Bioremediation and Phytoremediation: Chlorinated and Recalcitrant Compounds

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Chlorinated and Recalcitrant Compounds

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FOREWORD

Bioremediation and phytoremediation have progressed over the past decade from promising ideas to practical remediation approaches, especially with regard to the treatment of hydrocarbon-contaminated sites. Sites contaminated with chlorinated and recalcitrant compounds have proven more resistant to these approaches, but exciting progress is being made both in the laboratory and in the field. *Bioremediation and Phytoremediation: Chlorinated and Recalcitrant Compounds* brings together the latest breakthrough bioremediation research and field applications in chapters that cover cometabolic processes, aerobic and anaerobic mechanisms, biological reductive dechlorination processes, bioaugmentation, biomonitoring, and phytoremediation of recalcitrant organic compounds.

This is one of six volumes published in connection with the First International Conference on Remediation of Chlorinated and Recalcitrant Compounds, held in May 1998 in Monterey, California. The 1998 Conference was the first in a series of biennial conferences focusing on the more problematic substances—chlorinated solvents, pesticides/herbicides, PCBs/dioxins, MTBE, DNAPLs, and explosives residues—in all environmental media. Physical, chemical, biological, thermal, and combined technologies for dealing with these compounds were discussed. Several sessions dealt with natural attenuation, site characterization, and monitoring technologies. Pilot- and field-scale studies were presented, plus the latest research data from the laboratory. Other sessions focused on human health and ecological risk assessment, regulatory issues, technology acceptance, and resource allocation and cost issues. The conference was attended by scientists, engineers, managers, consultants, and other environmental professionals representing universities, government, site management and regulatory agencies, remediation companies, and research and development firms from around the world.

The inspiration for this Conference first came to Karl Nehring of Battelle, who recognized the opportunity to organize an international meeting that would focus on chlorinated and recalcitrant compounds and cover the range of remediation technologies to encompass physical, chemical, thermal, and biological approaches. The Conference would complement Battelle's other biennial remediation meeting, the In Situ and On-Site Bioremediation Symposium. Jeff Means of Battelle championed the idea of the conference and made available the resources to help turn the idea into reality. As plans progressed, a Conference Steering Committee was formed at Battelle to help plan the technical program. Committee members Abe Chen, Tad Fox, Arun Gavaskar, Neeraj Gupta, Phil Jagucki, Dan Janke, Mark Kelley, Victor Magar, Bob Olfenbuttel, and Bruce Sass communicated with potential session chairs to begin the process of soliciting papers and organizing the technical sessions that eventually were presented in Monterey. Throughout the process of organizing the Conference, Carol Young of

Battelle worked tirelessly to keep track of the stream of details, documents, and deadlines involved in an undertaking of this magnitude.

Each section in this and the other five volumes corresponds to a technical session at the Conference. The author of each presentation accepted for the Conference was invited to prepare a short paper formatted according to the specifications provided. Papers were submitted for approximately 60% of the presentations accepted for the conference program. To complete publication shortly after the Conference, no peer review, copy-editing, or typesetting was performed. Thus, the papers within these volumes are printed as submitted by the authors. Because the papers were published as received, differences in national convention and personal style led to variations in such matters as word usage, spelling, abbreviation, the manner in which numbers and measurements are presented, and type style and size.

We would like to thank the Battelle staff who assembled this book and its companion volumes and prepared them for printing. Carol Young, Christina Peterson, Janetta Place, Loretta Bahn, Lynn Copley-Graves, Timothy Lundgren, and Gina Melaragno spent many hours on production tasks. They developed the detailed format specifications sent to each author, tracked papers as received, and examined each to ensure that it met basic page layout requirements, making adjustments when necessary. Then they assembled the volumes, applied headers and page numbers, compiled tables of contents and author and keyword indices, and performed a final page check before submitting the volumes to the publisher. Joseph Sheldrick, manager of Battelle Press, provided valuable production-planning advice and coordinated with the printer; he and Gar Dingess designed the volume covers.

Neither Battelle nor the Conference co-sponsors or supporting organizations reviewed the materials published in these volumes, and their support for the Conference should not be construed as an endorsement of the content.

Godage B. Wickramanayake and Robert E. Hinchee
Conference Chairman and Co-Chairman

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BIOLOGICAL REMOVAL OF 1,2-DICHLOROETHANE AND TETRACHLOROETHENE FROM CONTAMINATED GROUNDWATER (LARGE-SCALE)

Gerhard Stucki, Markus Thüer (Ciba Specialty Chemicals Inc., Schweizerhalle, Switzerland)

ABSTRACT: The performance of two biological large-scale processes to purify groundwater contaminated with chlorinated hydrocarbons is presented. At the first site in Lübeck (Germany), 1,2-dichloroethane (DCA) is aerobically mineralised by microorganisms bread under laboratory conditions and inoculated into a groundwater purification plant to treat 5 to 20 m^3/h at 8 to 12°C. Average feed concentrations of 15 mg/l were degraded to below 10 µg/l. The initially conventional activated carbon process was biologically modified. As a result, the monthly activated carbon requirement (5 tons) became redundant, and the operation costs fell by a factor of seven.

At the second site in Albstadt (Germany), tetrachloroethene (TCE) is removed from the unsaturated zone by soil vapour extraction, and the contaminated groundwater (2 m^3/h at 12 to 14 °C containing 1.5 mg/l TCE and 25 mg/l nitrate) is pumped and treated in three 1 m^3 reactors run in series using anaerobic and aerobic microorganisms. The contaminated groundwater is amended with methanol before fed to the denitrifying reactor followed by an anaerobic fixed bed and a reactor filled with granular activated carbon. The latter reactor is fed with traces of H_2O_2 required for aerobic conditions. This groundwater treatment began in October 1995 using sludge of a digestor and small volumes of enrichment and several pure cultures able to convert chlorinated ethenes.

THE LÜBECK SITE

Aerobic removal of 1,2-dichloroethane (DCA). In our company, the first full-scale process using bacteria isolated in the laboratory to treat contaminated groundwater started in 1990. The polluted site of a former pharmaceutical production plant was located in Lübeck (Germany), where DCA served as the single solvent for the extraction of pancreatine. Its production lasted from the early fifties until 1987 when the plant was decommissioned. The groundwater was polluted with **DCA as single contaminant**. It was detected in the top aquifer only, ranging from 3 to 15 m below the surface and flowing into surface waters about 350 m east of the production site, passing an area of private homes.

The major sources were removed and a detailed hydrogeological survey of the site was conducted. The pump and treat technology was chosen as remedial action respecting the chemical and physical characteristics of pollutant and environment of the site. Alternatives were regarded as either impractical or less efficient. The groundwater treatment options evaluated were air stripping, activated

carbon adsorption, oxidation by UV-ozone/UV-peroxide and biological degradation. The chemical and physical properties of DCA favored the choice of the biological method, since the alternatives were considered unfavorable due to the high solubility, the low sorptive properties (both favoring also the extraction by pump and treat technology) and the chemical structure (DCA has no double bond thus resists attack by ozone or hydroxyl radicals). Microorganisms able to mineralize DCA had been isolated previously and studies supported the applicability of the laboratory strains for the purification of groundwater (Stucki et. al., 1994).

Groundwater treatment plant and its performance. Details of the first 5 years of the pump and treat activity at the Lübeck site have been published previously (Stucki et al., 1995). The groundwater (up to 20 m^3/h) is pumped from a gallery of wells to two parallel sand filters followed by two filters filled with granular activated carbon run in series. The treatment plant was inoculated with DCA degrading microorganisms, and H_2O_2 and nutrients (N- and P-salts) were fed in trace amounts. Two years after start-up, an 8 m^3 rotating biological disc was taken into operation as an additional process unit in front of the sand filters to increase the biological degradation potential. The electrical power requirement for the whole plant is in the range of 5 ± 1 kW. The key operational figures are shown in Tab. 1

The main reason for the **economic success** of the biological purification of the DCA polluted groundwater was that the use of activated carbon as a major contributor to the operation costs became redundant with the biological degradation potential developing: The content of one of the carbon adsorbers had to be replaced 3 weeks after start-up due to the exhausted adsorption capacity. At that time, the biological degradation potential was almost zero. Later, biodegradation rates improved, and the time period until the next activated carbon filter was exhausted increased to several weeks. A third and so far last carbon exchange was required in the following winter season (January 1992). Since six years, no further activated carbon had to be exchanged.

The remediation technology was also an **ecological success**. Almost no energy in addition to the pumping energy to extract the groundwater was used. Furthermore, DCA was completely mineralized *on-site* as indicated by the oxygen consumption, the pH decrease and the chloride ions production during the passage of the groundwater through the bioreactors (Stucki et al., 1995).

The survival of at least one of the strains (*Xanthobacter autotrophicus* GJ10) originally inoculated could be confirmed four years after the start-up of the plant, when a gene probe taken from the plant effluent hybridized positively with the cloned dehalogenase gene from that organism (D.B. Janssen, Groningen, NL, personal communication).

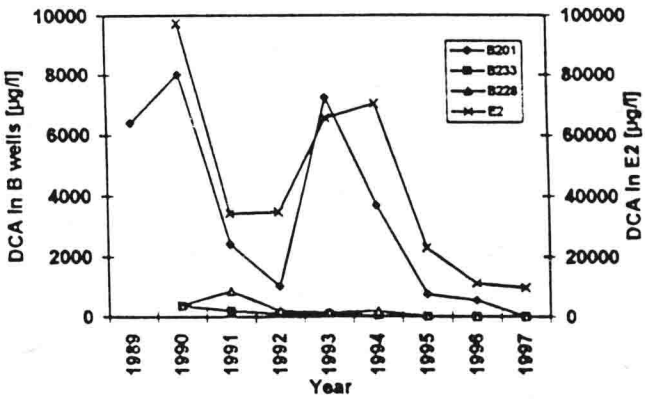
TABLE 1. Operational Key Figures

Parameter	Units	1990/91	1991/92	1992/93	1993/94	1994/95	1995/96	1996/97	Average	Total
Remediation time	years	1	2	3	4	5	6	7		8
Hydraulic load	m3/d	354	281	215	139	129	86	111	188	
Total water pumped + treated	m3/y	56000	103000	79000	50700	47000	28400	38300	57486	402400
Feed concentration	ug/l DCA	2614	4040	4666	9408	11421	3386	3444	5568	
DCA load	kg/d	0.9	1.1	1	1.3	1.3	0.5	0.4	0.93	
Total DCA removed	kg/y	146	415	369	477	500	195	150	322	2252
Activated carbon used	t/y	15	10	0	0	0	0	0		25
Peroxide used	t/y	11.7	20	10.8	9	8.5	4.2	4.3	10	68.5
Electrical power consumed	kWh	60000	20000	40000	41200	47000	43500	46500	42600	298200
Operation costs	DM/m3	3.2	1.01	0.46	0.54	0.68	0.72	0.42	1.00	
Costs saved	DM									883555

Operation costs = costs for energy (including pumping of groundwater), chemicals and sludge disposal, without manpower, maintain groundwater wells, analytics

During the past 9 years (1989 and 1990 observation, 1991 onwards remediation), almost 2.5 t of DCA was extracted and mineralized form the ground water at the Lübeck site. The contamination has moved in two waves through the site, with the top concentrations being measured in 1990 and 1993/94 (Fig. 1).

FIGURE 1. DCA concentrations in 3 observation wells (B 201 at the former production site, and B233/228 on the way towards the extraction well gallery) and in the most contaminated well E 2 of the extraction well gallery.



As a result of our remediation activities, the ground water situation at Lübeck has much improved. The DCA concentrations in plume have dropped considerable, and the average DCA concentration in the mostly polluted extraction well E2 has fallen by a factor of 10 (Fig. 1). Only 3 (E1 to E3) out of the 8 extraction wells are still in operation. The others were abandoned because they did not show any contamination for two or more years. The concentrations measured in control wells B 220 and B 221 downstream of the extraction gallery indicate that the spread of the groundwater contamination was successfully prevented. Most of the 13 private wells in the residential area up-stream the groundwater gallery show DCA concentrations of below 5 µg/l for the last 2 years.

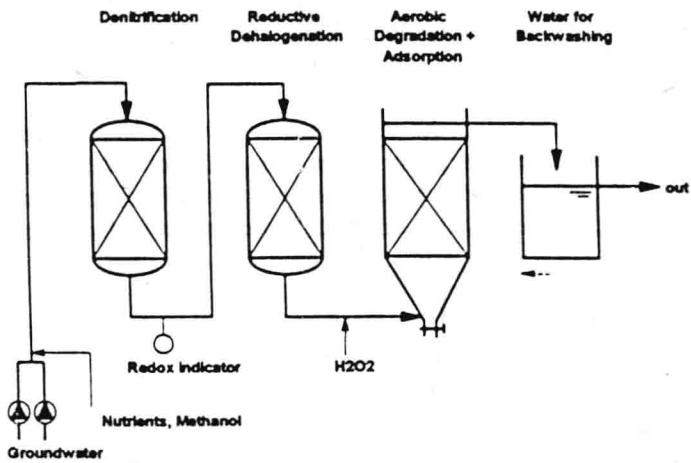
***In-situ* observations.** Groundwater samples from both observation and extraction wells show, that DCA may be partially mineralised *in-situ* even though the groundwater was never inoculated with the microorganisms used in the groundwater treatment plant. Groundwater samples showing the highest DCA concentrations also show the highest chloride ion concentrations, the pH is slightly lower than the pH in non-contaminated groundwater, and the oxygen concentration in DCA contaminated samples is depleted. In a few samples fed with additional DCA and kept under aerobic conditions, the chemical was mineralised, too. No DCA degrading microorganisms have been isolated from the Lübeck site so far. Ethene and ethane as potential anaerobic metabolites have not been analysed yet.

THE ALBSTADT SITE

Combined anaerobic-aerobic removal of TCE from groundwater. In a second case, a leaking concrete tank used as inverted siphon to collect and recycle TCE was identified as a source of soil and groundwater contamination. The treatment concept included the cleaning of the soil by soil vapour extraction and the pumping and treating of the groundwater containing up to 2 mg/l TCE using a patented biological process (Thüer et al., 1990), described in parts on laboratory scales by several other authors (e.g. De Bruin et al., 1992). Initially a target concentration of < 10 µg/l for the sum of the chlorinated ethenes was required since the treated water was intended to be re-infiltrated into the groundwater table. However, clogging of the infiltration well was feared due to the high calcium concentrations. Therefore, the environmental office agreed to divert the treated groundwater to the public sewer, where a target concentration of < 100 µg/l had to be reached.

Soil vapour was extracted from the unsaturated zone intermittently using a vacuum pump. It was treated by activated carbon. The condensate containing traces of TCE was fed to the groundwater treatment plant. The groundwater (temperature 12-13 °C, pH 7.5) contained less than 1 mg/l of iron and manganese ions, 150 mg/l of calcium ions, and 25-50 mg/l nitrate. It was pumped at a rate of 2 m^3/h through a series of three reactors each of a volume of 1 m^3 (Fig. 2).

FIGURE 2. Process to remove TCE.



Both the denitrification and the methanogenic reactor were inoculated with sludge of a digestor. The methanogenic reactor was further supplied with 0.3 l sludge obtained from pilot studies and adapted to reduce TCE and trichloroethene, and 0.2 l of a culture supplied by De Bruin (De Bruin et al., 1992). The aerobic activated carbon adsorber was inoculated twice with 2 l of a culture of *Methylosimus trichosporium* Ob3b grown on methane and with 1 l bacteria grown on cumene and isoprene, and 5 l material of a spontaneously operating anaerobic carbon adsorber which converted chlorinated solvents (Strohmeyer et al., 1991).

Anoxic conversion. Nitrate was removed to below 1 mg/l in the first reactor from the beginning of the groundwater treatment. The products built under anaerobic conditions were analysed after the second reactor. The redox potential as a very important process parameter (linked to a telephone alarm) is a reliable process indicator. A **potential of - 300 mV** indicates complete TCE conversion to *cis*-dichloroethene (DCE) via trichloroethene (TRI). About 80 to 90 % of the DCE expected was obtained. It was assumed that the remaining part of the substance might have been reduced further to ethene or ethane, both of which have not been analysed so far. Vinyl chloride was always below the quantification limit (10 µg/l). Traces of methane was detected as degradation product of the methanol fed.

Aerobic conversion. The moving bed activated carbon adsorber was supplied with H_2O_2 and was fed from the bottom (Fig. 2). Methane produced in the preceding steps was available as growth substrate for methanotrophs which have the potential to oxidised DCE. Based on isotherm studies, we calculated a biological DCE removal of about 60 to 90 %. The remaining part of the badly absorbable DCE was held back by the activated carbon. A few times, exhausted activated carbon was **partially exchanged** by virgin carbon added from the top of the reactor, thus keeping some of the grown DCE-oxidizing microorganisms in the system.

Outlook. The combined biological process is still insufficient to make the carbon adsorption redundant. The metabolic bottleneck both of the anaerobic and the aerobic metabolic route remains the DCE conversion. Is it possible to achieve better removal efficiencies if other carbon sources than methanol are fed? Or do we just miss those potent strains with improved catabolic rates?
The time required to maintain the plant amounts to about 30 to 60 min/week. The operation costs including energy for the pumps, methanol and peroxide were in the range of 1.3 DM/m3 when activated carbon was needed, and of about 0.8 DM during the last 15 months. About 35 kg TCE were removed from the site, so far.

REFERENCES

De Bruin, W.P., Kotterman, M.J.J., Posthumus, M.A., Schraa, G., and A.J.B. Zehnder. 1992. "Complete biological reductive transformation of tetrachloroethene to ethane." *Appl. Environ. Microbiol. 58:*1996-2000.
Strohmeyer, S.A., Winkelbauer, W., Kohler, H., Cook, A.M., and T. Leisinger. 1991. "Dichloromethane utilized by an anaerobic mixed culture: acetogenesis and methanogenesis." *Biodegradation 2:*129-137.
Stucki, G., and M. Thüer. 1994. "Increased removal capacity for 1,2-dichloroethane by biological modification of the granular activated carbon process. " *Appl. Microbiol. Biotechnol. 42:*167-172.
Stucki, G., and M. Thüer. 1995. „Experiences of a large-scale application of 1,2-dichloroethane degrading microorganisms for groundwater treatment." *Environ. Sci. Technol. 29*:2339-2345.
Thüer, M., Reisinger, M., and G. Stucki. 1991. "Ground water purification process." *European patent EP 0456 607 A1*, pp 1-4; and *US patent-5180495*.

REMOVAL OF DI-, TRI-, TETRA-, AND PENTACHLOROPHENOL MIXTURES IN A 5 L CONTINUOUS AEROBIC PACKED COLUMN

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ABSTRACT: A 5 L column packed with *tezontle* (a basaltic scoria) and inoculated with *Pseudomonas fluorescens* was operated at room temperature, under unaerated conditions. Three different chlorophenol mixtures were assessed: 200, 400 and 600 mg/L as total chlorophenols (with proportional concentrations of di-, tri-, tetra-, and pentachlorophenol). Both total (4-aminoantipirine method) and specific chlorophenols (GC/Mass spectrometry system), and total organic carbon (TOC analyzer) removals were measured. Of the total initial chlorophenols for the concentrations of 200, 400 and 600 mg/L, the packed column was able of removing 43-56, 75-80, and 77-86%, respectively. The biodegradation rates for each concentration range were 33-174 mg/L.day (for 200 mg/L), 118-494 mg/L.day (for 400 mg/L), and 179-725 mg/L.day (for 600 mg/L). These variations are due to the hydraulic residence time (HRT), controlled at values of 15.5, 31, and 62 hours. The lowest values correspond most of the time to HRT = 62 hours, and the higher values to HRT=15.5 hours. Total organic carbon TOC removals were around 38-59%. The system demonstrated to be a powerful tool in the bioremediation of waters contaminated with chlorophenols.

INTRODUCTION

Chlorophenols and derivatives have been widely used as pesticides, antifungal agents and herbicides. Due to the high toxicity of this xenobiotics, only a few biological treatment systems are suitable for the bioremediation of contaminated aquifers, water bodies or soils. The use of aerobic submerged filters, where specific microorganisms or mixtures of them have been immobilized, has been reported because of its high potential applications (Arvin, 1991, Seignez *et al.*, 1993, Fava *et al.*, 1996, Torres *et al.*, 1997b). Some of these experiences were carried out in small systems working batchwise. In most of the quoted papers the degradation of simple compounds and never of complex mixtures of them were assessed.

Objective. The aim of this work is to report the successful use of a 5 L continuous aerobic column with a low-cost packing material, for the biodegradation of di, tri, tetra, and pentachlorophenol mixtures in concentrations up to 600 mg/L as total chlorophenols. The relationship between chlorophenol's removal and the hydraulic retention time was investigated. TOC removals and cell loads in the packed column during the continuous process were also evaluated and discussed.

MATERIALS AND METHODS

Tezontle is a basaltic scoria abundant in the central Valley of México. It is a very cheap, light, resistant, porous and easy to handle construction material. Three different *tezontle* size distributions were characterized in terms of global porosity (%), apparent density (g/ml), and strength indirectly, as the solubilities in HCl and NaOH (%), as well as the suitability of being colonized by *Pseudomonas fluorescens* cells (as $FCU/g_{support}$). These mixtures were termed fine (particles between 0.84-1.19 mm), medium size (particles between 1.19-1.68 mm) and gross portion (particles between 1.68-3.36 mm).

The employed column is a perspex cylinder (95 mm in diameter, 900 mm in height). Active volume is around 3.5 liters according to the porosity of the employed packing material. The cross section is 0.007 m^2. It was packed with 100 mm height of gravel and 710 mm height of a given *tezontle* granulometry. The system was connected as shown on figure 1. A peristaltic pump was used in order to pump CPh solutions and circulate them upwards through the column. The system was started inoculating the *tezontle*. For this purpose, a 15 liters fermentation *of Pseudomonas fluorescens* in YPG (yeast extract, caseine peptone and glucose, 10 mg/L of each one) medium was used. Cell concentrations during the fermentation process were monitored by means of optical density. The rich culture was recirculated through the column for 24 hours and the wasted medium was discharged. Different solutions of chlorophenols in Dapaah medium (Dapaah and Hill, 1992) were fed to the system. The cell load in the column (as $CFU/g_{support}$) was evaluated periodically by platting dishes of YPG and King B (specially designed for fluorescent microorganisms as *P. fluorescens*) media as described by Salinas, 1997. Solutions containing mono-, di-, and trichlorophenols were fed consecutively to the column. The phenols and total organic carbon (TOC) were measured as detailed by Salinas, 1997 . Mixtures consisting of mono-di, mono-di-tri and mono-di-tri-pentachlorophenol were fed to the column. The procedure lasted 3 months. The results of this experimental work were reported elsewhere (Salinas, 1997). After this, di-, tri-, tetra-, and pentachlorophenol mixtures at 200, 400 and 600 mg/L were employed. Every total phenol's concentration was evaluated at three different hydraulic retention times, HRT, defined in the previous experiences. $FCU/g_{support}$, temperature, total and specific chlorophenol concentrations, and TOC were evaluated periodically. GC/mass techniques were employed in order to determine specific chlorophenols concentration in the effluents, the 4-aminoantipirine method, for the total chlorophenols evaluation, using the appropriate calibration curve. For more details, see Torres *et al.*, 1997a.

RESULTS AND DISCUSSION

The characterization of the packing material with different size distributions is given on table 1. Differences among the three size distributions were observed. Apparent densities were between 0.77 and 0.93 mg/L. Solubility values are an accelerated-test measurement of the decay of the material. The harder mixture is the gross portion, followed by the fine and medium sizes. Regarding the suitability for being colonized by *Pseudomonas*, it is interesting to note that fine and medium portions gave a very good cell load value (around 10^9 $FCU/g_{support}$), but the gross