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Ex-situ bioremediation of chlorobenzenes in soil

Turlough F. Guerin *

Telstra Corporation Limited, L33/242 Exhibition Street, Melbourne 3000, Australia

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Abstract

Chlorinated benzenes, including chlorobenzene (CB) and 1,2-dichlorobenzene (DCB) are widely used as chemical intermediates and solvents across industry. Soil contaminated with these compounds was treated in a pilot-scale trial in 6 m^3 cells. Air was drawn through each cell and exhausted via an activated carbon (GAC) filter system. The trial objective was to stimulate native microflora with nutrients and varying levels of organic amendments (0%, 12% and 35%). Initial soil DCB concentrations varied from <1 to 6 mg/kg in the three cells with an average of 2 mg/kg. Approximately 90% of the DCB mass present in the soil was removed over a period of 2–3 weeks. Up to 100-fold increases in total heterotrophs (THP), CB^+ and DCB^+ degraders were observed. Residual concentrations of chlorinated benzenes were generally below detection limits (0.2 mg/kg). Adding organic matter did not enhance the removal of CB and DCB under the trial conditions, which were set up to minimize losses from volatilization. Biodegradation estimation calculations indicated that <5% of the chlorinated benzenes were removed by volatilization and 90% removed by biodegradation. Laboratory shake flask trials confirmed that the soils in the pilot-scale treatment contained a microbial consortium capable of mineralizing CB and DCB. This consortium was capable of mineralizing both CB and DCB with up to 50% of carbon added as chlorinated benzene substrate being recovered as CO_2 and up to 44% of organic chlorine being released as chloride ion in mineralization tests, further confirming these chlorinated benzenes were biodegraded. The study confirms that vented ex-situ biotreatment processes for chlorinated benzenes can be achieved without excessive losses from volatilization and that naturally occurring microflora can be readily stimulated with aeration and nutrients.

Keywords: Chlorobenzenes; Volatilization; Biodegradation; Microbial; Pilot scale; Field scale; Laboratory; Microorganisms; Degradation; Biotreatment; Soil; Ex-situ; Venting; Organic matter; Organic amendments

1. Introduction

Chlorobenzenes are a priority environmental pollutant listed by the US Environmental Protection Agency and are used mainly as intermediates in the synthesis of pesticides and other chemicals. Chlorobenzenes are released to the environment during manufacture or use as intermediates in the production of other chemicals. They are also released during the disposal of chlorobenzene products, such as from incinerators and hazardous waste sites. Chlorobenzene (or monochlorobenzene, referred to as CB in this article) is released directly to the environment due to its use as a pesticide carrier, and is also released this way through its use in deodorizers, fumigants, degreasers, insecticides, herbicides, and defoliants. CB is used in the production of phenol and nitrochlorobenzene (*ortho* and *para* isomers), in the formulation of herbicides, to produce additional chlorobenzenes, and as a solvent in the manufacture of adhesives, paints, resins, dyestuffs, drugs and the production of diphenyl oxide, phenylphenol, silicone resin, and other halogenated organics [1]. 1,2-Dichlorobenzene (referred to as DCB in this article) is used primarily in the automotive and metal industries as a solvent for the removal of carbon and degreasing of metal parts. DCB is also used in the synthesis of organic chemicals such as toluene diisocyanate. The physico-chemical properties of these compounds are presented in Table 1.

The widespread use of these compounds has led to traces detected in soil and groundwater contamination [2]. CB levels in uncontaminated soils are generally less than 0.4 mg/kg for dichlorobenzene congeners and less than 0.1 mg/kg for other CB congeners. Levels of chlorobenzenes in sediments are generally in the ng/kg to μg/kg range, although levels in the mg/kg range have been reported in samples from industrial areas.

* Tel.: +61 418 391 646; fax: +61 3 96343413.
E-mail address: turlough.guerin@hotmail.com.

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Table 1
Physico-chemical properties of chlorinated benzenes[a]

Chlorinated benzene	Abbreviation	Molecular formula	Vapour pressure at 25 °C (Pa)	Aqueous solubility at 25 °C (mg/L)	Henry's law constant (kPa m^3/mol)	Log octanol/water partition coefficient (K_{ow})	Soil sorption coefficient (K_{oc})
Chlorobenzene	CB	C_6H_5Cl	1665	293	0.377	2.98	466
1,2-Dichlorobenzene	DCB	$C_6H_4Cl_2$	197	91.1	0.198	3.38	987
1,3-Dichlorobenzene	1,3-DCB	$C_6H_4Cl_2$	269	123	0.366	3.48	1070
1,4-Dichlorobenzene	1,4-DCB	$C_6H_4Cl_2$	90	30.9	0.160	3.38	1470

[a] Source: [1].

Chlorinated benzenes do, however, undergo biodegradation in the environment [3–5] though some remain recalcitrant even under conditions of enrichment [6]. These compounds are subject to degradation by various aerobic microorganisms [4,7–9]. Anaerobic microorganisms are also capable of degrading these compounds [10–12]. In the aerobic studies, degradation has been demonstrated with isolated pure cultures of both naturally occurring species and engineered strains. It is also apparent that mixed cultures or communities of microorganisms are able to completely degrade these compounds under both aerobic and anaerobic conditions [13]. Specific strains of the aerobic bacteria *Pseudomonas* sp. and *Alcaligenes* sp. have been isolated and grown on CB and DCB [4,14] and the genetic basis of degradation of these compounds in microorganisms has also been studied extensively [9,12,13,16–20]. Both mineralization [6,9,11,21–24] and cometabolism [25,26] are involved in CB and DCB biodegradation.

Mineralization is the complete degradation of a compound to form carbon dioxide and water. Mineralization, which can be an aerobic or anaerobic process [15], involves the release of CO_2 from the pollutant. In the case of chlorinated organic compounds, such as CB and DCB isomers, chloride ion is also a product of mineralization. Two main pathways are known for metabolism of aromatic compounds – the ortho and meta pathways. Only the ortho pathway leads to complete mineralization of chlorinated aromatic compounds. There are a number of steps involved in the mineralization of dichlorobenzene isomers [9,13,16,20,21,24,27,28]. Mineralization proceeds via different pathways, depending on the organism and on the position of the chlorine atoms. However, the most common pathway for dichlorobenzene isomer biodegradation can be categorised into five main steps:

1. Dichlorobenzene isomers are converted to a dihydrodiol by a dichlorobenzene dioxygenase enzyme.
2. A dehydrogenase converts the dihydrodiol to a chlorocatechol.
3. The chlorocatechol is then degraded by a non-specific Type II dioxygenase to form a dichloromuconic acid.
4. Chloromuconate cycloisomerases usually generate dienelactones from chloromuconates. These are then hydrolysed by dienelactone hydrolases to give maleylactates.
5. Maleylactates are reduced to 3-oxoadipic or beta-ketoadipic acid, which may still be chlorinated. Once dechlorinated, these metabolites are then converted to intermediates of the tricarboxylic acid (TCA) cycle for normal metabolism.

The operation of the *ortho*-cleavage pathway results in mineralization of chlorinated benzenes without the accumulation of chlorocatechols. Research on microorganisms from CB contaminated groundwater has shown that genetic recombination, between different species of microorganisms, can bring about the development of microorganisms with the capability of completely degrading chlorinated benzenes and that this genetic recombination can occur in contaminated environments [13]. This has implications for the natural attenuation of chlorinated benzenes in soil and groundwater environments contaminated with these compounds.

Cometabolism is the incidental, and usually only partial degradation, of a compound [29,30]. Dichlorobenzene isomers can undergo cometabolism [25]. Microorganisms such as *Brevibacterium*, *Corynebacterium*, *Rhodococcus* and *Xanthobacter* have been reported to cometabolise these compounds. When dichlorobenzene isomers are cleaved by peripheral enzymes such as mono- or di-oxygenases, and associated dehydrogenases, then chlorocatechols may form. This process does not occur by *meta*-cleavage enzymes. These degradation products can be toxic to microorganisms thus it would be desirable that the meta pathway be suppressed in a bioremediation process. There is limited evidence that this pathway can be readily suppressed in the field. Field and full-scale remediation projects, particularly those employing bioventing technologies, have also been shown to treat chlorobenzenes, though there are few providing extensive details of these projects in the public domain [31].

Literature on the biological degradation of chlorobenzenes has revealed that there was sufficient evidence from previous research to warrant evaluation of aerobic biodegradation processes in the field. While there is only limited information on field-scale bioremediation trials, there is sufficient microbial capability in the soil and water environment to warrant field-scale evaluation. Thus, soil composting (or co-composting) was considered as a potential, low cost approach for remediation of CB and DCB at the special chemicals manufacturing facility where these compounds were used as chemical intermediates. It was reasoned that composting, using a readily available and degradable source of organic matter, was likely to encourage naturally occurring microorganisms, to degrade these pollutants [32–35]. It was further reasoned that an inoculum selected to degrade DCB isomers and CB, could be added to the compost to augment degradation in a full-scale process if this was considered necessary at a later stage in the site cleanup.

Table 2
Pilot-scale treatment design

Treatment	% Soil (w/w)	% Mulch (w/w)	Soil volume (m^3)[a]	Mulch volume (m^3)	Est. cell mass (kg)
Cell 1	100	0	6	0	10,200
Cell 2	88	12	4	3.5	6,800
Cell 3	65	35	2	7	3,400

[a]Density of soil was 2000 kg/m^3 (ex-situ), density of mulch materials 250 kg/m^3 (loosely packed).

2. Objectives and scope of study

It was recognised that if a controlled composting approach were to be applied, increased losses of VOCs due to elevated soil temperatures, forced aeration and additional materials handling considerations associated with the organic material, required for the composting process, may present technical challenges. Thus, the pilot-scale process was designed to compare the removal rates of CB and DCB by the two major mechanisms, i.e. biodegradation and volatilization. The process employed three static pile soil treatments (referred to as cells) that were amended with varying amounts of inorganic nutrients and organic matter supplements.

Pilot-scale and laboratory trials were performed concurrently. The objective of the pilot-scale soil remediation work was to obtain field data on the relative extent of CB and DCB biodegradation and volatilization. The objectives of the pilot-scale soil remediation work were to:

- estimate the extent of CB and DCB degradation in the soil and reduce the *concentration* of CB and DCB in the soil to below the target of 0.2 mg/kg;
- estimate the losses of chlorobenzenes due to volatilization; and
- monitor the numbers of microorganisms in response to biostimulation (*viz*. venting, inorganic nutrient amendment and adding organic mater).

The objective of the laboratory trial was to demonstrate the presence of microorganisms in the soil with the capability of degrading chlorobenzenes. Specifically, these were to:

- test for the presence of microorganisms capable of degrading CB and DCB;
- develop and apply techniques for enumerating CB and DCB degrading microorganisms; and
- isolate microbial cultures capable of CB and DCB mineralization and demonstrate mineralization of chlorobenzenes by these cultures.

3. Materials and methods

3.1. Pilot-scale trial design

3.1.1. General

To achieve the trial objectives, it was required that the pilot-scale design be:

- of sufficient size to realistically assess the materials handling properties of the material at full scale and to provide sufficient thermal mass for composting;
- of suitable scale to assess the soil aeration system; and
- such that loss mechanisms be limited to volatilization via the air extraction system and the processes of biodegradation.

Treatment of the soil was in three cells, each of 6 m^3, with organic matter contents ranging up to 30% (w/w) (Table 2). The design provided favourable aerobic conditions to stimulate the removal of CB and DCB by the native microflora (and assumed the presence of such microorganisms). The trial was established in a covered building on the site.

The literature reviewed, prior to design, suggested that addition of organic material for composting is not, in principle, necessary to achieve mineralization of chlorobenzenes. If specific substrate utilisers (DCB or CB degrading microbes or consortia) are present in sufficient numbers in the soil, it should be sufficient to provide appropriate conditions (aeration, essential nutrient addition and maintenance of soil moisture) to stimulate the indigenous microflora and achieve degradation. The soil in each cell was supplemented with mineral nutrients and moisture. Note that the cell with 100% soil was not a control treatment. Rather, nutrient and moisture amendment and aeration of the soil represents the simplest form of bioremediation, *viz*. biostimulation.

Since chlorinated aromatic compounds are not present naturally in the biosphere in large quantities, organisms capable of degrading chlorobenzenes were not anticipated to be abundant in the organic supplement material. However, the possibility of their presence in the site soil, resulting from the long-term exposure to the compounds and the acclimation of the microflora, was recognised.

Removal of chlorobenzenes via microbial mineralization was determined as the difference between the measured concentrations of residual VOCs in the soil of the cells and the total VOC mass measured in the gas drawn through the cells.

3.1.2. Site and soil description

The building area on site containing the contaminated soil was approximately 3000 m^2. The overlying layer of gravel, sand and aggregate exhibits low concentrations of contamination in comparison to the clay materials. The contaminated soils were heavy clay soils and sub-soils of high plasticity. The in-situ bulk density of such clay material prior to excavation is typically 2000 kg/m^3. The soil ranged from mottled orange/red clay to a mottled whitish or grey clay with some iron oxide streaking, characteristic of extensive leaching with depth. The

Table 3
Initial concentrations of chlorinated benzenes in soil

Treatment	Mean (mg/kg)	No. samples	Minimum (mg/kg)[a]	Maximum (mg/kg)
In-situ (prior to trial)	15	10	<1	1300
Cell 1[b]	1.0	4	<1	2
Cell 2[c]	3.5	4	<1	6
Cell 3[d]	1.0	4	<1	<1
Total (Cells 1–3)	2.0	12	<1	6

[a] Field GC–PID identified chlorobenzenes in all soil cells (PQLs of 1 mg/kg DCB).
[b] Biostimulation (with no organic matter).
[c] Biostimulation (with organic matter) treatment, adjusted to mg/kg dry soil equivalent basis.
[d] Composting treatment (with high levels of organic matter) adjusted to mg/kg dry soil equivalent basis.

greater volume of the material was extensively leached, having been subjected to periodic or continuous waterlogging. This is consistent with the reported presence of shallow perched groundwaters in the area. The soil is essentially devoid of organic matter (0.15% total organic carbon) and pH ranged from 7.5 to 8.0.

3.1.3. Soil contamination

Samples from the main contaminated area at the site were found to contain mainly 1,2-dchlorobenzene (DCB), but smaller amounts of 1,3- and 1,4-dichlorobenzene, CB, other halogenated species, e.g. chloroform, dibromochloromethane, 3-methyl pyridine, xylene and other volatile organic compounds (VOCs). These samples were reported to have been collected from the near-surface soil, to a depth of 3.5 m. Soil concentrations ranged up to 80 mg/kg CB and 1300 mg/kg DCB, but were typically 10–20 mg/kg (for both CB and DCB). Other chlorinated compounds were in the range of 1–2 mg/kg, except for one sample with 10 mg/kg chloroform. The method used to determine the concentrations reported above were obtained using the GC–ECD method (described in Section 3.2.10). Soil for the trial was excavated from the area where the soil was expected to be representative of an average for the site of 10–20 mg/kg. Upon mixing of the soil, these concentrations decreased significantly (Table 3). A GC–FID (flame ionization detector) method was used to determine total CB and DCB isomers in the pilot-scale trial (see Section 3.2.8).

3.2. Pilot-scale procedures

3.2.1. Design of facility

The pilot-scale facility, located in a covered hard stand area, was designed to allow assessment of future full-scale options for bioremediation of the soil remaining at the site. The soil venting system consisted of a vacuum pump and manifold system connected to a set of air extraction pipes at the base of each of three soil cells. Air flow was measured and controlled by needle valves. Each soil cell was 3 m (long) × 2 m (wide) × 1 m (high). The cells were contained by a concrete wall and polyvinyl chloride (PVC) lined timber boxing. The vacuum created at the bottom of the cell induced airflow through the top surface of the cells to the venting pipes at the bottom. Points of air ingress to the cells were controlled by perforations in the PVC covers. The venting system was constructed with instrumentation to allow monitoring of volumetric gas flow, soil temperatures, extracted gas temperatures, vacuums, and VOC concentrations in extracted soil gas through each cell. The flow rates were adjustable to meet the oxygen consumption and cooling requirements of each soil mixture. Volatile traps consisting of granulated activated carbon (GAC) filters linked were installed at the extraction system outlet of each cell to capture airborne VOCs for the mass balance assessment.

3.2.2. Soil amendments

Due to the texture and structure of the soil to be used in the trial, organic matter amendments were considered necessary to improve its physical properties for bioremediation. The three soil treatments tested in the trial contained 0%, 12%, and 30% (w/w), respectively, of organic material (Table 2). Organic matter was provided by a local supplier of green tree waste. The soil was amended with 50 g/m^3 of soluble, controlled release inorganic nutrients with an N:P:K ratio of 22:5.7:0 supplied by Scotts [36], prior to mixing with organic material. Mixing was conducted using a 1 m^3 front end loader. Moisture content of the soil was 21–23% (v/v) prior to mixing. This stayed the same in the 12% organic matter treatment and increased by 5–7% in the 30% (v/v) organic matter treatment. Water was not added to the treatments.

3.2.3. Soil temperatures

Cell 1 (100% soil) tracked the average ambient temperature inside the building during the period of the trial. In Cell 2 (12% organic matter) temperature increased rapidly due to the thermal energy generated by composting. A decrease in the rate of composting activity (as measured by the rate of change of soil temperature) was apparent from 25 h until flow rates were increased at 51 h. The temperature peaked at 55 °C at 6 days (2.5 days after flow rates were increased) and then reduced until the air flow ceased at 13 days. An equilibrium temperature (heat generation/thermal radiation) was reached at 20 days after commission at a temperature of 40 °C in the absence of cooling. Cell 3 (35% organic matter) quickly increased to a maximum temperature of 55 °C at 48 h. The temperature of the soil then began to decrease under the cooling effect supplied by excess air. The temperature of the compost reduced until the gas flow was ceased at 13 days. An equilibrium temperature of 35 °C was maintained from 13 days to the end of the trial.

3.2.4. Airflows

Variations in the air flow rates were a source of variation in the concentrations of captured CB and DCB in the gas. The operational accuracy of the flowmeters (rotameters) utilized in the trial was quoted as 3% full-scale deflection (FSD). The FSD of the rotameters was 180 L/min thus the accuracy of the instruments was approximately ±5.4 L/min or ±1.7 L/min (at operational pressure). This represents a range of possible errors in the measurement of flow rates of 7–27% for each of the three streams measured. The operational accuracy of the pressure gauges placed on the reservoir pipe during the trial is quoted as ±2.5% FSD. Thus, the accuracy of the instruments was approximately 2.5 kPa, or ±3% at the operating pressures in the main reservoir pipe (150 mBar absolute). The flow in the gas lines exhibited some degree of turbulence causing oscillations in the rotameter. This was particularly evident at low flows with oscillations as high as ±5 L/min at 20 L/min flow (gauge reading). Readings were taken to be the average position of the meter when this occurred. The oscillations decreased with increasing flow rates and were generally absent at flows greater than 40 L/min. Average flow rates across the 3 cells were 25–40 L/min.

3.2.5. Soil sampling

For the analyses of VOCs and microorganisms, soil samples were removed from the mid depth of the cells at commissioning and on several occasions throughout the period of the trial. Sampling was performed using a 100 mm diameter hand auger. Samples were taken at three locations in each cell. A discrete sample from the mid depth (500 mm) of the soil profile was taken. A soil column 450 mm deep was removed from the cell and placed to the side prior to sampling the soil core from 450 to 550 mm depth.

3.2.6. Samples for VOC analysis

Three samples were taken from randomly selected positions across the soil surface in each cell. The soil core removed from between 450 and 550 mm was brought to the surface and the jar was pressed directly into the clay material to transfer material directly into a 150 mL pharmaceutical sample jar with a minimum of disturbance. The jar was tightly packed using a sample trowel and immediately sealed and labelled. The remainder of the auger core extracted from each hole at 500 mm depth was placed in a 1 L glass jar and sealed. Woodchips and twigs >25 mm diameter were removed from the sample.

3.2.7. Samples for microbiological and chemical analysis

The three discrete volumes of sample material remaining from the 500 mm depth cores were composited to form a single 1 L sample for the microbiological and chemical analyses. Each successive core was placed without mixing into a single 1 L clear glass sample jar and sealed. Woodchips and twigs >25 mm diameter were also removed from the sample.

3.2.8. Field analysis

VOCs removed by volatilization were measured in the off-gas at individual sample ports using a Photovac Model 10-S/55 portable photo-ionization gas chromatograph (GC–PID) and a Foxboro Model OVA 108 Century Organic Vapour Analyzer (FID). The activated carbon trap was sampled at the conclusion of the trial to allow quantitation of volatilized DCB, CB, and other VOCs from the soil, or from the organic materials introduced as soil amendments. The portable organic vapour analyzers used in the field trial were calibrated against a range of air mixtures of benzene, CB, and DCB (equivalent to 1 ppmv:1 ppmv:5 ppmv of each of these gases, respectively), twice a day. The limits of detection for CB and DCB were both 1 ppmv. Linearity curves were established for both CB and DCB using the field FID and GC–PID units.

3.2.9. Microbial monitoring

Samples from the contaminated soils were enumerated for aerobic microorganisms capable of using the chlorinated benzenes for growth (CB^+ and DCB^+) in the vapour phase. Inoculated mineral salts phosphate plates (10^{-2}–10^{-8} dilutions) were incubated in 20 L sealed containers with open 100 mL glass jars containing the chlorobenzenes. THP were monitored as referred to in Guerin [37]. Bacterial consortia were isolated on the vapour plates containing CB and DCB (Refer to Section 3.3).

3.2.10. Chemical analyses

Soils were prepared for GC–ECD analysis by extraction with hexane:acetone (3:1). Soils for the GC–FID analysis were placed in 150 mL head space vials and gas analyses were made directly from these. The methods used for quantitation of CB and DCB and semi-VOCs (as C_8 equivalent) were based on standard procedures using gas chromatograph–electron capture detection (GC–ECD) and gas chromatograph–flame ionization detector (GC–FID), respectively, using USEPA methods [38]. USEPA Method 8260B (GC–MS method for VOCs) was used to identify the presence of VOCs in the green tree mulch used in the trial [38]. Recoveries for all analytes varied between 80 and 110%.

3.2.11. Biodegradation estimation

A biodegradation estimation was conducted to account for all forms of VOC removal. The mass of VOCs measured in soil was compared with (1) the net mass of VOCs measured in extracted soil gas and (2) the VOCs captured and measured in the GAC volatile trap at the outlet of the recirculating air system. The net difference was inferred to represent removal by biodegradation. GAC was found to be highly effective in capturing the CB and DCB (98–99% removal efficiency). Desorption of the CB and DCB from the GAC was high with 98–100% recovery.

3.3. Laboratory scale procedures

3.3.1. General

Samples for these procedures were taken from locations on the site known to have high concentrations of chlorinated benzenes. These were collected at a range of depths and were used to prepare serial dilutions for the determination of microbial communities and to undertake chlorinated benzene utilization tests (see following subsections).

3.3.2. Determination of soil microbial communities

The microbial communities of each soil sample were determined using plating techniques for enumeration of the total heterotrophic population (THP) and, using selective media, to determine (presumptive) CB^+ or DCB^+ degraders. THP in the soils was determined by a standard method for total heterotrophic microorganisms using trypticose soy agar (Oxoid). Serial dilutions were made in phosphate buffered isotonic saline, pH 7.5. Aliquots were plated out using a spread plate technique [39]. The selective medium used for enumerating CB^+ and DCB^+ degraders was a mineral salts phosphate agar described elsewhere [37,40] with CB or DCB supplied in the vapour phase. This was achieved by incubating the plates in a sealed 20 L container in which was placed an open flask (100 mL) of CB or DCB. Colonies were counted after incubation at 28 °C. The incubation period was 4 days for THP plates and 14 days for the selective media. The CB^+ and DCB^+ utilizing communities were reported as number of organisms per gram of dry soil.

3.3.3. Isolation of CB and DCB utilizers

Populations of specific contaminant-degrading bacteria in soil samples are generally small, and they co-exist with numerous and diverse microflora [41]. It is therefore often necessary to enrich from the existing population to select specific contaminant degraders of interest, in order to acquire a culture for use in test work or as a soil inoculum. Four soil samples from the site were used as inoculum for culture enrichment flasks with CB or DCB present in vapour form. A consortium of microorganisms capable of growing on CB or DCB was isolated directly from colonies growing on the selective media plates. Six isolates were selected from colonies growing on CB^+ or DCB^+ plates inoculated with soil dilutions. Colony morphology and a microscopic description were recorded and the isolates numbered types 1–6. The six isolates were tested for the ability to grow on CB or DCB both individually and collectively. This was done both on plates and in liquid culture shake flasks. 100 mL of mineral salts phosphate pH 7.5 was inoculated with a single colony picked off a CB^+ or DCB^+ plate, and solvent added as vapour from liquid held in a tube within the flask. In these flasks, the chlorinated benzene was the sole carbon source.

3.3.4. Identification of microbial genera

Identification of soil organisms was by standard microbiological procedures followed by testing with the API kits 20NE and CHB (Bio-Merieux, France) and using the tests previously described [41,42]. Six isolates were selected by colony morphology from selective media. These were subcultured on THP plates and incubated for 1–2 days.

3.3.5. Metabolic capacity

The successful consortium of bacteria was tested for the ability to degrade various substrates: CB, DCB, 1,3-dichlorobenzene, 1,4-dichlorobenzene, toluene, benzene, phenol, and dichlorophenol. Fresh subcultures of the consortium were suspended in 100 mL mineral salts phosphate solution, pH 7.5 in eight 250 mL Erlenmeyer flasks. Each flask was supplied with a single metabolite as sole carbon source. Negative controls without carbon substrate were also run. Phenol was supplied to one flask as a direct addition at 100 mg/L. The other metabolites were supplied as vapour from liquid held in a test tube within the flask. The flasks were stoppered and a carbon filter was fitted to allow oxygen transfer without vapour loss. Growth was monitored as absorbance at 546 nm (i.e. turbidity).

3.3.6. Mineralization of CB and DCB

Fresh subcultures of the consortium were suspended in 1 L of mineral salts phosphate solution, pH 7.5, in sealed 2 L Erlenmeyer flasks. CB or DCB were supplied as sole carbon source as direct additions at 100 mg/L. Each treatment was set up in triplicate. Carbon dioxide generated was trapped in a NaOH solution held in a glass cylinder within the flask. The concentration of NaOH was determined by titration against a standard acid solution. Carbon dioxide produced was then calculated from the NaOH consumed, compared to the theoretical yield from the chlorinated benzene supplied. The degradation of CB and DCB was also determined by release of chloride ion (Cl^-). Samples of culture suspension were taken at regular intervals and frozen pending analysis of chloride by ion selective electrode using USEPA SW 845 Method 9212 [38].

4. Results and discussion

4.1. General

The approach in this study was to utilize soil composting, relying on the stimulation of native microflora with nutrients and varying levels of organic supplements. Two factors needed to be considered were: (1) the heat generated by the composting process would increase the mass transfer of DCB into the vapour phase (and thus losses to atmosphere or granular activated carbon trap); and (2) the additional cost, volume and materials handling challenges of the relatively large quantities of organic material to be added for composting. Under controlled and monitored conditions, mass transfer (by volatilization) can be a significant means of removing chlorinated benzenes from soil. However, mineralization of these compounds by naturally occurring microorganisms was recognised as a superior environmental outcome. In this pilot-scale process, it was sought to compare the removal rates of the volatile contaminants by the available mechanisms, *viz.* biodegradation and volatilization and these results are discussed in the following sections.

4.2. Fate of chlorinated benzenes in pilot-scale treatment

4.2.1. Losses due to volatilization in pilot-scale treatment

Losses due to volatilization were quantified and characterised using field measurements of VOCs in off-gas. Handling of soil would have led to losses of CB and DCB, depending on the extent of mixing. This was recognised as a loss mechanism and efforts were made during handling the soil to minimize these losses. Desorption was complete in the analysis of the GAC with 95–100% recoveries reported.

CB and DCB contaminants were confirmed in all three cells including Cell 3, where contaminant concentrations in soil were

below laboratory detection limits throughout the period of the trial. An estimate of DCB concentrations was derived which were compared with the measured loss from the soil mass, and also the mass collected in the GAC filter.

Initial soil DCB concentrations were as high as 6 mg/kg (average 2 mg/kg) (Table 3).

The extracted soil gas from Cell 1 (0% OM; 100% soil) exhibited a rapid decline in VOC concentrations in the first 24 h of system operation with VOC concentrations dropping from 34 to 4 ppmv. This was followed by a further slow decline to less than detection limits. The levels of VOCs in the extracted gas stream and in soil gas sampled from Cell 1 reached non-detectable levels 4 weeks after commissioning.

The extracted soil gas from Cell 2 (12% organic matter:88% soil) exhibited an apparent decline in VOC concentrations from 12 to 5 ppmv during the first 48 h. This was then followed by increasing VOC concentrations up to a peak of 67 ppm at 68 h. The temperature of the cell at that time was 46 °C. The concentration of VOCs then declined steadily to low levels similar to those exhibited by Cell 1 by the seventh day of operation. The concentrations of VOCs in the extracted gas stream and in soil gas sampled from Cell 2 reached non-detectable levels 4 weeks after commission.

The extracted soil gas from Cell 3 (35% organic matter:65% soil) exhibited an apparently static level of VOC concentrations during the first few hours at a concentration of 5–10 ppmv. This was then followed by an increase of VOC concentrations up to a peak of 49 ppmv at approximately 27 h. The temperature of the cell at that time was 48 °C. The concentrations of VOCs then declined rapidly to low levels similar to those exhibited by Cell 1 at around 48 h. The levels of VOCs in the extracted gas stream and in soil gas sampled from Cell 3 reached non-detectable levels 3 weeks after commissioning. Table 4 shows the components that were analysed in the GAC which captured the extracted gas from treatment 3.

4.2.2. Losses due to biodegradation

The initial concentrations of the chlorinated benzenes in the soil of the pilot-scale trial ranged from <0.2 to 6 mg/kg. The final concentrations of DCB in the soil were below the target concentration of 0.2 mg/kg. Removal of the chlorinated benzenes by volatilization was measured at <5%, compared with biodegradation of >90%. The results of the pilot-scale trial on DCB degradation, showing estimates of the final fate of the contaminants, are shown in Table 5, and Fig. 1 summarizes the data for both CB and DCB. The evidence for the predominant loss mechanism being biodegradation was that two independent estimates of losses by volatilization were obtained for the pilot trial (by direct measurement of off-gas and measurement of GAC absorbed CB/DCB). Both of these indicate that <5% was lost via this mechanism.

As shown in Fig. 1, the amount of loss of DCB from volatilization was substantially lower than the losses from CB. The extent of biodegradation of DCB was relatively high compared to that observed for CB. The explanation of the higher extent of volatilization of CB is likely to be a result of the relatively higher Henry's constant of CB compared with DCB (Table 1).

Microbial numbers (including DCB$^+$) increased in response to all the treatments (refer to Section 4.4):

- microbial cultures capable of utilizing chlorinated benzenes for growth were isolated from the site soil (refer to Section 4.5); and
- one consortium of bacterial strains was capable of mineralizing both CB and DCB.

The only losses in the trial were from volatilization and biodegradation. The chlorinated benzenes in the pilot-scale trial were removed predominantly by biodegradation.

Table 4
Concentrations of DCB and other VOCs in GAC[a]

VOC	Mean (mg/kg)	Minimum (mg/kg)	Maximum (mg/kg)
DCB[b]	3.8	<1	13
1,3-dichlorobenzene	<1	<1	<1
1,4-dichlorobenzene	1.0	<1	4
CB	11.7	<1	35
Other VOCs[c]	120	<1	370

[a] GAC = granulated activated carbon. Only one capture system was in place across the three cells.
[b] DCB = 1,2-dichlorbenzene.
[c] Based on a GC–MS scan and included volatile oils and non-chlorinated hydrocarbons.

Table 5
Biodegradation estimation of DCB from the pilot-scale trial

Parameter	DCB mass (g)
In initial soil	35.80
In final soil	3.45
VOC losses:	
1. Measured in off-gas	0.90
2. Measured in GAC filter	0.60
Total mass biodegraded	31.45–31.75
% Biodegraded	88–89

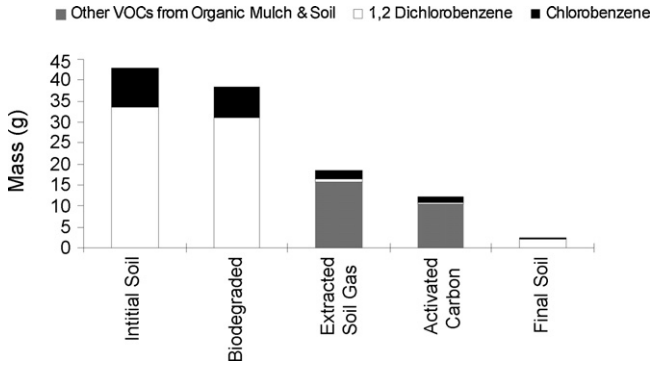

Fig. 1. Overall fate of chlorobenzenes in the pilot trial.

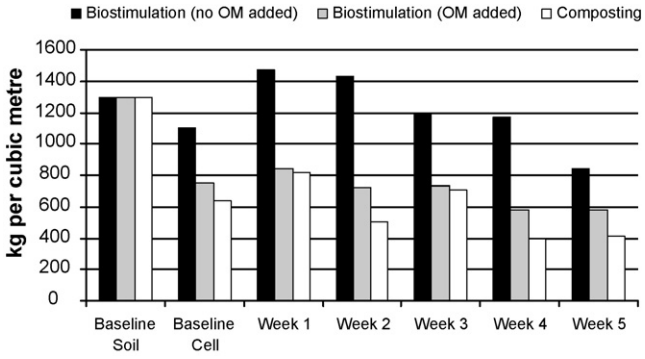

Fig. 2. Changes in treatment soil bulk densities.

4.3. Soil mix properties in pilot-scale trial

Bulk densities of the soil and soil mixtures tended to decrease in each of the treatments over the trial period (Fig. 2) which as expected as the added organic matter improved the aeration properties of the soil. Other soil properties are provided in Table 6 including total organic carbon (TOC), nitrogen, phosphorous and moisture content. Available nitrogen and phosphorus as well as moisture content were not limiting to microbiological populations in the treatment cells based on similar results from previously published bioremediation trials [34,35,40,43].

4.4. Microbiology of pilot-scale trial

4.4.1. Chlorinated benzene degraders

Weekly samples were collected from the three soil treatments for enumeration of DCB^+ and CB^+ degraders. Initial microbial counts in the soil, in all three treatment cells were in the range of 10^5–10^6 per g (Fig. 3). Initial populations in augmented soil from Cell 3 were highest at 6.1×10^6 to $8.1 \times 10^6\ g^{-1}$. Over the first 2 weeks of treatment, there was a 100-fold increase, to $10^7\ g^{-1}$, for both CB^+ and DCB^+ degrading types in both Cell 1 and Cell 2 (100% soil and soil amended with 15% organic matter, respectively). Cell 3 soil also showed a slight increase, to more than $10^7\ g^{-1}$ over this period. CB^+/DCB^+ degrader populations responded to biostimulation, and after 3 weeks, these increased to $10^8\ g^{-1}$ soil. CB^+ and DCB^+ populations then declined to $4 \times 10^6\ g^{-1}$ soil by the end of the trial. Over the following 4-week period (data not shown), the microbial numbers changed by less than an order of magnitude, in all three treatments. Both CB^+ and DCB^+ types declined in Cell 1. In Cells 2 and 3, the populations changed little up to the end of the treatment (Fig. 3).

4.4.2. Total heterotrophic populations (THP)

In-situ soil samples collected prior to the field trial contained THPs of 2×10^5 to $2 \times 10^6\ g^{-1}$ soil. The initial THP for the three treatments ranged from 8×10^5 to $6 \times 10^7\ g^{-1}$ soil (Fig. 3). The THP, which is a wide range of microorganisms, are capable of using numerous carbon sources for growth and grow at normal ambient temperatures. These THPs are in contrast to single degrader strains that may only use one or a few specific compounds as substrates for growth. Initial counts in the soil alone were $8 \times 10^5\ g^{-1}$. Initial populations in the augmented soils (Cells 2 and 3) were found to be higher, more than $10^7\ g^{-1}$. This reflects the contribution from the added organic matter. There was a very rapid 20- to 100-fold increase in THP in all treatments over the first week. The THP in Cell 1 increased to $4 \times 10^7\ g^{-1}$. The THP in Cells 2 and 3 increased to $10^9\ g^{-1}$ in the first week. These populations then declined slowly, in all treatments, to $10^5\ g^{-1}$ (i.e. the original THP), by the end of the treatment period.

Table 6
Characterization of pilot-scale trial soils[a]

Source	Sample type[a]	TOC (%)	Total N (mg/kg)	Av. N (mg/kg)	Total P (%)	Av. P (mg/kg)	Water (% w/w)
Baseline soil	Soil	0.15	575	351	0.02	253	23
Baseline Cell 1	Soil	0.16	495	231	0.02	3.7	23
Baseline Cell 2	Soil/organic matter	3.42	1200	149	0.03	17.1	21
Baseline Cell 3	Soil/compost	4.21	1120	187	0.02	21.9	22
Cell 1 – week 1	Soil	0.15	626	183	0.01	2.8	17.4
Cell 2 – week 1	Soil/organic matter	5.03	1380	63	0.04	29.0	22.8
Cell 3 – week 1	Soil/compost	8.25	2410	160	0.04	73.8	30.6
Cell 1 – week 2	Soil	0.18	539	178	0.02	3.42	16.9
Cell 2 – week 2	Soil/organic matter	6.86	1610	15	0.04	28.2	24.6
Cell 3 – week 2	Soil/compost	9.97	2350	46	0.04	87.3	30.7
Cell 1 – week 3	Soil	0.18	610	127	0.02	9.45	17
Cell 2 – week 3	Soil/organic matter	4.8	1600	55	0.04	22.8	22.7
Cell 3 – week 3	Soil/compost	5.9	1800	261	0.03	15.6	22
Cell 1 – week 4	Soil	0.24	469	227	0.02	3.5	17.2
Cell 2 – week 4	Soil/organic matter	7.22	769	32	0.04	37.1	27.7
Cell 3 – week 4	Soil/compost	10	862	108	0.03	35.6	30.3
Cell 1 – week 5	Soil	0.26	380	268	0.01	2.3	18.7
Cell 2 – week 5	Soil/organic matter	5.22	1230	66	0.04	21.1	25
Cell 3 – week 5	Soil/compost	10.6	1830	80	0.03	29.8	32.7

[a] Soil samples were heavy clays and were mixed thoroughly with a spatula for 20 min prior to analysis. Soil/compost blends were minced thoroughly for 30 min prior to analysis.

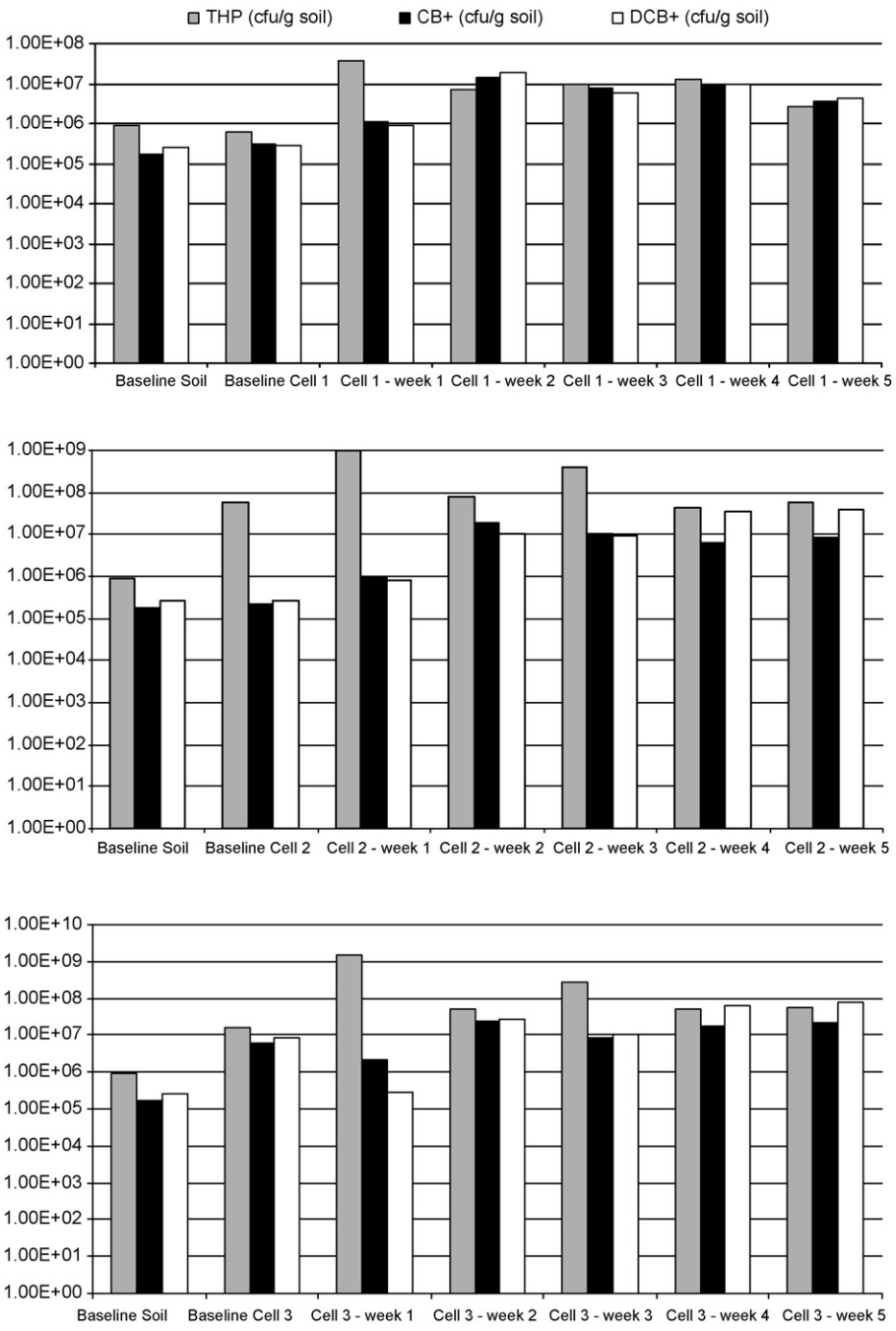

Fig. 3. Microbiological changes in treatment calls in units of colony forming units per gram soil (Cell 1: top; Cell 2: middle; Cell 3: bottom).

4.5. Microbiology of laboratory study

4.5.1. Chlorinated benzene degrading organisms in site soils

DCB^+ and CB^+ degrading organisms were found in four samples of DCB and CB contaminated soil collected from the site. These soils contained between 10^3 and $10^5\ g^{-1}$ of presumptive DCB^+ and CB^+ degraders (Table 7). These sub-populations accounted for between 1 and 50% of the measured THP.

4.5.2. A chlorinated benzene degrading consortium

A consortium of five isolates was capable of growing on both CB and DCB in liquid cultures. These were the colony types 1–5, isolated from the original soil plates. The cultures were sampled and counted at the end of the growth period. Microbial counts ranged from 2×10^7 to $2 \times 10^8\ mL^{-1}$, confirming that the increase in turbidity resulted from microbial growth and not chemical precipitation. The consortium was also able to grow on 1,4-dichlorobenzene, and phenol supplied as sole carbon and energy source. Benzene, toluene and 2,4-dichlorophenol were

Table 7
Microbial populations in soil (in situ)[a]

Sample location[b]	Sample depth (m)	Soil odour	THP (g^{-1})	CB^+ types (g^{-1})	DCB^+ types (g^{-1})
1	0.2–1	Slight	2.9×10^5	2.3×10^5	1.5×10^5
2	0.5–1	Slight	2.5×10^5	4.7×10^3	2.1×10^4
3	0.3–0.75	Slight	2.6×10^6	4.3×10^4	7.5×10^3
4	0.8–1.2	Intense	2.0×10^6	5.4×10^4	4.4×10^4

[a] Standard errors in the THP, CB^+ and DCB^+ analyses were 10–15%.
[b] Locations were representative of the highest contamination concentration at the site.

not used as growth substrates. In this test the consortium grew less vigorously on CB, than on the other compounds. The consortium grew strongly on CB but only weakly on DCB. Some substrate carbon is incorporated in microbial cell mass, so the results suggest a significant proportion (>50%) of the initial substrate supply had been utilised. Individual isolates were not capable of growing on DCB in liquid media. However, colony type 4 was able to grow on CB. The consortium consisted of five members. The strains were of the genera *Pseudomonas* and *Bacillus*. Evidence for mineralization of CB and DCB was obtained based on carbon dioxide production and chloride ion release in the liquid culture shake flasks (Table 8). Yields of carbon dioxide for both CB and DCB were consistent with mineralization. Between 40 and 50% of the carbon added as chlorinated benzene substrate was recovered as CO_2. Since carbon dioxide release is not definitive, and the result provides only indirect evidence for mineralization (for example, CO_2 may also be generated by endogenous metabolism), samples of culture medium were also analyzed for chloride. The chloride results confirmed that mineralization was occurring. The chloride yields indicated that 20% of the DCB and 40% of the CB were mineralized in these tests. Based on carbon dioxide and chloride ion release, it was likely that a consortium present in the soil was capable of mineralizing CB, and DCB to a lesser extent. The carbon dioxide generation and turbidity increases in the consortium cultures are illustrated in Figs. 4 and 5. The consortium was also found to also use phenol and 1,4-dichlorobenzene, but not 1,3-dichlorobenzene (data not shown).

4.5.3. Identification of consortium members

Three of the consortium members (colony types 3, 4 and 5) were isolates of the same species, identified as *Pseudomonas fluorescens*. The successful consortium was therefore made up of only three species: *Pseudomonas fluorescens, Bacillus cereus*, and a third (colony type 5), a Gram variable, oxidase negative

Table 8
Mineralization of chlorinated benzenes by the consortium

Parameter	CB	DCB
Initial substrate supplied (mmol)	1.09	1.15
Theoretical CO_2 yield (mmol)	6.54	6.90
Observed CO_2 yield (mmol)	3.55	2.78
Conversion (%)	54	40
Theoretical chloride yield (mmol)	1.09	2.30
Observed chloride yield (mmol)	0.48	0.56
Conversion (%)	44	24

[a]Standard errors in the THP, CB^+ and DCB^+ analyses were 10–15%.

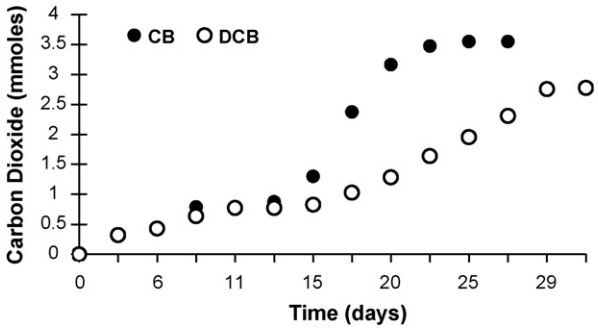

Fig. 4. Carbon dioxide accumulation in consortium cultures.

Bacillus, which was not further identified. The consortium culture was maintained by subculture at 2-week intervals, and used as inocula for further test work to characterize the metabolic activities of the consortium. The probability of correct identification of these microorganisms that were identified was within the range of 98–100%, based on the API test kit results. The consortia was not tested for its resilience in the environment as no tests were conducted to test their chlorinated benzene degrading activity (or survival rate) after adding to contaminated soil.

4.6. Limitations in estimating biodegradation in pilot-scale trial

The initial mass of VOCs in the soil was lower than expected, and the distribution of contaminants in the clay soils was found to be variable with standard errors as high as 15–20%. As a consequence, a significant proportion of the baseline samples contained no detectable chlorinated benzenes. Varying sensitivities or practical quantification limits (PQLs) were achieved for the analyses of the soils. A PQL of 0.2 mg/kg for dichloroben-

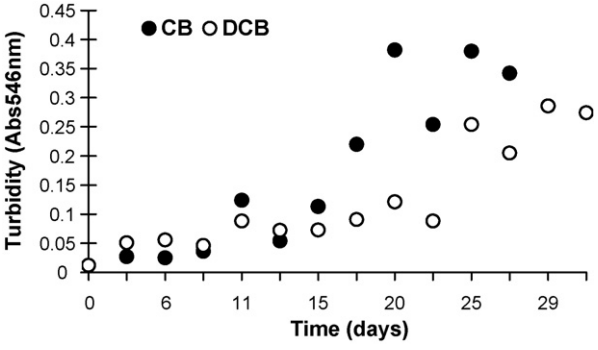

Fig. 5. Turbidity increases in consortium cultures.

zenes was reported for most analyses. The uncertainty in the initial mass of DCB arising from the method sensitivity is of the order of 10% of the total DCB mass. A PQL of 0.5 mg/kg was possible for CB, due to lower sensitivity of detection for this compound.

Analysis for VOCs in soil is preferably taken from undisturbed soil samples. Samples of soil taken from Cell 1 were obtained with a minimum of disturbance (sample vial pushed directly into undisturbed clay sample) with a minimum of VOC losses. Samples taken from treatment Cells 2 and 3 could not be obtained in an undisturbed form due to the effects of fibrous and rigid organic material mixed with the clay soil. While this is of importance with VOCs, it is less likely to be of significance for the DCB isomers (the principal contaminants), which have relatively lower volatilities compared with CB (Table 1).

4.7. Comparison of biostimulation and soil composting

One of the trial objectives was to compare biostimulation treatments (with and without added organic matter) with a soil composting technique. Although the initial concentration of chlorinated benzenes in the soil was lower than anticipated, it was apparent from Cell 1 results that effective removal occurred even though no organic matter was added. A direct estimate of the loss due to volatilization from this cell was possible and this demonstrates that not more than 20% of the DCB was removed in the extracted gas. The two treatments with added organic matter (Cell 2 and 3) were broadly similar in effect (with respect to CB and DCB degradation), with the proviso that the initial concentrations of chlorinated VOCs were significantly higher as shown in Table 3.

5. Conclusions

The experimental aims of the trials were accomplished as follows:

- during the trial, losses of chlorobenzenes by volatilization were measurable (<5%), but minor compared with biodegradation (>90%);
- the final concentrations of CB and DCB in the soil were below the target concentration of 0.2 mg/kg;
- a microbial consortium was obtained which could utilize CB and DCB as the sole sources of carbon and energy for growth in liquid cultures, however, CB mineralization was more extensive than that for DCB.

The findings from laboratory and pilot-scale trials demonstrated that CB and DCB can be degraded in soil. It is apparent from the pilot-scale trial that biostimulation, with inorganic nutrients and low levels of organic material (mulch), was as effective as soil composting in the removal of DCB and CB. The numbers of chlorinated benzene degrading microorganisms in the soil increased significantly (up to 100 folds) in response to stimulation in all of the treatments. The studies showed that the chlorinated benzenes could be removed from the contaminated soil using venting over a period of 5 weeks.

Statement of limitations

This article presents the views of the author only and does not necessarily reflect those of his employer, Telstra Corporation Limited.

References

[1] H.M. Malcolm, P.D. Howe, S. Dobson, Chlorobenzenes other than hexachlorobenzene: environmental aspects, in Concise International Chemical Assessment Document 60, Centre for Ecology and Hydrology, Monks Wood, United Kingdom (for World Health Organization), Geneva, 2004.
[2] M. Morita, Chlorinated benzenes in the environment, Ecotoxicol. Environ. Saf. 1 (1) (1977) 1–6.
[3] J.C. Spain, S.F. Nishino, Degradation of 1,4-dichlorobenzene by a *Pseudomonas* sp., Appl. Environ. Microbiol. 53 (5) (1987) 1010–1019.
[4] S.F. Nishino, J.C. Spain, L.A. Belcher, C.D. Litchfield, Chlorobenzene degradation by bacteria isolated from contaminated groundwater, Appl. Environ. Microbiol. 58 (5) (1992) 1719–1726.
[5] P.Y. Lu, R.L. Metcalf, Environmental fate and biodegradability of benzene derivatives as studied in a model aquatic ecosystem, Environ. Health Perspect. 10 (1975) 269–284.
[6] C.M. Aelion, C.M. Swindoll, F.K. Pfaender, Adaptation to and biodegradation of xenobiotic compounds by microbial communities from a pristine aquifer, Appl. Environ. Microbiol. 53 (9) (1987) 2212–2217.
[7] F.R. Brunsbach, W. Reineke, Degradation of chlorobenzenes in soil slurry by a specialized organism, Appl. Microbiol. Biotechnol. 42 (2–3) (1994) 415–420.
[8] F.R. Brunsbach, W. Reineke, Degradation of mixtures of chloroaromatic compounds in soil slurry by mixed cultures of specialized organisms, Appl. Microbiol. Biotechnol. 43 (3) (1995) 529–533.
[9] A.E. Mars, T. Kasberg, S.R. Kaschabek, M.H. van Agteren, D.B. Janssen, W. Reineke, Microbial degradation of chloroaromatics: use of the meta-cleavage pathway for mineralization of chlorobenzene, J. Bacteriol. 179 (14) (1997) 4530–4537.
[10] C. Holliger, G. Schraa, A.T.M. Stams, A.J.B. Zehnder, Enrichment and properties of an anaerobic mixed culture reductively dechlorinating 1,2,3-trichlorobenzene to 1,3-dichlorobenzene, Appl. Environ. Microbiol. 58 (1992) 1636–1644.
[11] B. Zacharias, E. Lang, H.H. Hanert, Biodegradation of chlorinated aromatic hydrocarbons in slow sand filters simulating conditions in contaminated soil—pilot study for in situ cleaning of an industrial site, Water Res. 29 (7) (1995) 1663–1671.
[12] A. Alfreider, C. Vogt, W. Babel, Expression of chlorocatechol 1,2-dioxygenase and chlorocatechol 2,3-dioxygenase genes in chlorobenzene-contaminated subsurface samples, Appl. Environ. Microbiol. 69 (3) (2003) 1372–1376.
[13] J.R. van der Meer, C. Werlen, S.F. Nishino, J.C. Spain, Evolution of a pathway for chlorobenzene metabolism leads to natural attenuation in contaminated groundwater, Appl. Environ. Microbiol. 64 (11) (1998) 4185–4193.
[14] H. Fritz, W. Reineke, E. Schmidt, Toxicity of chlorobenzene on *Pseudomonas* sp. strain RHO1, a chlorobenzene-degrading strain, Biodegradation 2 (3) (1991) 165–170.
[15] A. Kaschl, C. Vogt, S. Uhlig, I. Nijenhuis, H. Weiss, H.H. Richnow, Isotopic fractionation indicates anaerobic monochlorobenzene biodegradation, Environ. Toxical. Chem. 24 (6) (2005) 1315–1324.
[16] J.A. de Bont, M.J. Vorage, S. Hartmans, W.J. van den Tweel, Microbial degradation of 1,3-dichlorobenzene, Appl. Environ. Microbiol. 52 (4) (1986) 677–680.
[17] K. Pollmann, S. Beil, D.H. Pieper, Transformation of chlorinated benzenes and toluenes by *Ralstonia* sp. Strain PS12 tecA (tetrachlorobenzene dioxygenase) and tecB (chlorobenzene dihydrodiol dehydrogenase) gene products, Appl. Environ. Microbiol. 67 (9) (2001) 4057–4063.
[18] H. Raschke, T. Fleischmann, J.R. Van Der Meer, H.-P.E. Kohler, *cis*-Chlorobenzene dihydrodiol dehydrogenase (TcbB) from *Pseudomonas* sp.

strain P51, expressed in *Escherichia coli* DH5alpha (pTCB149), catalyzes enantioselective dehydrogenase reactions, Appl. Environ. Microbiol. 65 (12) (1999) 5242–5246.
[19] G. Schraa, M.L. Boone, M.S. Jetten, N.A.R. van, P.J. Colberg, A.J. Zehnder, Degradation of 1,4-dichlorobenzene by *Alcaligenes* sp. strain A175, Appl. Environ. Microbiol. 52 (6) (1986) 1374–1381.
[20] C. Sommer, H. Görisch, Enzymology of the degradation of (di)chlorobenzenes by *Xanthobacter flavus* 14p1, Arch. Microbiol. 167 (6) (1997) 384–391.
[21] K. Pollmann, V. Wray, D.H. Pieper, Chloromethylmuconolactones as critical metabolites in the degradation of chloromethylcatechols: recalcitrance of 2-chlorotoluene, J. Bacteriol. 187 (7) (2005) 2332–2340.
[22] S. Lee, J.H. Pardue, W.M. Moe, K.T. Valsaraj, Mineralization of desorption-resistant 1,4-dichlorobenzene in wetland soils, Environ. Toxicol. Chem. 22 (10) (2003) 2312–2322.
[23] P. Rapp, K.N. Timmis, Degradation of chlorobenzenes at nanomolar concentrations by *Burkholderia* sp. Strain PS14 in liquid cultures and in soil, Appl. Environ. Microbiol. 65 (6) (1999) 2547–2552.
[24] J.S. Yadav, R.E. Wallace, C.A. Reddy, Mineralization of mono- and dichlorobenzenes and simultaneous degradation of chloro- and methyl-substituted benzenes by the white rot fungus *Phanerochaete chrysosporium*, Appl. Environ. Microbiol. 61 (2) (1995) 677–680.
[25] C.M. Kao, J. Prosser, Intrinsic bioremediation of trichloroethylene and chlorobenzene: field and laboratory studies, J. Hazard. Mater. 69 (1) (1999) 67–79.
[26] M. Jechorek, K.D. Wendlandt, M. Beck, Cometabolic degradation of chlorinated aromatic compounds, J. Biotechnol. 102 (1) (2003) 93–98.
[27] R. Tchelet, R. Meckenstock, P. Steinle, J.R. van der Meer, Population dynamics of an introduced bacterium degrading chlorinated benzenes in a soil column and in sewage sludge, Biodegradation 10 (2) (1999) 113–125.
[28] A. Lehning, U. Fock, R. Wittich, K.N. Timmis, D.H. Pieper, Metabolism of chlorotoluenes by *Burkholderia* sp. Strain PS12 and toluene dioxygenase of *Pseudomonas putida* F1: Evidence for monooxygenation by toluene and chlorobenzene dioxygenases, Appl. Environ. Microbiol. 63 (5) (1997) 1974–1979.
[29] M. Alexander, Biodegradation of chemicals of environmental concern, Science 211 (1981) 132–138.
[30] M. Alexander, Some issues in biodegradation, Basic Life Sci. 45 (1988) 381–383.
[31] USEPA, Engineered Approaches to In Situ Bioremediation of Chlorinated Solvents: Fundamentals and Field Applications, 2000.
[32] T.F. Guerin, A comparison of mesophilic composting and unamended land treatment for the bioremediation of aged tar residues in soil, Remediation 9 (3) (1999) 59–77.
[33] T.F. Guerin, Co-composting of pharmaceutical wastes in soil, Lett. Appl. Microbiol. 33 (4) (2001) 256–263.
[34] T.F. Guerin, Co-composting of residual fuel contamination in soil, Soil Sediment Contam. 10 (6) (2001) 659–673.
[35] S.H. Rhodes, T.F. Guerin, P. Peck, Composting for the bioremediation of contaminated soil, in Solid Waste in; the Pacific. Common Issues—Common Solutions, in: 6th Annual Conference of the Waste Management Institute New Zealand, Dunedin, New Zealand, 1994.
[36] T.F. Guerin, Long term performance of a land treatment facility for bioremediation of non-volatile oily wastes, Resour. Conserv. Recycling 28 (1) (2000) 105–120.
[37] T.F. Guerin, Bioremediation of phenols and polycyclic aromatic hydrocarbons in creosote contaminated soil using ex-situ landtreatment, J. Hazard. Mater. 65 (3) (1999) 305–315.
[38] USEPA. SW-846 On-line Test Methods for Evaluating Solid Waste Physical/Chemical Methods, 1996 (cited; available from: http://www.epa.gov/epaoswer/hazwaste/test/tx3xxx.htm).
[39] T.F. Guerin, An improved technique for determining abundance of polycyclic aromatic hydrocarbon degrading microbial populations, Remediation 10 (1) (1999) 97–105.
[40] T.F. Guerin, The differential removal of aged polycyclic aromatic hydrocarbons from soil during bioremediation, Environ. Sci. Pollution Res. 7 (1) (2000) 19–26.
[41] M. Alexander, Introduction to Soil Microbiology, second ed., John Wiley and Sons, New York, 1977, pp. 467.
[42] D.L. Dindal, Soil Biology Guide, John Wiley and Sons, New York, 1991, pp. 1349.
[43] T.F. Guerin, S.H. Rhodes, P.C. Peck, Bioremediation of PAH contaminated soil. Laboratory and field studies, in: First Australasian Conference on Contaminants in the Soil Environment, Glenelg Press, Adelaide, 1996.