Reductive biodegradation of 1,2-dichloroethane by methanogenic granular sludge: perspectives for in situ remediation

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Abstract Granular methanogenic sludge was able to dechlorinate 1,2-dichloroethane (1,2-DCA) to ethene in UASB reactors. Ethanol served as the sole carbon and energy source. The average dechlorination rate measured on the basis of ethene production varied between 1.7 and 2.1 µmol 1,2-DCA/(h.gVSS) (46.7 and 57.4 mg/L.d). In order to elucidate the microbial origin of this bioconversion, enrichment cultures of the methanogenic sludge were prepared with different carbon and electron sources: pyruvate, lactate, H₂/CO₂, ethanol and formate. Dithiothreitol (DTT) was the strong reductant in order to increase the negative redox potential in the media. A homo-acetogenic gram-positive strain could be isolated in the presence of formate. 16S rRNA of the isolated strain showed that the bacterium was closely related (99.7%) to Acetobacterium wieringae. The strain also grew on pyruvate, lactate, H₂/CO₂ and ethanol, although dechlorination rates of 1,2-DCA were at least 5 times higher when formate was the (only) electron source. Average conversion rates reached 3 µmol/(h.gdry cells) and appeared to relate to cometabolic biocatalysis on the corrinoid centers of the homo-acetogenic strain. Some perspectives of anaerobic in situ bioremediation of groundwater polluted with chloroethanes are presented.

Keywords Acetobacterium sp.; anaerobic dechlorination; 1,2-dichloroethane; in situ bioremediation

Introduction

Chloroethanes are stable and volatile molecules that have been detected in the arctic, in oceans and in the atmosphere (Calahan et al., 1979). They are also present in sediments, soils and groundwater at high concentrations. Removal of these compounds by chemical conversion is a slow process since the observed half life is in the order of decades (Barbash and Reinhard, 1989). Due to the rather low oxidation state (~I or lower) of the carbon atoms in low chlorinated ethanes as 1,2-DCA, reduction by iron is not possible although this technique is used frequently to dechlorinate highly substituted ethenes as tetra- and trichloroethene (Gotpagar et al., 1997). Under anaerobic conditions, Howard et al. (1991) observed 1,2-DCA and 2-chloroacetic acid half lives of 1 year and 1 month respectively. In the atmosphere and in surface water, 1,2-DCA has estimated half lives of 111 days and 9 days, respectively.

The stability of chlorinated ethanes in anaerobic conditions depends on their chlorine substitution degree. Hexachloroethane, a very recalcitrant compound under aerobic conditions, was observed to be dechlorinated to tetrachloroethane in methanogenic sediment cultures (Vogel et al., 1987). Bouquey et al. (1995) also reported the biodegradation of hexachloroethane by a methanogenic culture. Further degradation of tetrachloroethanes based on reductive dechlorination was reported by other authors. Bouwer and McCarty...
(1983) and Chen et al. (1996) found that microorganisms in methanogenic soils were able to dechlorinate 1,1,2,2-TeCA to 1,1,2-TCA. 1,1,2-TCA could be dechlorinated further to 1,2-DCA and chloroethane. The latter compounds, containing only two or less chlorine atoms, have slower degradation rates in anaerobic conditions.

When chloroethane polluted groundwaters have to be cleaned, reductive dechlorination mediated by anaerobic organisms has several advantages. Firstly, the conditions of groundwater fit the need for a low reduction potential creating a good growth medium for 1,2-DCA cometabolizing organisms. Secondly, cost savings of a slow in situ bioremediation are attractive when time is not a restricted parameter. Thirdly, cometabolic conversion mediated by the corrinoids of anaerobic bacteria guarantees a broad spectrum dehalogenation capacity that is enhanced when the chlorine substitution degree of the chlorinated compound is increased (Vogel et al., 1987).

In this work, the Acetobacterium sp. strain isolated in the presence of formate, dithiothreitol (DTT) and 1,2-DCA was investigated for its 1,2-DCA and hexachloroethane degradation capacities. Additional enrichment cultures on chloroethanes have also been started on 1,2-DCA polluted sludge in order to find other dechlorinating strains.

**Materials and methods**

**Analytical methods**

Chlorinated ethanes were determined by analyzing 500 µL headspace samples on a Chrompack CP9001 GC (FID, 250°C). The GC contained a type CP-Sil 5CB column (50 m by 0.53 mm; film 1.25 µm) that was operated in the split injection mode. The carrier gas utilized was helium at a flow rate of 10 mL/min. Peak areas were calculated comparing them to standard curves and a standard mixture of 29 organohalogens (Sigma, EPA 601/602 Purgeables Kit). The oven (100°C) and injector (220°C) temperatures were constant. Samples were taken directly from the headspace (70 mL) of anaerobic serum bottles closed with VITON stoppers (Art. Nr. 7399, Rubber BV., The Netherlands). Qualitative determination was done on a quadrupole MS (Magnum, Finnigan) after running over a 30 metre Rtx5 column in a Varian 3700 series GC. The program started at 35°C (2 min), and increased at 5°C/min to a final temperature of 150°C. Injector/detector were set at 220°/230°C. He flow rate was 20 mL/min. Peaks were analyzed and compared with a LIST data stock. Ethanes/ethenes were quantified in 500 µL headspace samples on a packed column (10 m) in a Varian 3700 series GC. The He flow was 20 mL/min. Column temperature was kept constant at 50°C; injector/FID detector were set at 220/250°C.

**Chemicals and standards**

High-purity chlorinated ethanes were purchased from Fluka (Switzerland). Ethene, ethane, acetylene, CO, CO₂ and methane in nitrogen (1% each) were delivered by Scott Specialty Gases (Supelco Park, Bellefonte, PA) and were quantitatively injected in the GC-FID resulting in calibration curves ranging from 0 to 0.5 µmol/mL on the basis of ethene.

**Methanogenic granular sludge**

Dechlorinating granular sludge was grown in two UASB reactors which had originally been inoculated with granular methanogenic sludge from a full-scale UASB reactor treating potato processing water (Primeur, Waregem). This methanogenic sludge had a dry matter content of 142 g/l, a volatile suspended solids (VSS) value of 114 g/l and a mineral ash residue of 28.5 g/l. The reactor contained a blanket of 200 g sludge in a volume of 2.3 L. Ethanol was the only carbon and energy source.
Enrichment

The basal medium and supplement solution were described by Neumann et al. (1994). A 1 M NaHCO$_3$ buffer was made anaerobic and was mixed with the supplement solution at a volumetric ratio of 4 to 1 (40 mL 1 M NaHCO$_3$ with 10 mL supplement solution). 50 to 500 mg of pure DTT (corresponding to 0.005–0.05% w/v final concentration) was added. The resulting mixture was made anaerobic (N$_2$) and was autoclaved.

Media for growth of anaerobic bacteria were prepared in 120 mL serum bottles. 50 mL of a mixture containing (per L) deionized water (979 mL), basal medium (20 mL) and resazurin (1 mL, 0.2%) was brought in the bottles. Formate, pyruvate, lactate and ethanol were dosed at 40 mM, except for acetate (5 mM). The bottles were closed with Viton stoppers and were made anaerobic. After autoclaving the bottles and cooling down, the following solutions were dosed: (1) 2.5 mL of the anaerobic autoclaved mixture of NaHCO$_3$, supplement solution and reductant (DTT), and (2) 1,2-DCA stock solution in water (20 mM), corresponding to 350 or 700 µM. CO$_2$ and/or hydrogen were injected in the headspace (each 300 mbar overpressure) when these compounds served respectively as carbon or electron donor. The pH of the resulting media varied between 7.40 and 7.65. A 10 vol% of a bacterial suspension was injected in the medium. After this inoculum transfer, the media were set at 28°C and were stirred at 180 rpm.

Results and discussion

Degradation of 1,2-DCA in UASB reactors

Figure 1 shows the 1,2-DCA removal efficiency of a UASB reactor inoculated with 200 mL of unadapted granular methanogenic sludge. The corresponding loading rate of 1,2-DCA is given in Figure 1A. Further data concerning the COD removal efficiency (%) and the COD volumetric loading rate (g/L.d) are also given (Figure 1B). The hydraulic retention time during the period of operation varied between 10 and 20 hours. Before adding 1,2-DCA (start up period of 2 weeks), the granular methanogenic sludge used in the UASB reactor was brought into an active methanogenic state by adding a mineral medium and EtOH as the easily degradable carbon source. After this start up period of 28 days, the model compound 1,2-DCA was removed by the methanogenic sludge from the first day of dosing on.

Figure 1 1,2-DCA removal performance of UASB reactor inoculated with methanogenic sludge
The latter rapid removal start was probably due to the partial adsorption of 1,2-DCA on the sludge granules. Biogas production decreased and acetate formation in the effluent increased to maximum concentrations of 8 g/L. The pH varied between 6.9 and 7.1 due to the strong NaHCO₃ buffer (2.05 g/L). Applying an average volumetric loading rate of 49.4 mg/L.d of 1,2-DCA, the UASB reactor removed 1,2-DCA for 78.6%. From that average removal efficiency, 65–80% was found to be due to reductive dechlorination resulting in the formation of ethene. Ethene was measured in the biogas that was captured separately. Based on this ethene production, the rate of dechlorination occurring in the sludge blanket varied between 1.7 and 2.1 µmol 1,2-DCA/(h.gVSS).

**Enrichment experiments**

The unadapted methanogenic granular sludge, used as inoculum in the dechlorinating UASB reactor, was successfully enriched in terms of its 1,2-DCA dechlorination capability. After 6 enrichment steps and 2 roll-tube transfers, an isolate was obtained that was closely related to *Acetobacterium wieringae* (16S rRNA similarity of 99.7%). *A. wieringae* is a homo-acetogen and was isolated in 1982 by Braun and Gottschalk. In our experiments, formate was converted to acetate according to the carbon metabolism described by Diekert and Wohlfarth (1994), which confirmed the 16S rRNA taxonomic identification.

**Conversion of 1,2-DCA by the homo-acetogenic isolate Acetobacterium sp.**

After 6 enrichment steps and 2 roll-tube transfers, an isolate was obtained that dechlorinated 1,2-DCA to ethene at an average conversion rate of 3 µmol/(h.gdry cells) when formate was the electron and carbon source used as growth substrate and dithiothreitol as reductant. Cells were also grown in the presence of ethanol as electron and carbon source in place of formate. Although the ethanol grown culture obtained a higher OD₆₁₀ value (higher turbidity) than the formate grown culture, the difference in dechlorination performance was higher in the formate grown culture. During exponential growth (day 3 to 6) of the pure *Acetobacterium* sp. strain in the presence of formate, the maximum conversion rate reached 30 µmol/(h.gdry cells) (Figure 2).

Extraction of corrinoids in the biomass of the pure *Acetobacterium* sp. strain helped to elucidate the concept of dechlorination. The corrinoid concentration was almost 320 nmol/gdry cells. During growth, homo-acetogenic bacteria need these corrinoids to catalyze the reduction of 2 CO₂ to acetate gaining energy of this process (Diekert and Wohlfarth, 1994).

During the transfer of the methyl group on the corrinoid containing enzyme, the centered cobalt atom of the corrinoid reaches its oxidation state +1. Several studies have shown the

![Figure 2](https://iwaponline.com/wst/article-pdf/45/10/43/424841/43.pdf)

**Figure 2** Cometabolic reductive dechlorination of 1,2-DCA to ethene by an isolated *Acetobacterium* sp. strain grown with formate, respectively, ethanol as the sole electron source.
strong reducing power of these Co(I) corrinoids attacking halogen substituted carbon atoms of halogenated organic compounds, releasing halides (Gantzer and Wackett, 1991; Glod et al., 1997). Based on these data, dechlorination of the acetogenic strain is probably due to the cometabolic activity of its Co(I) corrinoids, normally ensuring the energy conservation in carbon metabolism.

The Acetobacterium sp. strain had the same growth characteristics in the absence or the presence of limited amounts of 1,2-DCA (tested to 700 µM), which is an indication for dechlorination cometabolism. Furthermore, the conversion rate of the 1,2-DCA is too low for a halorespiring process. Dehalorespiring organisms are reported to have conversion rates exceeding 60 µmol/(h.g$_{dry\,cells}$).

Perspectives for groundwater in situ purification

In principle, our results demonstrate that plain unadapted methanogenic sludge contains bacteria that are able to dechlorinate chlorinated ethanes. Hence, applying such sludge and supplementing it with ethanol or formate, offers a potential for in situ bioremediation.

In this work, the specific organism bringing about the reaction has tentatively been identified. It is a homo-acetogenic bacterium, very similar to Acetobacterium wieringae and to the best of our knowledge not imposing any threat to environmental health. Furthermore, ethanol and formate are good energy sources delivering electrons to the corrinoids of this homo-acetogen. There might be opposition against injecting methanogenic sludge, with an unidentified mixture of bacteria and organics. Therefore, the injection of a defined culture with determined characteristics can be preferred. This is a feasible option as the isolated Acetobacterium sp. can be grown easily in pure culture. Of course, the survival of this strain in natural soil and sediments must be examined in detail.

A very important point in practice is the dechlorination specificity of the injected bacterium. Indeed, the pollutant rarely exists as a single compound in the groundwater but several other chlorinated pollutants are also present. In other words, the injected bacterium should have versatile dechlorination capabilities.

The dechlorination of 1,2-DCA by the isolated Acetobacterium sp. strain is based on cometabolic reductive conversion mediated by corrinoids. These corrinoids are known to have a broad spectrum dehalogenation capacity (Gantzer and Wackett, 1991; Holliger and Schraa, 1994). Furthermore, increased chlorination increases the electrophilicity and oxidation state of a chlorinated ethane, making it more susceptible to dehydrohalogenation and reductive dechlorination reactions as dichloroelimination, hydrogenolysis or coupling. In contrast, these higher chlorinated chloroethanes are less susceptible to substitutions by nucleophilic reagents and to oxidation processes (Vogel et al., 1987).

As a result, bacteria reductively dechlorinating lower substituted chloroethanes as 1,2-DCA in a cometabolic process, are envisioned to be able to dechlorinate higher chlorinated ethanes, even at increased conversion rates. In this process, the difference in affinity of the corrinoid centers towards different chlorinated ethanes is estimated to be of minor importance. This is in contrast with the corrinoid centers in specific dehalogenases of halorespiring organisms. In these organisms, the optimized fit of some specific substrates towards the corrinoids is the main reason for the high turnover of these biocatalysts and the limited substrate spectrum. Dehalococcoides ethenogenes strain 195 is the only bacterium reported to reductively dechlorinate 1,2-DCA in a metabolic process (Maymo-Gatell et al., 1999). Other metabolizing organisms with high degradation rates of chloroethanes have not been reported yet. Therefore, anaerobic microbial dechlorination and detoxification of these compounds, even at low conversion rates, is a challenge for future research. Moreover, further research to demonstrate that Acetobacterium sp. qualifies as a versatile dehalogenator, is justified.
Conclusions

Methanogenic sludge can be an appropriate source of bacteria allowing the enrichment or isolation of anaerobic strains that dechlorinate 1,2-DCA. Based on the high corrinoid content of the isolated homo-acetogenic \textit{Acetobacterium} sp. strain and the moderate conversion rates obtained, the dechlorination process is probably due to cometabolic reduction reactions of the corrinoids. The environmentally safe nature of this strain and of its specific electron donors driving the dechlorination, make this combination an attractive option for bioaugmentation. Further experiments have to investigate whether cometabolizing anaerobic bacteria can be appropriate candidates for slow \textit{in situ} detoxification processes of aqueous chlorinated ethanes in groundwater.

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