IN-SITU BIOLOGICAL METHODS FOR CLEANUP OF CREOSOTE AND PENTACHLOROPHENOL IN GROUNDWATER

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INTRODUCTION

Contaminated groundwater has been found in all fifty states. Some of this water is contaminated with chlorinated organic pollutants such as pentachlorophenol (PCP) and polycyclic aromatic hydrocarbons (PAHs). The wood treating industry in Mississippi has been in operation for over one hundred years. Groundwater contamination by these compounds has been the result of leaching of these wood preservatives from the soil at waste disposal sites into groundwater reserves.

In-situ bioremediation is the biological treatment of soil and groundwater without removal from the contaminated site. This technology involves the use of microorganisms which can degrade the contaminating compounds in both soil and water. In-situ bioremediation requires an understanding of microbiological processes relative to biodegradation of the target contaminants as well as the soil's physical and chemical effects on these microbial processes. Typically, these systems utilize aerobic metabolism thus involving the addition of oxygen, usually as air, inorganic nitrogen, and phosphorus.

Several methods are available to remove organic pollutants from groundwater (1,2,3,4,5,6,7,8,9). Two of the most common methods are filtration and biological treatment. Both of these methods are effective in certain situations for groundwater cleanup of organic contaminants. Filtration involves the pumping of groundwater through carbon filters to remove contaminating organics. The cost for filtration of groundwater containing wood treating chemicals averages $1.25-$5.25 per 1000 gallons depending on influent concentration and type of carbon used. This method is labor intensive and the spent carbon used for filtration has to be disposed of in ways other than incineration. This only relocates the contaminants. Biological treatment (pump and treat) involves pumping the contaminated water into bioreactors where cleanup is carried out by means of microorganisms at a cost of $1.00 per 1000 gallons. Both bacteria and fungi have been shown to be important in the bioremediation processes.

In-situ bioremediation utilizes techniques for developing large populations of naturally occurring microorganisms that can detoxify parent compounds of interest to products that are no longer hazardous. By using this technology, which is even less labor intensive and more cost effective than pump and treat methods, a better and more permanent cleanup level can be achieved.

The objective of this research is to investigate the development of a rapid in-situ biological technique for the cleanup of organic wood preservatives in groundwater at a wood treating site in southern Mississippi. This involves the use of naturally occurring microorganisms as well as bacteria species previously isolated at our laboratory capable of degrading PCP and PAHs at a rapid rate. Since the bioremediation process is an aerobic process, the use of added oxygen and micronutrients will also be investigated as possible methods to enhance the bioremediation process. Analytical techniques applied to water samples obtained from wells already in place will be used to monitor the treatment effectiveness.

MATERIALS AND METHODS

A suitable site was selected at a South Mississippi location where creosote and PCP are present in both groundwater and subsoil. Three temporary recharge wells were established in upgrade and the same zone as recovery well (WC-5), 60 feet north of WC-5. The three wells were located with 15-foot spacing along a line approximately perpendicular to the direction of groundwater flow. The approximate locations of these wells, pumping wells (WCP-1, WCP-2, WCP-5), and treatment systems are shown in Figure 1. The recharge wells were installed to a depth of approximately 40 feet below land surface (ft bls) and screened from approximately 35-40 ft bls in the same zone screened by recovery well (WC-5). It should be noted that if constituents occur at greater depths, they may not be impacted by this experiment. The construction of the recharge wells were established by drilling a borehole to a depth of 40 ft bls with a rotary bit. The diameter of the borehole was between 6 and 8 inches. Soil samples derived from the drilling were inspected and later were

analyzed to provide information concerning contamination of subsoil. The wells were assembled from 2-inch diameter Schedule 80 PVC casing, a 10-foot long, 2-inch diameter Schedule 80 PVC well screen with 0.020 inch (20-slot) slot width, and a PVC bottom well cap. The wells were placed vertically in the center of the borehole. The casings were extended 1 foot above the ground surface.

A filter pack consisting of 20-40 grade silica sand was poured into the boreholes annuluses to a level at least 3 feet above the top of the screen. Approximately 15 pounds of 1/2-inch diameter bentonite pellets were poured into the boreholes annuluses with water and were allowed to hydrate for one hour. A cement slurry consisting of Portland cement and water were poured into the borehole annuluses until the ground surface was reached. The casings were capped with PVC caps. After allowing the cement slurry to set for 48 hours, the recharge wells were pumped to withdraw fine-grained particulates from well casings and filter pack.

A bacteria culture, previously isolated and known to efficiently degrade PCP and PAHs (Arthrobacter sp.) (10), oxygen (supplied as 0.005% hydrogen peroxide), and nutrients (as 0.040% nitrogen, 0.080% phosphorus, and 0.040% potassium), all of which were suspended in 500 gallons of water, were introduced into each recharge well. Hydrogen peroxide was used as an oxygen source because it can supply a much higher concentration of oxygen than can be achieved by dissolving air or even pure oxygen in water. This is important because the limitation of oxygen that can be supplied by aeration was the most common problem previously encountered in in-situ bioremediation work (9). Although hydrogen peroxide is cytotoxic at high concentrations, studies indicate it can be added to groundwater in concentrations up to 100 mg/L without adverse effects, assuming proper formulation and control (9). This suspension was infused into the subsoil and groundwater, which flows downgrade to the recovery well (WC-5) and pumping wells (WCP-1, WCP-2, WCP-5) Figure 1.

The charging system required for each well is by gravity feed and does not require pressurization. Each recharge well has been charged bimonthly since September 1993 and monthly since July 1994. Water samples from the recovery well are taken quarterly.

Special care was exercised to prevent contamination of the groundwater during sampling. Water contained within and adjacent to the well casing can potentially reflect chemical interaction with the atmosphere (by diffusion of gases down the casing) or the well construction materials (through prolonged residence adjacent to the casing). This water was removed from the well prior to sample collection. A bailer was used to extract water samples from the well. Much care was taken during insertion of sampling equipment to prevent undue disturbance of water in the well. The bailer was lowered into the water gently to prevent splashing and then extracted gently to prevent creation of an excessive vacuum in the well. The sample was poured directly into a one-liter teflon capped amber bottle. While pouring water from the bailer, the water was carefully poured down the inside of the sample bottle to prevent significant aeration of the sample. The sample was kept at 4°C and shipped within 24 hours of sampling. These samples have been analyzed for selected creosote components, chlorinated phenols (Table 1) and micro-organism populations.

EPA Method 3520 is used for extraction of groundwater (11). PAHs and PCP are analyzed using EPA Methods 8100 and 8140, respectively (11). The dilution plate method is being used to count bacterial colonies. The media used are nutrient agar, nutrient agar amended with 5 mg/L of technical grade pentachlorophenol (Vulcan Chemical Company, Wichita, Kansas) and nutrient agar amended with 20mg/L of whole creosote. The nutrient agar is autoclaved for 20 minutes at 15 psi and 121°C and then cooled to 55°C. Both creosote and PCP dissolved thoroughly in methyl alcohol is added to cooled nutrient agar. The number of colonies recovered on nutrient agar, nutrient agar amended with PCP, and nutrient agar amended with creosote represent the approximate number of colonies for total bacteria, PCP acclimated bacteria, and creosote acclimated bacteria, respectively.

RESULTS AND DISCUSSIONS

Concentration levels of total selected PAHs, tetrachlorophenol, and PCP for each sampling date are given in Table 2. A significant biodegradation of total selected PAHs has been occurring since this study began in September 1993. PAHs concentration has been reduced from 31.84 to 4.89 mg/L in 18 months. With the exception of samples collected in June 1994, this reduction has been gradual and consistent. Tetrachlorophenol degradation was very slow at first, but no detectable amount of this compound has been found since the end of the 1994 period. PCP degradation has been inconsistent, but overall PCP concentration has been reduced from 0.607 mg/L to around 0.400 mg/L during the last 18 months.

The bacterial population determined on selected media are given in Table 3. In comparison with the initial results, bacterial populations have shown a significant but inconsistent increase. More consistent increases began after June 1994 when recharge well injections were changed from a bimonthly to a monthly basis.

This is a three year project which began in September 1993. Current results are very promising if the current pattern continues. We believe that this technique will result in enhanced biodegradation of creosote as well as chlorinated phenols in both subsoil and groundwater.

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Table 1. List of selected polycyclic aromatic hydrocarbons (PAHs) and chlorinated phenols monitored in this study.

Benzo(b) fluoranthene
Benzo(k) fluoranthene
Carbazole
Chrysene
Cresols
Fluoranthene
Naphthalene
Phenanthrene
Pentachlorophenol (PCP)
2,3,4,6-Tetrachlorophenol (TeCP)

Table 2. Concentration of total selected PAHs and chlorinated phenols from the WC-5 recovery well taken since September 1993.

Date Sample Taken	Total Selected (PAHs mg/L)	TeCP (mg/L)	PCP (mg/L)
4-28-93 (before treatment)	31.840	.081	.607
9-13-93 (one month after first injection)	10.460	.047	.159
3-07-94	10.850	.052	.401
6-06-94	18.266	.089	.596
9-01-94	11.202	.065	.369
12-08-94	8.37	ND*	.275
1-26-95	4.89	ND	.426
Detection limit	---	0.020	.020

* ND = Non-detect

Table 3. Bacterial populations from the WC-5 recovery well isolated on selected media since September 1993.

Date Sample Taken	Nutrient Agar (Colony/mL)	Creosote Amended Nutrient Agar (Colony/mL)	PCP Amended Nutrient Agar (Colony/mL)
4-28-93	1500	900	100
9-13-93	800	700	900
2-01-94	115,800	114,400	136,800
3-07-94	16,000	16,000	13,000
6-06-94	5,000	4,000	4,000
10-04-94	78,700	78,200	67,900
12-15-94	107,000	90,000	72,000
1-26-95	238,000	273,000	202,000

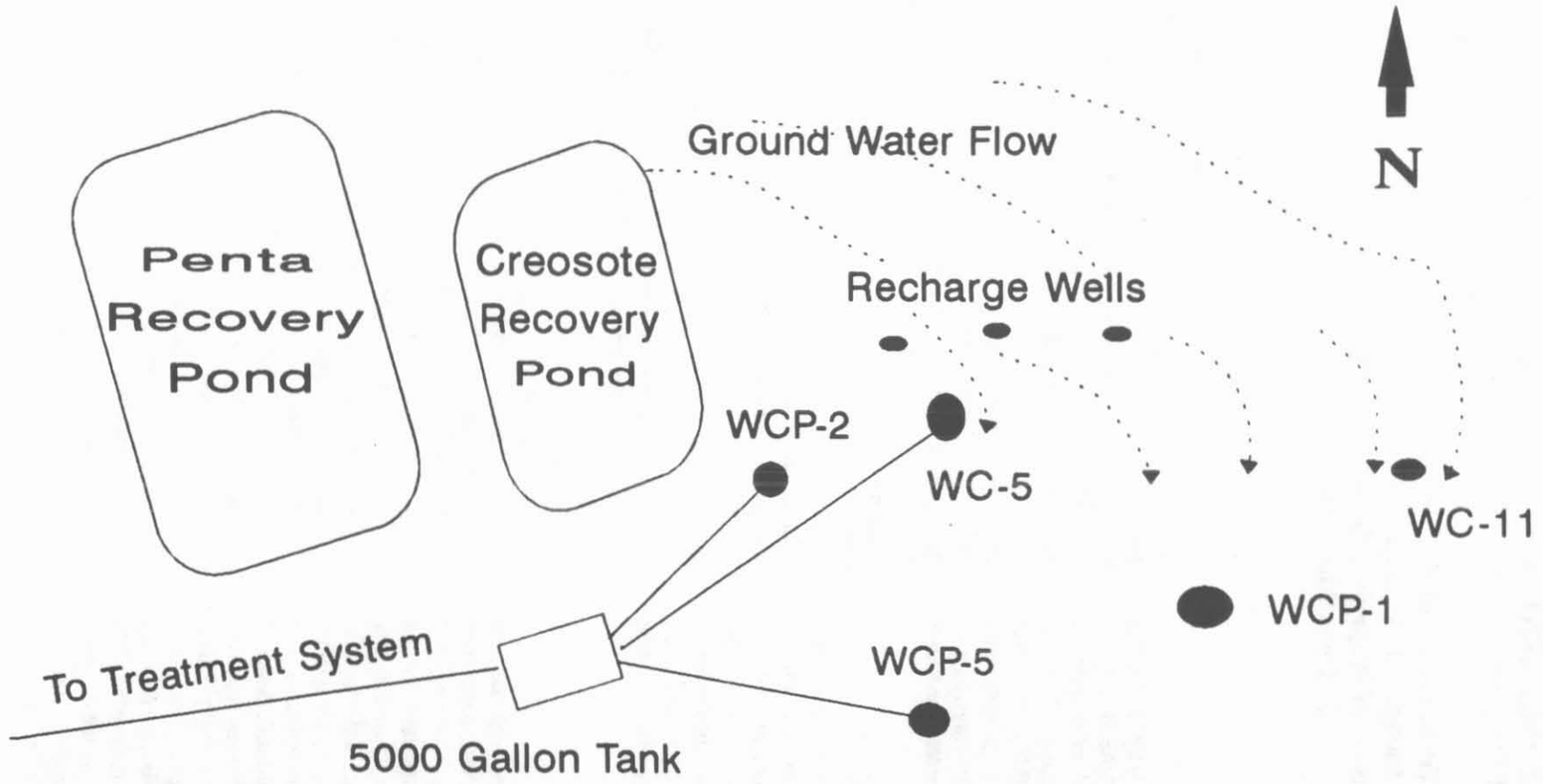

Figure 1. Recharge and Recovery Well Location at Wiggins.