ORIGINAL ARTICLE

Aerobic biotransformation of trichloroethylene, cis-dichloroethylene, and vinyl chloride by a mixed culture grown on a non-toxic substrate: Benzyl alcohol

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Abstract

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The aerobic transformation of trichloroethylene (TCE) and cis-dichloroethylene (cis-DCE) by tetrabutoxysilane-grown microorganism led to the investigation of other alternative substrates, including benzyl alcohol, a novel non-toxic substrate for promoting cometabolism. The levels of cis-DCE and TCE transformations achieved were dependent on the growth substrate. The culture demonstrated high cis-DCE transformation when grown on butyrate, 1-butanol, and glucose, and high TCE transformation when grown on benzyl alcohol and phenol. The culture when grown on benzyl alcohol was very effective in promoting TCE, cis-DCE, and vinyl chloride (VC) transformations. Approximate transformation yields based on the amount of benzyl alcohol utilized (mg of CAHs transformed per mg of substrate utilized) for VC, cis-DCE, and TCE was 0.35, 0.25, and 0.053, respectively. Since benzyl alcohol is not a regulated compound, such as phenol and toluene, it may have use in applications for in-situ bioremediation.

Key words : cometabolism, aromatic, phenol, TCE, bioremediation

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บทคัดย่อ

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การบำบัดทางชีวภาพแบบใช้อากาศของไตรคลอโรเอททีลีน ซิส-ไดคลอโรเอททีลีนและไวนีลคลอไรด์โดยใช้จุลินทรีย์ผสมโตจากสารอาหารปลอดพิษ เบนซิลแอลกอฮอล์

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การรายงานการบำบัดไตรคลอโรเอททีลีนและซิส-ไดคลอโรเอททีลีนแบบใช้อากาศโดยจุลินทรีย์โตจากเตตตระบิวทอกซีไซเลนเป็นจุดเริ่มต้นให้ค้นคว้าหาสารอาหารชนิดอื่น ๆ มาแทน รวมถึงเบนซิลแอลกอฮอล์ที่ไม่เป็นพิษและได้ผลดีในการร่วมย่อยสลายสารประกอบคลอรีนเหล่านั้น การวิจัยพบว่าประสิทธิภาพในการบำบัดขึ้นอยู่กับประเภทของสารอาหาร การบำบัดซิส-ไดคลอโรเอททีลีนที่ได้ผลดีมาจากการใช้บิวทีเรต 1-บิวทานอลหรือกลูโคสเป็นสารอาหาร ส่วนการบำบัดไตรคลอโรเอททีลีนที่ได้ผลดีมาจากการใช้เบนซิลแอลกอฮอล์หรือฟีนอลเป็นสารอาหาร จุลินทรีย์ที่โตจากเบนซิลแอลกอฮอล์สามารถร่วมย่อยสลายทั้งไตรคลอโรเอททีลีน ซิส-ไดคลอโรเอททีลีนและไวนีลคลอไรด์ได้อย่างมีประสิทธิภาพ ผลของการบำบัดคิดเป็นอัตราส่วนของปริมาณไวนีลคลอไรด์ ซิส-ไดคลอโรเอททีลีนและไตรคลอโรเอททีลีนที่บำบัดได้ต่อปริมาณเบนซิลแอลกอฮอล์ที่ใช้ (มก/มก) โดยประมาณคือ 0.35 0.25 และ 0.053 ตามลำดับ เนื่องจากเบนซิลแอลกอฮอล์เป็นสารที่ไม่เป็นพิษและไม่มีกฎหมายควบคุมในการใช้เหมือนฟีนอลและโทลูอีน ดังนั้น จึงน่าจะเป็นประโยชน์ต่อการบำบัดฟื้นฟูแบบชีวภาพของสารประกอบคลอรีนเหล่านี้ในภาคสนาม

ภาควิชาวิศวกรรมสิ่งแวดล้อม คณะวิศวกรรมศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย พญาไท กรุงเทพฯ 10330

Trichloroethylene (TCE) is one of the most frequently found suspected carcinogenic contaminants in groundwater (U.S. EPA, 2002). One of the promising remediation methods is *in-situ* biological treatment. Anaerobic biological process normally results in accumulation of cis-dichloroethylene (cis-DCE) and vinyl chloride (VC), a known carcinogenic compound (McCarty and Semprini, 1994). On the contrary, in a shallow unconfined aquifer or with oxygen implemented, numerous studies have demonstrated that aerobic bacteria can transform TCE via cometabolic oxidation to harmless end products, such as chloride ion and carbon dioxide (McCarty and Semprini, 1994; McCarty, 1997; Semprini, 1997b). Microorganisms grown on the following substrates have been reported as having TCE cometabolism potential: ammonia (Arciero *et al*., 1989), butane (Kim *et al*., 2000), cresol (Wackett and Gibson, 1988; Folsom *et al*., 1990), dichlorophenoxyacetic acid (Harker and Kim, 1990), ethylene (Ensign *et al*., 1992), isoprene (Ewers *et al*., 1991), isopropylbenzene (Dabrock *et al*., 1992), methane (Oldenhuis *et al*., 1991; Chang and Alvarez-Cohen, 1996), phenol (Folsom *et al*., 1990; Hopkins *et al*., 1993), propane (Wackett *et al*., 1989), propene (Ensign *et al*., 1992), and toluene (Wackett and Gibson, 1988; Shields *et al*., 1989).

Among those primary substrates mentioned above, aromatic substrates such as phenol and toluene have been reported to give higher TCE transformation yield (T_Y, mass of TCE transformed per unit mass of substrate consumed). However, both phenol and toluene are health-concerned regulated compounds with recommended maximum contamination levels for drinking water of 0 and 1 ppm, respectively (U.S. EPA, 2001). Therefore, obtaining regulatory approval for in-situ use may prove difficult in some cases. A number of studies have focused on finding non-regulated substrates for use in the in-situ treatment of TCE and other chlorinated aliphatic hydrocarbons (CAHs), such as dichloroethylene (DCE), vinyl chloride (VC), etc. These substrates included glucose (Gao and Skeen, 1999), methanol (Fan and Scow, 1993), ethanol (Kim *et al*., 1996), fructose (Muller and Babel, 1995), lactate (Munakata-Marr *et al*., 1996), and tetrabutoxysilane (Vancheeswaran *et al*., 1999).

In this study, the tetrabutoxysilane (TBOS) enrichment culture was tested for its ability to grow on a broad range of substrates, and cometabolize cis-DCE and TCE. Substrates tested included silicon-based organic compounds (TBOS, tetrapropoxysilane (TPOS), and tetraphenoxysilane), phenolic compounds (phenyl acetate, p-cresol, and phenol), alcohols (methanol, ethanol, 1-propanol, 1-butanol, 4-methyl-benzyl alcohol, 3-buten-2-ol, 3-buten-1-ol, 2-buten-1-ol, and benzyl alcohol), organic acids (formate, acetate, propionate, butyrate, and benzoate), aromatic compounds (benzene, toluene, and p-xylene), saturated and unsaturated hydrocarbons (methane, propane, butane, and ethylene), acetone, glucose, and methyl-*tert*-butyl-ether (MTBE).

Materials and Methods

1. Bacterial culture

An aerobic mixed culture was obtained from a TBOS-grown culture that had cis-DCE and TCE transformation ability (Vancheeswaran *et al*., 1999), which was originally started by using activated sludge of a wastewater treatment plant. To produce a reproducible culture for these studies, the enriched mixed culture was grown in 710-ml batch serum bottles (Wheaton Glass Co., Millville, NJ) on 400-mg/l TBOS in 400-ml basal salt medium (BSM) (Vancheeswaran *et al*., 1999) to an optical density of 1.0 at 550 nm (OD(550)). The culture was centrifuged, rinsed with BSM, and stored at an approximate concentration of 3 mg/ml at –80°C in 7% dimethylsulfoxide (DMSO).

To grow cells for the batch reactor tests, the frozen cells were thawed, rinsed with fresh BSM, and batch-grown on 400 mg/l TBOS or 1-butanol (Table 1) to an OD(550) of 0.6. Cells were centrifuged, rinsed with BSM, and diluted to concentrations of approximately 0.3 mg/ml for use in growth and cometabolic transformation tests.

2. Chemicals

All chemicals were purchased at the highest quality possible (98% purity or higher).

3. Experimental processes

TBOS-grown culture was first tested for TCE cometabolism potential by comparing between TBOS and its hydrolysis by-product, 1-butanol, as growth substrate. Then the culture was tested with listed substrates (Table 2) for growth and cis-DCE and TCE cometabolism potential. The three most effective non-toxic substrates were chosen and tested for VC, cis-DCE, and TCE cometabolic transformation.

4. Batch reactors

The experiments were conducted at 20°C in batch reactors consisting of 60-ml BSM in

Table 1. Growth substrates and cometabolic conditions of the batch experiments

Experiments	Cell Inoculate Growth Substrate	CAH Transformation During Growth on Specific Substrate	
		Growth substrate	CAH
Cometabolic transformation of TCE during growth on TBOS and 1-butanol	TBOS	TBOS (0-30 µmol), 1-butanol (0-160 µmol)	TCE (0.16 µmol)
Substrate tested for growth and TCE and cis-DCE transformation	1-butanol	See Table 2	cis-DCE (15 µmol), TCE (1.8 µmol)
Substrates tested for VC, cis-DCE, and TCE cometabolic transformation	1-butanol	Butyrate (97 µmol), Glucose (81 µmol), Benzyl alcohol (57 µmol)	VC (11 µmol), cis-DCE (4.1 µmol), TCE (0.7 µmol)

Table 2. Substrates tested for growth and cis-DCE and TCE transformation

Substrate	Amount[1] µmol	Final OD(550)	cis-DCE		TCE	
			% Removal[2]	T_y[3]	% Removal[2]	T_y[3]
Tetrabutoxysilane	20	0.34	45%	0.34	26%	0.023
Tetrapropoxysilane	27	0.29	40%	0.22	19%	0.012
Tetraphenoxysilane	17	0.21	100%	0.88	100%	0.106
Phenyl acetate	54	0.27	100%	0.28	81%	0.027
Methanol	324	0.02	0%	ND	ND	ND
Ethanol	162	0.24	32%	0.03	ND	ND
1-Propanol	108	0.24	44%	0.06	ND	ND
1-Butanol	81	0.24	98%	0.18	16%	0.004
Formate	973	0.02	0%	ND	ND	ND
Acetate	243	0.33	73%	0.05	ND	ND
Propionate	139	0.30	42%	0.04	ND	ND
Butyrate	97	0.40	93%	0.14	9%	0.002
Benzoate	65	0.24	84%	0.19	6%	0.002
Acetone	122	0.22	58%	0.07	ND	ND
Phenol	69	0.21	100%	0.22	100%	0.026
p-cresol	57	0.19	100%	0.26	100%	0.032
4-Methyl-benzyl alcohol	49	0.05	0%	ND	ND	ND
3-buten-2-ol	88	0.03	0%	ND	ND	ND
3-buten-1-ol	88	0.22	89%	0.15	21%	0.004
2-buten-1-ol	88	0.23	67%	0.11	20%	0.004
Glucose	81	0.40	93%	0.17	44%	0.010
Benzyl alcohol	57	0.18	100%	0.26	100%	0.032
Methane	243	0.07	0%	ND	ND	ND
Propane	97	0.04	0%	ND	ND	ND
Butane	75	0.04	0%	ND	ND	ND
Ethylene	162	0.02	0%	ND	ND	ND
Benzene	65	0.04	0%	ND	ND	ND
Toluene	54	0.02	0%	ND	ND	ND
p-Xylene	46	0.03	0%	ND	ND	ND
MTBE	65	0.03	0%	ND	ND	ND

[1] Substrate amounts were based on the equal electrons transferred in aerobic utilization.
[2] % Removal based on a single addition of 15 µmol cis-DCE or 1.8 µmol TCE
[3] Transformation yield (T_y) in µmol CAHs per µmol substrate
ND = not determined

125-ml crimp-sealed bottles (Wheaton Glass Co., Millville, NJ) with headspace air to maintain aerobic conditions. The reactors were autoclaved to prevent contamination, and BSM was added. After the addition of substrates and CAHs, bottles were inverted and shaken at 200 rpm overnight prior to cell addition. Simultaneous growth and cometabolism tests were initiated by adding 0.3 mg of cells (as described above) into batch reactors. Preparations and conditions of batch reactors are shown in Table 1. Substrates, CAHs, and growth (as observed by OD(550) unit) were monitored periodically until OD(550) and CAHs concentration remained constant. The batch reactors were operated in triplicate unless indicated otherwise. Pure oxygen was added when negative pressure

developed in the reactors. TCE and cis-DCE were added using a saturated stock solution in deionized water. VC was added as a pure gas. Control bottles containing CAHs alone and with resting cells (~0.3 mg-cells without substrate) were monitored along with active bottles.

5. Analytical methods

Calibration curves for all compounds were developed using external standards. The aqueous concentration of TBOS and 1-butanol was determined by dichloromethane (DCM) extraction and GC-FID analysis. Aqueous samples were extracted with DCM in a 1:1 volume ratio, by vigorously mixing for 5 min on a vortex mixer and centrifuging at 14,000 rpm for 3 min. The DCM extract (1 µl) was injected by a Hewlett Packard HP 7673A automatic sampler to a Hewlett Packard 5890 gas chromatograph (GC) equipped with a flame ionization detector (FID). Chromatographic separation was achieved with a 30 m × 0.32 mm × 0.25 µm RTX-5 column (Restek Corporation, Bellefonte, PA) and operated at the following temperature profile: 5 min at 35°C; gradient, 40°C/min; and 5 min at 300°C. Carrier gas was helium at a flow rate of 1.5 ml/min. The total mass of TBOS and 1-butanol was determined using aqueous concentration and liquid volume.

Headspace concentrations of cis-DCE and VC were determined on a HP5890-GC using a 30 m × 0.53 mm GSQ-PLOT column (J&W Scientific, CA), operated at 140°C, with a FID detector. The carrier gas was helium at a flow rate of 1.5 ml/min. TCE headspace concentration was determined on a HP5890-GC using a 30 m × 0.25 mm × 1.4 µm HP-624 column (Hewlett Packard, Wilmington, DE) operated at 140°C, with an electron conductivity detector (OI Analytical, College Station, TX). The carrier gas was helium at a flow rate of 1.5 ml/min with an argon/methane mixture (95%:5%) for make-up gas. The total mass of chlorinated compounds in the serum bottle was calculated by using published Henry's constants (Gossett, 1987) and equilibrium mass balances using the measured headspace concentration and the volumes of the gas and liquid phases.

The cell concentration was determined as total suspended solids (TSS) (American Public Health Association, 1985) using 0.2-µm membrane filter (Micro Separation Inc., MA). Cell growth was observed by monitoring the optical density at 550 nm (OD(550)) using a Hewlett Packard 8453 UV-Visible spectrophotometer.

Results and Discussion

1. Cometabolic transformation of TCE growing on TBOS and 1-butanol

In a first series of tests, TBOS ($Si(OC_4H_9)_4$) and its hydrolysis by-product, 1-butanol (C_4H_9OH), were compared as growth substrates promoting TCE transformation. The constant TBOS concentration in the control bottle indicated that the abiotic hydrolysis of TBOS was insignificant over the time scale of the growth experiments (data not shown). TCE was transformed during the degradation of both TBOS and 1-butanol. While greater amounts of TBOS and 1-butanol consumption led to more transformation of TCE, the relationship was not linear (Figure 1). Up to 20 µmol-TBOS or 80 µmol-butanol consumption, a similar amount of TCE was transformed (the hydrolysis of one mol of TBOS produces 4 mol of 1-butanol). The results indicate that cells grown on 1-butanol were responsible for TCE cometabolism, and TBOS mainly served as a source of 1-butanol upon hydrolysis. More TBOS consumption (30 µmol) led to higher TCE transformation but more 1-butanol consumption did not. The reason for this is not known, some possibilities are the result of enzymes during hydrolysis process of TBOS to 1-butanol, induction of TBOS, or inhibition effect from high concentration of 1-butanol.

2. Growth on a broad range of substrates and the cometabolic transformation of TCE and cis-DCE

Other potential growth substrates for this culture were evaluated along with the culture's ability to transform TCE and cis-DCE when grown

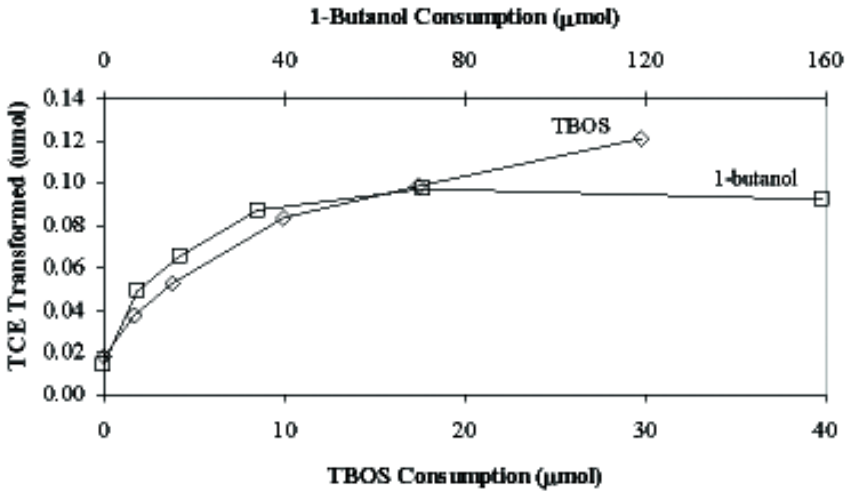

Figure 1. Comparison of TCE transformation by a culture growing on TBOS and 1-butanol

on each substrate. The amount of each substrate added to the batch growth reactors was based on an equal electron donor potential equivalent to 81 µmol 1-butanol (Table 2). Enrichments of 1-butanol-grown cells (0.3 mg dry weight) were added to triplicates of 125-ml bottles containing 60 ml media with cis-DCE or TCE (Table 1) and the specific growth substrate. Growth was observed by monitoring optical density. Results of cis-DCE transformation studies with formate, acetate, propionate, butyrate, and 1-butanol, as growth substrates, are shown in Figure 2. The culture grew to OD(550) of 0.3-0.4 on acetate, propionate, butyrate, and 1-butanol, but no growth occurred with formate. Transformations of cis-DCE were observed after OD(550) reached maximum value. Greater amounts of cis-DCE (13.9 - 14.7 µmol) were transformed with growth on 1-butanol and butyrate. Growth on acetate, and propionate resulted in 11.0 and 6.2 µmol cis-DCE transformed, respectively. Conservative estimates of T_Y on each of the substrates are as follows: 0.18 mol cis-DCE/mol 1-butanol, 0.14 mol cis-DCE/mol butyrate, 0.045 mol cis-DCE/mol acetate, and 0.045 mol cis-DCE/mol propionate. These estimates are conservative since cis-DCE was almost completely transformed with several substrates and exposure to more cis-DCE may have resulted in continued transformation.

The mechanism by which cells when grown on simple substrates, such as 1-butanol, butyrate, and glucose, promote cis-DCE cometabolism is not known. Gao and Skeen (1999) observed cis-DCE transformation in a glucose-induced microcosm, and reasoned that substrates like glucose can be aerobically degraded through multiple pathways, such as the Embden-Meyerhof-Parnas (EMP) pathway, the hexose monophosphate (HMP) pathway, and the tricarboxylic acid (TCA) cycle (Voet and Voet, 1990; Gao and Skeen, 1999). The enzymes involved in these pathways might be responsible for the cis-DCE transformation. In addition, Verce *et al*. (2002) reported the cometabolic transformation of cis-DCE by a VC-grown culture, and Coleman *et al*. (2002) recently reported a microcosm that can grow on cis-DCE and cometabolize VC and TCE. These results indicate that there are multiple natural-mechanisms for the removal of these chloroethenes in the aerobic environment.

The ability of the culture to transform cis-DCE with microbes grown on simple organic acids, such as butyrate and acetate, has potential implications on the intrinsic transformation processes. Anaerobic transformation of PCE and TCE often do not proceed beyond cis-DCE (McCarty

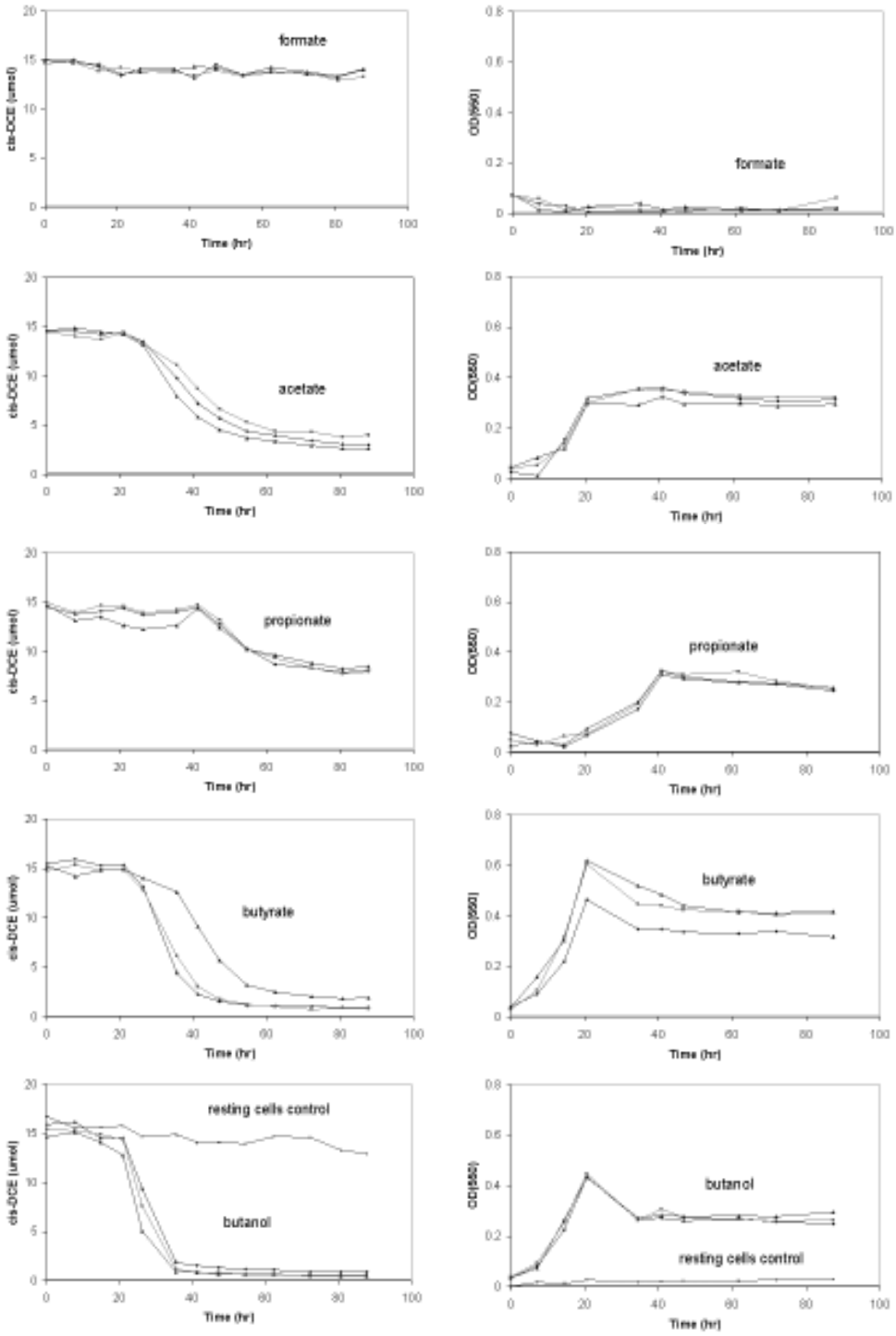

Figure 2. Cis-DCE transformation and growth (observed by OD(550)) of the enrichment grown on formate, acetate, propionate, butyrate, and 1-butanol, compared to resting cells control (x) with no substrate added. ◆ , ■ , and ▲ showed triplicate data of each condition.

and Semprini, 1994; Semprini, 1997a; Lee *et al*., 1998). Acetate, propionate, and butyrate may be produced by fermentation reactions in anaerobic portions of plume during the breakdown of hydrocarbons and other organic compounds (Fennell and Gossett, 1997, 1998). At the distal parts of the contaminant plume the growth of organisms on residual organic acids under aerobic conditions might promote intrinsic treatment of cis-DCE. The results presented here indicate more research is needed on this potential process.

The results for all substrates are summarized in Table 2. The mixed culture grew on tetrapropoxysilane (TPOS, $Si(OC_3H_7)_4$) and showed transformation of cis-DCE and TCE similar to that when grown on 1-propanol (C_3H_7OH), a TPOS hy-drolysis by-product. Tetraphenoxysilane ($Si(OC_6H_5)_4$) and phenyl acetate ($C_6H_5CH_2COOH$) were as effective as their hydrolysis by-product, phenol (C_6H_5OH), in serving as growth substrates for cis-DCE and TCE cometabolism. It is interesting to note that the optical density achieved with phenol and tetraphenoxysilane were similar, while that with phenyl acetate was higher probably due to the presence of acetate that is also generated during the hydrolysis of this compound. The culture also grew on glucose with effective transformation of cis-DCE and some TCE transformation. Butyrate and benzoate were shown to be effective substrates for cis-DCE cometabolism, although they did not induce much of the TCE transformation. Benzyl alcohol ($C_6H_5CH_2OH$) was an effective substrate that resulted in complete transformation of cis-DCE and TCE. Optical densities achieved with benzyl alcohol were in the range of those observed with growth on phenol.

Some interesting trends were observed from the tests with the different growth substrates (Table 2). Most of the growth-supporting substrates are very soluble in water. The culture did not grow on single carbon substrates, such as methanol or formate, saturated hydrocarbons (methane, propane and butane), unsaturated hydrocarbon (ethylene), or non-oxygenated aromatics (benzene, toluene, and p-xylene). The culture, however, grew on oxygenated ringed compound including phenol, p-cresol ($CH_3C_6H_4OH$), benzyl alcohol, and benzoate, but not 4-methyl-benzyl alcohol ($CH_3C_6H_4CH_2OH$). The culture also grew on 1-propanol, 1-butanol, acetone, acetate, and propionate, with all showing some cis-DCE transformation. Growth on acetate, butyrate, and 1-butanol gave a similar range (73-98%) of cis-DCE transformation (Table 2). Growth occurred on 3-buten-1-ol and 2-buten-1-ol, but not on 3-buten-2-ol, indicating the important characteristic of having a hydroxyl group at the terminal carbon.

Based on the growth substrates tested, the enrichment culture has growth characteristic similar to *Rhodococcus* strain R-22 (Fairlee *et al*., 1997). This *Rhodococcus* strain is able to grow on acetone, phenol and benzyl alcohol, but did not grow on benzene or toluene. One difference is that R-22 can also grow on propane, but our enrichment culture could not. We have not found any reports of the study of chlorinated ethene cometabolism by R-22. The *Rhodococcus* strain reported to have TCE transformation ability was *R. erythropolis* BD2, which was grown on isopropylbenzene and exhibited enzyme similar to toluene dioxygenase (Dabrock *et al*., 1994).

Effective cis-DCE transformation (100%) was correlated with good TCE transformation ability, while limited cis-DCE transformation (less than 50%) was correlated with limited TCE transformation potential. Effective transformation of both cis-DCE (100%) and TCE (100%) was achieved with the aromatic substrates, phenol, tetraphenoxysilane (that hydrolyzes to phenol), p-cresol and benzyl alcohol.

3. Test of cometabolic growth substrates for VC, cis-DCE, and TCE transformation

Butyrate, glucose, and benzyl alcohol were chosen as growth substrates to compare the cometabolic transformation of VC, cis-DCE and TCE based on the initial screening showing cis-DCE and TCE transformation potential (Table 2). The amount of butyrate, glucose, and benzyl alcohol was based on equal electron donor equiva-

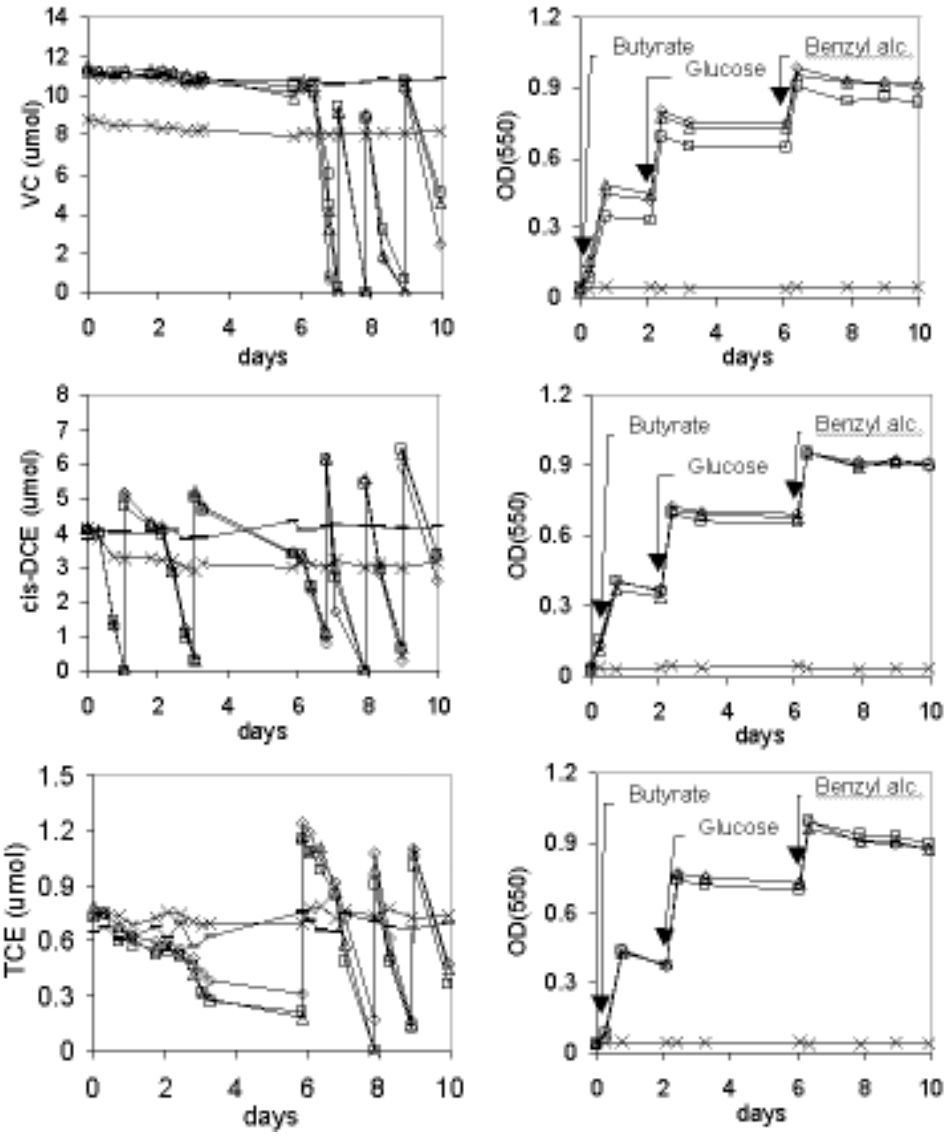

Figure 3. Transformation of VC, cis-DCE, and TCE, and growth observed by OD(550) after addition of butyrate, glucose, and benzyl alcohol at 0, 2.1, and 6.1 days respectively. ◊ , □ , and Δ showed triplicate data of each condition. Controls were: cells without substrate (x) and no-cells (-).

lents for growth (Table 1). Butyrate, glucose, and benzyl alcohol were added at 0, 2.1, and 6.1 days, respectively (Figure 3). Initial and additional amounts of VC and cis-DCE resulted in aqueous concentration of 70 – 85 μM, while TCE amounts resulted in 10 – 15 μM. The lower aqueous concentration of TCE was due to the reported inhibitory effect of TCE above this concentration range (Futamata *et al*., 2001). However, these concentrations were much higher than reported half-saturation concentrations of these compounds (Alvarez-Cohen and Speitel, 2001), indicating maximum

transformation rates were possible.

Similar increases in optical density were observed with growth on all the substrates. During growth on butyrate, no transformation of VC was observed, while effective cis-DCE and limited TCE transformation was observed. The total transformation of cis-DCE and TCE with growth on butyrate was 5 µmol and 0.2 µmol, respectively. Utilization of glucose, as a substrate, did not yield significant transformation of VC; however, 6 µmol of cis-DCE and 0.4 µmol of TCE were transformed. VC, cis-DCE, and TCE transformation was observed with the addition of benzyl alcohol at 6.1 days, and three repeated additions of VC, cis-DCE, and TCE were transformed without further addition of benzyl alcohol. Approximate transformation yields based on the amount of benzyl alcohol utilized (mg of CAHs transformed per mg of substrate utilized) for VC, cis-DCE, and TCE were 0.35, 0.25, and 0.05, respectively.

When cells were grown on butyrate and glucose, and then switched to benzyl alcohol, high transformation yields for vinyl chloride, cis-DCE, and TCE were achieved, and essentially no lag in the transformation was observed. It is likely that cells grown on butyrate and glucose were induced on benzyl alcohol to transform these compounds. The cometabolism of vinyl chloride was reported by both soluble and particulate methane monooxygenase (Chang and Alvarez-Cohen, 1996), alkene monooxygenase (Ensign *et al*., 1992), and in *in-situ* field tests when toluene *ortho*-monooxygenase was the dominant enzyme present (Hopkins and McCarty, 1995; Jenal-Wanner and McCarty, 1997). Toluene dioxygenase, expressed by *Pseudomonas putida* strain F1, did not transform vinyl chloride, whereas it transformed both TCE and cis-DCE (Wackett and Gibson, 1988). Therefore, the enzyme expressed for benzyl alcohol degradation is more likely a monooxygenase than dioxygenase. Current studies are underway to identify the oxygenase enzymes involved.

Conclusion

Cis-DCE and TCE were aerobically transformed by cells grown on a broad range of substrates having at least two carbons and were substituted with oxygen. Among tested substrates, 1-butanol, butyrate, glucose, and benzyl alcohol were non-regulated compounds and achieved more than 90% transformation of initial amount of cis-DCE present. TCE, however, was less effectively transformed. The only non-regulated compound to promote 100% transformation of TCE was benzyl alcohol. Phenol, p-cresol, and phenol-release compounds (tetraphenoxysilane and phenyl acetate) were very effective in both cis-DCE and TCE transformation, but both phenol and p-cresol are regulated chemicals that may not be acceptable for release in the environment, even though they are readily biodegraded.

The important characteristics of growth substrates for this enrichment culture are 1) high solubility; 2) the substrate must have two or more carbon atoms; and 3) have some oxygen or hydroxide substitution. The enrichment also has the ability to hydrolyze and utilize complex substrates, including TBOS, TPOS, tetraphenoxysilane and phenyl acetate. Similar effectiveness in the cometabolic transformation of cis-DCE and TCE was achieved as when their hydrolysis by-products (1-butanol, 1-propanol, and phenol) were used directly. This result might be useful for *in-situ* bioremediation where phenol could be replaced with relatively inert compounds such as tetraphenoxysilane and phenyl acetate.

Among the growth substrates tested, benzyl alcohol is of special interest since it is non-toxic and very effective in promoting the transformation of VC, cis-DCE, and TCE. These compounds are important since VC and cis-DCE are often present as anaerobic transformation products of perchloroethene (PCE) and TCE. The initial concentrations of VC, cis-DCE, and TCE were assumed to result in maximum transformation rates; however, more work on transformation kinetics is necessary.

Benzyl alcohol was found to be an effective substrate for the cometabolism of VC, cis-DCE, and TCE. It might be a potential substitute for toluene or phenol for *in-situ* bioremediation. Benzyl alcohol is in a liquid phase at room temperature, like toluene, with many advantages in physical, chemical, and toxicological properties (National Technical Information Service, 1989; National Toxicology Program, 2002). It has a higher boiling point (205°C), lower vapor pressure (0.15 mmHg @ 25°C), and higher solubility (40 g/l @ 20°C), compared to toluene (110.6°C, 22.0 mmHg @ 20°C, and 0.515 g/l @ 20°C), which potentially makes it more suitable in field site applications. For the safety of handling and transportation, benzyl alcohol has a moderate flammability with irritation effect in respiratory tract, while toluene is highly flammable with severe central nervous system effect from inhalation. For *in-situ* application, toluene is a common groundwater contaminant, with a recommended maximum contamination level (MCL) of 1 mg/l, while benzyl alcohol is a non-regulated compound and commonly used as food flavoring agent (Mallinckrodt Baker Inc., 2000; European Commission, 2002). More work is needed with direct comparison of this culture with phenol as a substrate. A broader evaluation is also needed on the microorganisms that can grow on benzyl alcohol and cometabolize chlorinated ethenes.

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