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Biostimulation and bioaugmentation enhances aerobic biodegradation of dichloroethenes

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Abstract

The accumulation of dichloroethenes (DCEs) as dominant products of microbial reductive dechlorination activity in soil and water represent a significant obstacle to the application of bioremediation as a remedial option for chloroethenes in many contaminated systems. In this study, the effects of biostimulation and/or bioaugmentation on the biodegradation of *cis*- and *trans*-DCE in soil and water samples collected from contaminated sites in South Africa were evaluated in order to determine the possible bioremediation option for these compounds in the contaminated sites. Results from this study indicate that *cis*- and *trans*-DCE were readily degraded to varying degrees by natural microbial populations in all the soil and water samples tested, with up to 44% of *cis*-DCE and 41% of *trans*-DCE degraded in the untreated soil and water samples in two weeks. The degradation rate constants ranged significantly ($P < 0.05$) between 0.0938 and 0.560 wk^{-1} and 0.182 and 0.401 wk^{-1}, for *cis*- and *trans*-DCE, respectively, for the various treatments employed. A combination of biostimulation and bioaugmentation significantly increased the biodegradation of both compounds within two weeks; 14% for *cis*-DCE and 18% for *trans*-DCE degradation, above those observed in untreated soil and water samples. These findings support the use of a combination of biostimulation and bioaugmentation for the efficient biodegradation of these compounds in contaminated soil and water. In addition, the results clearly demonstrate that while naturally occurring microorganisms are capable of aerobic biodegradation of *cis*- and *trans*-DCE, biotransformation may be affected by several factors, including isomer structure, soil type, and the amount of nutrients available in the water and soil.

Keywords: Bioaugmentation; Biostimulation; Degradation; Dichloroethene; Microcosm

1. Introduction

The frequently observed accumulation of the isomers of dichloroethenes (DCEs) as the dominant products of microbial reductive dechlorination activity in soil and water under anaerobic conditions and the lack of

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understanding of the factors controlling biodegradation of DCEs in situ represent significant obstacles to the application of bioremediation as a remedial option in many contaminated systems (Pavlostathis and Zhuang, 1993; Gibson et al., 1994; Bradley and Chapelle, 1997). Therefore, pump-and–treat systems using air stripping and adsorption onto granular activated carbon are the primary technologies used for removing volatile organic compounds (VOC), including DCEs. A decade of performance data, however, has demonstrated that these systems are not as efficient as once thought (Haley et al., 1991). In situ bioremediation has therefore been proposed as an alternative method in order to reduce time and cost for site restoration of systems contaminated with this group of compounds (Skeen et al., 1993; Saaty et al., 1995).

Along with deciding whether or not a site should be remediated using on site treatment or in situ treatment, it is necessary to decide the bioremediation options that needed to be employed. Three types of bioremediation are predominant in the industry today: natural attenuation, biostimulation, and bioaugmentation. The simplest method of bioremediation to implement is natural attenuation, where contaminated sites are only monitored for contaminant concentration to assure regulators that natural processes of contaminant degradation are active (Kaplan and Kitts, 2004). Biostimulation requires adjustments to the site (contaminated soil or water) in order to provide bacterial communities with a favourable environment in which they can effectively degrade contaminants. This includes the addition of nitrogen, phosphorus, and trace minerals while also making appropriate pH adjustments for the proliferation of indigenous microorganisms, hence speeding up the bioremediation process (Venosa et al., 1996; Salanitro et al., 1997). In cases where natural communities of degrading bacteria are present in low numbers or even absent, bioaugmentation, i.e., the addition of contaminant-degrading organisms, can speed up the degradation process (Al-Awadhi et al., 1996; Van Limbergen et al., 1998).

Partial dehalogenation of chloroethenes under anaerobic conditions has led to the speculation that a subsequent aerobic treatment may be necessary. Efforts have therefore been tailored towards investigating the biodegradation of DCEs under aerobic conditions (Malachowsky et al., 1994; Hopkins and McCarty, 1995). Several recent investigations indicate that microorganisms can oxidize DCEs to CO_2 in the absence of apparent alternative substrates in microcosms (Bradley et al., 1998; Bradley and Chapelle, 1998a,b; Klier et al., 1999). Significant aerobic oxidation of DCE was demonstrated for an organic-rich stream bed sediment (Bradley and Chapelle, 1998a,b), organic-rich surface soils (Klier et al., 1999), and organic-poor aquifer sediments (Bradley et al., 1998; Bradley and Chapelle, 1998a; Klier et al., 1999). However, to the best of our knowledge, no study has been conducted to investigate the potential for aerobic biodegradation of DCEs in microcosms incorporating soil and water from contaminated sites in Africa, with the view of providing baseline information for the possible natural attenuation of these compounds in contaminated sites in the African continent.

In this paper, we report the aerobic biodegradation of *cis*- and *trans*-DCE by indigenous microorganisms in soil and water collected from some contaminated sites in South Africa as well as the effects of biostimulation and/or bioaugmentation on the biodegradation of the compounds in soil and water microcosm settings under aerobic conditions pursuant to providing necessary information for the possible in situ bioremediation of these compounds in contaminated sites in South Africa.

2. Materials and methods

2.1. Sample collection

The soil samples used in this study were collected from separate locations to represent different soil types (clay and loam soil), while the wastewater samples were collected from the clarification tanks of the Northern and New Germany wastewater treatment plants in Durban, KwaZulu-Natal, South Africa. The soil samples were sieved using a 1.7 mm laboratory test sieve and transported immediately to the laboratory after collection and stored at 4 °C prior to the construction of the microcosms.

2.2. Bacterial cultures

The bacterial strains used in the bioaugmentation experiment were previously isolated using culture enrichment techniques from soil samples contaminated with chlorinated hydrocarbon compounds as described elsewhere (Olaniran et al., 2004b). Pure cultures of the isolates were stored on nutrient agar (Difco) slants at 4 °C as working stock cultures, and preserved from overnight broth cultures in 80% (v/v) glycerol at −20 °C.

2.3. Construction of microcosms

Soil microcosms were constructed in a laminar flow cabinet using synthetic groundwater as described by Klier et al. (1999). The synthetic groundwater contained 1.5 mM $MgCl_2$, 0.12 mM KCl, 0.03 mM NH_4NO_3, 1.0 mM $CaCl_2$, 1.5 mM $Ca(OH)_2$, and 8.5 mM $NaHCO_3$ in de-ionized water at pH 7.8. The reaction mixtures were prepared by combining 100 g of soil and 75 ml of the synthetic water in sterile 250 ml serum bottles (Wheaton). The headspace in each bottle was made up of approximately 75 ml of air. Aerobic conditions were

maintained by purging the reaction mixture with pure oxygen gas during the preparation, using a 0.2 μm filter and a syringe. A colorimetric redox indicator (resazurin, 0.0002%) was also added to the reactions to verify maintenance of aerobic conditions during incubation. One and a half grams of KOMPEL fertilizer (Chemicult products, Pty Ltd.), an agricultural fertilizer with N:P:K ratio of 3:1:6 was added to the microcosm used for the biostimulation experiments. Two milliliters of a mixed culture inoculum prepared from the standardized ($A_{600nm} = 1.0$) pure cultures of bacterial isolates (*Pseudomonas aeruginosa*, *Acinetobacter haemolyticus*, *Acinetobacter* sp, *Achromobacter xylosoxidans*, *Bacillus subtilis*, *Bacillus cereus* and *Klebsiella* sp.) previously described for DCE-degradation (Olaniran et al., 2004a,b) was added to all the bottles for the bioaugmentation experiments. The same amounts of both fertilizer and mixed culture inocula were combined and then added to the microcosms used for the biostimulation plus bioaugmentation experiments. Water microcosms were constructed with 150 ml of the different wastewater samples in 250 ml of the serum bottles (headspace, approximately 100 ml of air) and treated as described for the soil microcosms. Sufficient reaction mixtures were prepared for each set of conditions to permit "sacrifice" of replicate microcosms during sampling. Each series of reaction mixtures was amended with either *cis*- or *trans*-DCE to a final concentration of 0.5 mM and immediately crimp-sealed with Teflon-faced butyl rubber stoppers (Wheaton). The bottles were incubated at 25 °C on a rotary shaker at 150 rpm for at least 2 h before determining the initial concentration of the compounds, to allow for the equilibration of oxygen and the chlorinated ethenes between the gas and aqueous phases. Thereafter, the bottles were incubated at 25 °C, without shaking. Biologically inhibited controls were prepared for each series of reaction mixtures using autoclaved soil and water for the soil and water microcosms, respectively, and included in the study to measure abiotic losses of the test compounds. In addition, the control microcosms were amended with $HgCl_2$ to achieve a final concentration of 500 ppm. Microcosms were sampled weekly to measure DCE degradation.

2.4. Analytical methods

cis- and *trans*-DCE degradation in the different microcosms was monitored from the headspace sample analysis in a gas chromatograph (Varian model 3700) as described previously (Shim et al., 2001; Coleman et al., 2002). Using a gas-tight syringe (Hamilton), 100 μl headspace samples were injected into a gas chromatograph equipped with a flame ionization detector. Samples were analyzed with the injector and detector at 200 °C and the column at 100 °C. *cis*- and *trans*-DCE concentrations were quantified by comparison to a standard curve derived from known quantities of *cis*- and *trans*-DCE in serum bottles with the same gas and liquid volumes as the experimental bottles. The biodegradation rate constants in each microcosm were estimated according to LaGrega et al. (1994).

2.5. Soil and wastewater analysis

Soil and wastewater samples were analysed for pH, calcium, magnesium, sodium, iron, nitrate, nitrite, phosphate, sulphate, total organic carbon and total Kjeldahl nitrogen using standard methods (Black et al., 1965), while soil texture was determined mechanically by the hydrometer method (Gee and Bauder, 1979). The total heterotrophic bacterial populations in the soil and water samples were determined by standard spread plate counts (Gerhardt et al., 1991).

3. Results

3.1. Soil and wastewater characterization

The physicochemical properties of the soil and wastewater samples used for the microcosm experiments are listed in Tables 1 and 2, respectively. The soil sample AE was characterized as sandy clay soil, while soil sample BF was classified as a sandy soil based on textural analysis (Table 1). The pH of the soil and the water samples ranged between 4.92–5.02 and 6.94–6.98, respectively.

Table 1
Physico-chemical properties of the soil samples

Determinands	Soil-AE	Soil-BF
pH	4.92	5.02
Major cations/anions (μg/g)		
Calcium	22085	148
Magnesium	319	40.0
Sodium	2301	230
Potassium	893	299
Iron	11828	2111
Nitrate (soluble)	82.0	<24.6
Phosphate	762	15.3
Sulphate (soluble)	4590	<78.8
Total organic carbon	662	2960
Total Kjeldahl nitrogen	0.10	662
Texture[*]		
% Sand	52.0 ± 0.82	80.25 ± 0.50
% Clay	26.5 ± 1.29	8.25 ± 0.50
% Silt	21.5 ± 2.08	11.5 ± 0.58
[*]Total heterotrophic bacterial population (cfu/g × 10^5)	6.50 ± 1.15	3.0 ± 0.88

[*] Values are mean of results from triplicate analysis ± standard deviation.

Table 2
Physico-chemical properties of the wastewater samples

Determinands	NWW[a]	NGWW[b]
pH	6.98	6.94
Major cations/anions (μg/ml)		
Calcium	16.4	25.9
Magnesium	6.60	5.55
Sodium	62.5	93.0
Potassium	15.1	11.6
Iron	0.33	0.10
Nitrate (soluble)	18.2	15.0
Nitrite (soluble)	<0.05	0.39
Phosphate	4.2	4.86
Total Kjeldahl nitrogen	6.12	6.76
Total heterotrophic bacterial population[c] (cfu/g × 10^5)	13.25 ± 1.25	3.40 ± 0.70

[a] Northern wastewater.
[b] New Germany wastewater.
[c] Values are mean of results from triplicate analysis ± standard deviation.

Sodium concentration was found to be 10-fold higher, potassium threefold higher, calcium about one hundred and 50-fold higher and iron about sixfold higher in soil AE compared to soil BF. Phosphate and nitrate concentration were also higher in soil AE than in soil BF. However, soil BF was found to be richer in total organic carbon and total Kjeldahl nitrogen, with the total organic carbon concentration observed to be about fivefold higher than those obtained from soil AE (Table 1). Phosphate, total Kjeldahl nitrogen, calcium and sodium concentration were generally higher in New Germany wastewater than in Northern wastewater, with sodium and calcium concentration found to be 1.5-fold higher. Magnesium, potassium, iron and nitrate concentrations were generally slightly higher in the Northern wastewater sample than in New Germany wastewater sample (Table 2).

3.2. Biodegradation in soil microcosms

The degradation of *cis*- and *trans*-DCE in the soil microcosms are represented in Figs. 1 and 2. Both compounds were observed to degrade readily in both soil types with *cis*-DCE exhibiting a slightly faster rate of loss than *trans*-DCE in most cases. The degradation rate constants of *cis*-DCE ranged between 0.194 and 0.560 wk^{-1} while that of *trans*-DCE ranged between 0.215 and 0.401 wk^{-1}, for the various treatments of the soil microcosms (Table 3). The highest degradation rate constant was observed for *cis*-DCE in the biostimulation experiments while the combination of bioaugmentation and biostimulation resulted in the highest degradation rate constant for *trans*-DCE. However, the lowest degradation rate constant values were obtained in the microcosms with no additional nutrients and inocula (untreated soil microcosms) in both cases. Moreover, in the untreated soil microcosms, 43.72% of *cis*-DCE was degraded in soil type AE (Fig. 1a) and 32.1% in the soil type BF (Fig. 1b) after two weeks, while 36.73% (Fig. 2a) and 34.96% (Fig. 2b) of *trans*-DCE were degraded in the respective soil types for the same period. Furthermore, *cis*-DCE was observed to degrade faster in soil type AE, with 46.73% degradation in three weeks observed (Fig. 1a), compared to soil type BF where 33.61% decrease of compound was observed after the same period of time (Fig. 1b). However, there appeared to be no significant difference in the rate of loss of *trans*-DCE in the two soil types, with 63.27% (Fig. 2a) and 65.04% (Fig. 2b) of the compound still detected in the soil type AE and BF microcosm bottles, respectively, after two weeks. In soil type AE, the combination of biostimulation and bioaugmentation was observed to have a dramatic effect on the biodegradation of both compounds, with 14.44% increase in *cis*-DCE (Fig. 1a) and 18.35% increase in *trans*-DCE (Fig. 2a) degradation observed within two weeks, above those observed in untreated soil. This was followed by biostimulation alone, leading to 3.38% (Fig. 1a) and 18.11%

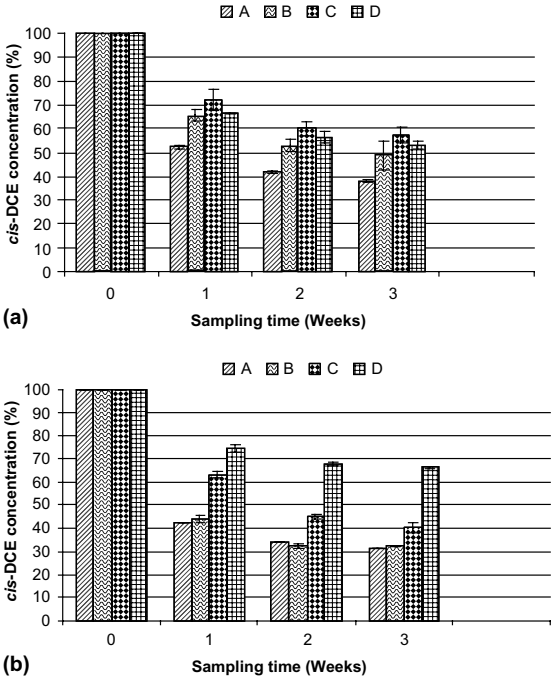

Fig. 1. Degradation profiles of *cis*-DCE in soil microcosms constructed with (a) soil type AE and (b) soil type BF under various treatments (A = biostimulation + bioaugmentation; B = biostimulation alone; C = bioaugmentation alone; D = soil with only the indigenous microorganisms). Bars indicate the average of triplicate samples while the error bars show the standard deviation.

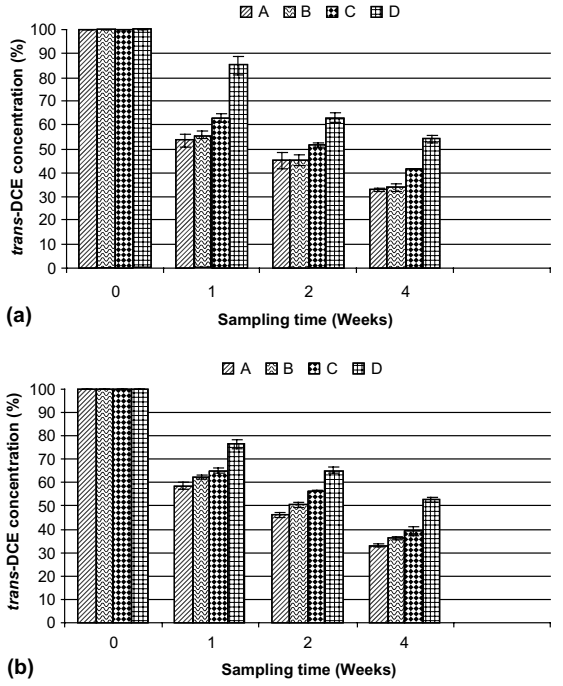

Fig. 2. Degradation profiles of *trans*-DCE in soil microcosms constructed with (a) soil type AE and (b) soil type BF under various treatments (A = biostimulation + bioaugmentation; B = biostimulation alone; C = bioaugmentation alone; D = soil with only the indigenous microorganisms). Bars indicate the average of triplicate samples while the error bars show the standard deviation.

(Fig. 2a) increase in *cis*- and *trans*-DCE degradation, respectively. Moreover, the combination of biostimulation and bioaugmentation was also found to result in 33.87% increase in *cis*-DCE (Fig. 1b) and 18.75% increase in *trans*-DCE (Fig. 2b) degradation in soil type BF in two weeks, compared to the untreated soils. In both cases, bioaugmentation alone had a lesser effect on the degradation of the compounds with only 11.6% and 8.71% increase in *trans*-DCE degradation observed for soil type AE (Fig. 2a) and BF (Fig. 2b), respectively, after two weeks.

3.3. Biodegradation in water microcosms

Figs. 3 and 4 illustrate the patterns of biodegradation of *cis*- and *trans*-DCE in the water microcosms. Both compounds were also observed to be readily degraded in the two water samples tested. The degradation rate constants of *cis*-DCE ranged significantly ($P < 0.05$), between 0.0938 and 0.529 wk^{-1} while those of *trans*-DCE ranged between 0.182 and 0.388 wk^{-1}, for the various treatments of the water microcosms (Table 3). In the untreated water microcosms (with no additional nutrients and inocula), *trans*-DCE was observed to disappear

Table 3
Biodegradation rate constants of *cis*- and *trans*-DCE in soil and water microcosms under various treatments (wk^{-1})

Treatments*	Soil microcosms				Water microcosms			
	AE		BF		NWW		NGWW	
	cis-DCE	*trans*-DCE	*cis*-DCE	*trans*-DCE	*cis*-DCE	*trans*-DCE	*cis*-DCE	*trans*-DCE
A	0.436 (0.00751)	0.401 (0.0365)	0.539 (0.00173)	0.385 (0.0125)	0.529 (0.0194)	0.257 (0.0146)	0.395 (0.00153)	0.388 (0.0242)
B	0.319 (0.0236)	0.397 (0.0234)	0.560 (0.0146)	0.341 (0.0113)	0.447 (0.0159)	0.338 (0.0636)	0.328 (0.0104)	0.347 (0.050)
C	0.257 (0.0252)	0.330 (0.010)	0.396 (0.0104)	0.287 (0.001)	0.190 (0.0159)	0.262 (0.0114)	0.0938 (0.00901)	0.298 (0.00896)
D	0.288 (0.0202)	0.229 (0.015)	0.194 (0.00551)	0.215 (0.0104)	0.103 (0.0164)	0.182 (0.0481)	0.136 (0.0150)	0.185 (0.00603)

* A = biostimulation + bioaugmentation; B = biostimulation alone; C = bioaugmentation alone; D = untreated samples (i.e., soil and water samples with only the indigenous microorganisms). Values in parentheses indicate the standard deviation from triplicate analysis.

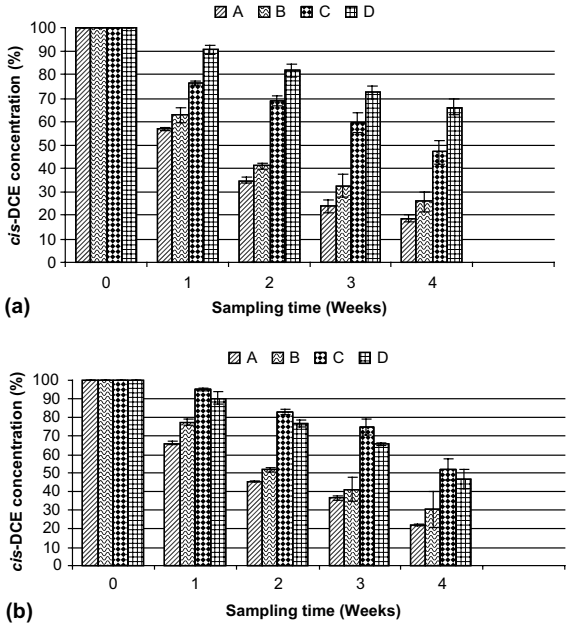

Fig. 3. Degradation profiles of *cis*-DCE in water microcosms constructed with (a) Northern wastewater and (b) New Germany wastewater under various treatments (A = biostimulation + bioaugmentation; B = biostimulation alone; C = bioaugmentation alone; D = soil with only the indigenous microorganisms). Bars indicate the average of triplicate samples while the error bars show the standard deviation.

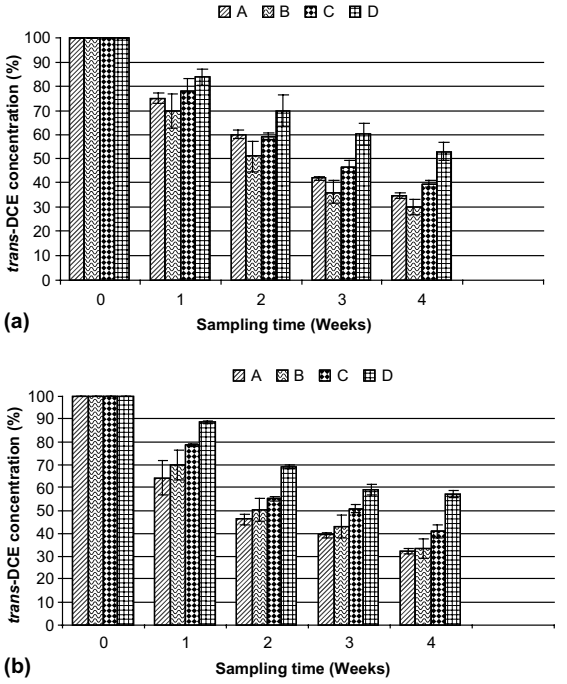

Fig. 4. Degradation profiles of *trans*-DCE in water microcosms constructed with (a) Northern wastewater and (b) New Germany wastewater under various treatments (A = biostimulation + bioaugmentation; B = biostimulation alone; C = bioaugmentation alone; D = soil with only the indigenous microorganisms). Bars indicate the average of triplicate samples while the error bars show the standard deviation.

faster than *cis*-DCE, with 39.84% and 40.91% found to be degraded in the microcosms constructed with Northern wastewater (Fig. 4a) and New Germany wastewater (Fig. 4b), respectively, after three weeks, while 27.6% (Fig. 3a) and 34.45% (Fig. 3b) of *cis*-DCE were degraded in the respective wastewater microcosms at the same incubation period. In microcosms constructed with the Northern wastewater, bioaugmentation alone was found to result in 12.78% increase in *cis*-DCE (Fig. 3a) and 13.31% increase in *trans*-DCE (Fig. 4a) degraded. However, biostimulation resulted in 39.97% increase in *cis*-DCE and 23.85% increase in *trans*-DCE degraded, while the combination of biostimulation and bioaugmentation led to 48.78% and 18.03% increase in *cis*-DCE and *trans*-DCE degraded, respectively, after three weeks.

Similarly, in those microcosms constructed with New Germany wastewater, the combination of biostimulation and bioaugmentation significantly affected the biodegradation of both compounds, resulting in 63.29% *cis*-DCE (Fig. 3b) and 60.65% *trans*-DCE (Fig. 4b), degradation in three weeks, values generally significantly higher than 34.45% *cis*-DCE and 40.91% of *trans*-DCE observed to be degraded for the same period in the untreated water microcosms. Biostimulation alone was also observed to have a marked effect on the biodegradation process, with 58.99% of *cis*-DCE and 57.26% of *trans*-DCE degraded, values which are 24.44% and 16.35% above the degradation percentages observed for *cis*- and *trans*-DCE, respectively, in the untreated water microcosms. However, addition of inocula alone was observed to have a negative effect on the biodegradation of *cis*-DCE and only contributed 8.21% increase to the amount of *trans*-DCE degraded.

4. Discussion

Some chlorinated compounds act as sole carbon source for some microorganisms under aerobic conditions and several microorganisms have been isolated that can use a number of chlorinated compounds as growth substrates (Stucki et al., 1983; Janssen et al., 1985). However, there is very little information on the potential for aerobic biodegradation with dichloroethenes as a sole carbon source, despite the observed accumulation of these compounds as products of reductive dechlorination of higher chlorinated ethenes in most contaminated sites. In this study, the effects of biostimulation and/or bioaugmentation on the biodegradation of

cis- and *trans*-DCE by naturally occurring organisms in soils and water was investigated in order to determine the best bioremediation option for aerobic biodegradation of these compounds in contaminated sites.

The experimental results indicate that *cis*- and *trans*-DCE were degraded to varying degrees by the natural microbial populations in the untreated soil and water samples used, with up to 43.72% of *cis*-DCE and 36.73% of *trans*-DCE degradation obtained in the soil samples after two weeks. However, up to 34.5% and 40.91% degradation of *cis*- and *trans*-DCE, respectively, was obtained in the water microcosms constructed with Northern wastewater without the addition of exogenous organic nutrients and inocula (untreated microcosms). Previous studies have also reported significant degradation of dichloroethenes by soil and aquifer microbial communities, with 3 weeks being required for the disappearance of 50% of *cis*-DCE, and 4 weeks for the same amount of *trans*-DCE to disappear (Klier et al., 1999). Similar results were observed in this study, since up to 46.73% of *cis*-DCE and 47.35% of *trans*-DCE was degraded after three and four weeks, respectively, in the untreated soil microcosms. Also, 47.01% and 43.02% of *trans*-DCE was degraded after 4 weeks in the untreated water microcosms constructed with Northern and New Germany wastewater, respectively. Differences in aerobic degradation rates between similar isomeric forms of chlorinated compounds as observed in this study have been noted previously for some other chlorinated compounds. In general, the rate of degradation is observed to be faster when the halogens have a greater distribution on the molecule (Semprini et al., 1990). Several studies have noted that methanotrophs degrade *trans*-DCE more efficiently than *cis*-DCE (Federle et al., 1990; Hopkins et al., 1993). However, the opposite was reported for phenol-utilizers (Hopkins et al., 1993) and propane-oxidizing bacteria (Malachowsky et al., 1994). It therefore appears that the rate of biodegradation is a function of the microorganisms involved and that a slight difference in structure can significantly affect transformation potential. More basic information on enzyme structure and functions is required in order to properly explain this observation (Malachowsky et al., 1994).

Chemical and physical properties of soil have a reflective influence on aeration, nutrient availability, water retention, and consequently on biological activity. Some of the important properties are the particle size, the chemical composition, porosity, moisture content, the aeration status, cation exchange capacity and the organic fraction. In particular, particle size affects the surface chemistry of soils and the size of the pores. Thus, the most advantageous pore structure is one in which water is retained but a considerable fraction of the pores remain packed with air. This probably explains the observed higher biodegradation observed in soil type BF because it has a greater percentage of sand and a lower percentage of clay compared to soil type AE. It could also be due to the very high organic carbon content of the soil and the fairly higher pH value. Furthermore, the lower rates of transformation observed in the microcosms constructed with the New Germany wastewater may be attributed to a fairly lower microbial populations compared to the Northern wastewater, which is about fourfold lower.

Although, *cis*- and *trans*-DCE were observed to be readily degraded in all the microcosms by the indigenous microorganisms, the results of this study also indicate that microorganisms in both the soil and water samples could be stimulated to enhance degradation of the compounds. This is evidenced by the higher degradation rates observed for treated microcosms compared to the untreated microcosms (Table 3) and the significant increase in the degradation of the compounds as illustrated in Figs. 1–4. Despite the presence of nutrients like phosphorus, nitrogen, trace metals and vitamins which are required for growth of microorganisms, the low bioavailability or competition for these nutrients may limit biodehalogenation. Addition of nutrients such as nitrogen fertilizers has therefore been observed to enhance biotransformation (Mohn and Tiedje, 1992). In general, microbes have an average C:N ratio of about 5:1 to 10:1 within their biomass, depending on the type of microorganism. Therefore, the general rule of thumb for biodegradation of hydrocarbons to cell mass is to add nitrogen and phosphorus at a ratio of carbon to nitrogen to phosphorus of 100:10:1 (Norris, 1994). This has been successfully implemented in the cleaning up of oil-contaminated sites from the Exxon Valdez in the Prince William Sound, Alaska, as early as 1989 (Button et al., 1992). Also, several studies on the effects of biostimulation with mainly N–P–K or oleophilic fertilizers have reported positive effects (Atlas, 1981; Margesin and Schinner, 1999). Addition of inocula to the microcosms (bioaugmentation) also led to an increase in the amount of the compounds degraded in all the microcosms constructed except for *cis*-DCE degradation in the set constructed with New Germany wastewater.

Biostimulation has been previously reported to enhance the degradation of *cis*-DCE at the Moffett Field groundwater test site (Hopkins et al., 1993), and bioaugmentation was observed to increase the rate of degradation of 3-chlorobenzoate (Gentry et al., 2001). However, two major problems have been associated with bioaugmentation. One is the rapid decline in numbers or death of the introduced microbes because of biotic or abiotic stress resulting from their competition with the established microbial community, resulting in decrease of the amount of inoculated cells (Goldstein et al., 1985). The other is the difficulty in dispersal of the introduced microorganisms throughout the contaminated site (Pepper et al., 2002). These probably explain why the effect of

bioaugmentation observed in this study is not as pronounced in the enhancement of the biodegradation process compared to those of the biostimulation process. However, it is worth noting that the combined effect of both biostimulation and bioaugmentation tend to have a more marked increase in the biodegradation of these compounds in both the soil and water microcosms. Major et al. (2002) also observed partial dechlorination of trichloroethene to *cis*-DCE in laboratory microcosms inoculated with soil and groundwater when amended with lactate or methanol. Following the addition of a dechlorinating enrichment culture, KB-1, the chlorinated ethenes in the microcosms were completely converted to ethene, thus confirming the significance of the combination of biostimulation and bioaugmentation in the effective degradation of chlorinated organics.

In conclusion, results from this study have clearly demonstrated the potential of naturally occurring microorganisms in soil and water systems in South Africa for aerobic biodegradation of *cis*- and *trans*-DCE. It also indicates that biotransformation of the compounds may be affected by several factors, including isomer structure, soil type, and the amount of nutrients available in the water and soil. Of particular importance is the significant increase in the biodegradation rate of the compounds obtained by the addition of fertilizers and inocula, suggesting that a combination of biostimulation and bioaugmentation may be required for the efficient biodegradation of these compounds in contaminated soil and water.

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