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Project Summary

Enhanced Bioremediation Utilizing Hydrogen Peroxide as a Supplemental Source of Oxygen: A Laboratory and Field Study

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Remedial actions at hazardous waste sites involving in-situ treatment are widely recognized as a preferred treatment option. Bioremediation, a treatment technology which can be implemented in-situ, utilizes available oxygen to metabolize organic contaminants. Laboratory and field scale studies were conducted to investigate the feasibility of using hydrogen peroxide as a supplemental source of oxygen for the bioremediation of an aviation gasoline fuel spill.

Field samples of aviation gasoline-contaminated aquifer material were artificially enhanced with nutrients to promote microbiological degradation of fuel carbon in laboratory columns. Oxygen provided from hydrogen peroxide decomposition was utilized biologically in the columns. However, the rapid rate of hydrogen peroxide decomposition at 100.0 mg/L resulted in the production of oxygen gas. Reduced rates of oxygen consumption during this period indicated that microbial inhibition may have occurred.

A mass balance of the fuel carbon indicated that approximately 36.0% of the initial mass leached out in the aqueous phase, 10.0% remained, and 54.0% degraded. The ratio of oxygen consumed to aviation gasoline degraded was greater than that predicted by the ideal stoichiometric conversion. Hydrogen peroxide breakthrough in the column effluent never exceeded 11.0% of the influent concentration which was increased up to 200.0 mg/L.

Field studies confirmed that there was a measurable increase of oxygen in the soil gas in the area where hydrogen peroxide was injected. This indicated that a significant fraction of hydrogen peroxide rapidly decomposed to oxygen gas and escaped into the unsaturated zone.

This Project Summary was developed by EPA's Robert S. Kerr Environmental Research Laboratory, Ada, OK, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

The Superfund Amendments and Reauthorization Act (SARA) of 1986 directed EPA to prefer remedial actions involving treatment that would permanently and significantly reduce the volume and toxicity or mobility of hazardous substances, pollutants, and contaminants over remedial actions not involving such treatment. Therefore, the off-site transport and disposal of hazardous substances or contaminated materials without treatment is the least favored remedial action where practicable alternative treatment technologies are available.

Bioremediation is a relatively new treatment technology that can be implemented in-situ in which microorgan-

isms metabolize organic contaminants generally into harmless byproducts. Aerobic bioremediation has been reported to degrade a wide variety of organic contaminants such as alkylbenzenes (benzene, toluene, xylene (BTX)), polynuclear aromatic hydrocarbons, heterocyclic organic compounds, and some of the simpler chlorinated compounds. Recently, researchers have found that transformation of trichloroethylene by methane-oxidizing bacteria under aerobic conditions is possible.

In aerobic respiration, free molecular oxygen accepts electrons from an electron donor, usually carbon, and is reduced to a lower oxidation state. An important aspect of these biochemical redox reactions is their irreversibility; therefore, dissolved oxygen is always consumed and never produced as a result of bacterial metabolism. Oxygen, if not present in adequate concentration, 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.

Due to the dissolved oxygen sink in biologically active contaminated aquifer systems, oxygen supplementation is required to maintain aerobic conditions. Several methods, such as air sparging, ozone injection, soil venting and liquid or gaseous oxygen injection, have been developed to increase and maintain the concentration of dissolved oxygen in the ground water.

Hydrogen peroxide injection into the ground water is a popular method of introducing oxygen to targeted contaminated low dissolved oxygen zones. Hydrogen peroxide decomposition reactions, ideally, yield one mole of water and one mole of oxygen thereby introducing pure oxygen into ground water. Hydrogen peroxide is highly soluble and potentially highly mobile, thus offering numerous operational advantages in the field. However, relatively little information concerning its utilization in a biologically active contaminated aquifer system is currently available.

The most important aspect of the decomposition reaction is the liberation of one mole of oxygen. The reaction product, oxygen, is the basis for injecting hydrogen peroxide due to the subsequent replenishment of oxygen in the ground water. The stoichiometry indicates that 47.1% by weight of decomposed hydrogen peroxide will be pure oxygen.

The two main mechanisms for hydrogen peroxide decomposition are enzymatic and non-enzymatic reactions. Enzymatic decomposition reactions are catalyzed by hydroperoxidases (catalases and peroxidases). Many scientific observations have been reported concerning catalysts; however, the greatest attention has been centered on the enzyme, catalase. Catalase, found in most bacteria, is primarily responsible for catalytically decomposing cell synthesized hydrogen peroxide, thus preventing the accumulation of hydrogen peroxide to a toxic level. Catalase is outstandingly effective in this process, being active at low hydrogen peroxide concentrations and at a rate far exceeding that of most other catalysts.

The most notable non-enzymatic decomposition reactions are those in the presence of iron salts and the generally accepted mechanism is a series of complex chemical reactions involving hydroxyl and perhydroxyl radical intermediates and both ferric and ferrous iron. Hydrogen peroxide decomposition was not observed to be significantly affected by pH in one laboratory study. However, the presence of a 0.01M solution of Potassium Phosphate (monobasic) was observed to significantly inhibit hydrogen peroxide decomposition. Phosphate inhibition of hydrogen peroxide decomposition is fortuitous since phosphate is also an important microbial nutrient.

A laboratory study where hydrogen peroxide provided the main source of oxygen for hydrocarbon degrading bacteria, researchers reported that the maximum concentration tolerated by a mixed culture of gasoline degraders was 0.05% hydrogen peroxide. The tolerance was increased to 0.2% by incrementally raising the hydrogen peroxide concentration. Tolerance was determined to occur when the number of colony forming units in the test column were essentially the same for the control column.

Objectives

The objectives of the laboratory study were to confirm that hydrogen peroxide can be used to supply oxygen in the bioremediation process, assess the tolerance of the system to hydrogen peroxide, and estimate the overall oxygen demand based on stoichiometric degradation of hydrocarbon. A field scale in-situ bioremediation pilot study in which hydrogen peroxide and nutrients were injected into contaminated aquifer material provided the opportunity to confirm laboratory observations in the field.

Methodology – Laboratory Stud

Contaminated aquifer material wa collected from the heart of an aviati gasoline plume using a modified hollc stem auger drilling tool. Approximate 476 g of wet, contaminated aquifer mate ial was placed in triplicate columns. A abiotic control column was not used this experiment. The glass columns we approximately 18 cm long (4 cm I.D Soil columns were kept in a consta temperature chamber at 12°C.

A mixture of feed water, nutrients, ar hydrogen peroxide was pumped (perista tic pump) through the columns. Th columns were operated in a continuo upflow mode. Feed solutions were mixe in an in-line mixing coil prior introduction to the columns. The influe nutrient concentrations to the columr was 400.0 mg/L Ammonium Chlorid 200.0 mg/L Potassium Phosphate (mon basic), 200.0 mg/L Sodium Phosphat and 100.0 mg/L Magnesium Sulfat Hydrogen peroxide was introduced 15.0 mg/L. Effluent samples from th column were retrieved in-line using syringe pump. This enabled the retriev of an aqueous sample in a closed syste without losing volatiles and witho aerating/deaerating the column effluen An inverted centrifuge tube was installe in-line to capture and quantify the ga produced from the column.

Available oxygen (Available Oxyge = [DO] + 0.471[H_2O_2]) from both th hydrogen peroxide and the dissolve oxygen (DO) was measured using th Winkler azide modification method (EP Method No. 360.2). Hydrogen peroxid analysis was determined using a perox titanic acid colorimetric procedure. Th columns were pretreated with a pho phate rich nutrient solution (128.0 mg Orthophosphate as P) prior to th introduction of hydrogen peroxide. Th pretreatment step was a precaution take to prevent iron decomposition of hydr gen peroxide.

Results - Laboratory Study

Hydrogen peroxide was initiall introduced at 15.0 mg/L. The oxyge demand exerted on the influent wa calculated as follows:

Oxygen demand

$$= (\text{Influent [DO]} + 0.471[H_2O_2]) - \text{Effluent [DO]}$$

where the effluent DO concentration, a determined by the Winkler metho detects both DO and oxygen fro hydrogen peroxide. Approximately tw weeks was required before significa oxygen consumption was observed in a

three columns. This response was interpreted as characteristic of microbial acclimation to a new chemical or physical environment.

Hydrogen peroxide was increased from 15.0 mg/L to 30.0 mg/L after the oxygen demand exceeded approximately 80% of the available oxygen. This increase corresponded to an additional 7.1 mg/L available DO. Hydrogen peroxide was increased to 100.0 mg/L after the oxygen demand exceeded approximately 80% of the available oxygen. Bubbles were observed in the in-line gas traps indicating that a loss of oxygen from the system was occurring.

During a period of fifteen days following the hydrogen peroxide injection of 100.0 mg/L, effluent DO remained constant (DO avg. = 24.6 mg/L, n = 29, st. dev. = 1.25 mg/L) in all three columns. In-line gas traps were used to capture and quantify the gas produced from the columns. The average rate of gas generation during this period from columns B and C was 1.17 ml/hr. Gas chromatograph analysis of the captured gas indicated that the gas composition was approximately 65%-70% oxygen and 30%-35% nitrogen.

A mass balance was performed on the influent and effluent available oxygen in he system. Mass balance results were converted to moles/hr and are included in Table 1. The oxygen mass balance indicates that roughly 44.0% and 45.0% of the influent oxygen was recovered in the aqueous and gaseous phases respectively for a total recovery of 89%. The unrecovered oxygen was assumed to be consumed both biotically and abiotically. The cumulative total and cumulative adjusted oxygen demand curves were prepared for each column. The cumulative adjusted oxygen demand curve is the difference between the cumulative total oxygen demand and the oxygen lost from the system due to degassing. The total and adjusted oxygen demand curves demonstrated the potential of hydrogen peroxide, at 100.0 mg/L to rapidly decompose resulting in the production of pure oxygen. The slopes of the cumulative adjusted oxygen demand curves during the 100.0 mg/L hydrogen peroxide injection period is less than the slope at lower hydrogen peroxide concentration periods during the study. A decrease in the oxygen consumption rate indicates that inhibition of bacterial respiration during this period may have occurred.

During the later stages of the study, degassing was noted to occur at hydrogen peroxide concentrations that

Table 1. Column Influent and Effluent Flux of Available Oxygen(1,2) in Both Aqueous and Gaseous Phase

	Columns		
	A	B	C
Influent			
aqueous	7.78E-5	7.78E-5	7.78E-5
Effluent			
aqueous	3.41E-5	3.4E-5	3.42E-5
	(43.8%)	(43.7%)	(44.0%)
gaseous	3.5E-5(3)	3.71E-5	3.29E-5
	(45.0%)	(47.7%)	(42.3%)

(1) Flux rate, moles oxygen/hr.
(2) Values in parentheses indicate percent effluent of total influent flux.
(3) Average of columns B and C.

previously did not result in degassing. It appears that this may have been due primarily to enzymatic decomposition associated with the additional biomass in the system and the short retention time of the bubbles in the column. Influent hydrogen peroxide concentration during the remainder of the experiment was incrementally adjusted as the oxygen demand changed with time.

The average cumulative total oxygen demand from the columns was 1940 + 127 mg oxygen and the average cumulative total oxygen demand, adjusted for the oxygen degassing was 1360 + 67 mg oxygen. During the various operating scenarios (i.e. varying influent hydrogen peroxide concentration) of this study, degassing accounted for approximately 30% of the total oxygen demand. The slope of the cumulative adjusted oxygen demand curve represents the overall rate of oxygen consumption during the study period.

Gas chromatograph analysis of the aquifer material and the column effluent was performed to maintain a mass balance of hydrocarbons in the system. The amount of fuel carbon (FC) degraded was estimated based on the mass of aquifer material in each column, the initial and final average FC concentrations, and an estimate of the effluent FC. Based on the average of all three columns, 36% of the initial mass of fuel carbon leached from the aquifer material, 10% remained on the aquifer material, and 54% degraded.

The empirical carbon and hydrogen content of the aviation gasoline was determined to be approximately 2.16 parts hydrogen per part carbon, or roughly 85% carbon and 15% hydrogen. The ideal stoichiometric biological conversion of hydrocarbon to carbon dioxide and water is approximately 3.48 parts oxygen per part hydrocarbon. The ratio of the estimated oxygen consumed to estimated aviation gasoline degraded in this study was greater than the stoichiometric conversion ratio (Table 2). Measured conversion ratios greater than the stoichiometric prediction was likely to occur due to errors in the mass balance analysis and the abiotic oxygen demand. It was not possible to differentiate between biotic and abiotic oxygen demand in this study.

Table 2. Conversion Ratios (O_2(mg)/ Aviation Gasoline (mg))

	Columns		
	A	B	C
Mass O_2 consumed(1) (mg)	1322.0	1307.0	1434.0
Mass Aviation gasoline(2) degraded (mg)	253.0	295.0	274.0
Conversion Ratio mg O_2/mg Av. gasoline	5.23	4.43	5.23

(1) Adjusted Cumulative Oxygen Demand
(2) Mass Aviation gasoline degraded = Mass FC Degraded/(0.85)

Colorimetric hydrogen peroxide analysis of the column effluent was performed to determine the persistence of hydrogen peroxide in the contaminated aquifer material. Prior to terminating the experiment, hydrogen peroxide was injected at 50.0, 100.0, and 200.0 mg/L. These concentrations were injected for 7, 10, and 13 hours, respectively, prior to collecting effluent samples. Breakthrough of hydrogen peroxide, for all three concentrations was less than 11% (Table 3).

Background - Field Study

Twenty years ago, aviation gasoline (25,000 gal.) spilled into a shallow, sandy, water table aquifer at the U.S. Coast Guard Station in Traverse City, Michigan. The spill site was cored extensively to determine the distribution of contamination in the subsurface. The majority of the contamination was found to be distributed within a narrow interval between 15 and 17 feet below the land surface (Table 4) corresponding to the seasonal low and high water table at the site.

Table 3. *Hydrogen Peroxide Breakthrough*

	Columns								
	A	B	C	A	B	C	A	B	C
$[H_2O_2]i$(mg/L)		50.0			100.0			200.0	
$[H_2O_2]e$(mg/L)	ND	ND	ND	9.6	10.4	2.5	16.5	17.0	9.3
H_2O_2 Breakthrough (%)	< 10	< 10	< 10	9.6	10.4	2.5	8.3	8.5	4.7

$[H_2O_2]i$, $[H_2O_2]e$ influent and effluent hydrogen peroxide concentration, respectively

Detection limits: $[H_2O_2]_{50.0}$ = 5.0 mg/L, $[H_2O_2]_{100.0,200.0}$ = 2.5 mg/L

Table 4. *Vertical Distribution of Contamination 50 feet Downgradient from the Injection Wells*

Depth Interval (feet below surface)	Fuel Hydrocarbons (mg/kg aquifer)
15.1-15.5	< 11
15.5-15.8	39
15.8-16.2	2370
16.2-16.5	8400
16.5-17.2	624
17 2-17.5	< 13
18.0-18.3	< 13

In 1988, the U.S. Coast Guard and the U.S. EPA began the operation of a pilot scale in-situ bioremediation project in the area of the original spill. A series of five deep wells were used to inject clean water beneath the plume area in an effort to raise the water table and subsequently saturate the contaminated "smear zone". Raising the water table was performed in order to allow the delivery of soluble nutrients to the targeted zones of contamination. Five chemical feed wells were used to inject nutrients and hydrogen peroxide in the shallow, contaminated layer. A series of downgradient monitoring wells and subsurface sampling points were installed to monitor the performance of bioremediation.

Nutrient and oxygen injection began in March 1988. A liquid oxygen source was used to inject approximately 40 mg/L dissolved oxygen. Approximately 3 months later (June 1988), hydrogen peroxide was injected. Prior to hydrogen peroxide injection, phosphate breakthrough had occurred in the nested monitoring wells. This was necessary to complex the iron found in the aquifer material with phosphates and therefore, minimize the iron catalyzed hydrogen peroxide decomposition reaction. Due to problems associated with the chemical feed system it was difficult to compare the concentration of available oxygen in the injected water with parameters measured in the monitoring wells.

Both soil gas and ground water analyses were used to investigate the fate of hydrogen peroxide in the in-situ bioremediation field scale pilot study.

Methodology - Field Study

Soil gas was sampled in the vicinity of the injection area and analyzed for the oxygen concentration. Soil gas samples were obtained using a series of stainless steel tubes (3/8 in. I.D.) that could be coupled together and driven into the subsurface to various depths. Soil gas sampling mainly occurred in the 0-10 ft range. Soil gas was pumped into a sample vessel which contained an oxygen detector using a hand held positive displacement pump. The oxygen detector is a GasTech Model LO2 OxyTechTor galvanic cell which measures the concentration of oxygen from 0-100% + 5% of the reading.

Results - Field Study

During the field investigation (8/89), hydrogen peroxide was injected (11 gpm) into ground water at 750 mg/L. The water table in the injection area was approximately 13.5-14.5 feet below grade. This data was used to map the horizontal distribution of oxygen as a function of depth (3, 6, 9-10 ft.). Oxygen was found in excess of 45% at the 9-10 ft. interval. The concentration of oxygen in the unsaturated zone was clearly greater than both atmospheric and background soil oxygen concentrations, 20.9% and 20.7% respectively. This indicated that a significant amount of oxygen was lost from the system and was not available for bioremediation of hydrocarbon in the saturated zone. This data also indicated that the oxyge concentration in the injection are increased with depth. Unlike the injectio area, the oxygen concentration in area further downgradient decreased wit depth.

The concentration of available oxyge in ground water downgradient from th injection zone clearly indicated that oxy gen was being delivered to the system. was difficult to predict the rate at whic hydrogen peroxide decomposed or th fraction of injected oxygen that wa liberated from the saturated zone. Thi was largely due to the variability of th influent hydrogen peroxide concentratio as a function of time.

Conclusions - Laboratory Study

Hydrogen peroxide was shown t rapidly decompose and produce pur gaseous oxygen. Due to precaution taken to minimize nonenzymatic decom position in this study, the data indicat that hydrogen peroxide decomposition t oxygen and water was mainly the resu of enzymatic catalysts. Oxygen provide by the hydrogen peroxide decompositio reaction was consumed in the columns. was not possible to distinguish betwee abiotic and biotic oxygen demand in thi study.

The injection of hydrogen peroxide a 100.0 mg/L had two observed notabl effects. The rapid rate of decompositio resulted in a high rate of oxygen ga production. The rate of oxygen ga production far exceeded the demanc and although the solubility of dissolve oxygen had not been exceeded, ga bubbles appeared to have insufficier time to diffuse from the gaseous phas into the aqueous phase. Approximatel 45% of the available oxygen injected int the columns was transferred into th gaseous phase. Secondly, the rate c oxygen consumption decreased indica

ng that bacterial inhibition may have occurred.

Mass balances of both oxygen and hydrocarbon was calculated to quantify the mass of oxygen consumed and the mass of hydrocarbon degraded. The ratio of estimated oxygen consumed to aviation gasoline degraded was found to be greater than the stoichiometric prediction.

Hydrogen peroxide, introduced into the biologically active columns at 50.0, 100.0, and 200.0 mg/L, never exceeded 11% breakthrough although the columns were only 18 cm in length.

Conclusions – Field Study

Injecting hydrogen peroxide into the aquifer at the pilot study area resulted in increasing the concentration of available oxygen in downgradient wells, rapid decomposition of hydrogen peroxide, and the liberation of oxygen gas into the unsaturated zone resulting in a concentration much greater than background. The rate of hydrogen peroxide decomposition at the site was unknown but was expected to be rapid due to the concentration of oxygen gas measured in the pilot study area.

Discussion

Results of the field investigation support observations from the laboratory study: hydrogen peroxide decomposed resulting in the liberation of oxygen at a rate faster than the oxygen could be utilized biologically and solubilized into aqueous phase. Subsequently, oxygen gas was liberated from the ground water into the unsaturated zone. The oxygen gas which was liberated into the unsaturated zone could be considered an oxygen sink from the biodegradation process in the saturated zone. This sink introduces a considerable element of uncertainty in estimating how much oxygen is actually delivered to the system and utilized in the biodegradation process. Consequently, predicting the amount of hydrocarbon degraded based on the amount of hydrogen peroxide delivered to the system is inaccurate. Additionally, the data from this study clearly dispels the concept that hydrogen peroxide will only decompose as a function of the biological oxygen demand.

Two areas addressed in the laboratory study that were not investigated in the field study are microbial inhibition due to hydrogen peroxide and the stoichiometry of the degradation of hydrocarbon based on the amount of oxygen consumed. An accurate mass balance of oxygen and hydrocarbon is critical to make an assessment of both processes. Neither an oxygen nor a hydrocarbon mass balance was accurately achieved at field scale. Therefore these two areas could not be investigated. An important note to make however, is that the conversion ratios found in the column study represent minimum values due to the oxygen and hydrocarbon mass balance of a carefully controlled system. In a field scale system, it is speculated that the amount of oxygen required will be significantly greater due to short circuiting of the oxygen with respect to the contaminant plume.

The decision to use hydrogen peroxide as a supplemental source of electron acceptor in bioremediation is an issue of economics and safety. Prior to selection of hydrogen peroxide as a source of oxygen for bioremediation systems, alternative sources should be considered. These alternatives include liquid oxygen and gaseous oxygen either shipped in or produced on-site, i.e. oxygen generation via molecular sieve. Additionally, when estimating the costs associated with using hydrogen peroxide, consideration should be given to the fraction of oxygen that is lost from the system.

Recommendations for Research

This study has focused on investigating the feasibility of utilizing hydrogen peroxide as a supplemental source of oxygen for the enhanced bioremediation of contaminated (aviation gasoline) aquifer material. Clearly, injection of hydrogen peroxide into the ground water will increase the concentration of dissolved oxygen which can be used as an electron acceptor in the bioremediation process. However, the rapid decomposition of hydrogen peroxide as observed in this study, and the subsequent liberation of the oxygen gas to the unsaturated zone will limit the effectiveness of hydrogen peroxide to supply oxygen to the saturated zone. The following recommendations are suggested to further evaluate the feasibility of utilizing hydrogen peroxide as a source of oxygen.

1 Research designed to differentiate between biotic and abiotic mechanisms of hydrogen peroxide decomposition will offer the opportunity to more completely understand the role of biological and chemical variables in this reaction. The application of this information could then be used to develop methods to control or minimize decomposition reactions. Understanding these processes may also help identify chemical and biological subsurface conditions that are not conducive to this method of oxygen supplementation.

2. The results of the laboratory column study found hydrogen peroxide at 100.0 mg/L to decrease the oxygen utilization rate. A decline in the oxygen utilization rate indicated that bacterial inhibition may have occurred. While other research has found the hydrogen peroxide microbial toxicity threshold to be much higher, these are usually based on bacterial enumeration. The oxygen utilization rate is a parameter which offers feedback on the performance of the biodegradation process. The concentration of hydrogen peroxide identified in this research which indicated bacterial inhibition had occurred has paramount importance since it is process oriented and is relatively low. Additional research is necessary both to verify this observation and to more closely examine the toxicity effect from a process perspective.

3. The feasibility of using hydrogen peroxide as a supplemental source of oxygen at a bioremediation site must consider also the economics and safety of this method compared to other candidate methods. Included in this evaluation are: the cost of the hydrogen peroxide; operation and maintenance costs of the equipment to store, mix, and deliver the hydrogen peroxide; the fraction of oxygen actually delivered to the saturated zone; toxicity; and safety. A comprehensive feasibility evaluation is necessary to identify the technical and economic benefits of using various techniques of oxygen supplementation.

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The complete report, entitled "Enhanced Bioremediation Utilizing Hydrogen Peroxide as a Supplemental Source of Oxygen: A Laboratory and Field Study," (Order No. PB 90-183 435/AS; Cost: $17.00, subject to change) will be available only from:

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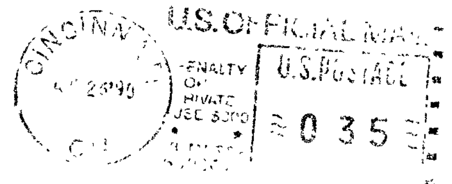

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