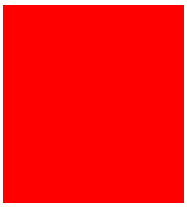
STUDY TITLE

INTRINSIC BIODEGRADATION OF CHLORINATED SOLVENTS IN AQUIFER MICROCOSMS FROM THE ALTONA CHEMICAL COMPLEX, ALTONA, AUSTRALIA

Author(s)



Study Completion Date

11 January 2001

Performing Laboratory

Environmental Chemistry Research Laboratory
Toxicology and Environmental Research and Consulting
The Dow Chemical Company
Midland, Michigan 48674

Laboratory Project Study ID

990242

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

Compounds: [redacted]
1,1,2-TRICHLOROETHANE

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[redacted]

SIGNATURE PAGE

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[redacted]

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[redacted]

ARCHIVING

Permanent records of all data generated during the course of this study, the protocol, protocol revisions/changes, and the final report are archived at Toxicology and Environmental Research and Consulting, The Dow Chemical Company.

ABSTRACT

Groundwater contamination thought to be associated with historical operations at the Dow plant site in the Altona Chemical Complex has been the subject of an extensive remediation investigation. Beneath this manufacturing facility exists a highly fractured basalt formation containing groundwater contaminated with [redacted] 1,1,2-trichloroethane (112-TCA), [redacted] and other chlorinated solvents. Results of recent field investigations have indicated that dissolved-phase contamination appears to be attenuating downgradient from the manufacturing facility. Examination of historical groundwater data for footprints of natural attenuation suggests that intrinsic biodegradation may play an important role in the fate and transport of chlorinated solvents at this site.

A laboratory study was initiated to evaluate the potential for intrinsic biodegradation of [redacted] Laboratory microcosms were prepared with crushed basalt and groundwater from the site to simulate actual field conditions, and were spiked with [redacted] Results show that approximately [redacted] of the parent [redacted] were degraded after 109 days. Compared to the observed [redacted] in sterile microcosms, this difference [redacted]) can be attributed to biological degradation of [redacted] by indigenous bacteria. Transient metabolites, namely [redacted] and [redacted], were occasionally observed over the course of the study. However, complete dechlorination of [redacted] was observed in most microcosms at the end of the one-year study. Conversely, biodegradation of [redacted] was not observed in aquifer microcosms when comparing results from viable microcosms to those of sterile (or control) microcosms.

Biotransformation potential for [redacted] and 112-TCA was assessed in microcosms amended with nutrients. Conclusive evidence for biodegradation of these compounds was not observed in the current study.

The results of this study indicate that microbial degradation of [redacted] is a significant mechanism for natural attenuation of chlorinated methanes in this fractured basalt aquifer system. Biodegradation of this test compound suggests that microbes indigenous to the Altona aquifer may have the ability to degrade other types of chlorinated solvents in the groundwater. As a result, monitored natural attenuation may be employed as a component of the remedy at this chlorinated solvent-contaminated site.

[redacted]

INTRODUCTION

Historical operations at Dow Chemical (Australia) Ltd manufacturing facility in Altona, Australia, are thought to have contributed to contamination of local groundwater. Dow Chemical (Australia) Ltd operated a small [redacted] plant from 1961 to the mid-1970's, which was coupled to a [redacted] plant operated by BF Goodrich on an adjacent site. Dow Chemical initiated a groundwater sampling program to understand the type and extent of groundwater contamination originating from the Dow Altona plant, and a groundwater sampling program has been carried out on a quarterly basis since 1997. The groundwater beneath the site contains elevated levels of chlorinated solvents, specifically [redacted] 112-TCA, and their lower-chlorinated homologs including [redacted]. Figure 1 details the regions where contamination has been measured in the groundwater. The chlorinated organic concentrations detected have generally been consistent over several sampling rounds, indicating no rapid advance of the groundwater plume. In addition, the overall distribution of chlorinated organic compound concentrations is consistent with the apparent groundwater flow direction, which is to the southeast of the site.

Natural attenuation has come under increased awareness as a potentially important process that can lead to the destruction of many subsurface contaminants. The most recently accepted regulatory definition for natural attenuation is "the biodegradation, dispersion, dilution, sorption, volatilization, and/or chemical and biochemical stabilization of contaminants to effectively reduce contaminant toxicity, mobility, or volume to levels that are protective of human health and the ecosystem" (USEPA, 1997). In addition to this USEPA definition, the Governor in Council of Victoria (Australia) declared in the State Environmental Protection Policy that "attenuation means the reduction in concentration of contaminants in a solution passing through a porous medium by natural mechanisms including removal by ion exchange, chemical precipitation, adsorption filtration or biodegradation and hydrodynamic dispersion…" (Victoria Government Gazette, 1997). Considering these definitions, it is evident that regulatory bodies view natural attenuation as a viable process that may result in the destruction and/or immobilization of groundwater contaminants, resulting in a process that acts to protect human health and the environment which may ultimately be supported as a remedy. One of the most important natural attenuation processes, intrinsic biodegradation, actually results in the destruction of the contaminant molecule to a

chemical form that is harmless to human health and the environment. The focus of this study was to assess whether or not intrinsic biodegradation of chlorinated solvents could be occurring in the Altona aquifer.

Prior to initiation of this study, groundwater was sampled from three wells and analyzed for chlorinated organic constituents. Table 1 shows a list of compounds detected in the groundwater obtained from off-site wells (OSW) 23, 5, and 8. OSW-23 is located near the heart of the contaminant plume, OSW-5 is located ~400 meters downgradient of OSW-23, and OSW-8 is located another 700 meters downgradient, near the leading edge of the plume. The monitoring well locations are indicated in Figure 1. Preliminary examination of these data suggests that intrinsic biodegradation of chlorinated solvents may be occurring in the subsurface. Evidence for this includes apparent loss of parent compounds in the field and presence of daughter products in the groundwater. These observations prompted further assessment of the role of biological processes in the removal of organic constituents from groundwater at the Altona site.

Because of the current interest in the use of natural attenuation as a remedy for contaminated sites, a number of protocols have been developed to facilitate implementation of this technology (Wiedemeier *et al*., 1999, and RTDF, 1997). Wiedemeier *et al*., authors of the most commonly used protocol, detail the lines of evidence needed to support natural attenuation as a remedy. The three lines of evidence that are generally required are:

1. Documented loss of contaminants at the field scale
2. Contaminant and geochemical analytical data
3. Direct microbiological evidence

The first and second lines of evidence require characterization of the groundwater chemistry with respect to nature and distribution of the contaminants, as well as determination of whether the biogeochemical conditions are conducive for biodegradation. The third line of evidence, direct microbiological evidence, requires demonstration that the indigenous microorganisms are capable of degrading the contaminants present at the site. This line of evidence is typically demonstrated by performing laboratory microcosm studies, which are often used to evaluate the ability of naturally occurring organisms to degrade groundwater and/or soil contamination.

These protocols also highlight recent literature, which show that a wide variety of groundwater contaminants, including chlorinated solvents, are susceptible to natural biologic and abiotic degradation mechanisms. Researchers first demonstrated the potential for anaerobic biotransformation of halogenated aliphatics in the 1980s (Bouwer and McCarty, 1983; Klecka *et al*., 1990; Parsons *et al*., 1983; and Vogel *et al*., 1987). Biodegradation of chlorinated ethanes, ethenes, and methanes have been demonstrated in the laboratory and field under a variety of redox conditions in the absence of oxygen. The primary mechanism by which transformation can occur is "reductive dechlorination", whereby Cl^- ions are released as the solvent molecule accepts electrons from an electron donor. Biodegradation of high concentrations of chlorinated solvents has been the subject of research in the recent past. Yang and McCarty (2000) have demonstrated that bacteria are capable of biodegrading saturation levels of chlorinated solvents (e.g. In fact, biological degradation of high-level solvent concentrations has been shown to be coupled with enhanced DNAPL dissolution rates into groundwater.

More specifically, chlorinated methanes, ethanes, and ethenes have been shown to biodegrade in groundwater environments. , the most highly has been shown to biodegrade under a variety of environmental redox conditions (Criddle *et al*., 1990; Egli *et al*., 1990). and have also been shown to biodegrade in aquifer environments. Major (1991) was the first to observe complete biological dechlorination of in groundwater near North Toronto, Ontario. Wilson (1994) has demonstrated that intrinsic biodegradation of occurs in groundwater at a NPL site near St. Joseph, Michigan. Chlorinated ethane biodegradation has been demonstrated by Chen (1996), where was observed to biodegrade to 112-TCA, Klecka *et al*. (1998) recently demonstrated that naturally occurring microbes have the ability to biodegrade to ethene in an aquifer environment. As a result, sufficient evidence exists which suggests that chlorinated solvent contamination is susceptible to biodegradation by microorganisms indigenous to aquifer environments.

Examination of the degradation pathways for the most highly chlorinated ethanes, ethenes, and methanes found at the site assisted in determining which compounds will be used for laboratory microcosm studies. Figure 2 details the expected pathways for transformation of and 112-TCA in an aquifer environment. Although it is recognized that and are parent compounds near the source area, it is apparent from these degradation pathways that possible sources of and in the

downgradient areas could be a result of the anaerobic biodegradation of [redacted] and/or 112-TCA. A primary contaminant in the field is 112-TCA, and is therefore an obvious choice for laboratory testing. [redacted] on the other hand, is not as prevalent in Altona groundwater, but it is a suitable model for the family of chlorinated ethenes – [redacted] and [redacted] – since these daughter products have been detected on-site. Therefore, [redacted] and 112-TCA are ideal candidates to be used in laboratory microcosm studies to assess the biodegradation potential of chlorinated ethanes and ethenes detected in groundwater beneath the Altona Chemical Complex.

In addition to these compounds, [redacted] was used as a test compound to assess the biodegradation potential of chlorinated methanes. Figure 3 details the expected pathways for transformation of [redacted] in a reducing aquifer environment. [redacted] was used as a model compound to test for biodegradation of chlorinated methanes such as [redacted] and [redacted] which can be biodegraded to CO_2 by aquifer bacteria (Davis and Madsen, 1991). [redacted] is a predominant compound found in Altona groundwater; therefore, use of [redacted] as a test compound will provide an assessment of the likelihood of [redacted] biodegradation.

The primary objective of this investigation was to determine if these natural processes of contaminant reduction/destruction are occurring in the subsurface beneath the Altona Chemical Complex. Laboratory studies were conducted to examine the biodegradation of representative chlorinated solvents in basalt/groundwater microcosms under conditions that simulate those in the aquifer. Biostimulation studies were also conducted to assess the potential for biological degradation of chlorinated solvents in the presence of nutrient amendments.

MATERIAL AND METHODS

Sample Collection

Basalt Samples

Basalt samples from the region of suspected contamination were obtained from new, temporary borings located near existing wells OSW-8 and OSW-23 (see Figure 1). The samples were obtained by advancing a borehole to the targeted sample interval using air rotary techniques. Once the borehole was advanced to the sampling location, wireline coring techniques were used to collect the basalt samples. One core was collected from each sampling location using a 1.5-meter stainless steel core

barrel. After each core was lifted to the surface, it was subdivided into three 50-cm sections, covered with plastic core sleeves, then placed within rigid PVC pipe to facilitate shipment. To assist in maintaining an anaerobic environment, pure nitrogen gas was used to purge the headspace inside two of the three PVC pipes prior to sealing the endcaps. The remaining section from each sampling location was sealed under ambient (aerobic) conditions. All sections were placed on ice in an insulated cooler (esky) and shipped overnight by Federal Express to the Environmental Chemistry Research Laboratory in Midland, Michigan.

Groundwater Samples

Groundwater samples were collected from wells OSW-5, OSW-8, and OSW-23 (see Figure 1). Fifteen liters were collected from OSW-23, ten liters from OSW-8, and seven liters from OSW-5. Groundwater was collected in one-liter polyethylene bottles and filled to zero headspace. The samples were placed on ice in an insulated cooler (esky) and shipped overnight by Federal Express to the Environmental Chemistry Research Laboratory in Midland, Michigan.

Test Chemicals

Intrinsic Biodegradation Study

The test chemicals used in the intrinsic bioremediation study were of the highest available purity. Radiolabeled (^{14}C) test chemicals were used during these experiments to facilitate determination of a material balance for each compound over the duration of the experiments, and also used to aid in identification and quantification of transformation products. Radiolabeled [redacted] and [redacted] were added to the microcosms as concentrated solutions in 1,4-dioxane.

Biostimulation Study

Non-radiolabeled test chemicals were also used to assess the biodegradation potential in laboratory microcosms. Non-radiolabeled 1,1,2-trichloroethane (Ultra Scientific Lot 070177) and [redacted] were added to microcosms as concentrated solutions in 1,4-dioxane.

[redacted]

All other chemicals used in preparation and analysis of the microcosms were of reagent-grade and obtained from commercial sources.

Preparation of Laboratory Batch Microcosms

Preparation of Crushed Basalt

Immediately prior to preparing the microcosms, solid basalt cores were crushed using a device constructed in the laboratory. This device consisted of a heavy stainless steel tube (4 inches in diameter and 12 inches long) with a stainless steel plug welded on one end of the tube. Pieces of the basalt cores were placed inside this tube and broken into smaller pieces by pounding the core with a foot-long stainless steel rod (3/4-inch diameter). Once the basalt pieces were crushed to a consistent size, the mixture was homogenized and portions were added to each microcosm as described below. This crushing procedure was performed in either an anaerobic chamber or in a fume hood (aerobic) in order to maintain the desired redox condition of the samples.

Intrinsic Biodegradation Study

Laboratory batch microcosms (LBM) were constructed using crushed basalt and groundwater collected near the Altona Chemical Complex. Table 2 details the experimental plan to test intrinsic bioremediation activity in groundwater and basalt native to the aquifer underlying Altona. For the intrinsic bioremediation studies, 5 grams of crushed basalt was placed in sterile 20 mL glass vial. Ten milliliters of authentic site groundwater were added to each bottle, resulting in ~50% headspace. Test chemicals were added by addition of 5 µL of stock solution prepared in 1,4-dioxane. Five milligrams per liter each of [redacted] and [redacted] were added to LBM, and each microcosm contained approximately 1.2 µCi of [^{14}C] activity ([redacted] and [redacted] After addition of the test chemicals, each microcosm was sealed immediately with a Teflon-lined red butyl rubber septum and an aluminum crimp seal. Sufficient quantities of LBM were prepared in order to allow for analysis of triplicate samples at six time points during the course of the study.

Biostimulation Study

These studies were performed using microcosms consisting of 250-mL glass serum bottles containing 75 grams of basalt and 150 mL of site groundwater. Because test chemicals were non-radiolabeled, groundwater was stripped to remove volatile organics prior to use. Stripping was performed by bubbling pure N_2 gas through site

[redacted]

groundwater for a period of 30 minutes. Test chemicals were added by addition of [redacted] of stock solution prepared in 1,4-dioxane. Non-radiolabeled test chemicals ([redacted] and 112-TCA) were added to LBM at approximately 5 mg/L final concentration. Toluene (15 mg/L) or a yeast extract (10 mg/L) and lactate (100 mg/L) mix was added to microcosms to provide microorganisms with co-substrates for chlorinated solvent biodegradation. Table 2 indicates which microcosms received these amendments. After addition of the test chemicals and co-substrates, each microcosm was sealed immediately with a Teflon-lined Mininert valve to allow for periodic sampling of the microcosm groundwater. Sufficient quantities of the LBM were prepared in order to allow for triplicate analyses throughout the course of this study.

Microcosms were also prepared using groundwater from OSW-5 and OSW-23 without adding crushed basalt. These studies were performed to assess the ability of groundwater bacteria to degrade chlorinated solvent contamination. The intent was to characterize any differences in the ability of groundwater bacteria and attached bacteria at biodegrading solvent contamination. LBM constructed with OSW-5 groundwater were prepared by adding 200 mL of native (or non-stripped) groundwater to each 250-mL serum bottle and sealing with a Teflon-lined Mininert valve. Similar LBM were also prepared with a 50:50 mix of stripped:non-stripped groundwater from OSW-23, which contained a higher total chlorinated organic concentration (TCOC) than the OSW-5 microcosms. Preparation of these LBM involved adding [redacted] of [redacted] or [redacted] to each microcosm. Toluene or yeast extract/lactate were added to these microcosms to stimulate microbial growth and/or biodegradation of the target compounds. Sufficient quantities of LBM were constructed in order to allow for triplicate analyses throughout the course of this study.

Biologically-Inhibited Microcosms

Loss of the test chemicals due to irreversible sorption, permeation of the septum barrier, or other abiotic processes were measured using biologically-inhibited (e.g. killed) LBM. These microcosms were constructed in a manner similar to that of the active microcosms, with the incorporation of sediment that was sterilized by autoclaving (1 hr for three consecutive days), filter sterilizing groundwater (0.22 µm membrane), and addition of a chemical sterilant (500 mg/L $HgCl_2$). A representative

[redacted]

set of biologically-inhibited microcosms were constructed for each of the aforementioned studies.

Redox Conditions

In order to evaluate the effects of redox potential on degradation, biotransformation of the test chemicals was examined under both aerobic and anaerobic conditions. Aerobic LBM were constructed as described above in laboratory fume hoods (under standard indoor atmospheric conditions). Anaerobic microcosms were prepared in a glove box under an anaerobic atmosphere equilibrated from a mixed gas containing 79% N_2, 29% CO_2, and 2% H_2. A redox indicator dye, resazurin, was added to each microcosm as a concentrated solution to result in a final concentration of 2 mg/L of the total aqueous volume. All LBM were incubated at 20 ± 2 °C. Aerobic microcosms were gently agitated during incubation while anaerobic microcosms remained static.

Microcosm Sampling

Microcosms prepared for the intrinsic biodegradation study were sacrificed and analyzed eight times over the course of this one-year study. On each of these dates, three microcosms were sacrificed and loaded onto an autosampler for headspace analysis by gas chromatography.

Biostimulation microcosms were sampled eight times during this study. For each sampling event, a representative 1 mL volume of groundwater was removed from each microcosm and added to a 20 mL vial containing 15 mL of 15% NaCl solution. Vials were then capped with Teflon-lined red butyl rubber septa and sealed with aluminum crimp caps. Vials were then loaded onto an autosampler for headspace analysis.

Analytical Methods

The distribution of ^{14}C-labeled parent compounds (and as well as intermediate and terminal degradation products, was determined by headspace gas chromatography with a radioactivity monitor. Samples were analyzed using a system composed of a Tekmar model 7000 headspace autosampler and a Hewlett Packard model 5890 gas chromatograph equipped with an INUS Systems, Inc. model GC3 GC Radioactivity Detector. The headspace autosampler operated as follows: 30-minute equilibration at 85 °C, sampling valve temperature of 105 °C, sample loop size of 3 mL at 105 °C, transfer line temperature of 110 °C with helium flow rate of 20 cm^3/min. The valve sequence used initially pressurized each vial to 10 psi for 10 seconds, vented to sample

loop for 8 seconds, and then injected the sample volume for 2 minutes. The gas chromatograph was operated according to the following specifications. The injector was held at 200 °C in continuous split mode with a 3 cm^3/min septum purge, 5 psi head pressure of helium, and an approximately 10:1 split ratio (35 cm^3/min total split flow). Separations were achieved using a Chrompack Poraplot Q HT column (25 m x 0.53 mm). The oven was operated at 45 °C for 3.5 minutes, ramped to 180° at 30°/min, held for 8 minutes, ramped to 195° at 25°/min, held for 4 minutes, ramped to 250° at 25°/min, and held for 10 minutes.

Non-radiolabeled test compounds were analyzed using a Hewlett Packard model 7694 headspace sampler and a model 5890 gas chromatograph equipped with a flame ionization detector according to the above specifications. The flame ionization detector was operated at 250 °C.

Total radioactivity was determined by liquid scintillation counting. LBM samples were mixed with 6 mL liquid scintillation cocktail (Aquasol by Packard Instrument Company) in a 7 mL vial prior to analysis. Samples were counted using a Beckman model 6000 liquid scintillation counter.

Groundwater samples were also submitted to Midwest Laboratories, Inc., Omaha, Nebraska, for analysis of major minerals and trace metals, inorganic anions, and volatile organic compounds to confirm parameter concentrations in the groundwater used for this study.

RESULTS

Groundwater Characterization

Groundwater samples were collected in September 1999, from wells OSW-5, OSW-8, and OSW-23 and analyzed prior to initiation of this study. As summarized in Table 1, field measurements indicated that the groundwater pH was neutral (6.7 to 7.8) and temperature varied between 17.2 to 17.9 °C. Dissolved oxygen concentrations varied from <1.0 ppm near the source area to greater than 2 ppm at a location approximately 400 meters downgradient of the suspected source area. These samples were also found to contain varying levels of chlorinated solvent contamination. At well OSW-23, which is in close proximity to a suspected source zone, greater than 1,100 ppm of total chlorinated organics were detected with 112-TCA (167 ppm) and [redacted] being the dominant chemical species present. At OSW-5, which is located ~400 meters

[redacted]

downgradient of OSW-23, the concentration of total chlorinated organics decreased to ~16 ppm. Here, the dominant chemical species were 112-TCA (8.5 ppm) and [redacted], which exist at much lower levels than are present in the upgradient location near OSW-23. Further downgradient of OSW-5 lies OSW-8, which contained ~2 ppm total chlorinated organics in September 1999. These results indicate a downward trend in total chlorinated organic concentration moving from the source zone towards downgradient well OSW-8, with higher ratios of (suspected) daughter products to parent compounds in the downgradient region.

Biological Activity in Subsurface Material

Studies were conducted to assess the potential for microbial degradation of chlorinated solvents under conditions that simulate those in the Altona aquifer. Biodegradation was examined under both aerobic and anaerobic conditions at 20 ± 2 °C in microcosms prepared with crushed basalt and groundwater from the Altona site. The test chemicals - [redacted] 112-TCA, [redacted] and [redacted] - were chosen to represent the principal contaminant groups found in groundwater at the site, namely chlorinated methanes, ethanes, and ethenes.

Intrinsic biodegradation study

Biodegradation of [redacted] and [redacted] were examined under aerobic and anaerobic conditions in microcosms containing crushed basalt and groundwater from two locations, OSW-8 and OSW-23. These microcosms were constructed to evaluate the potential for indigenous bacteria to biodegrade 5 mg/L each of [redacted] and [redacted] in groundwater from the two indicated well locations. The primary difference between the two well locations was the higher TCOC as the groundwater from OSW-23 contained approximately 1,100 mg/L TCOC versus 2 mg/L TCOC in groundwater from OSW-8. As a result, this experiment examined the potential for biodegradation of [redacted] and [redacted] at one location where these contaminants persisted and bacteria had presumably adapted to the presence of [redacted] and [redacted] (OSW-23), and at another location where these groundwater constituents were not detected (OSW-8) and bacteria had not adapted to the presence of these contaminants.

In anaerobic microcosms prepared using material from OSW-8, biological degradation of [redacted] was observed after 24 days when approximately 42% of the added [redacted] were degraded (see Figure 4). Five percent of the initial radioactivity was recovered as chloroform, 4% as methylene chloride, and 9% as $[^{14}C]CO_2$, indicating

[redacted]

that some degree of complete dechlorination had occurred. After one year, all of the [redacted] was degraded and 28% of the initial radioactivity was recovered as [^{14}C]CO_2. No chloroform or methylene chloride was measured after one year. The estimated time to achieve 50% disappearance of the parent compound in the anaerobic microcosms was ~35 days. No significant biodegradation of [redacted] was observed in aerobic microcosms after a one-year period (as compared to the sterile controls).

In anaerobic microcosms prepared using material from OSW-23, approximately 45% of the added [redacted] was degraded after 24 days (see Figure 5). Twenty-two percent of the initial radioactivity was recovered as [redacted] and [redacted] and 3% was converted to [^{14}C]CO_2. After one year all of the [redacted] was degraded while 1% was recovered as [redacted], 6% was recovered as [redacted] and 7% was recovered as [^{14}C]CO_2. The estimated time to achieve 50% disappearance of the parent compound in these microcosms was ~45 days.

As the study progressed, a decline in the material balance of [^{14}C]-labeled compounds in sterile (control) microcosms was observed. It appeared that a portion of the initial radioactivity had become incorporated into new biomass, was bound to the solid phase, or was lost from the test system. After 352 days of incubation, only 42% of the initial radioactivity were recovered as [redacted] in the aerobic (killed) controls and 1% was recovered as [redacted]. In the anaerobic (killed) control microcosms, 49% of initial radioactivity was recovered as [redacted] 1% as [redacted], and 5% as [^{14}C]CO_2. These are microcosms where all biological activity was halted by the presence of 500 mg/L $HgCl_2$. To assess how much, if any, radiolabeled compound was absorbed by the Teflon-lined septa; microcosms analyzed on October 25, 2000 (day 352 of study) were disassembled. The septa were placed in scintillation cocktail and analyzed on a liquid scintillation counter for total radioactivity. Results indicated that about 30% of the total radioactivity added to aerobic (killed) control microcosms were associated with radiolabeled compounds absorbed by rubber septa. Septa from viable aerobic microcosms also showed similar results (29% of total radioactivity recovered). For the anaerobic control microcosms 32% of total added radioactivity was associated with absorbed radiolabeled compounds, and 23% radioactivity was recovered from viable anaerobic microcosms. The total radioactivity recovered from anaerobic microcosm septa was lower as a result of the degree of biodegradation of the parent compounds in those active microcosms. Given these results, it is evident that much

[redacted]

of the radioactivity "lost" in the control microcosms was a result of ^{14}C-labeled compounds being absorbed by the Teflon-lined red butyl rubber septa.

The potential for intrinsic biodegradation of [redacted] was also evaluated during this study. Degradation of [redacted] was observed in a limited number of microcosms where [redacted] and/or [redacted] were recovered as daughter products, but levels were very low as compared to parent compound concentrations. As a result evidence for intrinsic biodegradation of [redacted] as evaluated during this microcosm study, was inconclusive.

Biostimulation Study

To determine if biotransformation was limited by availability of co-substrates, additional microcosm studies were conducted using biostimulation – a technique used whereby co-substrates and/or nutrient amendments are added to stimulate bacterial growth and biodegradation. Microcosms were amended with either 15 mg/L toluene or a mixture of 100 mg/L lactate and 10 mg/L yeast extract. These microcosms were incubated under both aerobic and anaerobic conditions and degradation of parent compounds were evaluated at selected time points throughout the study.

Consistent degradation of [redacted] was not observed in microcosms amended with either toluene or lactate/yeast extract. Trace concentrations of [redacted] and [redacted] were occasionally measured in random microcosms. However, a clear trend of [redacted] degradation associated with an increasing concentration of daughter products ([redacted], and [redacted]) was not observed.

The biodegradation of 112-TCA was also evaluated in this experiment. Amendments included toluene and yeast extract/lactate in both aerobic and anaerobic microcosms. Suspected daughter products (VC and EDC) were noted in select microcosms after six months incubation. VC was measured at levels as high as 0.5 mg/L after six months incubation. EDC was measured in a larger number of microcosms at concentrations as high as 6 mg/L, but these levels were only noted in microcosms prepared with groundwater and basalt from OSW-23. Groundwater used in these microcosms was stripped of volatile organics prior to study initiation, however chlorinated organics originally sorbed to basalt may have desorbed into the groundwater over the course of the experiment. As a result, it is possible that the observed EDC concentrations in OSW-23 microcosms were a result of EDC desorption from the solid phase (basalt) into the liquid phase (groundwater).

Evidence of lesser-chlorinated daughter products of 112-TCA biodegradation was not observed.

An additional component of the biostimulation study was to examine the potential for biodegradation of [redacted] and [redacted] in aerobic and anaerobic microcosms prepared with only groundwater and amended with co-substrates (crushed basalt was not added). The results of this component of the study are not conclusive with respect to biodegradation of [redacted] and [redacted] since recovered radioactivity was insufficient to draw any conclusions regarding the fate of the these compounds.

DISCUSSION

The importance of biological processes on attenuation of groundwater contaminants at the Altona Chemical Complex site was examined as part of an ongoing investigation. Chlorinated solvents have been detected in the groundwater both on and off the Dow Chemical (Australia) Ltd site, and the current study was requested to assess remedial alternatives for appropriate action at this site.

Analysis of the groundwater biogeochemistry suggests that anaerobic conditions are predominant in the vicinity of Dow Chemical (Australia) Ltd. Downgradient of the suspected source zone approximately 400 meters, dissolved oxygen levels increase to near 2 mg/L, indicating that slightly aerobic conditions predominate. Between these two regions exists a transition zone where mildly reducing conditions prevail. Ferrous iron concentrations were quite high in most wells near the source zone(s), with levels in excess of 20 mg/L being measured in groundwater from OSW-23. Nitrate levels were very low to non-detect. Sulfate levels near OSW-23 were measured at 90 mg/L, with levels further downgradient near OSW-8 at 388 mg/L. However, no sulfide was measured in this region, which suggests that sulfate reduction is not an active process in this portion of the aquifer. Dissolved methane levels were also very low. As a result the conceptual model of the biogeochemistry in the aquifer beneath the Altona Chemical Complex shows that slightly reducing conditions predominate, with iron reduction being the dominant microbial process.

The current laboratory study was initiated to assess the likelihood for intrinsic biodegradation of chlorinated solvents in microcosms prepared from material obtained from Altona. The test chemicals – [redacted] 112-TCA, and [redacted] – were chosen to represent the group of chlorinated methanes, ethanes, and ethenes found in groundwater

[redacted]

at the site. Although [redacted] is a compound that does not persist at the Altona site, a related compound, [redacted] exists at levels exceeding 11 mg/L.

Experimental results show that [redacted] was degraded in microcosms from two different regions of the site and under anaerobic conditions. The time required for 50% degradation of [redacted] ranged from 45 days in OSW-8 microcosms to 35 days in OSW-23 microcosms. Extensive mineralization of the test chemical [redacted] was observed, as approximately 28% of the initial radioactivity were converted to [^{14}C]CO_2 after 352 days of incubation. The balance of radiolabeled material in each microcosm was likely absorbed by microcosm components (e.g. septa) and may have been incorporated into biomass during growth. These results suggest that an active microbial community exist in the Altona aquifer that has the ability to dechlorinate [redacted] to non-hazardous endproducts, with perhaps the ability to dechlorinate other types of chlorinated solvent contamination.

Biodegradation of [redacted] and 112-TCA were not demonstrated during this study. [redacted] and 112-TCA, evaluated in a biostimulation study, were shown to degrade slightly under anaerobic conditions, but the expected accumulation of daughter products was not observed during this experiment. The expected degradation pathway of [redacted] as shown in Figure 2, included [redacted] and [redacted] and degradation daughter products. Elevated levels of [redacted] were observed in both aerobic and anaerobic microcosms, but it was not clear if this component was a degradation product or if it desorbed from basalt solids into the liquid phase. This suggestion is further supported by the fact that [redacted] was also measured in sterile (killed) microcosms where biodegradation was presumably halted.

Another indication of biodegradation of [redacted] is the formation of the [redacted] isomer. This isomer has been shown to be the dominant isomer that results from the biotransformation of [redacted] to [redacted] (Parsons *et al*, 1984). Since only very low concentrations of [redacted] were sporadically observed (<20 µg/L) in some microcosms, its general absence indicates that this pathway is not likely to predominate. [redacted] another key indicator of [redacted] (and 112-TCA) biotransformation, was only measured at low levels [redacted] and only in microcosms prepared using basalt and groundwater from OSW-23. Therefore, it is possible that the low level [redacted] measured in these microcosms was that which desorbed from the added solids into the water phase.

[redacted]

Figure 2 also shows the biodegradation pathways for 112-TCA. Transformation of this compound may proceed in one of two directions, by either a dehalogenation reaction that results in the production of EDC or by a haloelimination reaction that results in the production of VC. According to Chen (1996), approximately 80% of 112-TCA were converted to VC and the remaining 20% were converted to EDC. In the biostimulation study microcosms, fairly high levels of EDC (>6 mg/L) were measured, but only in those microcosms prepared using groundwater and basalt from OSW-23. These results suggest that OSW-23 solids likely contained EDC prior to the initiation of this study. In OSW-8 microcosms, only low levels of EDC were measured (<40 µg/L) after six months of incubation. Given that slightly higher concentrations of EDC were measured in sterile (control) microcosms on this same date, it is unlikely that EDC was produced as a result of biological degradation of 112-TCA.

The inconclusive results of the Altona biostimulation study support the need for further investigation into the biodegradation potential of [redacted] and 112-TCA. Results of this study neither support or deny evidence of microbial degradation of these two compounds due to the fact that the true source of daughter products (EDC and VC) was not established in the current experiment. A recommended direction of this study would include the use of radiolabeled test chemicals ([redacted] and 112-TCA), which would eliminate any interference from adsorbed chemical species associated with the basalt.

The results of field investigations, trends in contaminant levels, evaluations of biogeochemical and microbiological indicators, and laboratory microcosm experiments together provide some understanding of the importance of biological processes in the removal of groundwater contaminants at the Altona Chemical Complex site. Field results suggest that biological degradation may be occurring in the Altona aquifer and that a mixed source of highly chlorinated species ([redacted], [redacted] and 112-TCA) are possibly being degraded to less-chlorinated species. Biogeochemical parameters indicate that a reducing environment exists, which is typically needed to support microbial communities that have the ability to biodegrade highly chlorinated species. The results of this study show that [redacted] an organic compound found in the groundwater beneath the Altona Chemical Complex, can be biodegraded by bacteria indigenous to the underlying aquifer. These results also indicate the potentially important role that natural attenuation may have in the overall strategy for addressing groundwater contamination at the Dow Altona plant site.

[redacted]

ACKNOWLEDGEMENTS

The authors wish to thank [redacted] and [redacted] of IT Environmental (Australia) Pty Ltd for their assistance in obtaining the soil and groundwater samples. We also wish to acknowledge [redacted], [redacted], [redacted], and [redacted] for their technical input and for critically reviewing this manuscript.

[redacted]

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INTRINSIC BIODEGRADATION OF CHLORINATED SOLVENTS IN AQUIFER MICROCOSMS FROM THE ALTONA CHEMICAL COMPLEX, ALTONA, AUSTRALIA

Table 1. Characterization of Groundwater. All units are ppm (mg/L) unless otherwise noted.

Groundwater Characterization			
Parameter	Well OSW-23	Well OSW-5	Well OSW-8
pH	6.7	7.7	7.8
Temperature (°C)	17.9	17.4	17.2
Conductivity (mmhos/cm)	42.9	10.5	9.3
Dissolved Oxygen	0.9	0.7	2.1
Bicarbonate	427	493	374
Carbonate	0.2	2.3	2.1
Total Dissolved Solids	27,885	6,799	6,038
Nitrate-N	ND	ND	ND
Sulfate	90	192	388
Sulfide	ND	ND	ND
Chloride	15,263	2,806	2,302
Phosphorous	2.7	1.7	0.9
Ferrous Iron	27	ND	0.34
Manganese	33	0.62	0.03
Methane	ND	ND	ND
Ethane	ND	ND	ND
Ethene	ND	ND	ND

INTRINSIC BIODEGRADATION OF CHLORINATED SOLVENTS IN AQUIFER MICROCOSMS FROM THE ALTONA CHEMICAL COMPLEX, ALTONA, AUSTRALIA

Table 1. Characterization of Groundwater. All units are ppm (mg/L) unless otherwise noted. (Continued)

Chlorinated Organic Constituents			
[redacted]	[redacted]		
1,1,2-Trichloroethane	167	8.5	2.3
[redacted]	[redacted]		
TCOC	*1,172*	*16.5*	*2.3*

ND = not detected

[redacted]

INTRINSIC BIODEGRADATION OF CHLORINATED SOLVENTS IN AQUIFER MICROCOSMS FROM THE ALTONA CHEMICAL COMPLEX, ALTONA, AUSTRALIA

Table 2. Experimental Plan for Assessing Biodegradation Potential of [redacted] 112-TCA, and [redacted] in Altona Microcosms.

Sample I.D.	Source of Groundwater (Soil?)	Redox	Test Chemicals	^{14}C?	Amendments
INTRINSIC BIODEGRADATION STUDY					
1-24	OSW-8 (yes)	Aerobic	[redacted]	Yes	None
25-48	OSW-23 (yes)	Aerobic	[redacted]	Yes	None
49-72	OSW-8 (yes)	Anaerobic	[redacted]	Yes	None
73-96	OSW-23 (yes)	Anaerobic	[redacted]	Yes	None
97-111	OSW-23 (yes)	Aerobic	[redacted]	Yes	$HgCl_2$
112-126	OSW-23 (yes)	Anaerobic	[redacted]	Yes	$HgCl_2$
BIOSTIMULATION STUDY					
127-130	OSW-8 (yes)	Aerobic	[redacted]	No	Toluene
131-134	OSW-8 (yes)	Anaerobic	[redacted]	No	Toluene
135-138	OSW-23 (yes)	Aerobic	[redacted]	No	Toluene
139-141.5	OSW-23 (yes)	Anaerobic	[redacted]	No	Toluene
142-145	OSW-8 (yes)	Aerobic	[redacted]	No	Yeast Extract + Lactate
146-149	OSW-8 (yes)	Anaerobic	[redacted]	No	Yeast Extract + Lactate
150-153	OSW-23 (yes)	Aerobic	[redacted]	No	Yeast Extract + Lactate
154-157	OSW-23 (yes)	Anaerobic	[redacted]	No	Yeast Extract + Lactate
158-161	OSW-8 (yes)	Aerobic	1,1,2-TCA	No	Toluene
162-165	OSW-8 (yes)	Anaerobic	1,1,2-TCA	No	Toluene
166-169	OSW-23 (yes)	Aerobic	1,1,2-TCA	No	Toluene
170-173	OSW-23 (yes)	Anaerobic	1,1,2-TCA	No	Toluene
174-177	OSW-8 (yes)	Aerobic	1,1,2-TCA	No	Yeast Extract + Lactate
178-181	OSW-8 (yes)	Anaerobic	1,1,2-TCA	No	Yeast Extract + Lactate
182-185	OSW-23 (yes)	Aerobic	1,1,2-TCA	No	Yeast Extract + Lactate
186-189	OSW-23 (yes)	Anaerobic	1,1,2-TCA	No	Yeast Extract + Lactate

[redacted]

INTRINSIC BIODEGRADATION OF CHLORINATED SOLVENTS IN AQUIFER MICROCOSMS FROM THE ALTONA CHEMICAL COMPLEX, ALTONA, AUSTRALIA

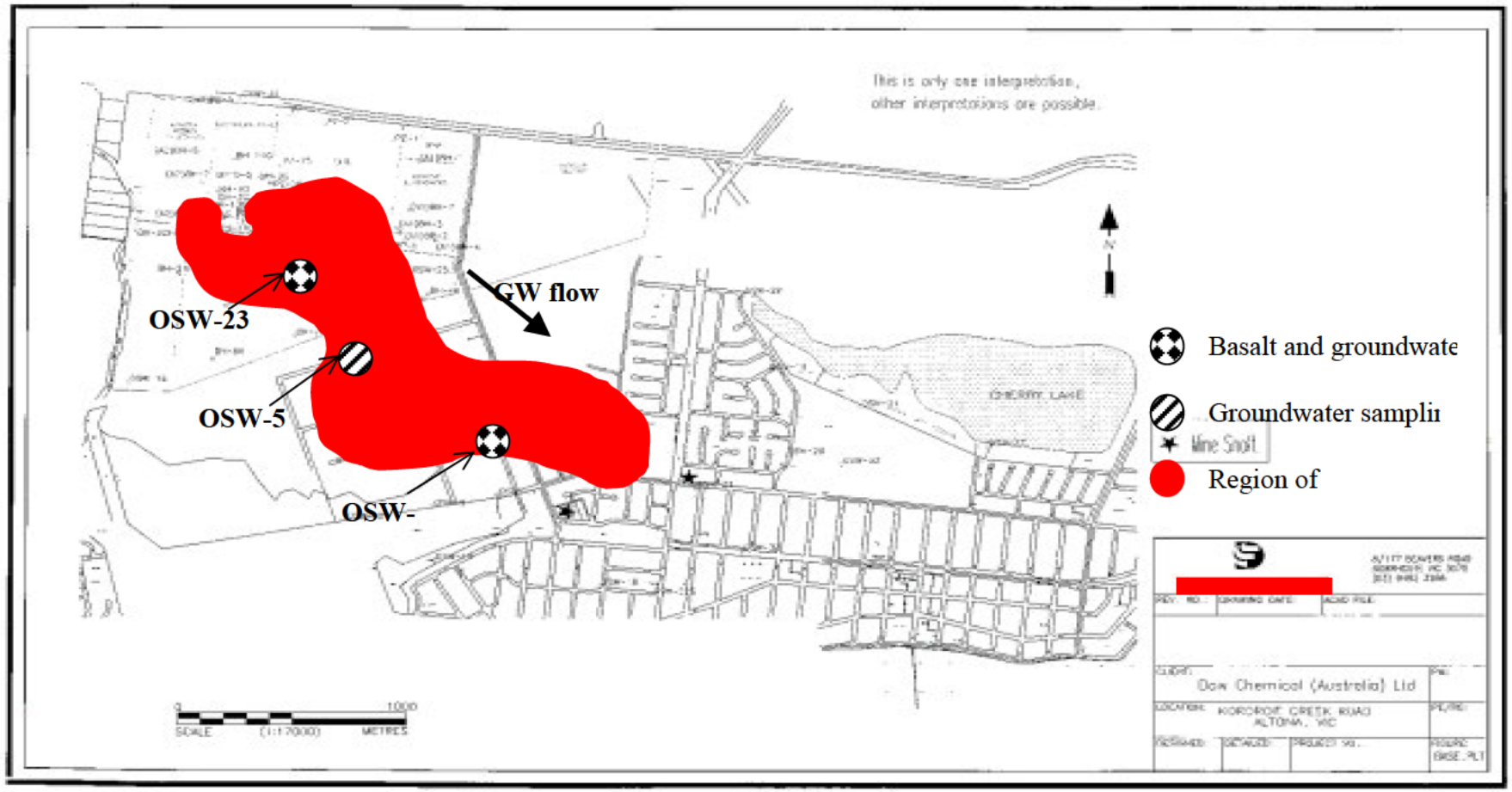
Table 2. Experimental Plan for Assessing Biodegradation Potential of [redacted] 112-TCA, and [redacted] in Altona Microcosms. (Continued)

190-193	OSW-23 (yes)	Aerobic	1,1,2-TCA, [redacted]	No	$HgCl_2$
194-197	OSW-23 (yes)	Anaerobic	1,1,2-TCA, [redacted]	No	$HgCl_2$
198-201	OSW-23 (yes)	Aerobic	1,1,2-TCA, [redacted]	No	$HgCl_2$ + Yeast Extract
202-205	OSW-23 (yes)	Anaerobic	1,1,2-TCA, [redacted]	No	$HgCl_2$ + Yeast Extract
BIOSTIMULATION STUDY – GROUNDWATER ONLY					
206-209	OSW-5 (no)	Aerobic	[redacted]	Yes	Toluene
210-213	OSW-5 (no)	Anaerobic	[redacted]	Yes	Toluene
214-217	OSW-23 (no)	Aerobic	[redacted]	Yes	Toluene
218-221	OSW-23 (no)	Anaerobic	[redacted]	Yes	Toluene
222-225	OSW-5 (no)	Aerobic	[redacted]	Yes	Yeast Extract
226-229	OSW-5 (no)	Anaerobic	[redacted]	Yes	Yeast Extract
230-233	OSW-23 (no)	Aerobic	[redacted]	Yes	Yeast Extract
234-237	OSW-23 (no)	Anaerobic	[redacted]	Yes	Yeast Extract
238-240,251	OSW-5 (no)	Aerobic	[redacted]	Yes	Toluene
252-255	OSW-5 (no)	Anaerobic	[redacted]	Yes	Toluene
256-259	OSW-23 (no)	Aerobic	[redacted]	Yes	Toluene
260-263	OSW-23 (no)	Anaerobic	[redacted]	Yes	Toluene
264-267	OSW-5 (no)	Aerobic	[redacted]	Yes	Yeast Extract
268-271	OSW-5 (no)	Anaerobic	[redacted]	Yes	Yeast Extract
272-275	OSW-23 (no)	Aerobic	[redacted]	Yes	Yeast Extract
276-279	OSW-23 (no)	Anaerobic	[redacted]	Yes	Yeast Extract

[redacted]

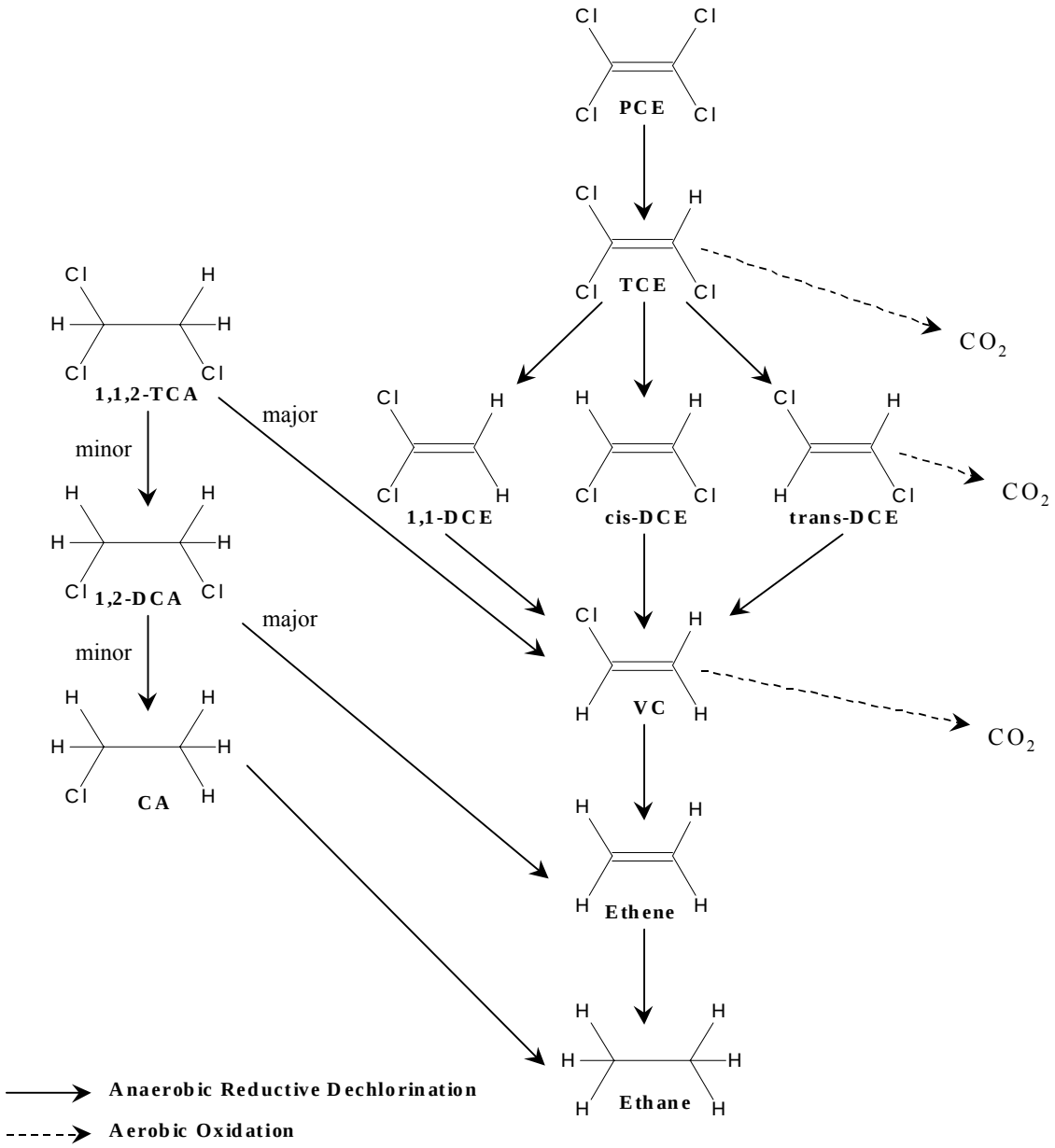
INTRINSIC BIODEGRADATION OF CHLORINATED SOLVENTS IN AQUIFER MICROCOSMS FROM THE ALTONA CHEMICAL COMPLEX, ALTONA, AUSTRALIA

Figure 1. Site Map Showing Region of Groundwater Contamination Near Dow Australia's Plant in the Altona Chemical Complex.



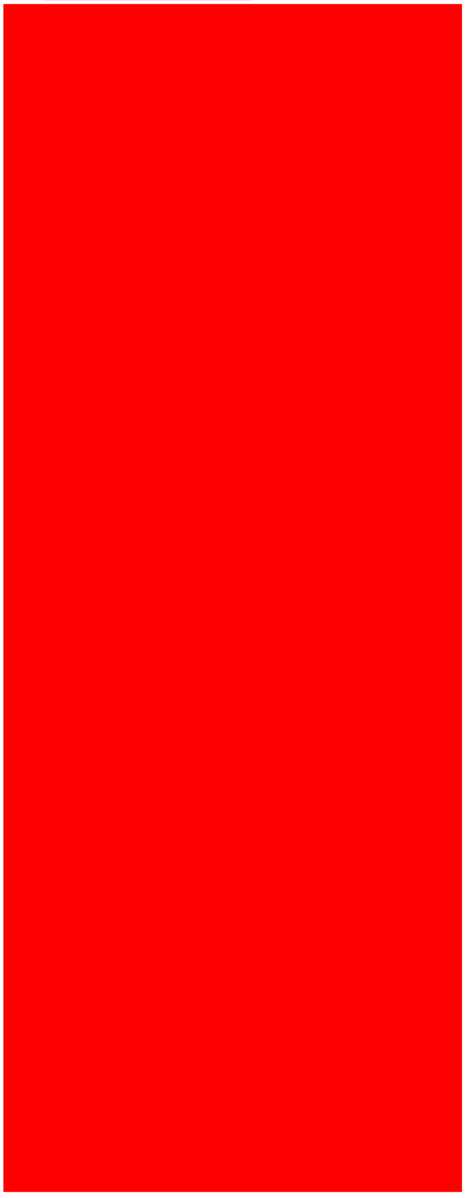
INTRINSIC BIODEGRADATION OF CHLORINATED SOLVENTS IN AQUIFER MICROCOSMS FROM THE ALTONA CHEMICAL COMPLEX, ALTONA, AUSTRALIA

Figure 2. Pathway for the Biological Transformation of Tetrachloroethylene (PCE) and 1,1,2-Trichloroethane (TCA). Daughter Products for PCE Include Trichloroethylene (TCE), Dichloroethylene (DCE), Vinyl Chloride (VC), Ethene, Ethane. Daughter Products for TCA Include Vinyl Chloride (VC), 1,2-Dichloroethane (EDC), Ethene, and Chloroethane (CA).



INTRINSIC BIODEGRADATION OF CHLORINATED SOLVENTS IN AQUIFER MICROCOSMS FROM THE ALTONA CHEMICAL COMPLEX, ALTONA, AUSTRALIA

Figure 3. Pathway for the Biological Transformation of [redacted] under Reducing Conditions. Daughters Include [redacted]



INTRINSIC BIODEGRADATION OF CHLORINATED SOLVENTS IN AQUIFER MICROCOSMS FROM THE ALTONA CHEMICAL COMPLEX, ALTONA, AUSTRALIA

Figure 4. Biodegradation of 5 mg/L [redacted] in OSW-8 Microcosms Under Anaerobic Conditions.

INTRINSIC BIODEGRADATION OF CHLORINATED SOLVENTS IN AQUIFER MICROCOSMS FROM THE ALTONA CHEMICAL COMPLEX, ALTONA, AUSTRALIA

Figure 5. Biodegradation of 5 mg/L [redacted] in OSW-23 Microcosms Under Anaerobic Conditions.

[redacted]