Appl Microbiol Biotechnol (1999) 52: 261–266

ORIGINAL PAPER

S. Chauhan · E. Yankelevich · V. M. Bystritskii
T. K. Wood

Degradation of 2,4,5-trichlorophenol and 2,3,5,6-tetrachlorophenol by combining pulse electric discharge with bioremediation

Received: 3 November 1998 / Received revision: 28 February 1999 / Accepted: 14 March 1999

Abstract Degradation of 2,4,5-trichlorophenol (2,4,5-TCP) and 2,3,5,6-tetrachlorophenol (TeCP) was studied using a two-stage approach that utilized efficient pulse electric discharge (PED) followed by biological degradation with a consortium from acclimated return activated sludge. The chlorinated phenols were treated in the PED reactor as an aerosol at a voltage of 55–60 kV, a frequency of 385 Hz, a current of 50–60, and with a 200-ns pulse. As determined by gas chromatography and mass spectrometry (GC/MS), the first stage converted 500 ppm 2,4,5-TCP to 163 ppm 2,4,5-TCP and dimethyldecene, dichloronaphthalenol, octyl acetate, and silyl esters. The total carbon content of 2,4,5-TCP after PED treatment was determined to be 228 ± 35 ppm. The remaining 2,4,5-TCP and the products formed were then mineralized by the acclimated activated sludge in shake flasks; the initial rate of degradation of 2,4,5-TCP was calculated to be 5 nmol min^{-1} mg $protein^{-1}$ at 163 ppm initial concentration (three orders of magnitude higher than the only rate found in the literature). By combining the two techniques, a synergistic effect (2.3-fold increase in the concentration of 2,4,5-TCP degraded and 3.3-fold increase in total carbon degraded) was observed, in that bacteria without any treatment degraded a maximum of 70 ppm 2,4,5-TCP but after PED treatment 163 ppm 2,4,5-TCP was degraded. TeCP was also mineralized by the acclimated activated sludge after treatment with PED. This two-stage approach was also evaluated using a continuous 1-l fluidized-bed reactor.

S. Chauhan · T. K. Wood (✉)
Department of Chemical Engineering, University of Connecticut, Storrs, CT 06269-3222, USA
e-mail: twood@uconnvm.uconn.edu
Tel.: +1-860-486-2483
Fax: +1-860-486-2959

E. Yankelevich · V. M. Bystritskii
Department of Physics and Astronomy, University of California, Irvine, California 92697-2575, USA

Introduction

Advanced oxidation technologies have appeared in the last 10 years as a result of defense-related research and development and offer real promise for degrading pollutants in both gas and liquid streams. Usually these technologies deal with electric pulsed discharges, high-energy electron beams, X-ray pulses, or powerful shock waves that involve production of free electrons, highly reactive radicals ($OH\cdot$, $H\cdot$, $O_2^-\cdot$), H_2O_2, and ozone (Getoff and Lutz 1985; Willibald et al. 1990; Woods and Pikaev 1994). These methods have been found to be very successful for treating flue gases and volatile organic compounds (Willibald et al. 1990). In liquids, electron irradiation has been shown to effect the degradation of a wide range of compounds in air-saturated aqueous solutions including oxidation of arsenite, ammonia, hydrogen sulfide, alcohols, amines, benzene, toluene, phenol, carboxylic acids (e.g., benzoic acid, formic acid), chlorinated aliphatics (e.g., carbon tetrachloride, perchloroethylene, trichloroethylene), acetonitrile, and alkenes (e.g., propene) (Getoff 1986; Getoff and Lutz 1985; Spinks and Woods 1990).

In the low-voltage pulsed-electric-discharge reactor, the energy of the reactive species (approx. 10 eV) is of the same or higher order than that of the chlorine ion bond strength (3.7 eV/molecule for chloromethane; Streitwieser and Heathcock 1981); hence, chlorine (and other halogens) can be removed as a result of cleavage of Cl-C bonds and substitution by these reactive species (note in air-saturated systems, the attack is primarily from $OH^{\cdot}$ and $O_2^-\cdot$ since oxygen quickly scavenges the solvated electrons; Getoff and Lutz 1985). For example, 30 chloride ions are produced for every 100 eV energy applied to a 0.07 M chloroform solution (Rezansoff et al. 1970). Similar results have been reported for other chlorinated aliphatics; for trichloroethylene, 9.1 chloride ions are produced for every 100 eV (for 0.1 mM aqueous solutions) (Getoff 1986), and for perchloroethylene, 17–31 chloride ions

are produced for every 100 eV (for 1 mM aqueous solutions) (Getoff 1986; Woods and Pikaev 1994). Dechlorination by irradiation is so reliable and linear with the applied energy that dechlorination of chloroform is used as a dosimeter (Rezansoff et al. 1970); thus, electron irradiation can be used to remove chlorine substituents.

Although electric discharge is capable of mineralizing many recalcitrant pollutants (and the diversity of compounds that can be remediated is one of its chief advantages), it requires great amounts of energy to mineralize compounds completely whereas the initial attack on pollutants (e.g., dechlorination) requires very little energy and is efficient. Bioremediation avoids the transport of hazardous materials, is well received by the public and, in many applications, is known to mineralize pollutants completely. It is also usually the least expensive remediation technique; for example, for eliminating compounds like trichloroethylene, this is potentially the most cost-effective alternative (McFarland et al. 1992; Winter et al. 1989).

The main disadvantage of using exclusively aerobic biotreatment is that chlorinated organic contaminants display extreme stability to biodegradation; highly chlorinated pollutants (two or more chlorine atoms per molecule) are in some cases completely resistant to aerobic biodegradation (Ensley 1991; Mondello 1989). In natural systems, slow, anaerobic dechlorination steps are required to reduce the number of chlorine atoms before aerobic bacteria may more rapidly metabolize the residual compounds.

To accelerate bioremediation significantly and to take advantage of the best of each of these independent technologies, pulsed electric discharge (PED) and bioremediation were combined recently into an innovative, two-stage technique of bioremediation to treat 2,4-dichlorophenol and perchloroethylene (Yee et al. 1998). In the first stage, a novel, efficient, low-voltage electrically pulsed discharge reactor is used to initiate degradation of recalcitrant pollutants, and bioremediation is used in the second stage to treat the dechlorinated intermediates. The PED reactor had the advantages that efficient dechlorination was achieved at lower voltages than those of high-energy systems by using an atomizer feed system, which increased efficiency by concentrating the pollutant at the air/liquid interface where the ionizing species are prevalent. This paper is the first report of these two techniques being combined to degrade 2,4,5-trichlorophenol (2,4,5-TCP) and tetrachlorophenol (TeCP). Chlorinated compounds have been widely used as herbicides, insecticides, fungicides and wood preservatives (Freiter 1997); however, their stability makes them resistant to biodegradation and ultimately contributes to their release into the environment, thus contaminating soil and ground water (Goerlitz et al. 1985; Kitunen et al. 1985; Valo and Salkinoja-Salonen 1984). 2,4,5-TCP is very persistent and is judged a priority pollutant by the U.S. EPA (Maltseva and Oriel 1997).

Materials and methods

Biodegradation

Return activated sludge was collected from the Irvine Ranch Water District, Irvine, Calif. A 20% inoculum of the activated sludge was added to a 250-ml conical flask containing 50 ppm 2,4,5-TCP and chloride-free M9 medium (Maniatis et al. 1982) supplemented with chloride-free 5% Luria-Bertani (LB) medium (Maniatis et al. 1982) (no NaCl in LB; $MgSO_4$ at 0.24 g/l and $CaSO_4$ at 0.17 g/l replaced $MgCl_2$ and $CaCl_2$ in M9 to facilitate the analysis of chloride released); incubation was aerobic at 30 °C and 300 rpm in an orbital shaker (New Brunswick Scientific, Edison, N.J.). After five transfers in 5 days into the same medium, the consortium was able to grow on agar plates containing 2,4,5-TCP (50 ppm).

Several colonies were scraped from the M9 + 2,4,5-TCP plate and inoculated into 25 ml chloride-free M9 medium with 5% LB + 50 ppm 2,4,5-TCP in a 250-ml conical flask and grown overnight at 30 °C and 300 rpm. The overnight culture was centrifuged (model J2-21 centrifuge, Beckman, Palo Alto, Calif.) at 2000 *g* for 15 minutes at 4 °C, washed with M9 medium (lacking chloride), and added to 25 ml PED-treated sample containing M9 medium (no chloride ions, no carbon source, absorbance of 0.7 at 600 nm). After 0, 4, 8, and 24 h, the culture was centrifuged, the aqueous layer extracted, and samples prepared for GC analysis.

Chemicals and analysis.

All chemicals used were of the highest purity available and were used without further purification. 2,4,5-TCP, 2,4,6-TCP, and 2,3,4,5,6-pentachlorophenol (PCP) were purchased from Sigma Chemicals (St. Louis, Mo.), 2,3,5,6-tetrachlorophenol (TeCP) was purchased from AccuStandard Inc. (New Haven, Conn.), and 2,4-dichlorophenol (DCP) was purchased from Eastman Kodak (Rochester, N.Y.).

The disappearance of chlorinated phenols during bioremediation was monitored after 16 h by a Beckman DU-640 spectrophotometer (Fullerton, Calif.) by measuring the wavelength of clarified media at 310 nm for 2,4,5- and 2,4,6-TCP, 306 nm for 2,3,5,6-TeCP, and 318 nm for 2,3,4,5,6-PCP. 2,4-DCP was assayed by a colorimetric assay (Martin 1949). Chloride released from the PED treatment of 2,4,5-TCP was measured in a spectrophotometer after addition of 200 µl 0.25 M ferric ammonium sulfate in 9 M HNO_3 and 200 µl saturated mercuric thiocyanate in ethanol to a 1-ml sample. The contents were incubated for 10 min, and the absorbance was then measured at 460 nm (Bergmann and Sanik 1957). Total carbon present after 2,4,5-TCP was treated in the PED reactor was measured by a Rosemount Dohrmann DC-190 total organic carbon analyzer (Arnold et al. 1992) (Santa Clara, Calif.).

The initial rates of bioremediation were determined by quantifying the concentration of chlorinated phenol over a 24-h period (after 0, 4, 8, 12, 24 h) in a gas chromatograph. Samples of 25 ml were harvested from shake flasks and centrifuged to remove cells, and the supernatant was extracted three times with equal volumes of ethyl acetate in the presence of naphthalene as an internal standard. The combined organic layers were dried over anhydrous sodium sulfate and evaporated in a rotary evaporator (Labconco, Kansas City, M.O). The contents were transferred to a sample vial, dried with a gentle stream of nitrogen and resuspended in 250 µl methylene chloride. A 1-µl sample of this was injected into the GC equipped with a flame ionization detector (5890 Series II; Hewlett Packard, Palo Alto, Calif.). A Hewlett Packard-5 capillary column (cross-linked 5% diphenyl 95% dimethylpolysiloxane, 10 m, 0.11 mm × 0.17 µm film thickness) with a temperature gradient from 80 °C to 220 °C at a rate of 20 °C min^{-1} was used to separate the compounds. The initial and final hold times were 2 min and 5 min, respectively, and the injector and detector temperatures were set at 180 °C and 220 °C respectively. Concentrations were determined by comparison of the peak heights to a standard curve.

The products formed after PED treatment of 2,4,5-TCP were identified by gas chromatography followed by mass spectrometry (GC/MS) performed by the Department of Chemistry and Biochemistry at the University of California, Los Angeles. Aliquots (1–4 μl) of the ethyl-acetate-extracted samples were loaded onto a solvent-free GC injector (dropping-needle type; Ray Allen Assoc., Boulder, Colo.) connected to a bonded-phase-medium, polarity-fused silica capillary column (DB-5, 30 m, 0.26 mm internal diameter, film thickness 0.25 μm, J & W Scientific, Folsom, Calif.) with helium as the carrier gas. The end of the column was inserted directly into the ion source of a modified HP 5985B GC/MS instrument. A head pressure of 10.3 kPa helium was maintained in the GC injector port. The injector port and transfer lines were maintained at 250 °C. The GC oven was held at 100 °C for 1 min following injection, and then increased linearly at 20 °C/min to a plateau of 300 °C. The mass spectrometer was operated in the electron ionization mode with a high-energy dynode detector (Phrasor Scientific, Duarte, Calif.) set at −5 kV. The MS ion source was held at 200 °C, and an ion current of 300 μA (70 eV) was used.

Pulsed-electric-discharge reactor

The PED reactor consisted of a glass chamber 150 cm in length and 25 cm inner diameter. The electrodes were made of stainless steel coated with chromium, oriented axially, intermeshed, and placed in the center of the chamber. 2,4,5-TCP (500 ppm) was treated in a 1-l volume as an aerosol at a voltage of 55–60 kV, a frequency of 385 Hz, a current of 50–60 A, and with a 200-ns pulse duration. An atomizer with a throughput of 7.6–37.9 l/h (at 0.2–0.5 MPa air) was mounted at the top or bottom of the reactor, and the effluent was collected at the bottom. After a single pass through the PED reactor, the effluent was collected and used for biological treatment.

Fluidized-bed reactor (FBR)

The FBR consisted of a glass cylindrical chamber 50 mm in diameter with an external water jacket for temperature control, 0.8 l fluidized-bed volume, and 0.7 l headspace volume. The carrier material was fluidized by a circulation pump (model AC-5C-MD; March Manufacturing Inc., Glenwood, Ill.), which was regulated by a rheostat. SIRAN-Carrier (Jaeger Biotech Engineering Inc., Costa Mesa, Calif.), open-pore sintered glass beads were used to immobilize the sludge bacteria (diameter 0.4–1 mm, pore size < 120 μm, surface area 90 m^2/l, void volume 60%). Solid glass beads at the bottom of the conical reactor were used to distribute the fluidizing liquid. The air flow rate was 35 ml/min and stripping losses of 2,4,5-TCP were 15%–20% (biodegradation not limited by oxygen as tripling the air flow rate did not alter 2,4,5-TCP degradation). The effect of adding salicylate as an additional carbon source was studied by varying the salicylate concentration (2, 4, 6, and 8 g/l at 1.5 ml/min) at 24 h intervals to the FBR at 30 °C (PED-treated feed fixed at 29 μg 2,4,5-TCP/min).

Results

Using shake flasks, the ability of acclimated activated sludge to degrade various chlorinated phenols (without PED) was investigated. 2,4-DCP (500 ppm) and 2,4,6-TCP (200 ppm) were degraded by the activated sludge without pre-treatment by PED whereas 2,4,5-TCP (250 ppm), 2,3,5,6-TeCP (100 ppm), and PCP (500 ppm) could not be degraded; therefore, 2,4,5-TCP and 2,3,5,6-TeCP were chosen for further study with PED. PED of the chlorinated phenolics samples at these concentrations resulted in 26%–56% of the total chloride being released as chloride ions; hence, PED was successful in dechlorinating these phenols. These results agree with the release of chloride demonstrated for 2,4-DCP and perchloroethylene by PED shown previously for this reactor (Yee et al. 1998).

GC/MS analysis of the PED-treated sample of 2,4,5-TCP (500 ppm initial concentration) indicated that the degradation products included a mixture of dechlorinated, straight-chain compounds as well as aromatic polymeric compounds. These compounds were tentatively identified as (retention times indicated and listed in order of prevalence): dimethyldecene (4.87 min), dichloronaphthalenol (17.79 min), octyl acetate (6.7 min), octamethylcyclotetrasiloxane (4.15 min), and trimethylsilyloxybenzoic acid (6.59 min). In a separate experiment, benzenedicarboxylic acid diisooctyl ester (21 min), hexanedioic acid diocytl ester (20 min), dichlorobenzenediol (9 min), dichlorophenol (20 min), and dichloronitrophenol (10 min) were detected. The silyl esters appear to be created by reaction of the PED-generated radicals with the glass cylinder of the PED reactor.

After treating 500 ppm 2,4,5-TCP with PED, the total carbon was found to be 228 ± 35 ppm of which 163 ± 18 ppm was determined by GC analysis to be 2,4,5-TCP. The acclimated activated sludge converted 22%, 59%, 76%, and 97% of the remaining 2,4,5-TCP in 4, 8, 12, and 24 h respectively; hence, it was able to convert most of the remaining 2,4,5-TCP and nearly all of the products formed after PED to cell mass and volatile compounds (0 and 24 h treatment shown in Figs. 1, 2). Similarly, the acclimated activated sludge converted 64%, 80%, and 92% of the TeCP in 8, 12, and 24 h respectively and was able to convert most of the remaining TeCP and nearly all of the products formed after PED to cell mass and volatile compounds (Figs. 3, 4).

The initial rate of degradation of the 2,4,5-TCP remaining after the first-stage PED treatment (163 ± 18 ppm) was found to be 4.95 nmol min^{-1} mg $protein^{-1}$ (Table 1). This initial rate was determined twice using two separate PED-treated samples with initial 2,4,5-TCP concentrations of 145 ppm and 181 ppm. The initial rate of TeCP degradation was 0.31 nmol min^{-1} mg $protein^{-1}$ (14 ppm TeCP remaining after PED, Table 1). No significant loss of the chlorinated phenol substrates in these shake-flask experiments was observed when heat-killed cells were used.

To determine whether an additional carbon source would enhance the bioremediation of PED-treated 2,4,5-TCP (163 ppm remaining), 19 shake-flask experiments were conducted using various carbon sources (10 mM salicylate, 5% LB broth, 0.2% yeast extract, 10 mM acetate, or 2% glucose), and the overnight chlorinated phenol concentration was measured after 16 h. For all these experiments, the most extensive degradation was found when no additional carbon was added, and the added carbon sources behaved similarly. For example, for 0.5 mM initial concentration after PED treatment, 90% 2,4,5-TCP was removed when no additional carbon source was present while 41%–51% was removed when the other carbon sources were added.

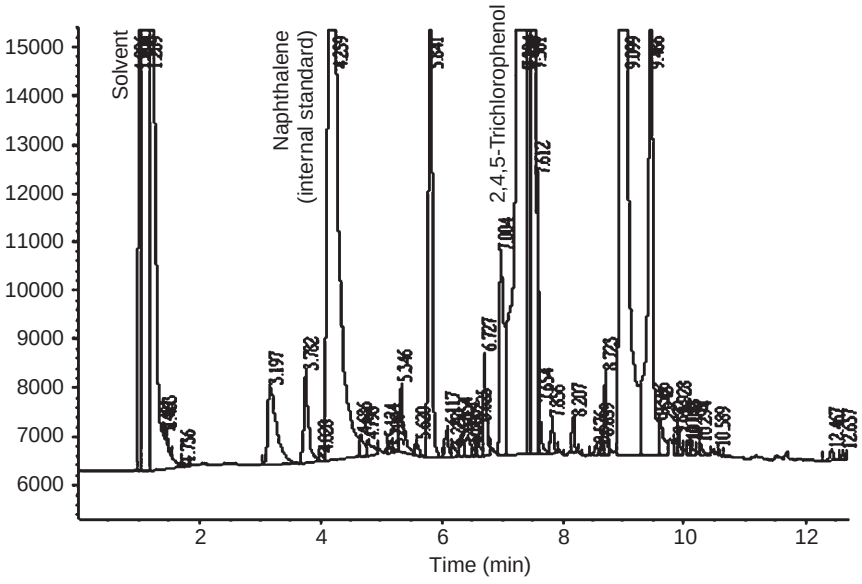

Fig. 1 Gas chromatograph of 500 ppm 2,4,5-trichlorophenol (2,4,5-TCP) after processing in the pulse-electric-discharge (PED) reactor. The 2,4,5-TCP and internal standard naphthalene peaks are labeled

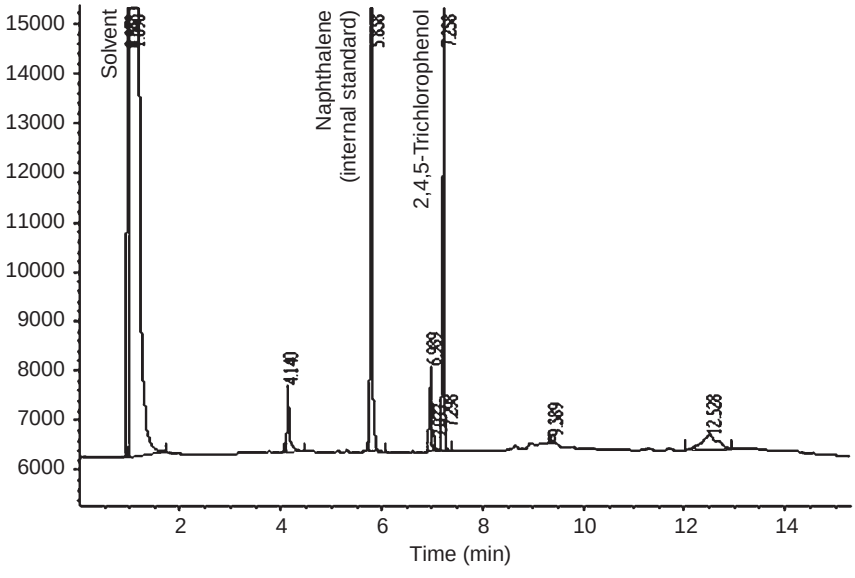

Fig. 2 Gas chromatograph of the PED-treated 2,4,5-TCP solution after 24 h processing with acclimated activated sludge. The 2,4,5-TCP and internal standard naphthalene peaks are labeled

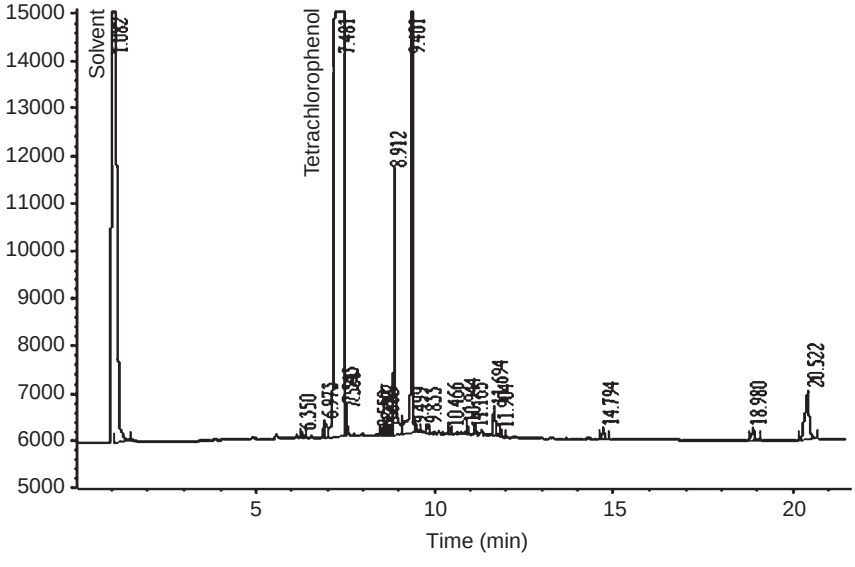

Fig. 3 Gas chromatograph of 100 ppm 2,3,5,6-tetrachlorophenol (2,3,5,6-TeCP) after processing in the PED reactor

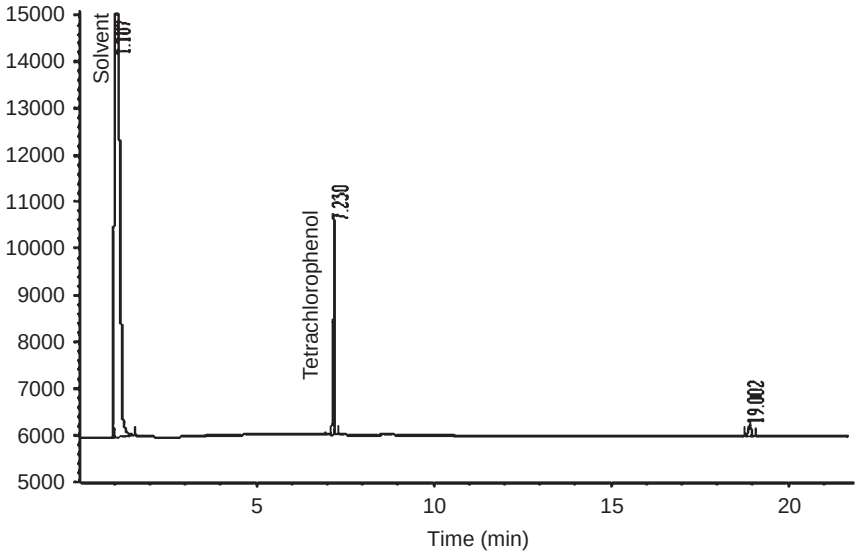

Fig. 4 Gas chromatograph of the PED-treated 2,3,5,6-TeCP solution after 24 h processing with acclimated activated sludge

Table 1 Degradation of 2,4,5-trichlorophenol (*2,4,5-TCP*) by acclimated activated sludge after pulsed electric discharge. The gas chromatography peak areas for the chlorinated phenols have been normalized using the internal standard (*std.*) peak areas of naphthalene, and the total protein concentration was 0.26 mg/ml at an absorbance of 0.70 (600 nm). *2,3,5,6-TeCP* 2,3,5,6-tetrachlorophenol

Compound	Time, h	Chlorinated phenol GC peak area	Internal std. GC peak area	Normalized peak area	Chlorinated phenol (ppm)	Degradation rates, (nmol min^{-1} mg $protein^{-1}$)
2,4,5-TCP	0	1083402	62325	1141166	181	3.1
	4	777556	59522	857582	143	5.1
	8	439658	63962	451247	80	1.5
	24	37965	76783	32459	6	
	0	904052	62660	877199	145	2.5
	4	769762	69854	669977	114	4.8
	8	267116	52972	306583	55	1.7
	12	173133	55271	190448	35	0.85
	24	19516	63237	18764	3	
2,3,5,6-TeCP	0	47689	35228	70850	14	0.31
	8	25731	55630	24208	4.8	0.13
	12	12651	45696	14489	2.9	0.04
	24	7567	70209	5640	1.1	0.03
	32	711	54925	677	0.1	

These results were corroborated, using a FBR. In this continuous reactor, the concentration of salicylate (as an additional carbon source) was varied from 0 to 8 g/l as the 2,4,5-TCP feed was kept at 58.5 μg/min. Two-thirds of the 2,4,5-TCP fed to the FBR was routinely degraded by the immobilized acclimated sludge (e.g., 58.5 ppm feed, 18.4 μg degraded/min). As the salicylate concentration increased, the amount of 2,4,5-TCP degraded in the FBR decreased by 43% (18.4 μg 2,4,5-TCP degraded/min at 0 g salicylate/l, 14.8 μg/min at 2 g salicylate/l, 15.1 μg/min at 4 g salicylate/l, 14.8 μg/min at 6 g salicylate/l, and 10.9 μg/min at 8 g salicylate/l).

Discussion

To date, very few results of biological degradation of 2,4,5-TCP have been reported (Maltseva and Oriel 1997; Ryding et al. 1994). Most studies for 2,4,5-TCP report the requirement for an additional carbon source (e.g., toluene, yeast extract), and the rates of degradation by the various bacteria are found to be very slow. After PED treatment, the rates of degradation of 2,4,5-TCP observed by us are considerably higher than those reported by other groups [5 nmol min^{-1} mg $protein^{-1}$ at an initial concentration of 163 ppm compared to 0.008 nmol min^{-1} mg $protein^{-1}$ at 10 ppm initial concentration (Ryding et al. 1994)]; although these rates are comparable to those reported for the isomers 2,3,6-TCP and 2,4,6-TCP [roughly 10 nmol min^{-1} mg $protein^{-1}$ at 30 ppm (Resnick and Chapman 1994)]. Furthermore, no additional carbon source was required in this study other than the PED discharge products (addition of salicylate was deleterious).

In addition to the faster rates of 2,4,5-TCP degradation afforded by PED treatment, the concentration of total carbon (228 ± 35 ppm) remaining after first-stage treat-

ment that could be degraded by bacteria was 3.3 times higher than the concentration that could be treated by bacteria without any pre-treatment (70 ppm 2,4,5-TCP). The concentration of 2,4,5-TCP that could be treated by bacteria after PED treatment was also found to be higher (70 ppm compared to 163 ± 18 ppm degraded after PED), indicating a synergistic effect. Thus this combination of PED and biotreatment appears to be an attractive method to degrade pollutants since it generates less-chlorinated intermediates, which serve to reduce 2,4,5-TCP toxicity and stimulate co-metabolic biodegradation.

This work expands the use of the two-stage remediation process to degrade additional chlorinated compounds. Whereas our previous study used pure cultures for bioremediation (Yee et al. 1998), use of a consortium will be more helpful since most contaminated areas contain a mixture of compounds and no single bacterium can handle all compounds.

Acknowledgments This study was supported by LG Industrial Systems Co. (LGIS-22936).

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