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BACTERIAL DEGRADATION OF ALIPHATIC HYDROCARBONS ENHANCED BY PULSED OZONE INJECTION

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ABSTRACT: Exceptional high rates of removal of aliphatic hydrocarbons found during on-site remediation of diesel and gasoline spills pulsed with microbubble air/ozone injection are coincidental with the presence of ozonophilic alkane-degrading bacteria. Growth of the bacteria (measured as hexadecane degraders) are stimulated by the addition of ozone in isolated groundwater/soil slurries removed from the regions of highest mass reduction of total petroleum hydrocarbons. Increases in the rate of degradation of TPH as pulsed ozone remediation continues reflects the change in composition of the microbial population to favor ozone-resistant, oxygen-utilizing bacterial species which thrive on the carbon fragments produced by ozone partial decomposition of the EPH fractions. Also, the low occurrence of expected carboxylic byproducts of ozone decomposition, normally acetates and formates, appear to be due to rapid bacterial decomposition to carbon dioxide.

INTRODUCTION

Special laminated Spargepoints® allow hydroperoxide to coat nano- to micro-sized gas bubbles containing air/ozone mixtures. Bench-scale tests showed the use of thin-layer microbubbles containing ozone with a coating of hydroperoxide improves the rate of oxidation of certain polyaromatic hydrocarbons (PAHs) commonly found in weathered fuel, when compared to ozone or hydroperoxides used separately or in combination without ultra-fine bubble formation. During injection of the gas fraction through the laminate membrane, the peroxide is siphoned by negative pressure to form coatings on the ultra fine bubbles. The coating creates a high surface area film of reactive hydroxyl radicals through which aliphatic and aromatic VOC compounds are drawn by Henry's Law of liquid-gas partitioning. A bubble continually releases ozone outwards creating a hydroxyl radical coating through which alkanes invade. The emphasis here is on bacterial changes associated with the chemical changes of the remediation process.

The remedial area was first treated with ozone/air microbubbles coated with hydroperoxide for specific bond cleavage or increase in oxidative potential. Diesel fuel contains primarily alkanes and alkenes (80%), simple aromatic ring compounds (BTEX), polyaromatic compounds (e.g., naphthalenes, benzoanthracenes, pyrenes), and occasionally ethers and additives. Ozone reacts in an aqueous and gaseous form to degrade aromatic ring compounds (BTEX) and certain ethers (MtBE) (Karpel vel Leitner 1994). It also breaks apart long-chain aliphatic compounds and polyaromatic hydrocarbons (PAHs) (Kerfoot, 2002; Hsu and Masten, 1997). (See detailed accounts: Kerfoot, 2002; Kerfoot, 2004.)

The capacity to produce simpler alkane chains and simple carboxylic acids within a soil saturated medium containing elevated oxygen ideal for aerobic biodegradation has several ramifications. For difficult organic compounds, the capacity to combine limited

sterilization and pre-digestion to promote biodegradation with optimal species presents an intriguing challenge. The use of partial oxidation through an Advanced Oxidation Process (AOP) to initiate breakdown of difficult complex corganics as a pre-step to biodegradation has been explored previously (Miller, 1988; Carberry, 1994). An AOP/biological oxidative strategy would be particularly advantageous if the initial oxidation steps of the sequential toxic chemical biological mineralization process is initially step-rate limited, and could be carried out chemically rather than biologically (Carberry, 1994). Previously the use of the aqueous hydroxyl radical has been explored by stoichiometric means (Bishop, 1968; Carberry and Benzing, 1991). The development of ozone reactions with microencapsulated gas bubbles with the addition of thin-film aqueous ozone/hydroxyl radicals offers a set of powerful and efficient oxidants which may also be employed as pre-digesters to compounds normally resistive to biodegradation. Carberry (1994) demonstrated that a PCP aqueous system, partially oxidized by Fenton's Reagent, increased maximum substrate uptake rate to a level four times greater than that of a non-pretreated system. The time to maximum growth rate was also enhanced from two to four days in a non-pretreated control to one to two days using preoxidation.

Site Description. A home fuel oil release at the site of a seashore inn on Cape Cod was discovered during an MCP 21E investigation. Laboratory analysis indicated exceedance of Commonwealth of Massachusetts groundwater standards for aliphatic hydrocarbons. The Commonwealth requires the breakdown of fuel components into compounds, both alkanes and alkenes, for the purpose of evaluating health risk and acceptable cleanup standards. The contamination extended from below a 275-gallon above-ground storage tank (AST), located in a basement near the furnace, outwards in a southwesterly direction across a patio and backyard of the inn. The source proved to be a copper feed line imbedded in concrete with microscopic holes that released fuel when under head pressure.

The site lies in the Mashpee pitted plain deposit, a sand and gravel aquifer extending about 150 feet (50m) below grade to a granidiorite bedrock (LeBlanc, 1980; Oldale and Barlow, 1986). Local hydrogeological characteristics beneath the property include: 1) interbedded mixed sandy soil from 1 foot below grade to 20 feet below grade; 2) local static water from roughly 7 to 9 feet below grade; 3) static water beneath the cellar floor from 1 to 2 feet; 4) southerly trend in shallow groundwater flow direction with a hydraulic gradient of 0.001 ft/ft and hydraulic conductivity (k) of 100 ft/day; 5) seasonal groundwater fluctuation of 1 ft elevation.

MATERIALS AND METHODS

A chemical oxidative remediation system to treat impacted soil and groundwater was installed on the site, and operation commenced on February 16, 2002. The system perfused air and ozone into the groundwater by means of Spargepoints® strategically located to treat the entire impacted area (Figure 1). During operation, negative pressure was applied to the basement to prevent invasion of fugitive vapors into the buildings. During a period of May 2 to June 7 2002, air/ozone gas and liquid hydrogen peroxide were added in areas of highest soil and groundwater contamination, MW7 and MW3. Approximately 60 gallons of a roughly 5% solution of hydrogen peroxide was injected to serve as a catalyst, with the expectation of enhancement of the chemical oxidation process of the

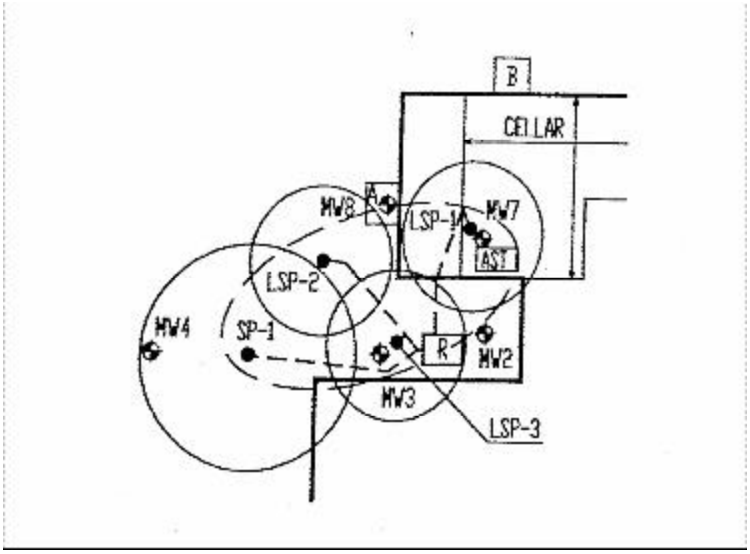

FIGURE 1. Site diagram showing the spill plume, monitoring wells (MW), spargewells (LSP and SP) and above-ground oil storage tank (AST) in basement.

petroleum-impacted soil and groundwater. Sampling for soil and groundwater extractable petroleum hydrocarbons, groundwater dissolved oxygen (DO), oxidation-reduction potential (ORP), and temperature was performed periodically to enable assessment of system performance. Since the treatment method utilizes chemical decomposition as the primary means of reducing contaminant levels, no remediation waste was generated.

System Operation. The system was operated during daytime only, in consideration of occupants of the inn. The gas/liquid introduction used a flow of 4 cfm, consuming about 5 gallons/day of peroxide in a 5% to 8% solution. Dosing was intermittent at one week per month. Daily deliverable oxygen can be broken down as follows, using the 5% to 8% solution:

Oxygen in air:	50 kg/day
Oxygen in ozone:	.3 kg/day
Oxygen in peroxide:	1.2 kg/day

The ozone gas concentration was measured from the head space of bubbles in solution by means of miniature sampling points placed at 10-foot intervals from injection points. Ozone depleted rapidly from the point of injection, decreasing from 250 ppmv to 6 ppmv ozone within 20 feet of injection.

By comparing DO and ORP results, the effective radius of influence was determined to be about 30 feet, maintaining a mean concentration of DO above 1.0 mg/L. At the center of the plume (MW3), the concentration of dissolved oxygen rose from 1.4 to about 6.4 mg/L during treatment. Redox potential rose from 45 to about 200 mv. The treated soil temperature rose from 9°C (48.2°F) to between 13° and 19.5°C across the site, about a 5° to 6°C rise (9° to 11°F).

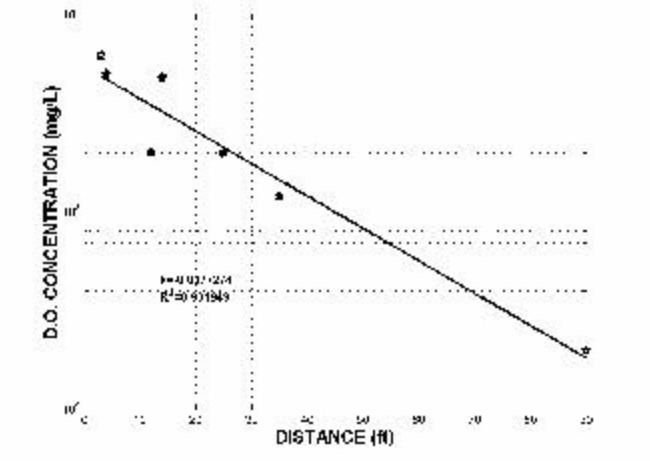

FIGURE 2. Dissolved oxygen (DO) content as distance from Spargepoint® locations.

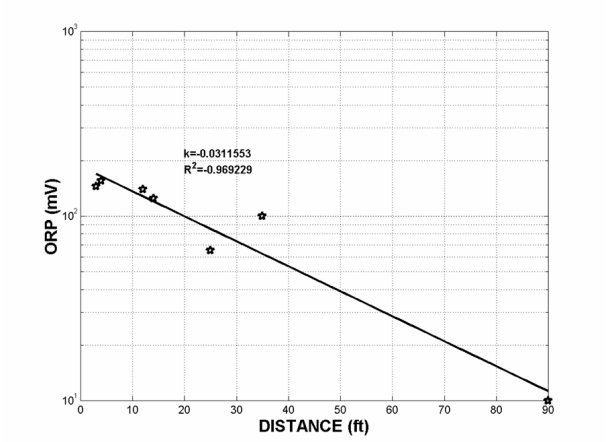

FIGURE 3. Oxidation/reduction potential (in millivolts) plotted as distance from Spargepoints®.

RESULTS AND DISCUSSION

The common belief is that ozone is a bactericide. The results exhibited the opposite effect. Ozone and air were supplied through a Spargepoint® at a concentration and mass that stimulate rapid growth of petroleum-degrading bacteria without killing them, except near (within 1 meter) of the point of introduction. The destruction near the Spargepoint® avoids surficial bacterial plugging of the point. Identification of the bacterial population that proliferates in the presence of ozone indicates the following genera and species (Leadbetter, 2002): *Pseudomonas sp*. 1, very small rod, oxidase positive, non-anaerobic; *Pseudomonas sp*. 2, motile, oxidase positive, anaerobic (nitrate respirer).

Bacteria in groundwater were enumerated by the plate count method according to Standard Methods (18th edition) 9215 C, using 1/3 concentration of Nutrient Agar. Hexadecane degraders were plated on Noble Agar and grown in an atmosphere of hexadecane as sole carbon source. Both total and specific degraders were incubated under aerobic conditions.

TABLE 1. Bacterial enumeration in well samples (colony forming units/ml [cfu]). Samples collected 7/23/02; analyzed 7/24/02.

	Total Heterotrophs, cfu/ml	Total Hexadecane Degraders, cfu/ml	Ratio Hexadecane Total Heterotrophs
MW1	18,000	13,000	0.7
MW2	1,000	500	0.5
MW3	100,000	100,000	1.0
MW4	10,000	3,300	0.3
MW5	1,800	800	0.4
MW6	1,200,000	600,000	0.5
MW7	20,000,000	6,000,000	0.3
MW7 (dup)	5,000,000	3,500,000	1.4
Detection limit	400	400	

TABLE 2. Anion analysis, including VFA, by capillary ion electrophoresis (mg/L), EPA Method 6500.

	Det. Limit	Cl	NO_2	NO_3	SO_4	Formate	Acetate	Proprionate	Butyrate
MW7	0.3	16	<0.3	<0.3	42	<0.4	<0.3	<0.3	<0.3
MW3	0.3	15	<0.3	<0.3	8	<0.3	<0.3	<0.3	<0.3

The number of total heterotrophic bacteria in samples MW1, MW2, MW4, and MW5 is very low compared to typical groundwater. The number of bacteria in MW3 falls in the low to low-normal range. The MW6 and MW7 samples contain unusually high numbers of bacteria, indicating high rate of degradation. High numbers are not surprising, since it is known that soil bacteria contain inducible peroxidase enzyme to destroy H_2O_2. The nearly 1:1 ratio between heterotrophic and hexadecane degrader bacteria in most wells indicates that bacteria are acclimated to fuel containing normal alkanes such as hexadecane. The lack of volatile fatty acids (VFA) is not surprising, since high numbers of aerobic bacteria would rapidly consume VFA products of petroleum biodegradation.

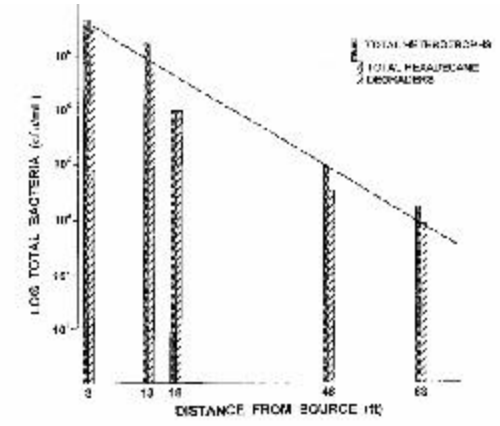

FIGURE 4. Bacterial content of groundwater well samples plotted with distance from source area.

Bench Scale Test Procedure. To offer conclusive proof that the bacteria were ozonophilic, bench-scale tests were conducted on groundwater collected from monitoring well MW7. The groundwater was expected to contain dissolved petroleum components from the fuel release. The tests were run on August 7, 2002 over a period of 6 hours. The tests included treating five (5) groundwater subsamples from MW7 with a combination of micro-sparged gases and hydrogen peroxide. All test cell contents were stirred using a Teflon®-coated stir-bar set to 5.0. The tests were run according to the parameters in Table 3.

TABLE 3. Batch test parameters.

Name	Gas Flow Rate (1 L/m)	Ozone Conc. (ppmv)[2]	Peroxide Flow Rate (mL/Min)[3]	End of Test Groundwater Sample Name
Background[1]	NA	NA	NA	B1
Test 1	0.8	Na	Na	A
Test 2	0.8	100	NA	AO100
Test 3	0.8	300	NA	AO300
Test 4	0.8	300	3.5	AOP300
Test 5	0.8	10	NA	AO10

Notes: (1) Subsample of collected groundwater from MW7
(2) Measured using "Kitagawa" Type SB ozone detector tubes
(3) 9% solution of hydrogen peroxide

Each test was conducted in a 1.5 liter glass reaction cell. Approximately 1,300 mL of contaminated groundwater was poured into the reaction cell and subjected to 30 minutes of treatment that included the above treatment parameters. Following the conclusion of each test, groundwater samples were collected from the test cell and refrigerated until they were submitted within 24 hours of collection to two laboratories for different types of analyses (Table 4).

TABLE 4. Bacterial enumeration in Shorehaven well MW-7 groundwater additional test

Samples collected 8/7, Samples analyzed 8/7 colony forming units/ml(cfu)			
	Total Heterotrophes cfu/ml	**Total Hexadecane degraders,cfu/ml**	**Ratio Hexadecane to Total Heterotrophs**
A(Air)	200,000	100,000	2
A010 (Air/ozone 10ppmv)	1,300,000	1,000,000	1.3
A0100 (Air/ozone 100ppmv)	480,000	500,000	1
A0300 (Air/ozone 300ppmv)	<400	<400	
A0P300 (Air/ozone/peroxide 300ppmv)	<400	<400	
Background (MW7) B1	100-1000	<500	
B1 dup	<400	<400	
Blank	<400	<400	
detection limit	400	400	

Total Heterotrophic and Specific Degrader Bacteria. Bacteria in ground water were enumerated by the plate count method according to Standard Methods (18th Edition) 9215C, using 1/3 concentration of Nutrient Agar. Hexadecane degraders were plated on Noble Agar and grown in an atmosphere of Hexadecane as sole carbon source. Both total and specific degraders were incubated under aerobic conditions.

TABLE 5. Anions by capillary ion electrophoresis mg/L

	det limit	C1	NO_2	NO_3	SO_4	Formate	Acetate	Propionate	Butyrate
A	0.3	16	0.9	4	48	0.1	0	0	0
B1	0.3	16	0	0	48	0.1	0	0	0
A010	0.3	16	1.2	4	48	0.1	0	0	0
A0100	0.3	16	0.9	4.5	48	0.1	0	0	0
A0300	0.3	16	0	6	48	1	0	0	0
A0P300	0.3	15	1	5	44	3	3	0	0

When elevated levels of ozone (300 ppmv) and ozone and peroxide (5%) were used, formate and acetate were detected.

The groundwater for the bench-scale test was removed from MW7 48 hours after completion of treatment. The background number of hexadecane degraders was low (<500), before addition of ozone. When micro-sparging for only 30 minutes with ozone (10 to 100 ppmv), the bacterial population increased to 500,000 to 1,000,000 cfu. Concentrations of 300 ppmv appeared lethal to the bacteria, but 10 ppmv was exceptionally stimulating. Considering normal attenuation with distance from injection, pulsing with up to 300 ppmv would create a radius from 5 ft to 30 ft of ozone within the .5 to 100 ppmv range – ideal for encouraging bacterial breakdown at the same time that ozone would also be fragmenting the aliphatic chains.

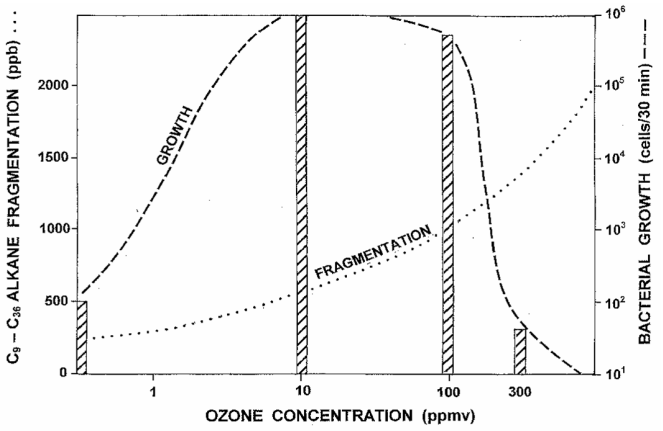

FIGURE 5. The observed growth of total hexadecane degrading bacteria exposed to varying ozone concentrations compared to rate of fragmentation of alkane fractions

Simultaneous analysis of aliphatic C9-C36 hydrocarbons showed an increase in small carbon chain (C9-C16), while substantial reduction of the longer chain aliphatics (C-18-C36) occurred with ozone and ozone/peroxide addition. The bacterial populations were utilizing the fragments as carbon sources during their spectacular growth under the 10 and 100 ppmv ozone concentrations. Reduction of methyl naphthalene, acenaphthalene, anthracene, benzo(a) anthene, benzo (k) fluorathene, and benzo (a) perylene was also occurring in the aromatic fraction.

CONCLUSIONS

The results of the site remediation, post treatment well and soil samples, as well as a bench-scale test on water sample removed from MW7 showed that application of peroxide-coated ozone bubbles to spilled fuel oil results in rapid carbon removal. The rapid rate appears related to carbon chain fragmentation, insertion of oxygen on alkane chains, and enhanced oxygen supply. Ozonophilic bacteria, specifically *Pseudomonas sp.*, were isolated from regions of rapid carbon loss. Contrary to the common perception that ozone is bactericidal, growth of the ozonophilic hexadecane degrading bacteria was rapid when low-level ozone, 1 to 200 ppmv, was supplied. The carbon loss closely matched twice the total oxygen supply.

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