

Dechlorination and organohalide-respiring bacteria dynamics in sediment samples of the Yangtze Three Gorges Reservoir

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Abstract Several groups of bacteria such as *Dehalococcoides* spp., *Dehalobacter* spp., *Desulfomonile* spp., *Desulfuromonas* spp., or *Desulfitobacterium* spp. are able to dehalogenate chlorinated pollutants such as chloroethenes, chlorobenzenes, or polychlorinated biphenyls under anaerobic conditions. In order to assess the dechlorination potential in Yangtze sediment samples, the presence and activity of the reductively dechlorinating bacteria were studied in anaerobic batch tests. Eighteen sediment samples were taken in the Three Gorges Reservoir catchment area of the Yangtze River, including the tributaries Jialing River, Daning River, and Xiangxi River. Polymerase chain reaction analysis indicated the presence of dechlorinating bacteria in most samples, with

varying dechlorinating microbial community compositions at different sampling locations. Subsequently, anaerobic reductive dechlorination of tetrachloroethene (PCE) was tested after the addition of electron donors. Most cultures dechlorinated PCE completely to ethene via *cis*-dichloroethene (*cis*-DCE) or *trans*-dichloroethene. Dehalogenating activity corresponded to increasing numbers of *Dehalobacter* spp., *Desulfomonile* spp., *Desulfitobacterium* spp., or *Dehalococcoides* spp. If no bacteria of the genus *Dehalococcoides* spp. were present in the sediment, reductive dechlorination stopped at *cis*-DCE. Our results demonstrate the presence of viable dechlorinating bacteria in Yangtze samples, indicating their relevance for pollutant turnover.

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Introduction

The Yangtze River is the third largest river in the world, with a mean annual water discharge of 29,400 m³/s and a sediment load of 500 million tons/year (Zhang 1995). In the last decades, pollutant discharge into Chinese rivers has increased because of growing industrial development and the widespread application of fertilizers, pesticides, and herbicides in agriculture (Müller et al. 2008). Additionally, changes in water quantity, water quality, and the aquatic ecosystems have to be expected from large dam constructions, e.g., the Three Gorges Dam (TGD) at the Yangtze. A joint Sino-German research project was initiated to obtain more insight into the environmental processes under these highly dynamic conditions (Bergmann et al. 2012).

In this study, the dehalogenation of chlorinated pollutants and monitoring the growth of dechlorinating bacteria are reported. Chlorinated contaminants such as polychlorinated biphenyls (PCBs) and chlorinated benzenes (CBs) have been detected in the Yangtze River water and sediments in low concentrations (Jiang et al. 2000; Wang et al. 2009). PCBs are anthropogenic environmental pollutants, which originate from the recycling procedures or dumping of electronic wastes (Shen et al. 2009; Liu et al. 2008). CBs, especially hexachlorobenzene, had a lot of applications in industry and agriculture (Barber et al. 2005). In China, it was used as a cheap broad spectrum insecticide (Jiang et al. 2000). Chlorinated ethenes belong to the most commonly found contaminants in the groundwater worldwide, because they had been widely used as cleaning and degreasing agents (Schmidt and Tiehm 2008; Tiehm and Schmidt 2011). There are several groups of bacteria which are able to dehalogenate chlorinated aliphatic and aromatic hydrocarbons under anaerobic conditions. Remarkably, similar groups of bacteria have been reported to be capable of dechlorination of CBs (Field and Sierra-Alvarez 2008; Taş et al. 2011), PCBs (Bedard 2008), and chlorinated ethenes (Löffler et al. 2000; Schmidt et al. 2006). In this study, chloroethenes were used as model compounds. Microorganisms which are known to be able to reductively dechlorinate pollutants are *Desulfomonile* spp., *Desulfitobacterium* spp., *Dehalobacter* spp., *Desulfuromonas* spp., and *Dehalococcoides* spp., but only bacteria belonging to *Dehalococcoides* spp. are capable of complete dehalogenation of tetrachloroethene (PCE) to ethene (Holliger et al. 1993; Smidt and de Vos 2004; Schmidt et al. 2006; Aktaş et al. 2012; Löffler et al. 2012).

Previously, in most laboratory studies on anaerobic dechlorination, only one or two groups of bacteria were

investigated and monitored by polymerase chain reaction (PCR) analysis, e.g., *Desulfitobacterium* sp. and *Dehalococcoides* spp. (Lohner and Tiehm 2009), *Desulfitobacterium dehalogenans* and *Desulfomonile tiedjei* (El Fantroussi et al. 1997), *Desulfuromonas* spp. and *Dehalococcoides mccartyi* (Löffler et al. 2000; Löffler et al. 2012), *Dehalobacter* spp. and *Dehalococcoides* spp. (Smits et al. 2004; Grostern and Edwards 2006) or *Dehalococcoides* bacteria (Schmidt et al. 2006; Aktaş et al. 2012). It has been shown recently that in the field, often several dechlorinating bacteria are present simultaneously (Rouzeau-Szynalski et al. 2011). In the environment, the reductively dechlorinating bacteria have been detected in different compartments, mainly in contaminated soil (Löffler et al. 2000; Hendrickson et al. 2002) and polluted groundwater (Schmidt et al. 2006; Schmidt and Tiehm 2008; Vancheeswaran et al. 1999). For river sediments, their occurrence in the Dutch part of the Rhine River (Holliger et al. 1993), the Red Cedar, Père Marquette (Löffler et al. 2000), Tahquamenon and Pine Rivers in the USA, and the Perfume River in Vietnam (Griffin et al. 2004) was reported. However, no reports were available addressing the anaerobic dechlorination potential in the Yangtze River. The objectives of this study are (1) to assess the dechlorination potential in Yangtze sediment samples and (2) to monitor the changes of the dechlorinating microbial community during PCE dechlorination.

Materials and methods

Sampling and sample storage

Eighteen sediment samples were taken in the Three Gorges Reservoir (TGR) catchment area (Fig. 1). Seven samples (MP1, MP2, MP3, MP4, MP5, MP6, and MP7) are from a sediment core with a total length of 0.7 m taken 76.5 m below the water level in the Yangtze River near the TGD. The sediment core was collected with a stainless gravity sediment core sampler (100 cm in length and 25 cm in diameter). Three samples (YCQ1, YCQ4, and YEND) originate from the Yangtze River close to and within Chongqing city, and another sample was also taken within Chongqing from the tributary Jialing River (JL1). These four samples are surface sediment samples from the littoral zone and were collected manually. Six samples (XX01, XX02, XX04, XX07, PYK, and WJW) were taken from the tributary Xiangxi River in a depth between 3 and 71 m below the water level, and another sample (DH2) originated from the tributary Daning River (depth 35 m). These samples have been collected with a sediment grabber. All samples were stored in plastic bags, which were put into gastight tins. After sampling, the sediments were stored at 4 °C until usage.

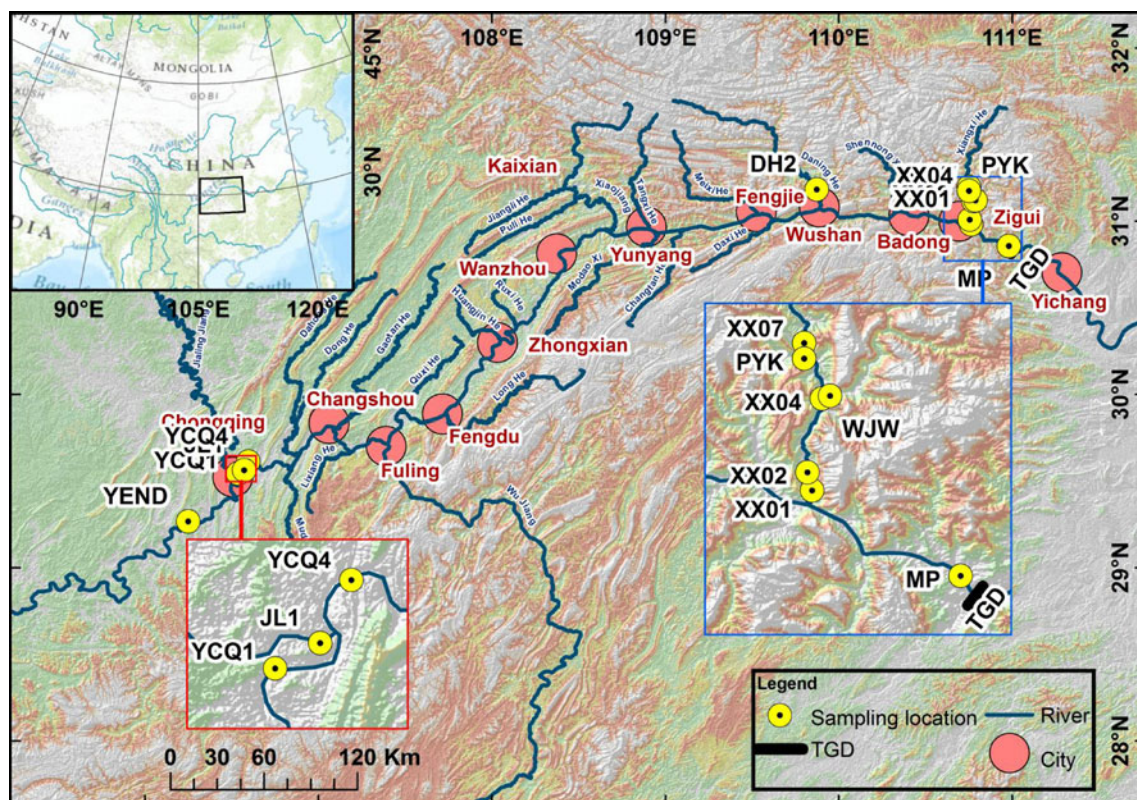


Fig. 1 Map of sampling points within the Three Gorges Reservoir

Biodegradation studies

Experiments were performed in 2-L laboratory glass bottles capped with teflon-coated septa, held in place with screw caps. The mineral medium contained the following mineral salts (analytical grade, Merck, Darmstadt, Germany) per liter of demineralized water: 3.17 g of KH_2PO_4 , 14.33 g of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 0.45 g of $(\text{NH}_4)_2\text{HPO}_4$, 0.12 g of $\text{MgHPO}_4 \cdot 3\text{H}_2\text{O}$, 5 mL of trace element solution 1 (200 mg of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 20 mg of $\text{MnSO}_4 \cdot 5\text{H}_2\text{O}$, 4 mg of CoCl_2 , 20 mg of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 20 mg of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 530 mg of CaCl_2 , 3 mg of H_3BO_3 , 4 mg of $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$, and 1 mL of concentrated H_3PO_4 per liter), and 100 μL of trace element solution 2 (50 mg of $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$, 50 mg of $\text{Na}_2\text{SeO}_3 \cdot 5\text{H}_2\text{O}$, and 250 mg of NiCl_2 per liter). The medium was adjusted to $\text{pH} 7.2 \pm 0.2$ and autoclaved at 121°C for 20 min. Oxygen was removed by repeatedly applying vacuum and flushing with nitrogen. Additionally, all cultures received filter-sterilized 1 mL vitamin solution (2 mg of biotin, 2 mg of folic acid, 10 mg of pyridoxamine, 5 mg of riboflavin, 5 mg of thiamine, 0.1 mg of cyanocobalamin, 5 mg of nicotinamide, 5 mg of *p*-amino benzoic acid, 5 mg of lipoic acid, 5 mg of pantothenic acid per 100 mL). Sodium acetate (40 mg), 40 mg of sodium pyruvate, 0.5 g of yeast extract, and 10 mL H_2 per liter were added in order to provide the electron donors required for reductive

dechlorination. Depletion of acetate and pyruvate was followed by ion chromatographic analysis. All handling, except H_2 addition, was performed inside the anaerobic gas chamber flushed with nitrogen. All bottles were amended with 50 μM (8.3 mg/L) PCE (99 %, Fluka, Steinheim, Germany). Each batch test was inoculated with 20 g of wet sediment. According to the dehalogenation of chloroethenes and consumption of electron donors, the cultures have been repeatedly spiked with 50 μM (8.3 mg/L) PCE and amended with acetate, pyruvate, and hydrogen. Bottles were kept at room temperature ($22\text{--}24^\circ\text{C}$) in the dark. In order to avoid volatilization of chloroethenes during sampling, the teflon-coated septa were pierced with stainless steel needles. Aqueous phase samples were taken using glass syringes, and septa were quickly changed subsequently. Chlorinated ethenes and ethene were analyzed by gas chromatography. Chloride formation was monitored via ion chromatography. Samples for gas chromatographic analyses were acidified to $\text{pH} 2$ with phosphoric acid in order to stop biological reactions and stored at 4°C until analysis.

Analytical methods

Chloroethene and ethene concentrations were determined using a gas chromatograph (Series II 5890, Hewlett Packard, Waldbronn, Germany) equipped with headspace

sampler, flame ionization detector, and electron capture detector. Separation was accomplished in a capillary column (Hewlett Packard PONA, 50 m, 0.2 mm inside diameter, crosslinked methyl siloxane, 0.5- μm film thickness). The injector and detector temperatures were 180 and 220 °C, respectively, and the following temperature program was used: held at 35 °C for 15 min, heated gradually to 60 °C (1.5 °C/min), heated gradually to 130 °C (15 °C/min), held for 7 min, heated gradually to 200 °C (30 °C/min), and held at 200 °C for 5 min. Concentrations were determined using external standards ($R^2=0.998$). Reproducibility on standard analyses was $\pm 6\%$. Each reported value was the average of at least duplicate samples and represents the aqueous phase concentration.

Chloride concentrations were determined via ion chromatograph (Metrohm 761 compact IC, Metrohm, Filderstadt, Germany) equipped with a Metrohm A-Supp-5 column conductivity detector. Concentrations were determined using external standards ($R^2=0.996$). Reproducibility on standard analyses was $\pm 1\%$.

All samples were taken from the aqueous phase. At each time point, oxidation–reduction potential (ORP) and pH were determined with a multimeter WTW Multiline P4 (Weilheim, Germany), and oxygen was measured using a WTW oximeter Oxi 330.

Polymerase chain reaction

For the analysis of total DNA from the sediment, the gas-tight tins were opened in the anaerobic gas chamber. Per sample, 1 g of soil was removed and extracted using the Ultra Clean Soil DNA Kit (MoBio Laboratories, Carlsbad, USA) according to the manufacturer's protocol. For PCR analysis of the culture liquid, 50 mL of the culture was transferred to a conical centrifuge tube (VWR, Darmstadt, Germany) and afterwards filtered through a 0.2 μm membrane filter (47 mm diameter) (PALL, Michigan, USA). The membrane was stored at $-20\text{ }^\circ\text{C}$ until DNA extraction and analysis. The DNA of the frozen filters was extracted using the Fast DNA[®] Spin Kit for soil (MP Biomedicals, OH, USA).

The pre-screening of sediment samples for the *16S* rRNA gene sequences specific for dechlorinating microorganisms *Desulfomonile* spp., *Desulfitobacterium* spp., *Dehalobacter* spp., *Desulfuromonas* spp., and *Dehalococcoides* spp. was done with nested PCR containing 2 μL of the template, 13.4 μL of DNase free water, 2 μL of 10 \times reaction buffer, 0.2 μL of Taq polymerase, 200 μM of each nucleotide, and 500 nM of each primer, using a Biometra thermocycler (Biometra, Goettingen, Germany). First, a PCR with bacterial primers was performed using different dilutions of DNA extracts as templates. Thus, false negative results caused by PCR inhibitors could be precluded. Second, a 1 to 10 dilution of the bacterial PCR product was used as template

for a second PCR with primers specific for the five dechlorinating groups. The used primers and temperature programs are listed in Table 1. Amplicons were tested with agarose gel electrophoresis, and gels were documented with a GeneFlash camera (Syngene, Cambridge, UK).

For enumeration of the *16S* RNA gene copies in the degradation experiments, quantitative PCR (qPCR) was performed in an Eppendorf Realplex² qPCR machine (Eppendorf, Hamburg, Germany) with primers and temperature profiles listed in Table 1. The qPCR was done with a Master Mix containing 8.5 μL of RNase and DNase free water (Invitrogen, Carlsbad, USA), 12.5 μL of 2 \times Sensi Mix including Hot Start Taq Polymerase (Bioline, Luckenwalde, Germany), and 500 nM of each primer. All samples and standards were analyzed in duplicates, and gene concentrations were reported as gene copies per liter of culture liquid. Calibration was performed with serial dilutions of a known quantity of linearized plasmid containing *16S* gene fragments. The quantification limits (QL) for all qPCR assays were 1E+5 *16S* RNA gene copies/L.

Results

The pre-screening of the sediment samples revealed that dechlorinating bacteria were present in most of the samples. In 16 of the 18 sediment samples, positive PCR signals for at least one of the 5 different dechlorinating groups were detected (see supplementary data Table T1). For subsequent dehalogenation studies, nine sediment samples from different areas and with different dechlorinating microbial communities were selected.

All nine batch cultures were able to reductively dechlorinate PCE, thus demonstrating the viability of the bacteria. During 28 weeks, in all batch cultures, the pH stayed at a constant level between 7.0 and 7.4. The oxygen concentration in the medium never exceeded the limit of detection (0.2 mg/L), and the ORP values decreased at the beginning and afterwards remained between -24 and -124 mV (supplementary Figs. S1 and S2). Thus, the O₂, pH, and ORP values confirmed suitable conditions for reductive dechlorination. All batch cultures were spiked at least two times with PCE and showed repeatedly reductive dechlorination. qPCR analysis showed different patterns of dechlorinating microorganisms during the dehalogenation tests (Table 2). The growth of *Dehalococcoides* spp. was observed in all cultures showing complete dechlorination of PCE to ethene. Three of the batch experiments are shown exemplarily in Figs. 2, 3 and 4. The remaining six batch experiments are shown in the supplementary data Figs. S3–S8.

The culture MP1 transformed PCE after the first addition of PCE mainly to trichloroethene (TCE). Initially, only a small amount of about 5 μM *cis*-DCE was formed (Fig. 2a).

Table 1 Primers and temperature programs used for the nested PCR and qPCR

Detection of	Primer sequence (5'–3')	Amplicon size (bp)	Reference for primer sequence	Temperature program nested PCR	Temperature program qPCR
Bacteria (16S)	F-AGAGTTTGATCCTGGCTCAG R-GGTACCTTGTACGACTT	1,500	Hendrickson et al. (2002)	3 min 95 °C 35 cycles: 30 s 94 °C/30 s 55 °C/90 s 72 °C 10 min 72 °C	–
<i>Dehalococcoides</i> spp. (16S)	F-GATGAACGCTAGCGGCG R-TCA GTGACAACCTAGAAAAC	690	Hendrickson et al. (2002)	3 min 95 °C 35 cycles: 30 s 94 °C/30 s 55 °C/60 s 72 °C 10 min 72 °C	–
<i>Dehalococcoides</i> spp. (16S)	F-AAAGCGGTTTTCTAGGTTGTAC R-CTTCAATGCAATGTCAAAT	215	Smits et al. (2004)	–	15 min 95 °C 45 cycles: 15 s 94 °C/20 s 55 °C/20 s 72 °C Melt
<i>Desulfomonile</i> spp. (16S)	F-GGGTCAAAGTCGGCCTCTCGACG R-GCTTTCACATTCGACTTAICG	360	El Fantroussi et al. (1997)	3 min 95 °C 35 cycles: 30 s 94 °C/30 s 55 °C/60 s 72 °C 10 min 72 °C	15 min 95 °C 45 cycles: 15 s 94 °C/ 20 s 55 °C/20 s 72 °C Melt
<i>Dehalobacter</i> spp. (16S)	F-GTTAGGGAAGAACGGCAICTGT R-CCTCTCCTGTCCCTCAAGCCATA	250	Smits et al. (2004)	3 min 95 °C 35 cycles: 30 s 94 °C/30 s 58 °C/40 s 72 °C 10 min 72 °C	15 min 95 °C 45 cycles: 15 s 94 °C/30 s 55 °C/30 s 72 °C Melt
<i>Desulfuromonas</i> spp. (16S)	F-AACTTCGGGTCCTACTGTC R-GCCGAACTGACCCCTATGTT	820	Löffler et al. (2000)	3 min 95 °C 35 cycles: 30 s 94 °C/ 30 s 55 °C/ 60 s 72 °C 10 min 72 °C	–
<i>Desulfotobacterium</i> spp. (16S)	F-GTACGACGAAGGCCCTTCGGGT R-CCCAGGGTTGAGCCCTAGGT	210	Smits et al. (2004)	3 min 95 °C 35 cycles: 30 s 94 °C/ 30 s 58 °C/ 40 s 72 °C 10 min 72 °C	15 min 95 °C 45 cycles: 15 s 95 °C/ 30 s 58 °C/30 s 72 °C Melt

Table 2 qPCR results after 28 weeks of incubation and dechlorination products

	TGD area			Xiangxi River			Yangtze River		Yangtze River
	MP1 Gene copies/L	MP3 Gene copies/L	MP7 Gene copies/L	XX01 Gene copies/L	XX04 Gene copies/L	XX07 Gene copies/L	YCQ1 Gene copies/L	YCQ4 Gene copies/L	DH2 Gene copies/L
qPCR analysis after 28 weeks									
<i>Dehalococcoides</i> spp.	<QL	2.45E+10	8.59E+10	4.15E+10	4.76E+10	4.10E+10	7.18E+10	6.50E+10	3.17E+10
<i>Desulfomonile</i> spp.	2.25E+06	1.93E+06	1.41E+06	7.79E+06	8.74E+06	4.10E+06	2.29E+06	1.99E+06	3.93E+06
<i>Dehalobacter</i> spp.	1.58E+07	2.57E+06	8.31E+06	6.18E+06	1.56E+06	1.61E+06	2.05E+06	2.62E+06	1.92E+06
<i>Desulfitobacterium</i> spp.	1.11E+09	<QL	3.62E+07	9.00E+07	2.14E+08	2.58E+08	5.58E+07	5.07E+09	6.70E+06
Dechlorinating activity to									
<i>cis</i> -DCE	+	+	+	+	+	+	+	+	+
<i>trans</i> -DCE	-	-	-	-	+	+	-	-	+
VC	-	+	+	+	+	+	+	+	+
Ethene	-	-	+	+	+	+	+	+	+

All values are in copies/L

Plus sign (+) detected, minus sign (-) not detected, QL (quantification limit = 1E+5 16S RNA gene copies/L)

No further dechlorination of TCE to *cis*-DCE was observed within the first 15 weeks. After addition of PCE and electron donors, the dehalogenation of TCE started also, and TCE was completely dechlorinated to *cis*-DCE after 22 weeks.

Repeated spiking with PCE resulted in an accumulation of *cis*-DCE. No further dechlorination products such as vinylchloride (VC) or ethene were measured. In this culture, a nearly stoichiometric chloride formation was observed.

Fig. 2 PCE dechlorination and growth of dechlorinating bacteria in culture MP1. **a** Aqueous phase concentrations of chloroethenes and ethene; **b** results of qPCR (*n.d.* not detected)

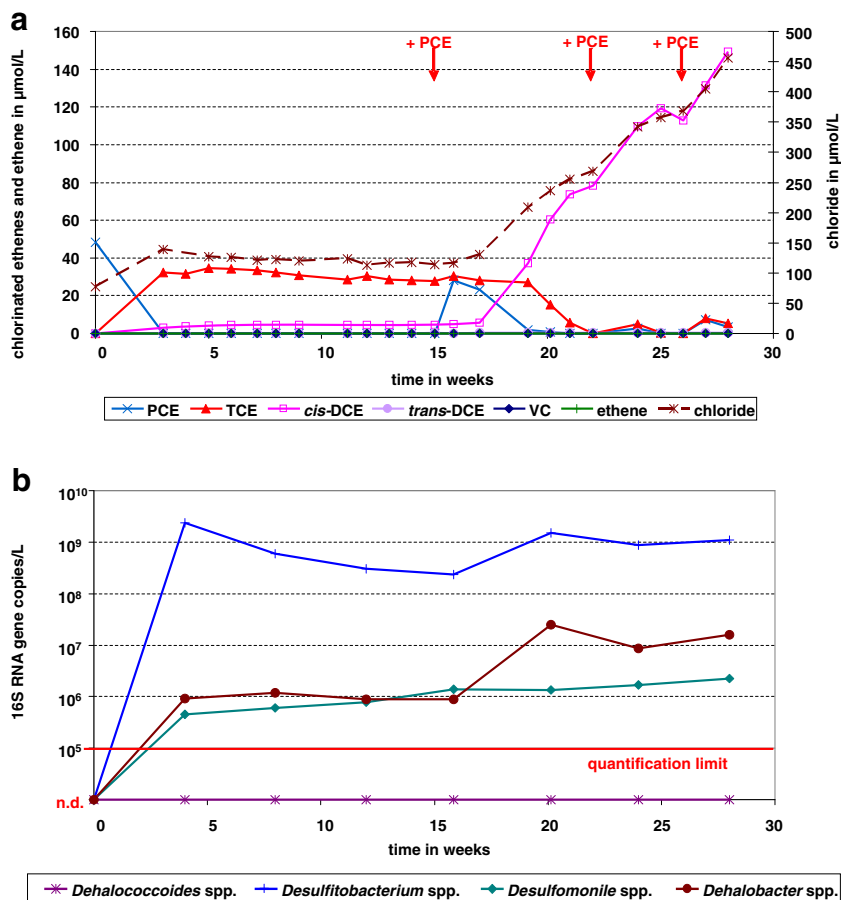
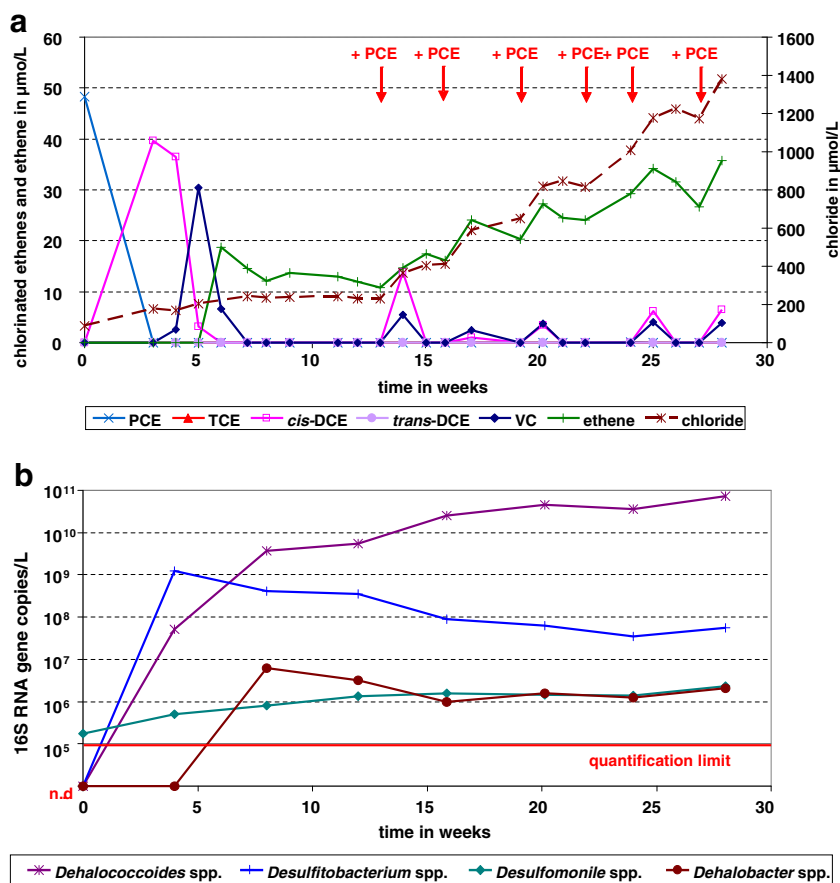


Fig. 3 PCE dechlorination and growth of dechlorinating bacteria in culture YCQ1. **a** Aqueous phase concentrations of chloroethenes and ethene; **b** results of qPCR (*n.d.* not detected)



The dechlorinating microbial community analysis is shown in Fig. 2b. The main increase in bacterial genes was detected for *Desulfitobacterium* spp. within the first 16 weeks. During this time, the gene copies per liter of *Desulfitobacterium* spp. increased about four orders of magnitude. In the case of *Dehalobacter* spp., the main growth of one order of magnitude occurred in the first 4 weeks. After the second spiking, again a growth of about one order of magnitude to $10E+7$ 16S RNA gene copies/L was observed. *Desulfomonile* spp. showed a weak growth, and *Dehalococcoides* spp. was below the quantification limit during the whole investigation period.

One culture (YCQ1) which completely dechlorinated PCE via TCE, *cis*-DCE, and VC to ethene is shown in Fig. 3a. The culture was spiked six times with PCE and showed a complete dehalogenation to ethene each time. The dechlorination of PCE started quickly, and after 3 weeks, PCE was completely transformed to *cis*-DCE. At the end of the experiment, the time for complete dechlorination of 48 µM PCE via *cis*-DCE and VC to ethene was less than 2 weeks.

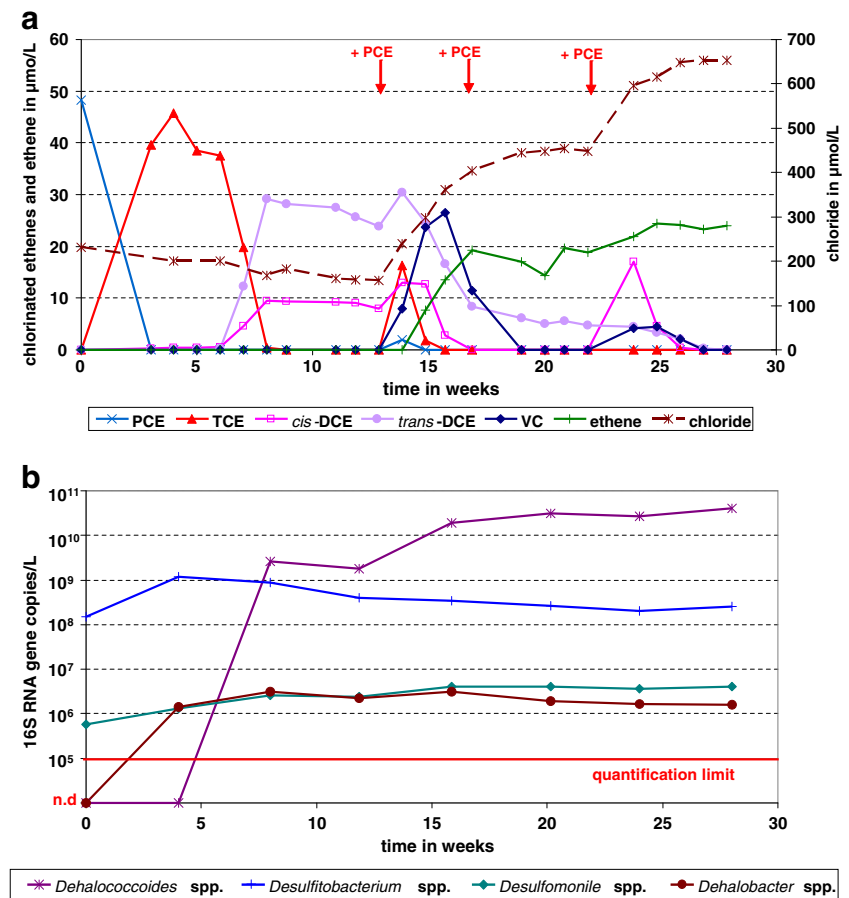
The growth of the dechlorinating microorganisms is shown in Fig. 3b. Within 4 weeks, the number of *Dehalococcoides* spp. increased by at least three orders of magnitude, and in the following 24 weeks, it increased by another

three orders of magnitude. Finally, $10E+11$ gene copies/L was reached. In the case of *Desulfitobacterium* spp., first, an increase of four orders of magnitude was observed, and then a slow decline to about $10E+8$ 16S RNA gene copies/L occurred. *Dehalobacter* spp. and *Desulfomonile* spp. showed a small incline of about 1 and two orders of magnitude.

In Fig. 4a, the results for the culture XX07 are shown. In contrast to the other cultures, the dehalogenation of PCE to *trans*- and *cis*-DCE in a ratio of 3:1 was observed. After repeated addition of PCE and electron donors, a complete dechlorination of PCE and the lower chlorinated metabolites to ethene and stoichiometric chloride formation were observed.

The growth of *Dehalococcoides* spp. was observed concomitantly with the reduction of TCE to *cis*- and *trans*-DCE (Fig. 4b). The gene copy number per liter increased at least four orders of magnitude within 8 weeks. After 28 weeks, a value of approximately $1E+10$ 16S RNA gene copies/L was reached. The gene copy numbers of *Desulfitobacterium* spp. were already high initially and increased about one order of magnitude. *Desulfomonile* spp. and *Dehalobacter* spp. showed growth in the first 4 weeks about one order of magnitude, but afterwards remained constant until the end of the experiment.

Fig. 4 PCE dechlorination and growth of dechlorinating bacteria in culture XX07. **a** Aqueous phase concentrations of chloroethenes and ethene; **b** results of qPCR (*n.d.* not detected)



The results of the batch studies are summarized in Table 2 (for more detailed information see supplementary data Figs. S3–S8). Obviously, different bacteria known for their organohalide respiring potential developed during the incubation of Yangtze sediment samples. In particular, increase of gene copy numbers over four orders of magnitude was observed for *Desulfitobacterium* spp. (Figs. 2 and 3, Fig. S5), *Dehalobacter* spp. (Figs. S4 and S6), and *Dehalococcoides* spp. (Figs. 3 and 4, Figs. S3–S8). If bacteria of the *Dehalococcoides* group were not present, the reductive dechlorination stopped at *cis*-DCE. In the presence of *Dehalococcoides* spp., in all samples except one, a complete reductive dechlorination to ethene occurred. Only in one sediment sample (MP3), VC was observed as the end product of reductive dechlorination (Fig. S3).

Discussion

In all batch tests incubated with sediments from the TGR catchment area, anaerobic biological dechlorination was demonstrated (Table 2). Although *Dehalococcoides* spp. were below the limit of detection in the initial nested PCR screening (Table T1), these bacteria were detected in most of the batch dechlorination tests. However, differences in the

biochemical pathways and end-products of reductive dechlorination were observed, most probably due to different halo-respiring bacteria community compositions. In nearly all sediment samples, *Desulfuromonas* spp. was detected with the nested PCR approach (Table T1). However, growth of *Desulfuromonas* spp. was not studied by qPCR in the batch tests because of the lack of suitable primers.

The culture MP1 repeatedly dechlorinated PCE via TCE to *cis*-DCE (Fig. 2a). In this culture, *Dehalococcoides* spp. was below the quantification limit during the whole incubation time. In contrast, *Dehalococcoides* spp. were detected with increasing numbers over investigation time in the YCQ1 (Fig. 3b) and the XX07 cultures (Fig. 4b). These cultures showed complete dechlorination, thus confirming previous reports that bacteria of the *Dehalococcoides* group are the only known microorganism to completely dehalogenate PCE to ethene (Holliger et al. 1999; Löffler et al. 2000; Maymó-Gatell et al. 2001; Hendrickson et al. 2002; Smidt and de Vos 2004; Schmidt et al. 2006). During 28 weeks and anaerobic transformation of approximately 60 mg/L PCE, the *Dehalococcoides* spp. gene copies/L increased by five orders of magnitude. Also, Aktaş et al. (2012) reported a several orders of magnitude increase of gene numbers during the reductive dechlorination of approximately 100 mg/L PCE to ethene.

Although in both cultures, YCQ1 and XX07, complete dechlorination to ethene was observed, the temporarily detected metabolites differed. As in most previous studies (Löffler et al. 2000; Schmidt and Tiehm 2008; Aktaş et al. 2012), dechlorination via *cis*-DCE without any detection of *trans*-DCE was observed in culture YCQ1 (Fig. 3a). The culture XX07 dechlorinated PCE via TCE to *trans*-DCE and *cis*-DCE in a ratio of 3:1. Similar ratios were already reported in some other studies (Griffin et al. 2004; Kittelmann and Friedrich 2008; Cheng and He 2009; Cheng et al. 2009; Marco-Urrea et al. 2011). Chow et al. (2010) and Marco-Urrea et al. (2011) found that the *Dehalococcoides* strains MB and CBDB1 dechlorinate PCE and TCE to mainly *trans*-DCE. In our study, mainly the growth of *Dehalococcoides* spp. and smaller increases of *Desulfitobacterium* spp., *Desulfomonile* spp., and *Dehalobacter* spp. were observed. The *D. mccartyi* group encompasses the six bacterial isolates of strains 195, BAV1, CBDB1, FL2, GT, and VS (Löffler et al. 2012). Most probably, different strains of the genus *Dehalococcoides* spp. with different dechlorinating abilities were present in the cultures YCQ1 and XX07. In order to obtain more insight into the dechlorinating strains of *Dehalococcoides* spp. within the Yangtze samples, further investigation focusing on chloroethene reductive dehalogenase genes such as *pceA*, *tceA*, *vcrA*, and *bvcA* are promising (Behrens et al. 2008; Maphosa et al. 2010).

In culture MP1, *Desulfitobacterium* spp. was the predominant growing bacterium in the absence of *Dehalococcoides* spp. The transformation of approximately 50 mg/L PCE to *cis*-DCE resulted in an increase of *Desulfitobacterium* spp. over four orders of magnitude, and an increase of *Dehalobacter* spp. over two orders of magnitude (Fig. 2b). Obviously, the addition of PCE and the electron donors acetate/pyruvate and H₂ could stimulate the growth of the different dechlorinating bacteria. However, a small amount of sulphate and Fe(III) was available in the mineral medium, and it is also possible that fermentation of the yeast extract resulted in the formation of fumarate. As reviewed recently by Maphosa et al. (2010), these compounds might also have been used as electron acceptors by *Desulfitobacterium* spp. or *Desulfomonile* spp. in the cultures.

Mass balance calculations revealed that the formation of chloride corresponded to the dechlorination of PCE to DCE or ethene (Figs. 2, 3 and 4). Also, the amount of *cis*-DCE detected in the aqueous phase samples of the culture MP1 reflected the amount of PCE added to the flask in total, i.e., by several spikings. However, in all cultures with complete dechlorination, less ethene formation was observed than calculated from the total amount of spiked PCE. As discussed previously (Aktaş et al. 2012), the repeated aqueous phase sampling results in an increase of the gas/liquid ratio in the bottles. Therefore, the high volatility, in particular, of ethene leads to a transfer into the gas phase resulting in lower concentrations in the aqueous phase.

In the case of incomplete dechlorination of PCE to the end-product *cis*-DCE, as observed in culture MP1, bioaugmentation with a *Dehalococcoides* spp.-containing culture can stimulate further dechlorination to ethene (Ritalahti et al. 2005). On the other hand, sequential anaerobic/aerobic degradation (Tiehm and Schmidt 2011) might be important in the field. Higher chlorinated compounds like PCE and TCE are more easily dechlorinated in an anaerobic reductive environment (Schmidt et al. 2010; Tiehm and Schmidt 2011). Lower chlorinated compounds like DCE and VC also can be degraded by aerobic degradation (Tiehm et al. 2008; Schmidt et al. 2010; Zhao et al. 2010; Zhao et al. 2011). At contaminated field sites, anaerobic dechlorination could stop at *cis*-DCE or VC, followed by aerobic mineralization of the lower chlorinated metabolites (Schmidt and Tiehm 2008; Lohner et al. 2011; Tiehm and Schmidt 2011). A similar sequential anaerobic/aerobic degradation might be possible also in the Yangtze TGR, taking into consideration the periodical water level fluctuations and expected spatio-temporal dynamics of the hydrochemical parameters (Bergmann et al. 2012; Kranzioch and Tiehm 2012). However, additional studies are required to prove this hypothesis.

Conclusions and outlook

In this study, the presence of viable dechlorinating bacteria in Yangtze sediment samples was demonstrated, thus indicating their relevance for pollutant turnover in the catchment area. The applied PCR methods proved to be suitable for the detection and quantification of the different dechlorinating bacteria. The qPCR analysis showed, in several samples, that the dehalogenation of PCE corresponded to the increasing numbers of *Dehalococcoides* spp., *Desulfitobacterium* spp., *Desulfomonile* spp., or *Dehalobacter* spp. In this study, the focus was on different bacteria, which are capable growing on chlorinated ethenes as electron acceptor. Studies focusing on the dehalogenase genes and dynamic environmental conditions are in progress.

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