Inhibition of tetrachloroethene and trichloroethene on methanogenesis in anaerobic sludges from various origins

A. M. Wang, C. S. Hwu and C. H. Wu

ABSTRACT

Nine anaerobic sludges were screened to obtain the most effective methanogenic inoculum for the anaerobic treatment of groundwater that is contaminated with tetrachloroethene (PCE) or trichloroethene (TCE). The selection was based on the toxicity of PCE or TCE to acetoclastic methanogens in different sludges. The effects of two biological factors, sludge origin and specific acetoclastic methanogenic activity, and a physical factor, specific surface area of sludge, on the degree of inhibition were examined and compared. The fifty percent inhibition concentrations (IC_{50}) of PCE and TCE that were obtained from 30 °C batch inhibition tests ranged from 0.18 to 0.41 and 1.71 to 3.31 mM, respectively, for the examined sludges. The toxicity of the contaminants to anaerobic sludges did not depend on the two biological factors but was closely correlated with the specific surface area of sludge. Suspended sludges, which have higher specific surface areas than granular sludges, suffered much greater inhibition. This paper suggests the use of anaerobic granular sludges as inocula in bioreactors for treating PCE- and TCE-contaminated groundwater to reduce the effect of their inhibition.

Key words | anaerobic sludge, inhibition, methanogenesis, tetrachloroethene, trichloroethene

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INTRODUCTION

Volatile chlorinated ethenes (VOCls) are among the most common contaminants in groundwater environments globally. Due to their widespread use as degreasing and dry cleaning solvents in industry, tetrachloroethene (PCE) or trichloroethene (TCE) are the VOCls that appear in groundwater with the greatest frequency and in the highest concentrations (Ni *et al.* 2014; Bretón-Deval *et al.* 2016; Mao *et al.* 2017). Currently in Taiwan there are 121 publicly announced groundwater pollution control sites that are contaminated with PCE and/or TCE. The highest concentration of PCE and TCE in groundwater streams was 40.5 and 445 mg/L, respectively (TEPA 2019). PCE cannot be converted under aerobic conditions because of its high electronegativity but it has been demonstrated to be anaerobically degraded by reductive dechlorination to less chlorinated ethenes, including TCE, dichloroethenes (DCEs), vinyl chloride (VC) and ethene (Tiehm & Schmidt 2011; Cheremisinoff 2017). Obviously, a treatment that can completely dechlorinate PCE and TCE to ethene is required for groundwater bioremediation.

Numerous investigations have reported that PCE can be completely transformed to non-chlorinated compounds by anaerobic microorganisms. Several bacteria that can reductively dechlorinate PCE have been identified. Of these, *Dehalococcoides ethenogenes* is the most well-known microorganism that can completely dechlorinate PCE to ethene (Fennell *et al.* 2004; Ni *et al.* 2014; Matturro *et al.* 2016). This information is critical to achieving complete PCE and TCE dechlorination in continuous-flow reactors under optimal conditions, including the presence of suitable inocula. However, research has demonstrated that success is uncertain when an anaerobic sludge that is acclimated to one substrate is used as an inoculum for treating other substrates (Morgan *et al.* 1990; Yang & Anderson 1993). Significant differences in reactor performance have been observed using different seed sludges in the treatment (Hendriksen & Ahring 1992; Peng *et al.* 1994). According to research and practical experience, a suitable seed sludge must be chosen prior to the treatment of PCE/TCE-containing groundwater.

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The toxicity of PCE/TCE to acetoclastic methanogens was selected because these methanogens are of great metabolic importance (Gujer & Zehnder 1983) and highly sensitive to inhibition (Bereded-Samuel *et al.* 1996; Chen *et al.* 2014) during anaerobic biodegradation. On the other hand, reports on biodegradation of chlorinated compounds using anaerobic bioreactors are mostly related to methanogenic conditions (Guerrero-Barajas *et al.* 2014). In comparing sludges from different origins, the effects of two biological factors – sludge origin and specific acetoclastic methanogenic activity – and one physical factor – specific surface area of the sludge – on the extent of inhibition to acetoclastic methanogens was examined (Hwu *et al.* 1996).

MATERIALS AND METHODS

Materials

Nine anaerobic granular or suspended sludges from full-scale wastewater treatment plants were collected. Table 1 lists the characteristics of the anaerobic sludges that were used in this study. Before the experiment, all sludges had been stored at 4 °C in gas-tight plastic containers for less than two weeks. After reactivation (see below), sludges A, B and C were placed in test tubes containing a few glass beads (diameters 3–5 mm). The tubes were then vigorously vortexed for 5 min under anaerobic atmosphere. The crushed sludges A, B and C are denoted as A′, B′ and C′.

The basal medium that was used in the reactivation of sludges and the inhibition assay was composed of (in mg/L final concentration) NH_4Cl (280), K_2HPO_4 (250), $MgSO_4{\cdot}7H_2O$ (100), $CaCl_2{\cdot}2H_2O$ (10) and yeast extract (100). The medium was made up in demineralized water and buffered by adding 2 g/L $NaHCO_3$. One milliliter of a trace element solution (Zehnder *et al.* 1980) was added per liter of medium. The pH of all media was adjusted to 7.0 ± 0.1 by adding few drops of HCl.

EXPERIMENTAL METHODS

Reactivation

Since the test sludges had various origins and were stored at 4 °C, the sludges had to be reactivated at 30 °C under the same conditions to obtain the methanogenic activity of each sludge in a way that allowed for comparison. Prior to use, each sludge was reactivated in a 1 L serum bottle by fed-batch incubation using 2 g COD/L acetate, with the exception of the crushed sludges A′, B′ and C′, which were directly prepared from sludges A, B and C. The bottles that were used for reactivation were intermittently shaken. Residual concentrations of acetate were monitored to evaluate acetoclastic methanogenic activities. The reactivation of a sludge was terminated when the activity reached 0.26 ± 0.09 g COD per g volatile solids (VS) per day. The reactivated sludges were subsequently used as seed materials in inhibition tests (see below).

Inhibition test

The approach and experimental design in this study differed slightly from those of Hwu *et al.* (1996) and Van Eekert (1999). Reactivated granular sludges were elutriated to remove floating matter and fine particulates. Both suspended and crushed sludges were repeatedly washed and settled five times. The sediments were then pipetted into

Table 1 | Sources and characteristics of anaerobic sludges used for PCE and TCE inhibition tests

Sludge code	Wastewater type	Reactor type	Sludge appearance	Diameter (mm)	VS[a] (%)	TS[b] (%)	VS/TS (%)
A	Pulp paper	UASB[c]	Granular	2.49 ± 0.83	7.25	11.49	62.6
B	Starch	UASB	Granular	2.86 ± 1.25	4.98	5.69	87.5
C	Starch	UASB	Granular	1.92 ± 0.74	6.20	7.89	78.6
D	Beverage	UASB	Granular	1.21 ± 0.43	8.98	9.35	96.0
E	Food	UASB	Granular	1.86 ± 0.97	10.06	13.74	73.3
F	Food	UASB	Granular	2.38 ± 1.04	8.21	9.36	87.8
G	Chemical	UASB	Granular	1.84 ± 0.55	5.89	6.34	92.9
H	Brewery	BIMA[d]	Suspended	0.13 ± 0.09	2.46	2.57	95.9
I	Domestic	UASB	Suspended	0.1 ± 0.05	1.92	5.63	34.0

[a]VS: volatile solids; [b]TS: total solids; [c]UASB: upflow anaerobic sludge bed; [d]BIMA: biogas induced mixing arrangement.

serum bottles. Each bottle had a working volume of 120 ± 1 mL and was equipped with a viton rubber septum and an aluminum screw cap. The treatments (elutriation or washing and settling) minimized the background methane production during the experiments. A sludge concentration of approximately 2 g VS/L was used in each test throughout the study. The final liquid volume, comprising the volumes of the sludge (2 g VS/L), basal medium, acetate (2 g COD/L), and PCE or TCE solution, was 25 mL. The 2 g COD/L acetate was used to recover the anaerobicity and the methanogenic activity, which possibly had changed during elutriation. Subsequently, the headspace of each bottle was flushed with N_2/CO_2 gas (70/30, v/v) for 3 min. The bottles were then placed in a reciprocating shaker water-bath at a fixed temperature of 30 °C and stirred using approximately 50 strokes per minute. After 1 day of incubation, varying concentrations of PCE or TCE were fed using microsyringes into all bottles, except for the control. The control was injected with the same volume of demineralized water. After overnight exposure to the toxicants, all bottles were fed with 1 g COD/L acetate to assay the methanogenic activity. Each headspace was flushed and the bottles were reincubated for 1 h. The methane content in the headspace of each bottle was then determined intermittently (2 h in general) over a 24 h period. Preliminary experiments over 120 h revealed the absence of a lag phase (data not shown). Accordingly, a period of 24 h was used to determine the maximum specific acetoclastic methanogenic activity. The maximum methanogenic activity was computed from the slope of the curve of cumulative methane production as a function of time. The relative activity in the inhibition experiment was expressed as a percentage of the control activity. The fifty percent inhibition concentrations (IC_{50}) are defined as the toxicant concentrations that caused a 50% loss of relative activity. All experiments were performed in duplicate, except that experiments on the control sample in each batch were conducted in triplicate or quintuplicate.

Analyses

Surface areas of the test sludges were analyzed using an image analyzer (Magiscan, Applied Imaging, UK). The scanner was set such that only particles with diameters that exceeded 10 μm were scanned and analyzed. Two-dimensional data were converted into three-dimensional data based on the assumption that all sludge particles were spherical. Sludge samples received the same treatment as used in the inhibition tests and quintuplicate images were obtained for analysis. The methane content of the gas samples was determined as previously described (Hwu *et al.* 1996). The TS and VS were measured using standard methods (APHA 1998).

RESULTS AND DISCUSSION

Overnight exposure to the individual toxicant was used, based on our previous finding (data not shown) that PCE/TCE dechlorination does not begin within a day. Accordingly, dominance of PCE/TCE degradation in the inhibition assessment experiments was ruled out; the methane was produced merely from the acetate; all inhibition could be attributed to the toxicant and none was attributable to any of its degradation products. The inhibition of PCE/TCE to anaerobic sludges of various origins is expressed using IC_{50} values. Table 2 summarizes the experimental data concerning PCE/TCE IC_{50} values that were obtained from the inhibition tests together with the sludge activity of each control in the inhibition tests and the specific surface area of the sludges that was used in the tests. The IC_{50} values reveal that PCE inhibited acetoclastic methanogenesis more than did TCE. The granular sludges were less susceptible to the toxic effects of PCE/TCE than were the suspended (H, I) and crushed (A′, B′ and C′) type sludges. The sludge from the UASB reactor that was used to treat starch processing wastewater (sludge C) was the

Table 2 | Activities, specific surface areas and IC_{50} values of various sludges

Sludge code	Activity[a] (g CH_4-COD/g VS·d)		Specific surface area (x 10^5 mm^2/g TS)	IC_{50} (mM)	
	PCE	TCE		PCE	TCE
A	0.75	0.73	0.18	0.38	2.80
B	0.45	0.50	0.21	0.34	2.56
C	0.56	0.55	0.06	0.41	3.31
D	0.45	0.45	0.12	0.39	2.85
E	0.37	0.37	0.41	0.32	2.56
F	0.39	0.39	0.14	0.40	2.52
G	0.48	0.48	0.22	0.38	2.46
H	0.52	0.52	2.66	0.19	1.93
I	0.45	0.45	4.79	0.18	1.71
A′	0.73	0.73	2.69	0.21	2.13
B′	0.52	0.52	2.26	0.23	1.90
C′	0.51	0.51	1.10	0.28	2.28

[a]Acetoclastic methanogenic activity of control (without receiving contaminants), determined during the inhibition test.

most resistant to the toxicity of both PCE and TCE. Biological and physical factors that might have affected the order of resistance are discussed below.

The findings herein regarding the inhibition of PCE/TCE to methanogens are generally supported by investigations in which similar concentrations thereof were used (Bereded-Samuel *et al.* 1996; van Eekert 1999). The biological characteristics of the sludge may importantly influence the degree of toxicity of numerous toxicants. Therefore, the effects of the origin and activity of the sludge on the toxicity of PCE/TCE to acetoclastic methanogens were investigated. Sludges A, B and C had the same originals as sludges A′, B′ and C′, respectively. As their microbial compositions can be regarded as identical, sludges with the same origin might be expected to be similarly susceptible to each toxicant. In contrast, the IC_{50} values for sludges A, B and C differed from those for sludges A′, B′ and C′ (Table 2). These disordered behaviors and different responses imply that the origin of the sludge does not affect the toxicity of PCE/TCE.

Figure 1 plots the specific methanogenic activity on acetate of each sludge as a function of its PCE/TCE IC_{50} value. The correlation coefficients (R^2) and best-fit curves are obtained using the linear least-squares regression method. Apparently, no clear correlation between PCE/TCE inhibition and the methanogenic activity of the sludge exists ($R^2 = 0.0209$ for PCE and 0.0021 for TCE). Yu & Smith (2000) suggested that the mechanism of the toxicity of halogenated aliphatics to methanogens is determined by the extent of their chlorination and their molecular structure. They observed that TCE inhibited wastewater consortium more than did PCE. However, since PCE was found herein to be more inhibitory than TCE to anaerobic sludges, the mechanism that explains the irrelevance of toxicity to their chlorination in this study is unclear.

In addition to the aforementioned biological conditions, some physical factors, such as the particle size of the sludge, may importantly influence the degree of inhibition. Figure 2 displays the correlation between the specific surface areas and PCE/TCE IC_{50} values of the 12 tested sludges. The computer-fitted curves ($R^2 = 0.9485$ for PCE and 0.8961 for TCE) were closely fitted by all data. The inhibition of PCE/TCE clearly increased with the specific sludge surface area. Notably, the suspended (H, I) and crushed (A′, B′ and C′) type sludges had higher specific surface areas than those of the granular sludges (Table 2) so they experienced greater inhibition. Hwu *et al.* (1996) conducted comparative experiments on both flocculent and granular sludges to determine the inhibition of acetoclastic methanogenesis by oleic acid. They found that the process in suspended and flocculent sludges was more strongly inhibited than in granular sludges. This was also evidenced by Fang (2000) that biogranules are more resistant than suspended sludge to the toxicity of pollutants. We attributed these results to the higher specific surface area of flocculent sludges. Furthermore, the toxicity of oleic acid in Hwu *et al.* (1996) was consistent with that observed by Pereira *et al.* (2002).

Granular sludge can be used very favorably as an inoculum in upflow reactors because of its high specific contaminant removal rate and high settleability (Lettinga 1996). It can be maintained on wastes that do not allow granulation (Rinzema *et al.* 1993). Granular sludge becomes increasingly attractive because this study verified that granular sludge is less susceptible than suspended sludge to the PCE/TCE toxicants. However, regarding a full-scale

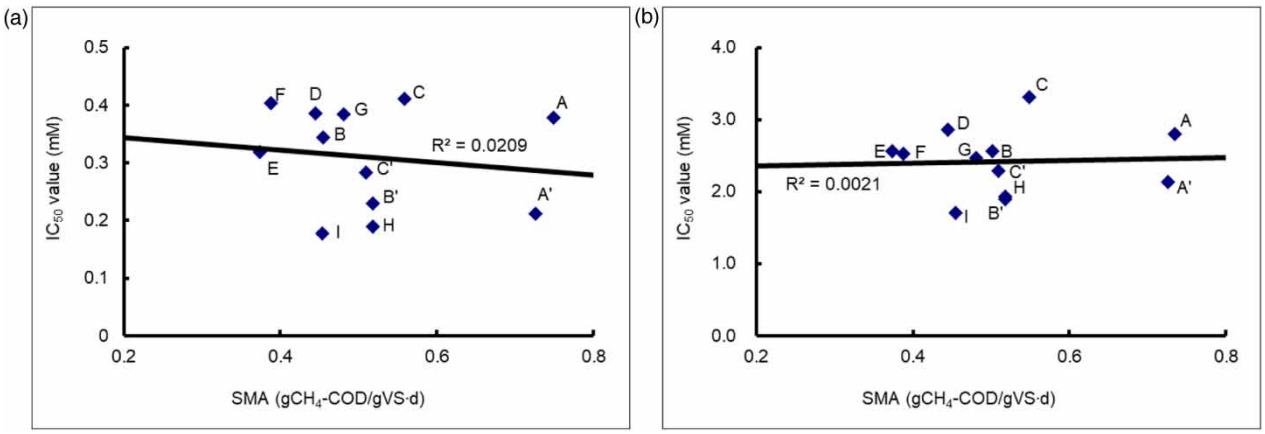

Figure 1 | Relation between specific methanogenic activities (SMA) and IC_{50} values of anaerobic sludges exposed to (a) PCE and (b) TCE. The bold line (—) represents the computer fitted curve. Letters next to filled symbols indicate the codes of sludges, which can be found in Table 2.

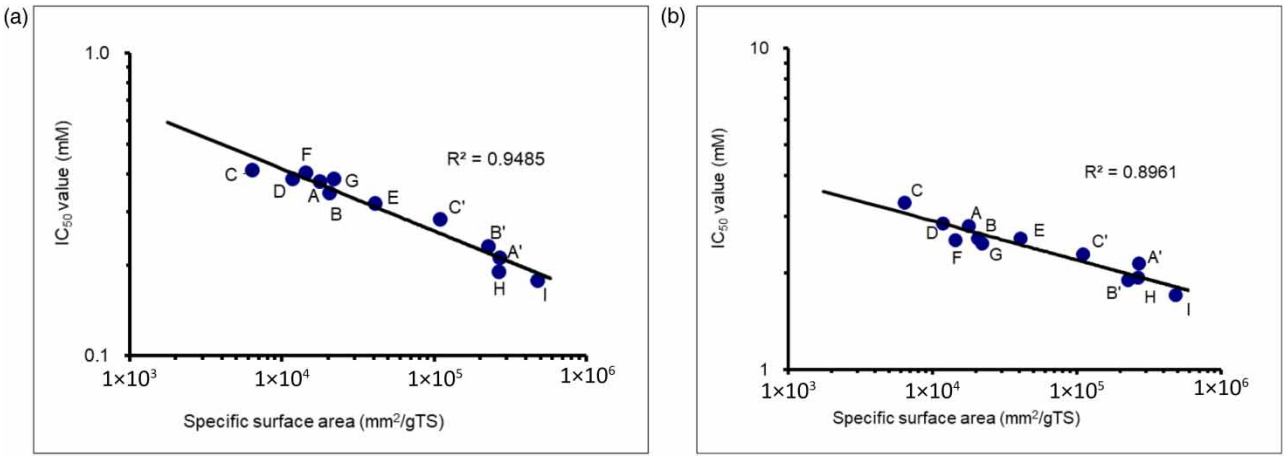

Figure 2 | Correlation of specific surface areas and IC_{50} values of anaerobic sludges exposed to (a) PCE and (b) TCE. The bold line (—) represents the computer fitted curve. Letters next to filled symbols indicate the codes of sludges, which can be found in Table 2.

application of wastewater treatment plant, the availability of granular sludge must be taken into consideration. Over 2,000 full-scale UASB reactors have been built and more are under construction. Hence, increasing amounts of granular sludges will become available in the near future. The use of granular sludges as inocula for UASB reactors for treating PCE/TCE-containing groundwater is, therefore, appropriate. In the selection of seeding material from granular sludges, origin is not a major concern. Granule size and other physical properties such as settleability are more important considerations regarding PCE/TCE toxicity.

CONCLUSIONS

Inhibition of two major groundwater contaminants, PCE and TCE, on acetoclastic methanogenesis is much more related to specific surface areas of anaerobic sludges than to their methanogenic activities and origins. Granular sludges suffer less inhibition than suspended ones. Selection of anaerobic granular sludge is recommended to serve as seeding materials for the treatment of PCE/TCE-contaminated groundwater. These novel findings are important for on-site bioremediation of PCE/TCE-contaminated groundwater using bioreactors in which reductive dechlorination under methanogenic conditions is prevalent. The nine anaerobic granular sludges investigated in this study were taken from full-scale wastewater treatment plants. Supply of the sludges as seeding materials for dechlorinating bioreactors is sufficient in practice.

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