BIOREMEDIATION OF LOW LEVEL PCP AND PAHS CONTAMINATED WATER USING BIOFILTRATION

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INTRODUCTION

Polluted groundwater and soil have increasingly become the focus of clean-up efforts in the past several years. In the past, many wood treatment plants have generated large volumes of process water and soil contaminated with pentachlorophenol (PCP) and creosote. Micklewright (1990) reported that wood products such as crossties, utility poles, marine piles, and structural lumber were treated in the United States during 1990 with 76 million gallons of creosote and 27 million pounds of PCP. Wood preserving process water can contain very high concentrations of PCP (up to 5000 ppm) and creosote compounds (200-300 ppm) that must be recovered or treated before disposal. Creosote contains over 200 polycyclic aromatic hydrocarbons (PAHs), of which sixteen are listed by the EPA as being priority pollutants. PCP is toxic at low concentrations to both plants and animals, and its effects are cumulative. PCP is also listed as an EPA environmental priority pollutant. Current methods for wastewater and soils detoxification include incineration, physical and chemical treatments, and biological treatments (Brown 1989).

Two of the most common decontamination methods for process wastewater are carbon adsorption and biological treatment. Carbon adsorption involves the pumping of wastewater through activated carbon filters to remove contaminating organic compounds. This method, although efficient in contaminant removal, is labor intensive, often cost prohibitive, and the spent carbon must be disposed of in ways other than incineration because of the potential for dioxin production. Biological treatment involves processing contaminated water in bioreactors where clean-up is carried out by microorganisms.

Microorganisms, including bacteria, fungi, cyanobacteria and eukaryotic algae, play an important role in the natural degradation of PCP and PAHs. Biological detoxification has the ability of a microorganism to produce enzymes that metabolize a specific organic compound, usually to carbon dioxide and water (mineralization). Bioremediation has been a recommended clean-up treatment process at over 20 abandoned wood treatment sites (Dasappa and Loehr 1991).

Many commercial aerobic bioreactors are now successfully treating process water containing PCP and PAHs. Bioremediation is the method of choice primarily because it is often less expensive to initiate and operate than other methods and is more easily controlled; however, the rate of degradation is slow, especially for low concentration levels. To maintain a continuous and efficient bioconversion reaction, air, nutrients and a renewed supply of microorganisms have to be added to the bioreactors periodically. One possible way to overcome this problem and possibly enhance the bioconversion rate, even under anaerobic conditions, is to have a higher concentration of degradation enzymes present and to provide more intimate contact between the enzymes and substrate. This can be accomplished by immobilizing the enzyme producing microorganisms onto an insoluble support through which the contaminated water is passed.

Immobilization of microorganisms that degrade PCP and PAHs onto an inert matrix provides several advantages over free cell bioreactors. Immobilization allows for a higher cell density compared to a free cell bioreactor and offers the potential for much higher biodegradation rates. It may also provide greater thermostability for the enzymes involved in biodegradation and may allow a higher tolerance of the microorganisms to the toxic compound being degraded (Heitkamp et al. 1990; O'Reilly and Crawford 1989; Wong and Wong 1992). Disadvantages include increased resistance of the toxic compound through the matrix and increased oxygen limitation due to high cell densities and low oxygen solubility (O'Reilly and Crawford 1989). Knowledge of the physical, chemical, and nutritional requirements of the

degrading microorganism is essential for maintaining high cell densities (Heitkamp et al. 1990).

The objectives of this study are to optimize a biofiltration system that contains a highly efficient PCP and PAHs degrading bacteria for commercial application. Optimization will be done in the laboratory and involve maintaining the flow rates at commercial levels, increasing the efficiency of the system with higher cell densities, and testing PCP and PAHs in the same wastewater, since they are often mixed in an industrial setting. At the end of the study, a small-scale biofiltration unit, consisting of the most efficient parameters as determined by laboratory studies, will be tested at a commercial facility in the field. At this site, the retention volume to flow rate for their pump-and-treat system is 10:1, while the PCP concentrations average at 2 ppm.

METHODS AND PROCEDURES

Different laboratory-scale biofiltration systems have been developed at the Mississippi State Forest Products Laboratory. The current biofiltration units are presented in Figure 1. The packed bed columns, which are being used to evaluate the performance of different biocarriers, are three-foot-long cast acrylic columns with a one inch interior diameter and a 0.25-inch wall thickness. The bottom of the column is sealed with a PVC coupling containing an air dispersion frit. A stainless steel wire screen is placed two inches from the bottom of the column to support a 24-inch packed bed. The synthetic waste is pumped into the column at a controlled preset flow rate (20 ml/hour). The retention volume to flow rate at this setting is 26:1, which means our system currently runs 2.6 times slower than the commercial facility. However, because our PCP concentrations are 10 times higher, a longer retention time is required. An influent port is located one inch above the supporting screen. The feed pump is a Cole-Parmer model 50000-078. The effective bed volume of each column is 18.84 cubic inches. Each test unit, including the control test unit, consists of a three-column series. Initially, a single column with recirculation of the effluent back through the column was tested. However, degradation was occurring in the reservoir as well as in the column, which is not acceptable. A three column series is also more representative to what is currently used by the wood treating industry.

An *Arthrobacter* sp. which has been proven to be a very efficient degrader of both PCP and PAHs (Walker 1992) is currently being tested against different matrices. This particular bacterium was isolated from a PCP and creosote wood treatment plant in Joplin, Missouri, and is being grown on mineral salts media (MSM) with PCP as the only carbon source. This eliminates the possibility of interference from extraneous compounds that are found in nutrient media and also stimulates maximum production of the enzyme complexes. A recently isolated unidentified bacterium will also be tested. This bacterium was isolated from a bioreactor unit treating PCP and PAH contaminated water. Preliminary tests have shown that this bacterium degrades the PCP in contaminated water very rapidly to non-detectable levels (less than 20 ppb). In addition, the bacterium prefers PCP-contaminated water to nutrient broth solutions, making it a very good candidate for water biofiltration studies.

Matrices under consideration include sand, perlite, two commercial biocarriers, and glass beads. Each matrix is being evaluated using the three-column test series with bacteria bound to the matrix (treatment) and without bacteria bound to the matrix (control). Each matrix is being evaluated from preparation to completed column test series a minimum of three times (= 3 replications). Control column series are always run at the same time as a treatment column series. Initially, the matrices are conditioned, if required. The commercial carriers were conditioned with sodium hydroxide to neutralize the matrix. All matrices are pre-washed with deionized water and autoclaved (35 min, 2x). The matrix is added to a Fernbach flask containing MSM pH 7.2 with 20 ppm PCP (plus or minus bacteria) and then mixed with a stirring shaft for approximately one week. PCP is checked and re-added as needed to provide substrate for the bacteria and allow the PCP to absorb onto the matrix. The matrix is then washed with MSM plus 20 ppm PCP (MSM-P).

The columns are then packed with the conditioned matrix and the flow rate is calibrated to 20ml/hour. MSM-P is fed upwards through the column series. Water samples are taken daily from each test column plus influent. Samples are acidified and extracted with methylene chloride and analyzed by gas chromatography (GC). Each test series is run until the PCP disappears in the column effluent or reaches a stable plateau. Some matrices will be run for an extended three-month study to determine bacterial survival and degradation efficiency over time. The best matrices will also be tested using groundwater from a wood treating site containing both PCP and PAHs. Once a test run is completed, the matrix in the columns will be extracted and analyzed by GC for PCP concentrations. This will provide an indication of

PCP adsorption by the different matrices. Microtox analyses and bacteria enumerations are also being performed on initial and final samples.

Microbial enumerations are done by viable plate counts in order to assess the rate of microbial reproduction and to determine the numbers of microorganisms present. In this procedure, serial dilutions are plated onto a suitable solid growth medium using the spread plate technique. Media for bacterial counts are nutrient agar, nutrient agar supplemented with PCP (5 µg/ml), creosote (20 µg/ml), or both; for fungal counts potato dextrose agar amended with antibiotics (PDAA), PDAA supplemented with PCP (5 µg/ml) or creosote (20 µg/ml), and actinomycete isolation agar.

The toxicity of the wastewater samples is determined with a Microtox model 500 toxicity analyzer (Microbics Corporation, Carlsbad, California) according to the manufacturer's specifications. Microtox evaluates the toxicity of a given substance by measuring the change in light level of viable luminescent bacteria upon their exposure to test substrates. The results are expressed as EC_{50} which is the effective concentration of the toxicant causing a 50% decrease in the light output by the bacteria under exposure time (t = 5 minutes) and test temperature (T = 15 degrees Celsius).

RESULTS AND DISCUSSION

Sand was initially tested as a support matrix. Flow rates could only be pushed to 1.7 units per hour, which is 30 times slower than one commercial wood treating facility. MSM-P was run at both 20 ppm and 50 ppm and the columns were maintained for up to 90 days. Results show that there was an immediate and rapid decrease in PCP concentration in the treatment columns and below detection limit levels (<20 ppb) of PCP were maintained throughout the study (Table 1). In comparison, control columns showed no degradation throughout this study. One problem with sand is the plugging of the matrix pores with bacterial organic matter, slime, and cell debris such that flow rate efficiency decreased over time.

Perlite, a heat-expanded volcanic material manufactured by Hyponex Corporation, was tested next. Perlite has an approximate diameter of 3 mm and length of 3-5 mm. Pore size is unknown. The biofiltration column was packed with 18.5 in^3 of perlite and flushed with 10 mg/l PCP in MSM. Flow rates through perlite were faster (37 ml/min), and rates had to be adjusted down. The flow rate was held at approximately 20 ml per hour, and 156 ml of effluent was collected every 24 hours for 120 hours. Concentrations seemed to plateau at 1 ppm by 96 hours (Table 2). Absorption of PCP in the control column was evident through 120 hours of run time. At 120 hours, the overall decrease in PCP concentration was 82% compared to the control. Bacteria continued to wash from the perlite into the effluent through 120 hours.

A new matrix from W. R. Grace Company, a ceramic catalyst support, is currently being tested. This matrix is conditioned with 0.4% sodium hydroxide, rinsed, and the pH neutralized to 7.2. Flow rates were held at 20 ml per hour and flushed with 20 ppm MSM-P. amples were taken every 24 hours. No decrease in PCP concentrations were seen between control and treatment columns (Table 3). Adsorption of PCP in both column series was evident through 144 hours of run time. Bacteria were present on the matrix in sufficient numbers to support degradation. This matrix is being tested one last time with the bacteria interacting with the matrix for two weeks before sampling begins.

SIGNIFICANCE

The costs associated with the removal and disposal of PCP and creosote wastes are becoming prohibitive to the wood treatment industry because of the large volumes involved and the economically marginal nature of many wood preserving operations. Biofiltration offers the potential for much higher biodegradation rates, greater thermostability for the enzymes involved in biodegradation, and a higher tolerance of the microorganisms to the toxic compound being degraded, as well as cheaper overall clean-up costs. Successful development of this cost efficient and highly effective bioremediation method could be applied to industry for a more rapid process water clean-up and detoxification of contaminated groundwater. This type of biofiltration technology would apply to many other industrial groundwater pollutants including pesticides and petroleum products.

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Table 1. Decrease in PCP concentration during biofiltration experiment where sand was tested as the support matrix. PCP levels were tested at both 20 and 50 ppm.

	PCP Concentration ppm			
Day	Treatment	Control	Treatment	Control
0	20	20	50	50
1	2	20	8	51
2	BDL*	17	2	48
3	BDL	19	BDL	44
4	BDL	19	<1	48
5	BDL	22	BDL	49
6	BDL	17	BDL	47
7	BDL	18	BDL	50
14	BDL	18	BDL	47
21	BDL	18	BDL	50
28	BDL	19	BDL	45
35	BDL	17	BDL	49
42	BDL	20	BDL	47
49	BDL	20	BDL	50
56	BDL	16	BDL	49
63	BDL	20	BDL	48
70	BDL	19	BDL	49
77	BDL	18	BDL	45
84	BDL	19	BDL	49
90	BDL	17	BDL	46

* BDL = Below the Detection Limit of 20 ppb.

Table 2. Decrease in PCP concentrations (ppm) during biofiltration experiment where perlite was tested as the support matrix.

Time	PCP Concentration ppm	
Hours	Treatment	Control
0	8.8	8.8
24	5.2	4.8
48	4.0	3.1
72	4.5	2.5
96	3.7	0.7
120	5.8	1.1

Table 3. PCP concentrations (ppm) during biofiltration experiment where ceramic biocarrier was tested as the support matrix.

Time	PCP Concentration ppm	
Hour	Treatment	Control
0	20.0	20.0
24	10.0	8.6
48	11.3	11.5
72	12.8	12.5
96	14.7	14.2
120	14.8	15.1
144	15.5	13.7

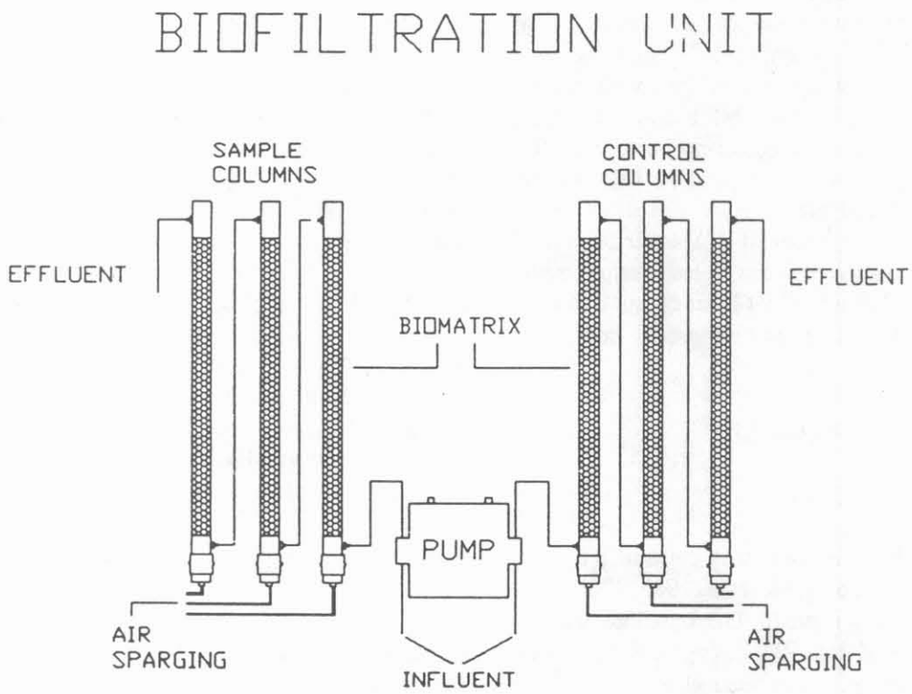

Figure 1. Diagram of laboratory-scale biofiltration columns currently in use to test parameters influencing degradation rates and cell densities.