (typically the contaminant), which is reduced to a lower oxidation state. Oxygen, if not present in adequate concentrations within biologically active zones, will limit the ability of aerobic microorganisms to actively degrade contaminants.[13,17] The rate of biotransformation, and thus contaminant persistence, has been reported to be controlled by the transport of oxygen into the contaminated groundwater, indicating that oxygen is usually the most limiting factor within contaminated aquifer systems.[13,18] The importance of adequately supplying oxygen into targeted, aerobic, biologically active zones cannot be overstated. Unfortunately, the oxygen demands of an active in situ biotreatment system can be quite high.

Fogel et al.[19] estimated that 3 lb of oxygen are required for every lb of petroleum product degraded. Goldsmith and Balderson[20] estimated a stoichiometric requirement of 8.6 mol oxygen for every mol of diesel fuel degraded. Bajpai and Zappi[15] estimated a typical requirement of 0.5–1 g oxygen/g hydrocarbon biodegraded. They further stated that oxygen to hydrocarbon dosing (w/w) requirements exceeding 4:1 are possible. Huling et al.[18] estimated a 5:1 ratio of oxygen to gasoline (w/w) requirement based on laboratory column testing. It can easily be seen from these references that the effective introduction of oxygen into highly biologically active zones is paramount in the design and maintenance of an effective in situ biotreatment system.

There are several techniques that may be used for effectively introducing oxygen into targeted biologically active zones within an in situ bioremediation system treating a saturated aquifer. Potential oxygen application options include down-hole air sparging, aboveground aeration/reinjection, or injection of $H_2O_2$ directly into the aquifer.[5,6,12] Dissolved oxygen, when introduced via aeration, has a limited solubility in aqueous solutions (~9 mg/L at 25 °C and 11 mg/L at 5°C). This solubility limitation can severely hinder establishment of areas of high biological activity due to the suppressed oxygen levels associated with the inherent mass transfer problems found with aeration-based equilibrium concentrations. Alternatively, oxygen may be delivered to the subsurface in the form of $H_2O_2$, which is a clear, odorless liquid that is readily available in large quantities and is fully immersible in water. The natural decomposition of $H_2O_2$ provides the molecular oxygen needed for aerobic microbial metabolic activity.[21] Within

subsurface systems, $H_2O_2$ typically dissociates to produce 1/2 mol dissolved oxygen/mol $H_2O_2$, as shown in the scheme illustrated below:[18]

$$H_2O_2 + H_2O \rightarrow 0.5\ O_2 + 2\ H_2O \qquad (1)$$

Molecular oxygen, which can be supplied using $H_2O_2$ or pure oxygen sparging, has a solubility of 40–50 mg/L,[22] representing at least a 4-fold increase in available oxygen within a saturated aquifer environment. Other attributes of $H_2O_2$ as an oxygen source for in situ biotreatment are that $H_2O_2$ is (1) reasonably inexpensive; (2) nonpersistent; (3) a stable liquid, which eliminates problems with storage and introduction into the aquifer; and (4) generally environmentally benign.[21] Zappi et al.[5] reported that some in situ systems initially using groundwater injected back into the aquifer and charged with oxygen via above-ground aeration were forced to convert to $H_2O_2$ injection due to the inability to keep up with the high oxygen demands of the subsurface biomass. Several reports on the use of $H_2O_2$ injection to supply oxygen into subsurface biologically active zones indicated various degrees of success.[17,19,22,23,24,25] Fogel et al.[19] reported the successful remediation of a petroleum hydrocarbon-contaminated aquifer using $H_2O_2$ injection. Flathman et al.[26] evaluated $H_2O_2$ injection using a series of column experiments and reported a high potential for the use of $H_2O_2$ as an oxygen source for in situ treatment.

The primary problem with the use of $H_2O_2$ as an oxygen source is the excessive reaction of the $H_2O_2$ with various components of the aquifer environment, which can dramatically impede the effective transport of the $H_2O_2$ into the biologically active zone.[5,15,18,23] Spain et al.[23] reported significant losses of $H_2O_2$ attributed to catalase degradation via the indigenous microorganisms that were present within the aquifer. Catalase is a biological enzyme (hemetin-containing) with an average molecular weight of 240,000 that is produced by living cells as a removal mechanism for excessive $H_2O_2$ produced during aerobic respiration.[27,28] Hinchee et al.[17] reported pseudo-first-order $H_2O_2$ decomposition rate constants ranging from 0.10 to 0.01 $min^{-1}$ during field tests of $H_2O_2$ injection. They reported that the predominant $H_2O_2$ sink appeared to be biologically mediated catalase decomposition.

Reaction of $H_2O_2$ with naturally occurring cations within soils has been reported.[18,22,29] Transition metals appear to be the most reactive metallic species. Schumb[29] reported that Mn and Fe are very reactive with concentrated $H_2O_2$ solutions. Morgan and Watkinson[22] reported that Fe-based reactions between $H_2O_2$ and soil particles have been problematic in terms of effectively transporting the $H_2O_2$ into targeted treatment zones. They reported that chelating and/or sequestering agents have been used within $H_2O_2$ amending solutions to curb cationic reactions.

Bajpai and Zappi[15] suggested the use of phosphate stabilizers to enhance the transportability of $H_2O_2$ through subsurface systems. Aggarwal et al.[30] reported limited success in stabilizing $H_2O_2$ solutions from both biotic and abiotic reactions within a soil system. Schumb[29] reported significant decomposition of $H_2O_2$ at both low (<3) and high (>7) pH values. This is not surprising when considering the increased disassociation of $H_2O_2$ at higher pH values (i.e., pKa = 11.6).[31] However, most research efforts that evaluated the fate of $H_2O_2$ within biologically active systems agreed that biologically based reactions (i.e., catalase) were the primary mechanism for $H_2O_2$ degradation. Reaction of $H_2O_2$ with naturally occurring organic matter is also possible based on reactions reported between $H_2O_2$ and organically rich soils;[30,32] plus, the reaction of $O_3$ with humic acids is well documented as an oxidizer sink during water treatment, indicating the susceptibility of most chemical oxidizers to reaction with natural organic matter.[33] Clearly, excessive losses of $H_2O_2$ to nonbeneficial reactions (i.e., reaction with soil constituents or excessive biotic reactions) can result in significant increases in the overall cost of remediation using in situ biotreatment.

An improved understanding of what aquifer systems, in terms of geobiochemical composition, are best suited for the use of $H_2O_2$ as an oxygen source is key to the design of effective aerobic in situ biotreatment systems. Therefore, a series of laboratory experiments was performed to assess what $H_2O_2$ sinks within aquifer systems undergoing aerobic biotreatment most control $H_2O_2$ fate within these subsurface environments. This was accomplished through the study of the reaction of $H_2O_2$ with various biologically (biotic) and nonbiologically (abiotic) active soil types that were considered characteristic of various geochemical constituents found in aquifer systems.

## MATERIALS AND METHODS

The general approach to this study was to divide the aquifer environment into two separate physical compartments (groundwater and soil) that contain naturally occurring biological and chemical species that may react with $H_2O_2$. The reactants found in either compartment in most in situ biotreatment application scenarios were taken to be natural soil chemical constituents (cations, high pH, and organic matter) and active bacterial populations. In order to segregate soil constituent effects based on a single predominant soil constituent, soils were collected from across the United States based solely on their having a characteristic chemical constituent that was dramatically higher than the

other constituents commonly found in U.S. soils.[15] The rationale for evaluating many soil constituents was to develop an empirical database that can be used to assess the relative difficulty that may be encountered if $H_2O_2$ injection was attempted at a given site. By using a suite of constituents and respective soil types, this database would have a universal appeal for use by environmental engineers when designing in situ biotreatment systems.

## Materials

Water used in all experiments was distilled and deionized (DDI water) prior to use. Solutions of $H_2O_2$ were formulated from a 50% (w/w) solution (Fisher Scientific). A stock solution of $H_2O_2$ was made by diluting the 50% $H_2O_2$ solution with the DDI water to formulate an aqueous solution of 10,000 mg/L $H_2O_2$ (w/w). Several serial dilutions of known concentrations of $H_2O_2$ were made on an as-needed basis from this stock solution during testing.

Several soil samples, each having a unique characteristic geochemical component that dominated its composition, were selected based on review of U.S. Geological Survey databases. The soil samples were collected from their native environments and shipped to the laboratory, where they were air-dried and sieved through a No. 4 sieve to remove debris and oversized detritus. Table 1 lists gradation data for the soil specimens used in this study. Table 2 lists additional details on the geochemical and biological characteristics of the soil samples used in this study. Table 3 provides a listing of the dominant soil characteristic and respective sample ID coding used throughout this paper. The rationale for including each soil specimen in the experimental design and the original location of the sample are detailed below.

(1) Control (purchased from U.S. Silica Inc.)—a nonreactive soil that allows for evaluation of experimental losses.
(2) HI-FE (Pope County, AR)—evaluation of elevated levels of both Fe and Mn.
(3) HI-TOC (Newton County, MS)—evaluation of reaction with organic matter and associated microbial consortia.
(4) HI-NA (LeFlore County, MS)—evaluation of Na, plus, this specimen had average constituent levels compared to the characteristics of the reported composition of U.S. soils.[15]
(5) AVG (Warren County, MS)—another soil considered generally representative of an "average" U.S. soil.
(6) HI-PH (Custer County, OK)—evaluation of both elevated pH and high biomass density.

**Table 1.** Soil specimen gradation data.

| Soil Specimen[a] | % Sand | % Silt | % Clay |
|---|---|---|---|
| Sand Control | 96 | 4 | 0 |
| Tellico Loam | 38 | 40 | 22 |
| Gessie | 48 | 46 | 6 |
| Aligator Clay | 13 | 65 | 22 |
| WES | 8 | 76 | 16 |
| Crot | 56 | 24 | 20 |

[a]Specimens are named after the location from which they were collected.

## Analytical and Microbiological Methods

A variety of analytical techniques for $H_2O_2$ were evaluated. The reasons for evaluating several methods were that poor light transmittance, complex soil chemical matrix, and the rapid reactions associated with the soil-water slurries used during this study made analysis of $H_2O_2$ using traditional colorimetric techniques difficult at best. A reflective colorimetric measuring system marketed as the RQFlex Reflectometer by EM Scientific Inc. was selected for water-phase $H_2O_2$ analysis because of ease of operation, accuracy of results, and flexibility in terms of water color/turbidity variation. This system uses a color-change reaction based on $H_2O_2$ concentration that is reflected off of a reactive strip into the detector of a hand-held colorimeter with a preset wavelength emission band. The soil-water slurries were phase-separated using centrifugation, and the $H_2O_2$ concentration within the liquid phase was measured using the RQFlex system. Due to the rapid decomposition of $H_2O_2$ within some of the experimental matrices tested in this study, the centrifugation and analysis of liquid centrates were performed using an organized protocol of rapid centrifugation and analysis that only took a few minutes to complete and that resulted in $H_2O_2$ concentrations reflective of actual sampling time increments.

Microbial enumerations were determined using the acridine orange direct count method, which gives total bacterial populations using epifluorescent microscopy. Epifluorescent microscopy allowed the direct observation and total enumeration of viable versus nonviable microorganisms within the soil specimens in less time than that required for other culturing methods (i.e., total heterotrophs). This technique is admittedly not as truly reflective of the total microbiological character and population density present in each soil specimen as are other bacterial enumeration techniques, such as fatty acid/phospholipid analysis using a gas chromatography system; however, it was considered a good choice for its intended use within the framework of this study because it provided a comparative assessment of bacterial activity and relative populations within a rapid analytical time frame and an easy, well-established laboratory procedure.

The bulk mineralogy and the clay minerals content in the five test soils (excluding the Ottawa sand used as a nonreactive control) were determined by X-ray diffraction

**Table 2.** Soil chemical and microbiological data.

| Soil Specimen | Mineral Content | Microbial Enumeration (#/mL) | Elemental Analysis (mg/kg) | | Other Analytes (mg/kg) | |
|---|---|---|---|---|---|---|
| Sand Control | Quartz[a] | – | Very Low | | Very Low | |
| | | | | | pH 6.8 | |
| Tellico Loam | Quartz[a] | $7.0 \times 10^6$ | 51600 | Fe | 6033 | TOC |
| | Hematite[b] | | 3850 | Mn | 11 | CEC |
| | | | 671 | P | pH 6.6 | |
| | | | 580 | K | | |
| | | | 416 | Ca | | |
| | | | 22 | Na | | |
| Gessie | Quartz[a] | $1.4 \times 10^7$ | 17900 | Fe | 14296 | TOC |
| | K-Feldspar[a] | | 13300 | Ca | 15 | CEC |
| | Na-Feldspar[b] | | 983 | K | pH 7.2 | |
| | Dolomite[b] | | 655 | P | | |
| | | | 647 | Mn | | |
| | | | 42 | Na | | |
| Aligator Clay | Quartz[a] | $2.0 \times 10^7$ | 16400 | Fe | 7227 | TOC |
| | K-Feldspar[b] | | 7503 | Na | 17 | CEC |
| | Na-Feldspar[b] | | 2560 | Ca | pH 5.5 | |
| | | | 1560 | K | | |
| | | | 514 | P | | |
| | | | 462 | Mn | | |
| WES | Quartz[a] | $2.1 \times 10^7$ | 21100 | Fe | 5320 | TOC |
| | K-Feldspar[b] | | 1440 | Ca | 11 | CEC |
| | Na-Feldspar[2] | | 1140 | K | pH 5.3 | |
| | | | 606 | P | | |
| | | | 449 | Mn | | |
| | | | 29 | Na | | |
| Crot | Quartz[a] | $1.4 \times 10^8$ | 59500 | Ca | 4746 | TOC |
| | K-Feldspar[b] | | 13500 | Fe | 14 | CEC |
| | Na-Feldspar[b] | | 5570 | Na | pH 10.0 | |
| | Amphibole[b] | | 4470 | K | | |
| | Calcite[b] | | 514 | P | | |
| | | | 255 | Mn | | |

*Note*: Soil pH measured using slurry technique; [a]Primary mineral component; [b]Secondary mineral component.



(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. In preparation for XRD of the bulk sample, a portion of each sample was ground in a mortar and pestle to pass through a 45-μm (no. 325) mesh sieve. For analysis of the clay-size fraction, an aqueous slurry of the powder was made, placed on a substrate, and allowed to air-dry overnight at room temperature (23 °C). An XRD pattern was collected on glycol for each sample. Bulk sample, random powder mounts were analyzed using XRD to determine the mineral constituents present in each soil specimen.

**Table 3.** Summary of dominant soil specimen characteristics.

| Soil Specimen | Dominant Feature | Sample ID |
|---|---|---|
| Sand | Clean Media | Control |
| Tellico Loam | Fe and Mn | HI-FE |
| Gessie | TOC | HI-TOC |
| Aligator Clay | Na | HI-NA |
| WES | Average Soil | AVG |
| Crot | pH/Ca | HI-PH |

### The Fate of $H_2O_2$ in the Groundwater Compartment

The reaction of $H_2O_2$ with solubilized chemical constituents from each soil specimen was evaluated by monitoring the reaction of $H_2O_2$ with waters equilibrated with each soil sample. This allows for evaluation of any chemical solute that derived from the soil phase that may degrade $H_2O_2$ [i.e., cations, humics, or enzymes (both inter- and intracellular

enzymes)]. Equilibrated waters were produced by mixing 20 g of each soil specimen with 80 mL DDI water for 24 hr in a 250-mL plastic bottle, and were mixed using a recipritating shaker table. After 24 hr, the samples were removed and centrifuged at 13,000 × g for 15 min, and the centrate was filtered through a 0.45-μm membrane filter. A second set of bacterial-free equilibrated water samples were prepared just as the first set of samples, except that these samples were autoclaved at 121 °C for 25 min after equilibration. Autoclaving was found by Spain et al.[23] to provide very efficient deactivation of catalase activity within aquifer soils. Their results indicated that effective deactivation was provided by autoclaving on a similar scale to that achieved using $HgCl_2$ amending. Aggarwal et al.[30] reported that autoclaving was more efficient in deactivating catalase activity within soils as opposed to $HgCl_2$ amending.

Hydrogen peroxide was dosed into the equilibrated waters by adding a sufficient amount to achieve a final dose of 20 mg/L. These tests were performed in triplicate using 250-mL plastic bottles (autoclaved where appropriate). Mixing within the reaction bottles was accomplished by agitating the bottles using the reciprocating table. Samples were collected at various intervals, depending on observed rate of $H_2O_2$ depletion and the respective $H_2O_2$ concentrations measured during the previous sampling event (none exceeded 20 min lapsed reaction time).

### Fate of Hydrogen Peroxide within the Soil Compartment

The fate of $H_2O_2$ within the soil compartment was evaluated by reacting $H_2O_2$ with the various soil specimens using 25% (w/w) soil slurries. Soil slurries were formulated by adding 20-g dry weight of each soil sample to 80 mL of a sterilized 20-mg/L $H_2O_2$/DDI water solution. Two sets of experiments were performed—one using the prepared soil samples as is and a second set using soil samples that were autoclaved prior to slurrying. This was done to segregate the impact of active biodegradation from abiotic reactions. The soil slurries were shaken for 24 hr using the reciprocating table. The $H_2O_2$ was monitored over time via periodic analysis (<45 min for total experimental run time) of the aqueous phase of the slurries for $H_2O_2$ concentration. Aqueous samples were collected from each bottle, centrifuged, then filtered through a 0.45-μm membrane filter, and the filtrate was analyzed for $H_2O_2$ concentrations using the RQFlex system. These experiments were also performed in triplicate.

### Reaction of Hydrogen Peroxide with Biocatalysts

The fate of $H_2O_2$ during reaction with both viable bacterial cells (biomass) and pure catalase enyzme was evaluated using batch shake experiments. Using the reflectometric technique, the $H_2O_2$ was analyzed. The source of the biomass, as measured by volatile suspended solids (VSS) according to Standard Methods (1995), was an activated sludge chemostat operated during a separate, yet concurrent study which involved acclimation of an aerobic consortia to acetone to be used for the biodegradation of high levels of ketones from a contaminated groundwater source. Waste sludge taken from the chemostat was diluted to targeted concentrations of VSS using distilled water. Reaction with various levels of VSS were reacted with 200 mL of a 20-mg/L $H_2O_2$ solution contained in 250 mL plastic bottles that were agitated using the shaker table.

Reaction with catalase was evaluated by dosing various enzyme units of bovine catalase (Aldrich Chemicals Inc.) into a 20-mg/L $H_2O_2$ solution contained in a 250-mL plastic bottle also agitated using shaker table. During the active biomass, VSS, and the catalase reaction experiments, $H_2O_2$ levels within the liquid samples were monitored over time using the reflectometric technique described above. Both experiments were performed in triplicate.

## RESULTS AND DISCUSSION

### Soil Characterization

Tables 1–3 present information concerning the biogeochemical characteristics of the soil specimens used in this study. From Table 1, it can be seen the specimens ranged from well to uniformly graded soils. Most of the soils were generally a silty clay, except for the sand control. Table 2 lists various information about each specimen. From the list in Table 2, Table 3 was drafted to summarize the perceived predominant characteristic of each specimen and to list the specimen ID codes that will be used herein during discussion of the various soils. As seen in Table 2, all specimens had nominal biomass densities that are typical of most healthy, non-contaminated soils.[34] Not surprisingly, quartz was the main building-block mineral, with several other minor minerals, for the soil specimens used in this study (Table 2).

### Degradation of Hydrogen Peroxide in Equilibrated Water Solutions

Table 4 lists the results of chemical analysis of the equilibrated waters that were used during $H_2O_2$ fate experiments ($[H_2O_2]_o$ = 20 mg/L) within the groundwater compartment. The concentrations shown are generally reflective of the primary constituents of the soil specimens used in the equilibration experiments (see Table 2).

The results of these experiments are presented in Figures 1 and 2 for the nonautoclaved and autoclaved water samples, respectively. From both figures, only the HI-PH water that was not autoclaved resulted in appreciable $H_2O_2$ degradation (Figure 1); however, this removal appears to

**Table 4.** Chemical analysis of equilibrated waters.

| Analyte | Control | HI-FE | HI-TOC | HI-NA | AVG | HI-PH |
|---|---|---|---|---|---|---|
| Ca | 1.92 | 1.51 | 24.9 | 15.4 | 7.27 | 63.8 |
| Fe | 0.21 | 0.034 | 2.76 | 86.4 | 60.4 | 712 |
| Mn | ND | 0.025 | 0.096 | 0.683 | 9.52 | 9.52 |
| Na | 2.95 | 1.09 | 0.286 | 0.7 | 4.28 | 442 |
| Alka | <10 | 99.3 | 11.8 | 64.2 | 275 | 2590 |
| $NH_3$-N | <0.02 | 0.10 | 0.066 | 0.562 | 0.08 | 0.345 |
| Total-P | <0.2 | 2.13 | <0.2 | 0.239 | 2.01 | 1.51 |
| TOC | 58.1 | 64.2 | 50.7 | 65.9 | 75.2 | 97.6 |
| pH | 6.81 | 5.25 | 6.58 | 7.20 | 5.52 | 10.0 |

be biotic in nature because this extent of removal was no longer active after the sample was autoclaved (Figure 2). This extent of reaction with the biocatalysts in the HI-PH water is attributed to the microbial consortia that are present in this highly alkaline soil. Skujins (1976) concluded that bacteria living in alkaline systems have high catalase activity, which is supported by the high reactivity of the $H_2O_2$ observed with the HI-PH water experiments. The other equilibrated waters resulted in negligible $H_2O_2$ degradation. The AVG soil equilibrated water that was not autoclaved indicated a slight $H_2O_2$ removal potential (5 mg/L) that was expended within the first 5 min of testing. A review of the microbial enumeration data presented in Table 2 indicates the HI-PH soil contained bacterial populations that were an order of magnitude higher than the next highest populations of bacteria enumerated in the other soil specimens. The AVG soil had the next highest bacterial counts of the other soil specimens, indicating a high reactivity of the $H_2O_2$ with the active biomass (or enzymes) that was desorbed from the soil into the equilibrated waters.

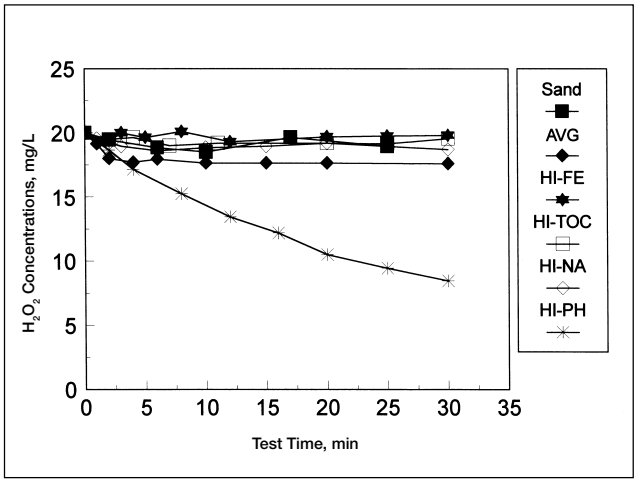


**Figure 1.** Reaction of $H_2O_2$ with equilibrated water (not autoclaved).

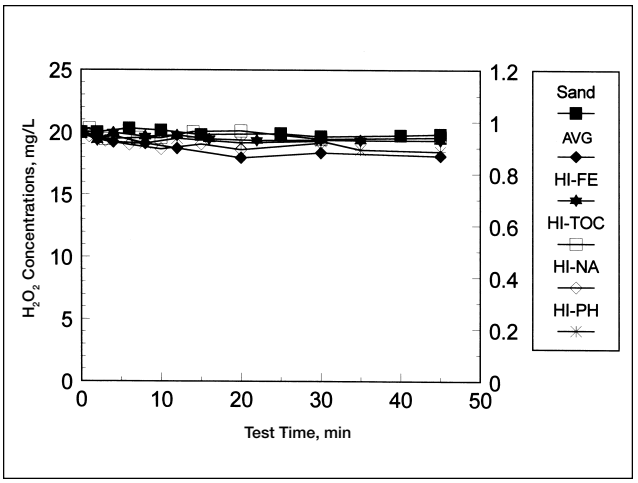


**Figure 2.** Reaction of $H_2O_2$ with equilibrated water (autoclaved).

These results indicate that chemical constituents (minerals) that were desorbed from the soil specimens were present at levels that were not adversely reactive to the dosed $H_2O_2$. Therefore, the reaction of $H_2O_2$ with chemical constituents found in typical groundwater appears to be minimal, except for the presence of biologically active agents such as viable bacterial cells.

It is realized that the organic contaminants within the groundwater and soil compartments also may pose an $H_2O_2$ sink. However, without some form of degradation into OH, $H_2O_2$ is not very reactive with most organic contaminants.[35] Zappi et al.[36] evaluated the fate of $H_2O_2$ as an oxygen source for a suspended growth bioreactor treating groundwater contaminated with high levels of benzene. Their results indicated that the $H_2O_2$ was very reactive to the biomass, but hardly reactive with the high levels of benzene present in the bioreactor. Benzene has been traditionally considered a very oxidizable organic compound[37] using powerful chemical oxidizers, such as $O_3$ and OH, but not $H_2O_2$, which has a much lower oxidation potential. Therefore, based on these past efforts, most of the organic contaminant(s) present in the aquifer system undergoing treatment can be considered a minor fate mechanism. However, a careful review of the reactivity of the contaminant is recommended using methods described by Zappi et al.[36] for each contaminant type under investigation for a given site or a series of simple reactivity experiments performed using actual site samples to assess the overall reactivity of the total contaminant matrix present in the aquifer system with $H_2O_2$. One interesting aspect to the fate of $H_2O_2$ within a contaminated aquifer system is that Alyea and Pace[38] report that the presence of organic chemicals, such as phenol, catechol, and *p*-cresol, all had a stabilizing effect on $H_2O_2$ reaction with catalase. Therefore, it is possible that some stabilizing effect may be realized when supplying $H_2O_2$ into contaminated aquifers (albeit minor, as witnessed by the

reports discussed earlier concerning excessive $H_2O_2$ losses during actual in situ bioremediation activities).

### Degradation of Hydrogen Peroxide within the Soil Compartment

Results of the experiments evaluating $H_2O_2$ fate within the soil compartment are presented as Figures 3 and 4. Figure 3 presents the results of $H_2O_2$ reactivity studies using soil samples that were not autoclaved, making them biologically active. These data indicate that the HI-FE and HI-TOC soils provided the most rapid $H_2O_2$ sinks of all the soils tested. Both resulted in complete removal of $H_2O_2$ within 8 min of testing. The HI-FE soil was expected to provide a significant sink due to the Fe-$H_2O_2$ reactions. Based on the results from the equilibrated water experiments, the high levels of $H_2O_2$ reactivity observed with HI-TOC soil were surprising.

However, Aggarwal et al.[30] reported that soil containing high TOC levels provides conditions conducive to the growth of microorganisms with high catalase-production capability. The reason that this level of activity was not observed in the equilibrated water experiments is likely due to strong sorptive bonds of the enzymes on the elevated humic fractions found on soils containing high levels of organic matter.[39,40] The HI-PH soil provided the next highest level of $H_2O_2$ degradation by achieving complete removal within 12 min of reaction. The ability of the HI-PH soil specimen to degrade the $H_2O_2$ supports the data generated during the $H_2O_2$ fate experiments using the nonautoclaved equilibrated waters, in which the HI-PH soil was the only sample to exhibit an appreciable $H_2O_2$ loss. The HI-NA and AVG soils were the next most reactive, respectively. The only soil sample not to show significant $H_2O_2$ decay was the sand control. This is not surprising, considering the lack of reactive sites on a quartz sand particle.

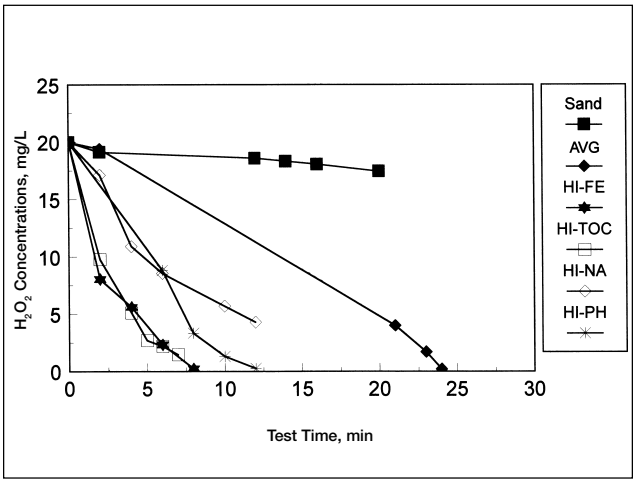


**Figure 3.** Reaction of $H_2O_2$ with soil (not autoclaved).

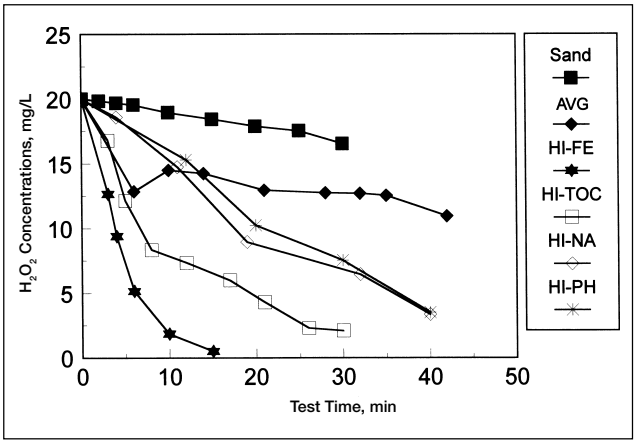


**Figure 4.** Reaction of $H_2O_2$ with soil (autoclaved).

The soil specimens were then reacted with the $H_2O_2$ after autoclaving to eliminate the presence of biologically active agents. These data are shown in Figure 4. The impact of autoclaving (i.e., deactivation of biocatalysts) on the extent and rate of $H_2O_2$ degradation was dramatic. The HI-FE soil exhibited the least change, yet the time required to remove the $H_2O_2$ to below detection limits was increased from 8 min to over 15 min reaction time (see Figures 3 and 4). With the other soil specimens, the impact of autoclaving on $H_2O_2$ removal appeared to be even more significant. This strongly suggests that a large fraction of the $H_2O_2$ loss observed in the nonautoclaved soils is attributable to biotic mechanisms. This observation is supported by those of others who evaluated the fate of $H_2O_2$ within soil systems.[23,41,42] Unfortunately, none of these studies reported rate constants or rigorously separated soil compartment reactions. However, their reported conclusions do support the present conclusion that biotic reactions appear to dominate $H_2O_2$ fate within soil systems.

Table 5 lists the pseudo-first-order rate constants calculated from the data presented in Figures 3 and 4. Clearly, deactivation of the biological activity via autoclaving dramatically reduced, but not eliminated, the $H_2O_2$ decay reactions. Using the assumption that autoclaving fully deactivated all of the biological agents, it can be stated that Fe clearly appears to most impact the abiotic fate of $H_2O_2$ within soil matrices. This observation is not surprising, given that the reaction of $H_2O_2$ with ferrous iron to form ferric iron is well-documented as "Fenton's reaction," shown below, which has been used for water and soil treatment due to the production of OH as an advanced oxidation mechanism.[43,44,45]

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\cdot} + HO^{-} \quad (2)$$

$$Fe^{3+} + H_2O_2 \rightarrow HO_2^{\cdot} + H^{+} + Fe^{2+} \quad (3)$$

$$Fe^{2+} + OH^{\cdot} \rightarrow OH^{-} + Fe^{3+} \quad (4)$$

**Table 5.** First-order rate constants for soil-slurry experiments.

| Soil System | Rate Constant, $min^{-1}$ ($r^2$) Autoclaved | | Rate Constant, $min^{-1}$ ($r^2$) Not Autoclaved | | d$K$ [a] ($min^{-1}$) |
|---|---|---|---|---|---|
| HI-FE | 0.26 | (0.99) | 0.51 | (0.89) | 0.25 |
| HI-TOC | 0.075 | (0.98) | 0.38 | (0.99) | 0.305 |
| HI-NA | 0.043 | (0.97) | 0.13 | (0.99) | 0.087 |
| HI-PH | 0.043 | (0.95) | 0.34 | (0.87) | 0.297 |
| AVG | 0.0092 | (0.63) | 0.14 | (0.77) | 0.131 |
| Control | 0.0061 | (0.98) | 0.0057 | (0.93) | –0.0004 |

[a] $dK = K_{not\text{-}auto} - K_{auto}$.

$$HO_2^{\cdot} + Fe^{3+} \rightarrow O_2 + H^+ + Fe^{2+} \quad (5)$$

$$H_2O_2 + OH^{\cdot} \rightarrow H_2O + HO_2^{\cdot} \quad (6)$$

Fenton's reaction is maximized at an acidic pH because of increased solubility of soluble, reduced Fe species (i.e., $Fe^{2+}$) that initiate radical production. This reaction does occur at neutral to slightly basic pH and is not limited to acidic pH; however, the rate is reduced because of lower aqueous phase, reduced Fe species concentrations. The above reactions highlight several $H_2O_2$ fate mechanisms associated with Fenton's reaction that are possible within soil media. Several sources of naturally occurring Fe species capable of mediating these reactions that can be attributed to $H_2O_2$ degradation have been detailed in the literature.[46,47,48] Candidate species reported include simple ferrous iron ($Fe^{2+}$), geothite (a-FeOOH), and ferrous sulfates (e.g., $FeSO_4$). Soils typically serve as a good reservoir of Fe compounds, as discussed by Dragun and Chiasson.[49] They list Fe concentrations within U.S. soils ranging from 100 to over 100,000 mg/kg. Additionally, OH produced via Fenton's reagent could also degrade organically bound Fe, which, in turn, once liberated can further serve as a scavenger for more $H_2O_2$ as shown in the above reactions.

The most dramatic change in $H_2O_2$ decay reactions was found to be with the HI-TOC and HI-PH soils [$K_{no\text{-}auto}$ vs. $K_{auto}$ (see Table 5)]. Based on a review of Table 2, it can be seen that the HI-PH soil had the highest biomass concentration of all the soil specimens by almost a full order of magnitude; therefore, a dramatic change in $H_2O_2$ decay rate due to biocatalyst inactivation is not unexpected. A similar fate must have been experienced by the biocatalysts associated with the HI-TOC soil. It is also possible that the high heat and oxidizing conditions present within the autoclave may have oxidized much of the oxidizable TOC within the soil, resulting in the removal of a significant oxidizer sink. However, it would be expected that the reduced iron ($Fe^{2+}$) present in the HI-FE soil would also be oxidized to $Fe^{3+}$, resulting in the loss of a Fenton's reactant, if autoclaving provided that significant of an oxidative step, which was not the case, as seen on review of the HI-FE data in Figure 3.

The evaluation of the $H_2O_2$ reaction data showed that the degradation of $H_2O_2$ follows first-order kinetics (i.e., all $r^2 > 0.9$). Therefore, assuming that the degradation of $H_2O_2$ within a soil slurry follows first-order kinetics for both biotic and abiotic reactions, the removal of $H_2O_2$ can be presented as

$$\frac{dC_{hp}}{dt} = (K_{biotic} * C_{hp}) + (K_{abiotic} * C_{hp}) \quad (7)$$

where $C_{hp}$ is $[H_2O_2]$, mg/L; $K_{biotic}$ is the first-order rate constant for biotic reactions, $min^{-1}$; and $K_{abiotic}$ is the first-order rate constant for abiotic reactions, $min^{-1}$.

The above equation can be rearranged as follows:

$$\frac{dC_{hp}}{dt} = (K_{biotic} + K_{abiotic}) * C_{hp} \quad (8)$$

and

$$\frac{dC_{hp}}{dt} = K * C_{hp} \quad (9)$$

where $K = K_{biotic} + K_{abiotic}$. Therefore, the rate constant for the abiotic reaction can be calculated from the first-order constants calculated from each $H_2O_2$ fate experiment using the following relationship:

$$K_{biotic} = K - K_{abiotic} \quad (10)$$

By assuming autoclaving inactivated all or most of the biological activity, for the nonautoclaved soil

$$K_{no\text{-}auto} = K = K_{biotic} + K_{abiotic} \quad (11)$$

Thus, the first order rate constant for the experiments using the autoclaved soil can be expressed as for each soil specimen as

$$K_{auto} = K_{abiotic} \quad (12)$$

The difference between the rate constants generated during the autoclaved and nonautoclaved soil specimens can be assumed to be $K_{biotic}$ for each soil specimen. These values are listed as "d$K$" in Table 5. The values of $K_{biotic}$ calculated using this method are relatively consistent between

each soil specimen, ranging from 0.087 to 0.305 $min^{-1}$. These values indicate that the biomass present in all soil specimens had significant activity toward $H_2O_2$. To evaluate if biomass density (microorganism populations) correlated directly with $H_2O_2$ activity, regardless of the possible difference in microbial consortia physiological composition, a plot of acridine orange counts versus $H_2O_2$ degradation rate constant was drafted as Figure 5. The data imply that the soil microbial consortia established under the unique conditions associated with that soil ecosystem can have vastly differing $H_2O_2$ degradation activity (assumed to be catalase activity). This agrees with some of the published efforts indicating soil ecology can have an impact on the magnitude of catalase activity.[31,50]

Based on a review of the rate constants listed in Table 5, it can be stated that biotic mechanisms appear to be the major mechanism of $H_2O_2$ degradation. As stated earlier, this conclusion is supported by other studies. The rapid degradation of $H_2O_2$ in the HI-FE soil indicates that the degradation of $H_2O_2$ via Fe-based catalysis (likely a Fenton's reaction) also is a major removal mechanism when compared with results obtained for the other soil specimens. The presence of high TOC (HI-TOC) appears to have an equal impact with high pH (HI-PH) on $H_2O_2$ degradation (see Table 5). The HI-NA and AVG soil specimens resulted in rate constants of approximately one-third that for the other soils, but within the range reported by Hinchee and Downey.[51] The lesser degree of $H_2O_2$ degradation observed with the HI-NA and AVG soil specimens is probably more realistic for most soil undergoing biotreatment, since the other soil specimens were selected because they represent extremes in terms of at least one of the soil constituents. Therefore, the $H_2O_2$ degradation rate constants calculated for the HI-NA and AVG soils can be used as good estimates when designing in situ biotreatment systems for soil of "average" composition, if testing on the actual site soil is not planned.

### Reaction with Biocatalysts

Figure 6 presents the results from the reactivity experiments of $H_2O_2$ with bovine catalase. These data indicate that the higher the catalase concentration, the more rapid the degradation of $H_2O_2$. From these data, first-order rate constants of 0.54, 0.051, and 0.0092 $min^{-1}$ for catalase concentrations of 1, 0.1, and 0.01 enzyme units, respectively, were calculated with correlation of fit values all in excess of 0.9, indicating acceptable statistical fit. These rate constants were then plotted on an X-Y plot of catalase concentration versus rate constant (Figure 7). The straight line indicates that the overall reaction of $H_2O_2$ with catalase is a second-order reaction (first order with respect to both catalase and $H_2O_2$ concentrations) with the overall kinetic rate constant represented by the slope

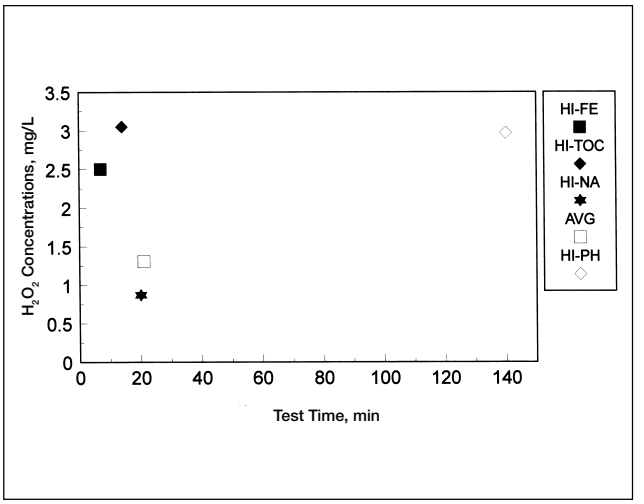


**Figure 5.** Degradation rate of $H_2O_2$ vs. soil biomass level.

of this plot, which was calculated to be 0.539 L/(min*enzyme units). This method of kinetic data analysis is described in more detail by Kuo et al.[52]

Figure 8 presents the reaction data for $H_2O_2$ with various levels of active biomass (represented as VSS). The rate constants for VSS levels of 20, 50, 100, and 200 mg/L VSS were calculated to be 0.027, 0.078, 0.11, and 0.27 $min^{-1}$, respectively. The correlation of fit constants for the semi-log plot regressions were all in excess of 0.9, indicating acceptable fit. Figure 9 presents an X-Y plot of VSS versus respective $H_2O_2$ rate constant. The calculated overall rate constant is 0.0013 L/(min*mg).

These data present pseudo-first-order $H_2O_2$ degradation rate constants of similar magnitude to those estimated for the biotic activity observed with the soil specimens. These data collectively indicate the high level of reactivity that biomass and associated enzymes possess for $H_2O_2$ degradation. It is interesting to ponder that the biochemical reactions deemed of targeted interest (i.e., biotic utilization of the oxygen from the $H_2O_2$) are also the same reactions that can account for excessive degradation of

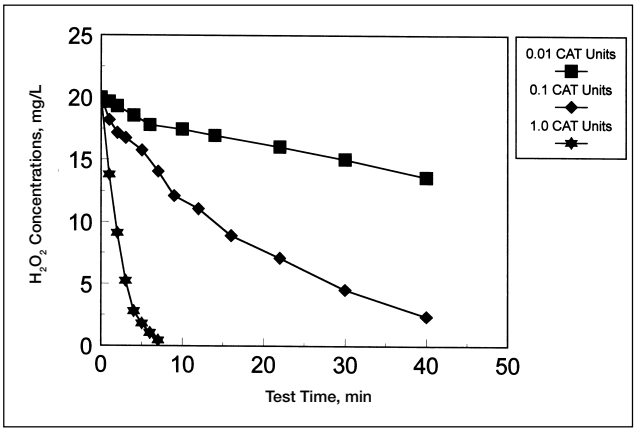


**Figure 6.** Reaction of $H_2O_2$ with catalase (CAT).

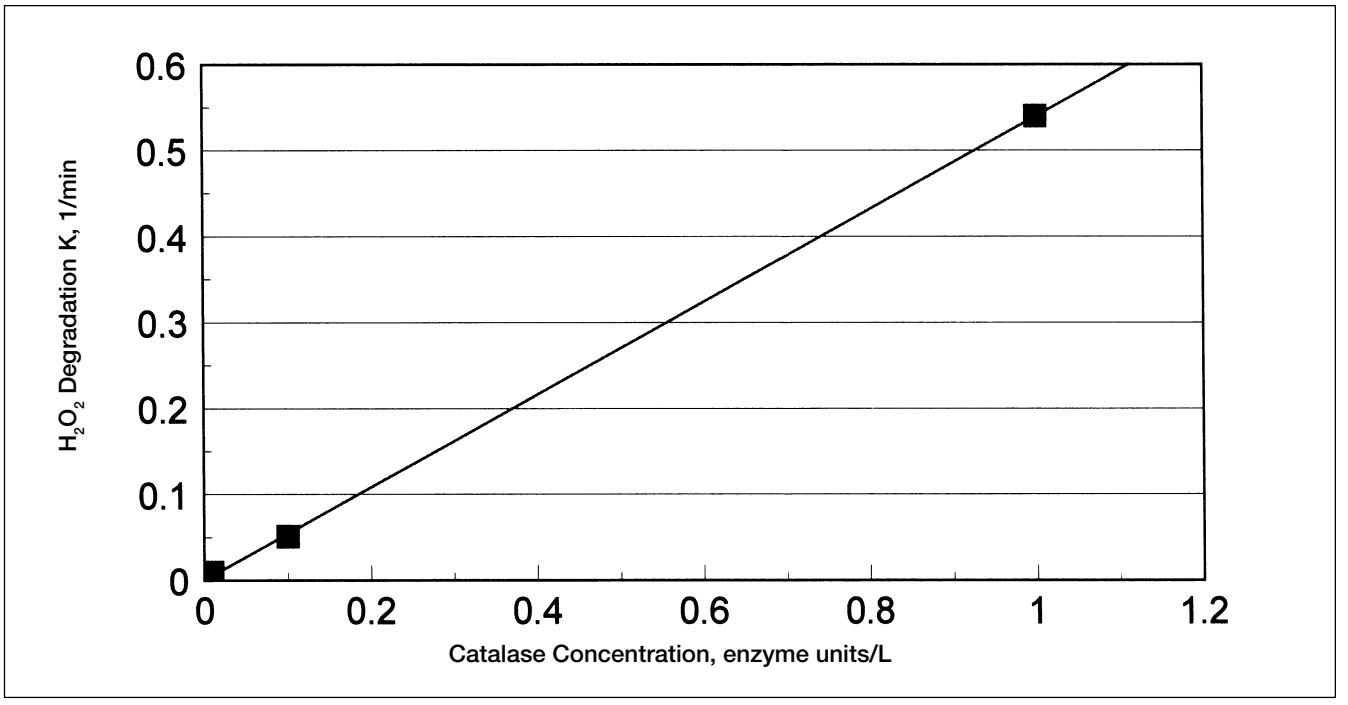


**Figure 7.** Determination of overall rate constant. Reaction of $H_2O_2$ with catalase.

the $H_2O_2$ upon introduction into biologically active zones within an in situ biotreatment system.

## ENGINEERING SIGNIFICANCE

Understanding the fate of $H_2O_2$ 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 the use of $H_2O_2$ as an oxygen source for bioremediation of a variety of contaminants.[15,18,21,36,51] However, many of these sources express concern over the rapid decomposition of $H_2O_2$ as well as its toxic effects on microorganisms when used in large concentrations (i.e., greater than 2500 mg/L).[12] Our findings are supported by previous studies pertaining to $H_2O_2$ decomposition. Biological sinks attributed to high catalase activity pose the greatest impediment to $H_2O_2$ transport. Therefore, as biomass production is stimulated within the targeted subsurface treatment zones, the difficulties with maintaining effective oxygen tensions will heighten as the oxygen levels exceed solubility (~40 mg/L), causing excessive molecular oxygen losses via production of bubbles, which tend to float upward into useless aquifer zones overlying the targeted active areas. This loss prevents the oxygen from being beneficial to downstream bacterial populations. In essence, as oxygen demand increases with increased biomass, so does the reactivity of the $H_2O_2$ with the biotic component of the aquifer matrix.

In terms of abiotic reactivity, Fe appears to be the most reactive species evaluated, followed closely by TOC, pH, and monovalent cations. The impact of these chemical constituents on $H_2O_2$ fate generally followed the impacts of these constituents on the fate of $O_3$ (another oxidizer) within water matrices containing similar chemical species.[33]

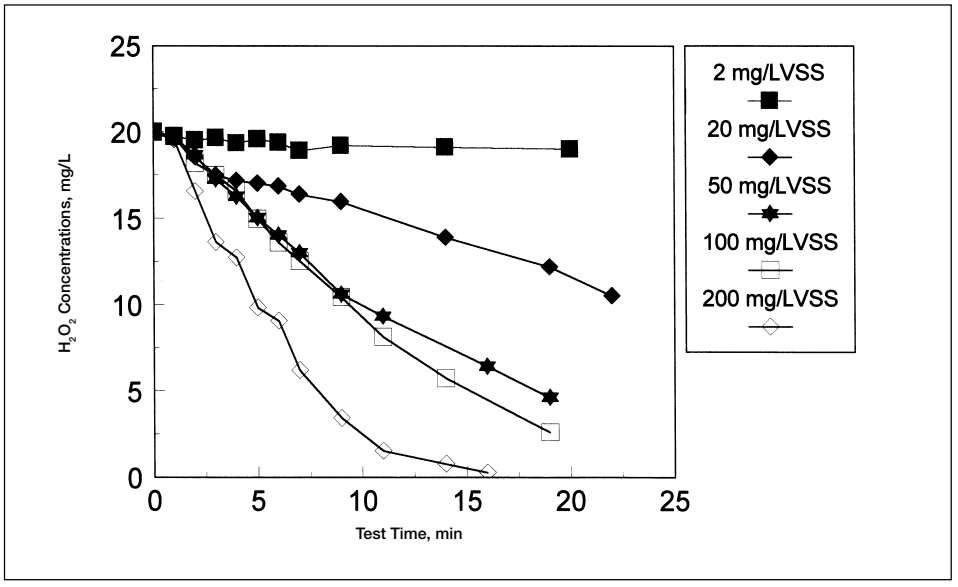


**Figure 8.** Reaction of $H_2O_2$ with biomass.

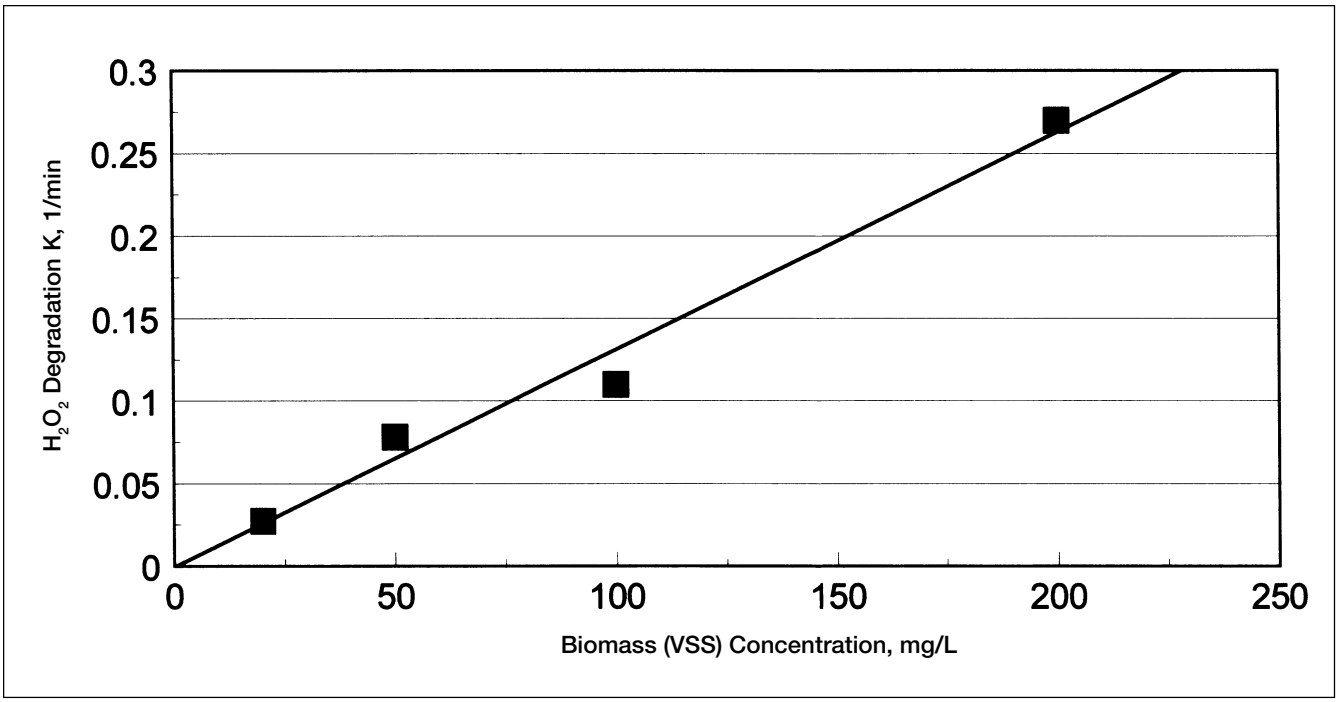


**Figure 9.** Determination of overall rate constant. Reaction of $H_2O_2$ with biomass.



## CONCLUSIONS

The results of this study indicate that the groundwater compartment of an aquifer system will generally have a small impact on the fate of $H_2O_2$, except when large quantities of suspending bacteria are present. The soil-slurry experiments clearly indicate that biotic mechanisms are the predominant fate mechanism for $H_2O_2$. Reaction with Fe and, although not directly, other studied reactive cations, such as Mn, also appears to be a significant sink for those soils containing high levels of these cations. The degradation of $H_2O_2$ within all of the systems tested were adequately modeled using the pseudo-first-order kinetic model. The reaction of $H_2O_2$ with biocatalysts was found to be an overall second-order reaction that is first order with respect to either reactant. The overall implication of this study was that reaction with biomass will greatly impact the transport of $H_2O_2$ as compared with those abiotic reactions associated with soil chemical constituents, with the possible exception of reaction with the cations.

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