

Full Length Research Paper

Aerobic cometabolic degradation of *cis*- and *trans*-dichloroethene by a consortium of bacteria isolated from contaminated sites in Africa

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Biodegradation of dichloroethenes (DCEs) to innocuous products such as ethene and carbondioxide is often a limiting factor in the use of reductive dechlorination as an approach for the remediation of chloroethene-contaminated sites. In this study, the effect of some water-soluble, non-toxic and non-flammable substrates on the biodegradation of DCEs by a mixed culture of aerobic bacteria isolated from contaminated sites in Africa was investigated. A general increase of 2.13 – 22.18% and 0.39 – 12.61% in the amount of *cis*- and *trans*-DCE degraded, respectively, was observed in the presence of the different primary substrates. Glucose had the most significant effect resulting in 91.68% removal of *cis*-DCE and 86.98% removal of *trans*-DCE after 7 days. These values corresponded to 22.18 and 12.61% above the amount of *cis*- and *trans*-DCE degraded, respectively, in the absence of primary substrates. Results from this study indicate that the biodegradation of both compounds is enhanced by cometabolism. This finding is significant for active bioremediation systems, since the addition of these substrates to groundwater has several advantages over other cometabolic electron donors presently in use.

Key words: Bacterial consortium, biodegradation, cometabolism, dichloroethene.

INTRODUCTION

The widespread use of higher chlorinated ethenes; tetrachloroethene (PCE) and trichloroethene (TCE), as dry-cleaning solvents and degreasing agents for military and industrial applications, is of serious concern to human health, owing to their carcinogenicity and toxicity (Milde et al., 1998; Squillace et al., 1999). Through natural attenuation, these industrially-important solvents can be degraded anaerobically by pure and mixed cultures of microorganisms, via reductive dehalogenation to less-chlorinated ethenes; dichloroethenes (DCEs) and

vinyl chloride (Gao et al., 1997; Maymó-Gatelle et al., 1995). However, the frequently observed accumulation of DCEs in subsurface ecosystems, as predominant daughter products of anaerobic biotransformation of PCE and TCE, has resulted in significant environmental contamination worldwide and is a cause for concern (Maymó-Gatelle et al., 1995). Furthermore, the biodegradation of DCEs to innocuous products such as ethene and carbondioxide is often a limiting factor in the use of reductive dechlorination as an approach for the remediation of chloroethene-contaminated sites. Therefore, an understanding of the factors affecting the transformation of DCEs is critical for evaluating natural attenuation and for establishing engineering procedures for chlorinated-solvent remediation (Löffler et al., 1997). Cometabolic degradation of DCEs under aerobic

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Table 1. List of bacterial isolates used in this study.

Isolate code	Source	Tentative identity
NG ₁	Refuse dumpsite (Nigeria)	<i>Bacillus cereus</i>
NG ₂	Soil sample (Nigeria)	<i>Bacillus</i> sp
NG ₃	Soil sample (Nigeria)	<i>Pseudomonas aeruginosa</i>
SA ₁	Soil sample (South Africa)	<i>Acinetobacter haemolyticus</i>
SA ₂	Soil sample (South Africa)	<i>Pseudomonas</i> sp.
SA ₃	Activated sludge (South Africa)	<i>Klebsiella</i> sp
SA ₄	Activated sludge (South Africa)	<i>Acinetobacter</i> sp.

Values are the means of triplicate data \pm standard deviation.

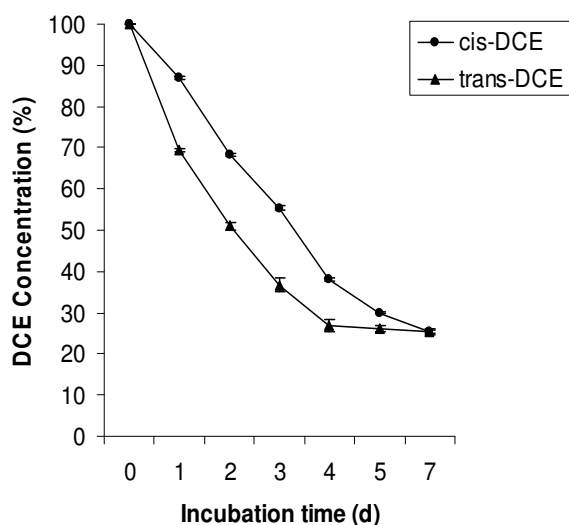


Figure 1. Biodegradation profiles of *cis*- and *trans*-DCE by the mixed culture of the bacterial isolates. Data points are the mean of triplicate cultures and the error bars indicate one standard error above and below the mean.

conditions has been observed in a number of microorganisms, including toluene-, phenol- and propylene- oxidizers, methanotrophs, and nitrifying bacteria, in which the addition of specific substrates is required for induction of the enzymatic processes (Dolan and McCarty, 1995; Ensign et al., 1992; Hopkins et al., 1993; Strandberg et al., 1989; Vanneli et al., 1990). However, some of these primary substrates such as phenol and toluene are themselves hazardous and not acceptable for release into the subsurface environment while others, such as propylene, methane and propane, are gaseous substrates with low water solubility (Gao and Skeen, 1999). Therefore, there is a need to evaluate water-soluble and non-toxic substrates that can induce microbial degradation of DCEs. Previously, we reported the aerobic dechlorination potential and dehalogenase activities of some bacteria isolated from contaminated sites in Africa (Olaniran et al., 2004, 2005). In this study, the effect of some water-soluble substrates on the biodegradation of DCEs by a mixed culture of these bacteria, under aerobic conditions, was

investigated. It is believed that these substrates could serve as safer and more acceptable alternatives for the substrates previously reported for cometabolic degradation of this group of compounds.

MATERIALS AND METHODS

Chemicals and media

Cis-dichloroethylene (*cis*-DCE) [97%] was obtained from Fluka and *trans*-dichloroethylene (*trans*-DCE) [98%] from Aldrich Chemical Company Inc. (Milwaukee, WI). The minimal salts medium (MSM) used for enrichment cultures was modified from that reported by Hartmans et al. (1992) by reducing the concentration of phosphate to 20 mM, ammonium to 10 mM and chloride concentration to 0.02 mM.

Isolation and identification of the bacterial isolates

The bacterial strains used in this study were isolated from samples collected from contaminated sites in South Africa and Nigeria using culture enrichment techniques, and identified as described elsewhere (Olaniran et al., 2004) and listed in Table 1. Pure cultures of the isolates were grown in nutrient broth (Difco), stored on nutrient agar (Difco) slants at 4°C as working stock cultures.

Measurement of DCE degradation

Two milliliters of potassium phosphate buffer cell suspension of the mixed culture of the bacterial isolates prepared from equal amounts of the standardized ($OD_{600} = 2$) culture of the individual isolates were used to inoculate 150 ml MSM in 250 ml serum bottle (headspace; 98 ml of air). The serum bottles were crimp-sealed with Teflon-faced butyl rubber stoppers (Wheaton). *Cis*- and/or *trans*-DCEs were added as the sole carbon source at a final concentration of 1 mM and monitored daily by injecting 100 μ l headspace sample with a 100 μ l gas-tight syringe (Hamilton) into a gas chromatograph equipped with a flame ionization detector and injector at 200°C and a packed column at 100°C. For the cometabolic degradation studies, one of the following substrates was added to the medium containing either *cis*- or *trans*-DCE: glucose, sucrose, lactose, yeast extract, and fertilizer (KOMPEL; Chemicult products, PTY LTD) at a concentration of 0.1%; toluene and phenol at a concentration of 50 mM before inoculated with the mixed culture of the bacterial isolates. The degradation of *cis* and *trans*-DCE was monitored in the different flasks by gas

Table 2. Average degradation rate constants of *cis*- and *trans*-DCE in the presence of the different primary substrates.

Primary substrate	Average degradation rate Constant (d^{-1})	
	<i>cis</i> -DCE	<i>trans</i> -DCE
None	0.170 ± 0.00603	0.195 ± 0.011
Glucose	0.356 ± 0.0115	0.291 ± 0.008
Sucrose	0.269 ± 0.0397	0.236 ± 0.0035
Lactose	0.184 ± 0.005	0.214 ± 0.0026
Toluene	0.184 ± 0.0121	0.219 ± 0.0012
Phenol	0.181 ± 0.011	0.207 ± 0.0058
Fertilizer	0.234 ± 0.011	0.196 ± 0.0031
Yeast extracts	0.247 ± 0.0512	0.201 ± 0.0066

Values are the means of triplicate data ± standard deviation.

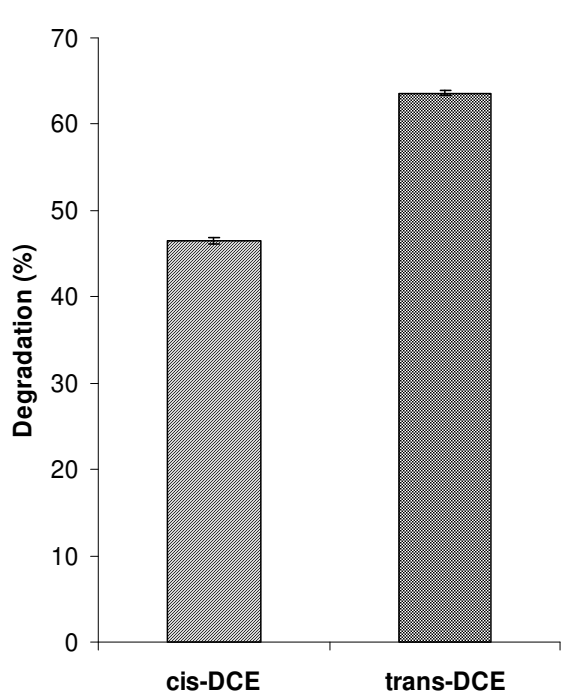


Figure 2. Amount of *cis*- and *trans*-DCE simultaneously degraded by the mixed culture of the bacterial isolates when present as a mixture. Data points are the mean of triplicate cultures and the error bars indicate one standard error above and below the mean.

chromatographic analysis as described above. Abiotic (no cells plus *cis* and/or *trans*-DCE) was used as a negative control and the percentage removal of the compounds indicated above that of losses of the negative controls. The degradation rate constant in each enrichment was estimated according to LaGrega et al. (1994).

RESULTS

The list of bacterial isolates used in this study is shown in Table 1. Both *cis*- and *trans*-DCE were effectively degra-

ded by the mixed culture of these bacterial isolates resulting in about 75% degradation in both cases (Figure 1). Also, 46.45% *cis*-DCE was simultaneously degraded with 63.63% of *trans*-DCE in a mixture of these compounds (Figure 2). A general increase of 2.13 – 22.18% and 0.39 – 12.61% in the amount of *cis*-DCE (Figure 3a) and *trans*-DCE (Figure 3b) degraded, respectively, was observed in the presence of the different primary substrates. Glucose has the most significant ($P < 0.05$) effect on the biodegradation of both compounds, resulting in 91.68% removal of *cis*-DCE and 86.98% removal of *trans*-DCE after 7 days, values corresponding to 22.18 and 12.61% above the amount of *cis*- and *trans*-DCE degraded, respectively, in the absence of primary substrates. Sucrose was also found to markedly affect the biodegradation of *cis*-DCE, followed by yeast extract, and fertilizer in that order, resulting in 14.89, 12.05 and 10.96% increase in biodegradation after 7 days, compared to the control [without any primary substrate] (Figure 3a). However, lactose, toluene, and phenol do not seem to have any significant effect on the biodegradation of *cis*-DCE by these organisms, resulting in only 2.84, 2.88 and 2.13% increase in degradation, respectively (Figure 3a). The presence of sucrose also led to an increase of 6.52% in *trans*-DCE degradation, followed by 4.02% increase in the presence of fertilizer, and 3.27% increase by lactose, while the presence of toluene, yeast extract and phenol only resulted in 2.15, 1.17 and 0.39% increase in *trans*-DCE degradation, respectively, above that of the control (Figure 3b). This is supported by the significantly high values of the degradation rate constant obtained in the presence of glucose and sucrose compared to those in the absence of the primary substrates (Table 2).

DISCUSSION

Despite activities of a wide variety of halogenated hydrocarbon-utilizing bacteria, several halogenated aliphatics, such as chlorinated ethenes are still believed to remain resistant to biodegradation under aerobic conditions. However, several microbial mechanisms for chloroethene transformation have been recently identified (Bradley and Chapelle, 2000), but little or no reports exists on the use of microorganisms that are indigenous to contaminated sites in Africa. Cometabolic oxidation of chloroethenes by microorganisms has been successfully exploited for engineered remediation of chloroethene contaminants in groundwater (McCarty and Semprini, 1994; Semprini, 1995). Also, aerobic cometabolism of dichloroethenes has been demonstrated by a variety of organisms, such as methanotrophs, phenol-oxidizers, nitrifying bacteria and propylene-oxidizers by adding specific substrates needed to induce the enzymatic processes (Bielefeldt et al., 1995; Dolan and McCarty, 1995; Ensign et al., 1992; Hopkins et al., 1993; Oldenhuis et al., 1991; Strandberg

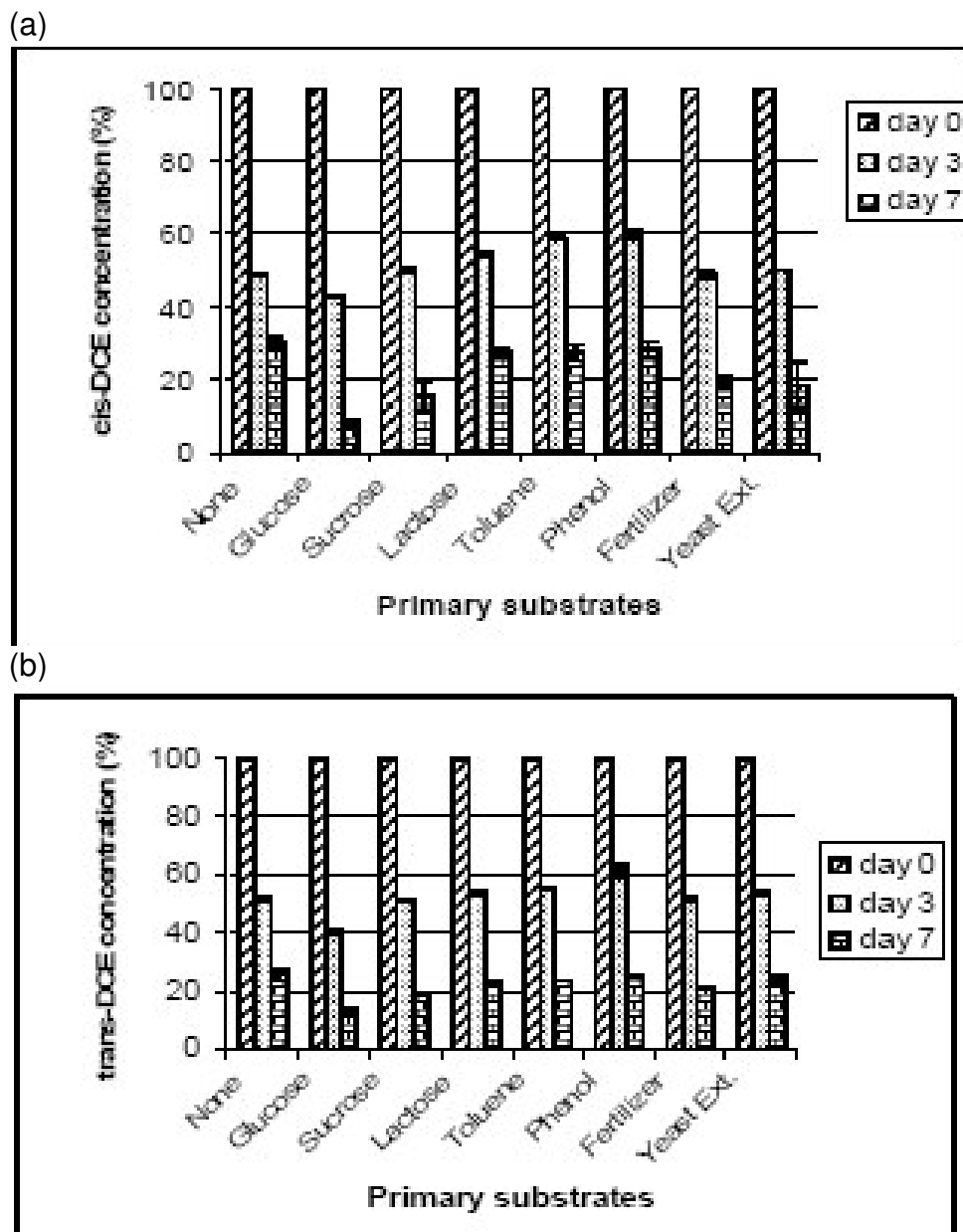


Figure 3. Degradation profiles of (a) *cis*-DCE and (b) *trans*-DCE in the presence of the different primary substrates. The data points are averages based on three replicate cultures, and the error bars indicate standard deviation.

et al., 1989; Vannelli et al., 1990). However, some of these substrates are themselves not safe in the environment, hence the need for the use of more environmentally-friendly primary substrates. The observed high degradation of DCE observed in the presence of glucose corroborates previous report of glucose-induced biodegradation of *cis*-DCE in subsurface soil under aerobic conditions (Gao and Skeen, 1999).

Results from this study indicate that the biodegradation of both compounds are enhanced by cometabolism, as seen by the effect of the different primary substrates

tested. It is very promising to note that the addition of non-toxic and water-soluble substrates such as glucose, sucrose and yeast extracts had significant effect on the biodegradation of *cis*- and *trans*-DCE. This is significant for active bioremediation systems, since the addition of these substrates to groundwater has several advantages over other cometabolic electron donors previously investigated. The observed simultaneous degradation of a mixture of these compounds also has important implications for their removal in sites contaminated with mixtures of chlorinated aliphatic hydrocarbons.

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