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THEMATIC ISSUE

# Microbial dechlorination of HCB, PCP, PCB180, HCH and PCE in a Yangtze Three Gorges Reservoir enrichment culture, China

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**Abstract** Highly chlorinated pollutants are often found in river sediments, such as in the Yangtze River. In this study, the transformation of hexachlorobenzene, pentachlorophenol, polychlorinated biphenyl (PCB), hexachlorocyclohexane and perchloroethene was demonstrated in a mixed microbial culture enriched from Xiangxi River sediment [a tributary of Yangtze River belonging to the Three Gorges Reservoir (TGR)] which contained halo-respiring bacteria. Reductive dehalogenation by the Yangtze bacteria resulted in the formation of lower chlorinated metabolites, such as tri- and dichlorobenzenes, tri- and dichlorophenols, vinyl chloride and ethene. In case of PCB180, the lesser chlorinated metabolite PCB146 was detected and is recommended for future monitoring programs. Increased gene copy numbers of dechlorinating bacteria, e.g. *Dehalococcoides* spp. and *Desulfitobacterium* spp., were observed after the transformation of the chlorinated pollutants. In conclusion, the study demonstrates the capability of Yangtze River bacteria to dechlorinate several important chlorinated pollutants, indicating efficient pollutant turnover in the TGR area.

**Keywords** Chlorinated pollutants · Three Gorges Reservoir · Metabolites · PCB146 · Reductive dechlorination · Yangtze

## Introduction

Chlorinated organic compounds have been released to the environment through their extensive use in agriculture and chemistry over the past 50 years. Chlorinated substances served as biocides [e.g. hexachlorocyclohexanes (HCHs), hexachlorobenzene (HCB) and pentachlorophenol (PCP)], electrical fluids [e.g. polychlorinated biphenyls (PCBs)], intermediates for chemical synthesis [e.g. HCB and vinyl chloride (VC)], solvents and degreasing agents [e.g. tetrachloroethene/trichloroethene (PCE/TCE)] and have functions in many other applications (Abramowicz 1995; Adrian and Görisch 2002; Kaufhold et al. 2013; Lal et al. 2010; Olaniran and Igbinosa 2011; Schmidt et al. 2010; Wu et al. 2015). The extensive industrial and agricultural applications resulted in the deposition of chlorinated organic compounds in various environments, especially in soils, groundwater aquifers and sediments of lakes and rivers (Dohmann et al. 2016; Liu et al. 2010; Taş et al. 2010a; Xi et al. 2015). Due to their physicochemical properties, exposure to these compounds can have carcinogenic or toxic effects. Therefore, the elimination of chlorinated pollutants from water resources is highly relevant all over the world (Olaniran and Igbinosa 2011; Wang

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and He 2013; van Doesburg et al. 2005; Taş et al. 2010b; Schmidt and Tiehm 2008).

The Yangtze River, with a length of 6300 km, is one of the biggest rivers in the world. It has been a source of life for the Chinese people for centuries and is a habitat for a remarkable variety of aquatic species (Floehr et al. 2013). In the past decade, rapid industrial and economic growth in China resulted in increasing discharge of pollutants into the environment (Dohmann et al. 2016; Tao et al. 2013). In the Yangtze River, with the impoundment of the 630-km-long Three Gorges Reservoir (TGR), upstream of the Three Gorges Dam, huge industrial, residential and agricultural areas were flooded. As a consequence, notable amounts of organic and inorganic pollutants were released to the reservoir (Bergmann et al. 2012; Wolf et al. 2013). The polychlorinated pollutants of HCB (Jiang et al. 2000; Wang et al. 2009), PCP (Tang et al. 2007), PCB180 (Jiang et al. 2000; Wang et al. 2009) and α-, β-, γ- and δ-HCH (Liu et al. 2015; Wang et al. 2009) have been detected in the Yangtze River.

Anaerobic dechlorination of higher chlorinated organic compounds has been observed at many contaminated sites, in particular in Europe and the USA (Aulenta et al. 2005; Carreón-Diazconti et al. 2009; Demarest 2013; Köber et al. 2014; Schmidt and Tiehm 2008; Semprini 1995). *Dehalococcoides* spp. represent the only bacteria capable of complete dechlorination of chloroethenes and can also transform other chlorinated compounds (Demarest 2013; Kaufhold et al. 2013; Löffler et al. 2013; Taş et al. 2010a; Wang and He 2013). However, several other microorganisms, such as *Dehalobacter* spp., *Desulfomonile* spp., *Desulfuromonas* spp. and *Desulfitobacterium* spp., are also able to degrade chlorinated organic contaminants through organohalide respiration (Maphosa et al. 2010; Tiehm and Schmidt 2011; Wang and He 2013). Studies into the capability of halo-respiring bacterial communities to use several different chlorinated pollutants are limited. Only Kaufhold et al. (2013) and Wang and He (2013) studied the substrate spectrum of a dechlorinating culture, and other studies are restricted to only one of the chlorinated pollutants (Adrian et al. 2007; Field and Sierra-Alvarez 2008; Middeldorp et al. 2005; Schmidt and Tiehm 2008). Kaufhold et al. (2013) studied dehalogenation of halogenated substrates using a highly enriched *Dehalococcoides*-containing culture from the contaminated site in Bitterfeld, Germany. Wang and He (2013) reported dechlorination of multiple halogenated compounds using a culture containing *Dehalococcoides* spp. and *Dehalobacter* spp. enriched from a wastewater treatment plant in Gehua, P. R. China. Recent studies with dechlorinating bacteria enriched from Yangtze River sediments focused on chloroethene degradation. The Yangtze River cultures were shown to contain several dechlorinating bacteria, such as *Dehalococcoides* spp., *Desulfitobacterium* spp., *Dehalobacter* spp. and *Desulfomonile* spp. (Kranzioch et al. 2013, 2015).

The aim of this work was (a) to examine the capability of a mixed microbial culture enriched from Three Gorges Reservoir sediment to dehalogenate HCB, PCP, PCB180 and HCHs and (b) to identify dominating transformation pathways.

## Materials and methods

### Biodegradation studies

All experiments were performed in 2-L laboratory glass bottles capped with Teflon-coated septa, held in place with screw caps. Cultivation was performed in duplicate in a mineral salt medium amended with electron donors (40 mg of sodium acetate, 40 mg of sodium pyruvate, 0.5 g of yeast extract and 10 mL $H_2$/L) as described previously (Kranzioch et al. 2013). All handling, except $H_2$ addition, was performed inside an anaerobic gas chamber flushed with nitrogen. The bottles were amended with 0.35 µM (0.1 mg/L) HCB (99.9 %), 6 µM (1.5 mg/L) PCP (99.9 %), 0.25 µM (0.1 mg/L) PCB180 (99.5 %), 3.44 µM (1.0 mg/L) HCH ($\alpha$:$\beta$:$\gamma$:$\delta$ = 1:1:1:1, 99.5 % purity) and 45 µM (7.5 mg/L) PCE (99.9 %) (Sigma-Aldrich, Steinheim, Germany). Each batch test was inoculated with 20 mL of the culture XX01 enriched from Xiangxi River (tributary of Yangtze River) sediment (Kranzioch et al. 2013). Further one bottle was prepared as sterile control with all primary substances and was poisoned with 1 g/L sodium azide. Bottles were stored at room temperature (22–24 °C) in the dark. The aqueous phase samples were taken using glass syringes.

### Analytical methods

HCHs and PCBs were determined using a gas chromatograph (PerkinElmer Autosys XL AUSXA 53006, Massachusetts, USA) equipped with an electron capture detector, according to the German standards of DIN 38407, part F2: 02/1993. Other dechlorination products not included in DIN 38407 (such as PCB146) were identified and determined using a gas chromatograph (Agilent 7890A, USA) equipped with a mass spectrometer (Agilent 5975C, USA) and a gas chromatograph (Agilent 7890A, USA) equipped with an electron capture detector, according to the PCB single congener or mixtures of Aroclor from the AccuStandard. CBs were analysed with a gas chromatograph (PerkinElmer Autosys XL, Massachusetts, USA) equipped with a TurboMass Gold spectrometer, according to the German standards of DIN 34807, part F17:1999-02. CPs were analysed using a gas

chromatograph (Agilent 6890N, Waldbronn, Germany) equipped with a mass spectrometer. A 500 mL diluted sample volume was preconcentrated with 200 mg of Strata X material (Phenomenex, Aschaffenburg, Germany); elution was conducted with 5 mL of acetone. After reducing the volume of the organic solvent to 200 µL, an aliquot of 2 µL was injected into the GC/MS system. Chloroethenes and ethene concentrations were determined using a gas chromatograph (Series II 5890, Hewlett-Packard, Waldbronn, Germany) equipped with a headspace sampler, flame ionisation detector and electron capture detector. Samples for gas chromatographic analyses were acidified to a pH of 2 with phosphoric acid and stored at 4 °C until analysis. The standard deviation coefficients of the analysis were 30 % in case of the CPs and 25 % in case of HCHs, PCBs and CBs. For chloroethenes, reproducibility on standard analysis was ±6 %. Chloride concentrations were determined using ion chromatography (Metrohm 761 Compact IC, Metrohm, Filderstadt, Germany) with ±1 % reproducibility on standard analysis.

### Polymerase chain reaction (PCR)

For PCR analysis, 50 mL of the culture was filtered through a 0.2-µm membrane filter (47 mm diameter) (PALL, MI, USA). The membrane was stored at −20 °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 end-point PCR and qPCR of the culture samples for the detection of the dechlorinating bacteria *Desulfomonile* spp., *Desulfitobacterium* spp., *Dehalobacter* spp. and *Dehalococcoides* spp. were conducted as described previously (Kranzioch et al. 2013). The qPCR for *Desulfuromonas* spp. was performed with DeSuF205 (AACCTTCGGGTCC TACTGTC) and DeSuR1033 (GCCGAACTGACCCCT ATGTT) primers with the following qPCR protocol: 95 °C for 15 min; 45 cycles of 95 °C for 15 s, 63 °C for 30 s and 72 °C for 50 s; and after 45 cycles, a melting curve from 63 to 99 °C. The end-point PCR was previously described in Kranzioch et al. (2013).

## Results and discussion

The dechlorination of HCB by the Yangtze culture is shown in Fig. 1a (first flask) and Fig. S1a (second flask). In the two duplicate active cultures, the metabolites PeCB and tetrachlorobenzenes (TeCBs) were detected after 4 weeks of incubation time. After 10 weeks, higher concentrations of

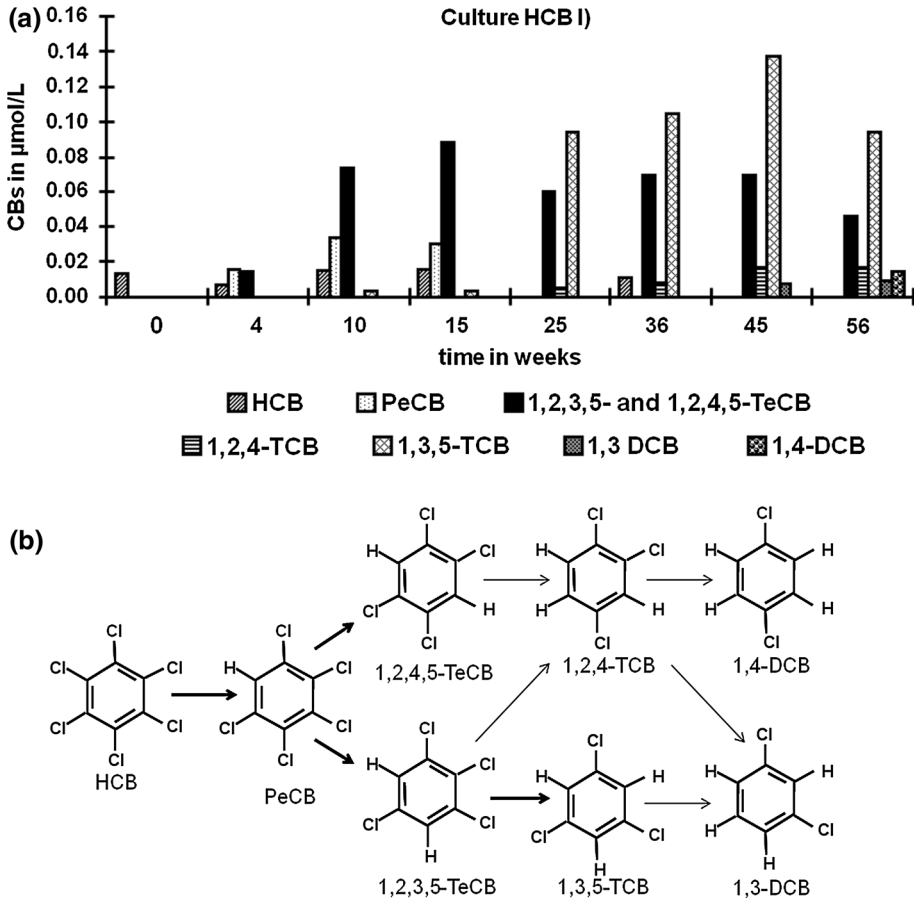


**Fig. 1** **a** Chlorobenzene aqueous phase concentrations, **b** HCB dechlorination pathway in the Yangtze River culture. (*Bold arrows*) main dechlorination pathway, (*light arrows*) secondary dechlorination pathways

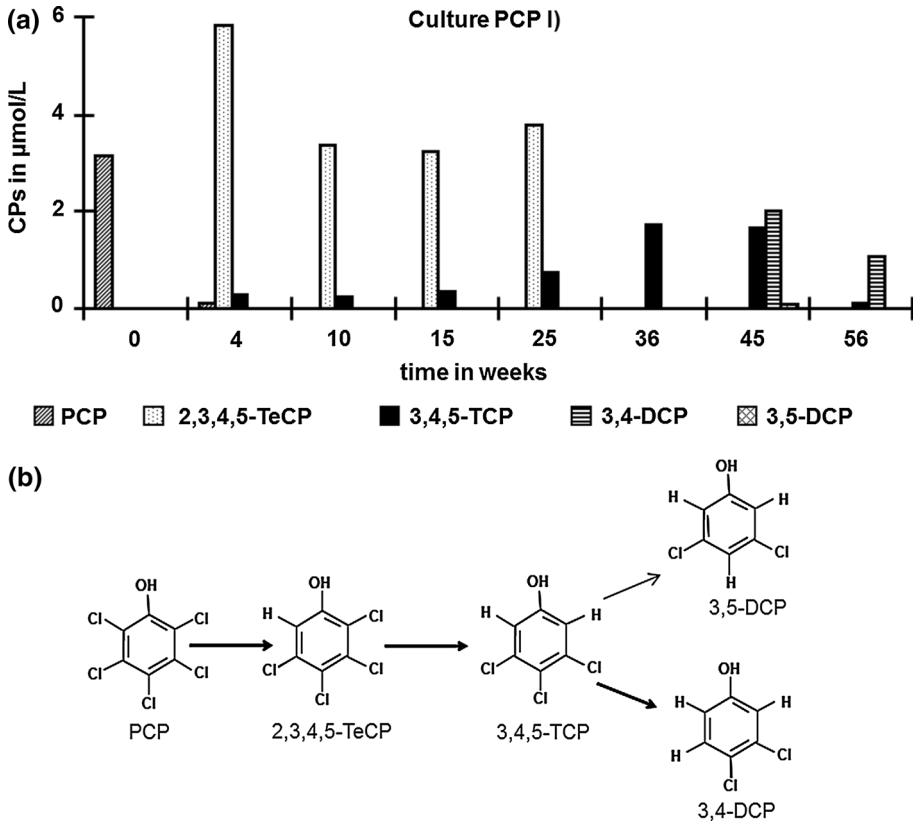


**Fig. 2** **a** Chlorophenol aqueous phase concentrations, **b** PCP dechlorination pathway in the Yangtze River culture. (*Bold arrows*) main dechlorination pathway, (*light arrows*) secondary dechlorination pathways

dissolved TeCBs and trichlorobenzenes (TCBs) were detected. 1,2,4-Trichlorobenzene (1,2,4-TCB) and 1,3,5-TCB reached maximum concentrations of 0.01 and 0.14 µmol/L after 45 weeks, respectively. After 45 and 56 weeks, two different DCBs (1,3- and 1,4-DCB) were detected at concentrations of 0.01 µmol/L. Due to limited water solubility (Table S6), the aqueous phase concentrations of HCB were below 0.02 µmol/L during the whole incubation time. The less hydrophobic metabolites reached higher dissolved concentrations (Figs. 1a and S1a). The maximum concentration of the detected dissolved chlorobenzenes was approx. 0.25 µmol/L in both flasks. Dechlorination kinetics were most likely triggered by the dissolution rates, and solid-phase HCB was still present at the end of the experiments. Additionally, co-metabolic processes might have affected pollutant transformation but were not examined in this study. Although experimental conditions differ, similar time frames of HCB dechlorination were reported in previous studies (Duan and Adrian 2013; Kaufhold et al. 2013; Taş et al. 2010b, 2011). No dechlorination products were observed in the abiotic control (data not shown).

The observed dechlorination pathway for HCB is illustrated in Fig. 1b. 1,3,5-TCB and 1,2,4-TCB were detected, followed by 1,3- and 1,4-DCB after the next dechlorination step. The degradation pathway from HCB over PeCB and 1,2,3,5-TeCB to mainly 1,3,5-TCB was also observed previously (Kaufhold et al. 2013; Duan and Adrian 2013).

The degradation pattern of PCP is shown in Figs. 2a and S2a. After 4 weeks, 2,3,4,5-tetrachlorophenol (2,3,4,5-TeCP) was detected. Obviously, elimination of the chlorine atom occurred at the ortho-position. The next chlorine was again preferentially removed at the ortho-position, resulting in the formation of 3,4,5-TCP. However, small amounts of 2,4,5-TCP were also detected in the duplicate degradation test (Fig. S2a), resulting from dechlorination at the meta-position.

The observed dechlorination pathway (Fig. 2b) of this study is consistent with the pathway proposed previously by Adrian et al. (2007). However, formation of a specific TeCP isomer was not demonstrated in the previous study. Here, dechlorination of PCP via 2,3,4,5-TeCP, mainly 3,4,5-TCP to 3,4-DCP and 3,5-DCP, is clearly shown. In the sterile control, no metabolites were formed within 56 weeks (data not shown).

The results for PCB180 are shown in Figs. 3a and S3a. Due to the low water solubility and slow dissolution kinetics, the concentration of dissolved PCB180 was increased with increasing incubation time, from starting values of approx. 4 nmol/L to the highest values of 150 nmol/L after 25 weeks. In the sterile control, the concentration of PCB180 also varied during the experiment, from values of

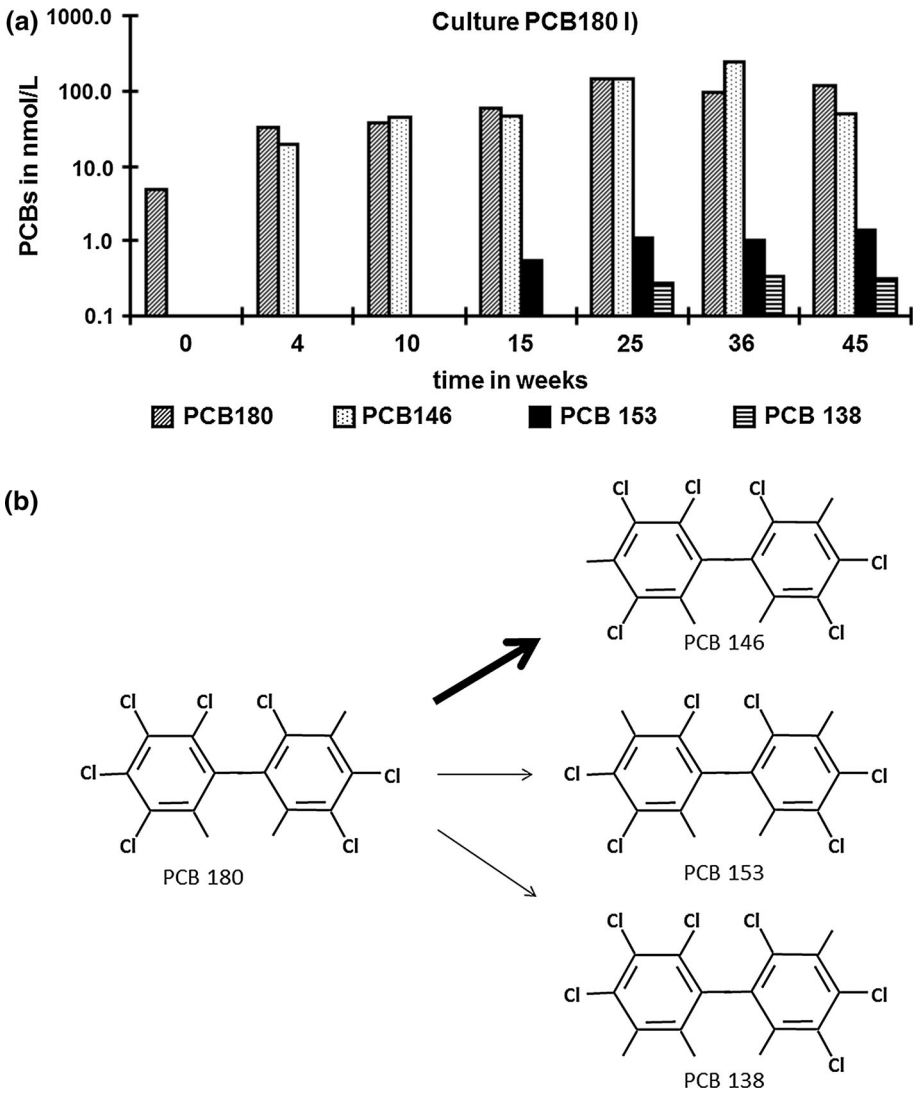


**Fig. 3** **a** Polychlorinated biphenyl aqueous phase concentrations, **b** PCB180 dechlorination pathway in the Yangtze River culture. (*Bold arrows*) main dechlorination pathway, (*light arrows*) secondary dechlorination pathways

6 nmol/L to approximately 300 nmol/L. In the active cultures, the formation of PCB146, PCB153 and PCB138 was observed. For PCB146, an increase from 19 nmol/L to more than 200 nmol/L could be detected between weeks 4 and 36. For PCB153, a small increase could be detected between weeks 15 and 56, from 0.5 to 1 nmol/L. These results are in accordance with the transformation pathways observed previously, i.e. dechlorination in the para- or meta-position (Field and Sierra-Alvarez 2008). Meta- and para-dechlorination was also observed during the dechlorination of PCB180 through the XX01 culture. The preferred dechlorination position was the double-flanked para-position, resulting in PCB146 as the main dechlorination product. Furthermore, the double-flanked meta-position was preferred over the single-flanked because higher amounts of PCB153 compared to PCB138 were detected. None of the lower chlorinated PCBs were detected in the sterile control (data not shown).

The pattern of $\alpha$-, $\beta$-, $\gamma$- and $\delta$-HCH transformation was followed for 56 weeks for the two cultures and the abiotic control. For the HCHs, only analysis of the original compounds was performed, and detection of catabolites was beyond the scope of this study. Therefore, transformation processes were evaluated by comparison of the active cultures with sterile controls. The active cultures showed a fast decrease in $\gamma$-HCH (also known as lindane) in the first 4 weeks to 20 % of the concentration in the sterile control (Figs. 4 a and S4a). After 10 weeks, the amount of $\gamma$-HCH decreased to below the detection limit. Badea et al. (2009), Cui et al. (2012), van Doesburg et al. (2005) and Middeldorp et al. (2005) reported similar dechlorination rates for $\gamma$-HCH. Additionally, a rapid decrease in concentration was found for $\alpha$-HCH. The concentration of $\alpha$-HCH after 10 weeks in the active cultures was 5 % of the concentration in the sterile control. After 15 weeks, $\alpha$-HCH was below the detection limit. The results confirm previous

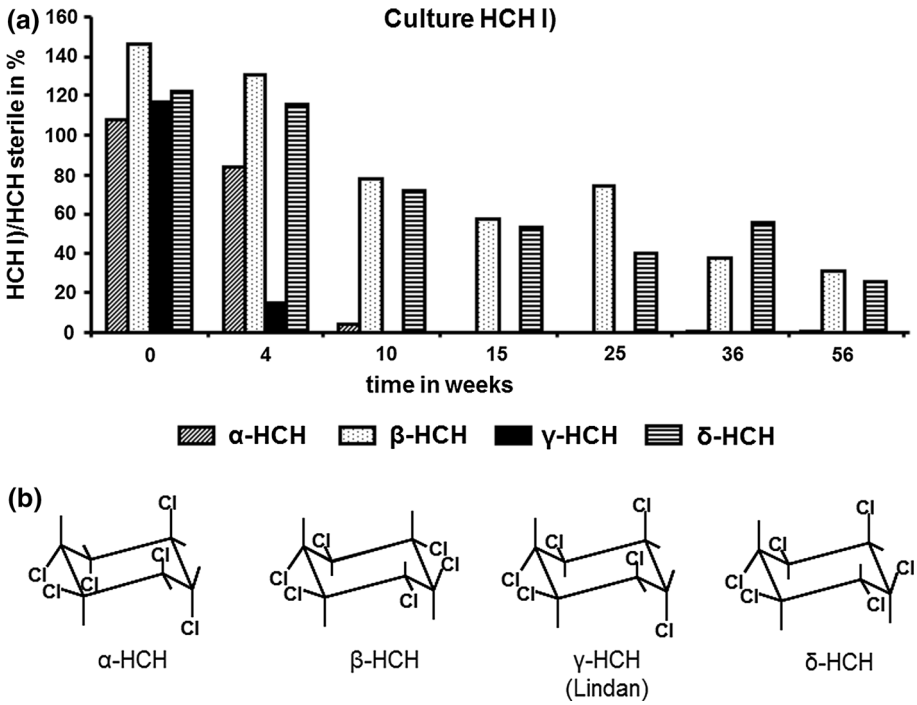


**Fig. 4** **a** Decrease in $\alpha$-, $\beta$-, $\gamma$- and $\delta$-HCH aqueous phase concentrations in the Yangtze River culture compared to the sterile control, **b** structures of $\alpha$-, $\beta$-, $\gamma$- and $\delta$-HCH

**Table 1** qPCR results of the dechlorinating culture XX01, with different chlorinated pollutants at the end of the experiment after 56 weeks

| | *Dehalococcoides* spp. gene copies/mL | *Desulfitobacterium* spp. gene copies/mL | *Dehalobacter* spp. gene copies/mL | *Desulfomonile* spp. gene copies/mL | *Desulfuromonas* spp. gene copies/mL |
|---|---|---|---|---|---|
| HCH I) | <QL | 8.9E+5 | 1.6E+3 | <QL | 2.7E+2 |
| HCH II) | <QL | 1.2E+5 | 2.3E+3 | <QL | 1.1E+3 |
| HCB I) | 3.9E+4 | 7.1E+4 | <QL | <QL | 2.9E+2 |
| HCB II) | 7.5E+4 | 8.0E+4 | <QL | <QL | 1.2E+5 |
| PCP I) | 2.7E+5 | 2.9E+5 | <QL | <QL | <QL |
| PCP II) | 4.4E+4 | 1.0E+5 | 1.3E+3 | <QL | <QL |
| PCB180 I) | 1.8E+3 | 7.1E+4 | <QL | <QL | 4.7E+2 |
| PCB180 II) | 3.3E+3 | 7.7E+4 | 2.2E+3 | <QL | 1.9E+2 |
| PCE I) | 1.9E+6 | 1.8E+5 | <QL | <QL | <QL |
| PCE II) | 2.2E+6 | 1.1E+5 | <QL | <QL | 4.6E+2 |
| Sterile control | 1.4E+2 | 1.0E+2 | <QL | <QL | <QL |

The average values at the beginning of the experiment were 9.8E+3 copies/mL for *Dehalococcoides* spp. and 1.7E+3 copies/mL for *Desulfitobacterium* spp. The other dechlorinating groups, i.e. *Dehalobacter* spp., *Desulfomonile* spp. and *Desulfuromonas* spp., were at the beginning of the experiment below the quantification limit. <QL: below the quantification limit of the qPCR method (1.0E+2 copies/mL)

reports that $\alpha$- and $\gamma$-HCH are transformed rapidly under anaerobic conditions (Lal et al. 2010). However, $\beta$- and $\delta$-HCH degradation was also reported (Quintero et al. 2005). In this study, a decrease in $\beta$- and $\delta$-HCH in the active cultures was observed compared to the sterile control.

To proof the activity of the dechlorinating culture, a PCE control was prepared. The dechlorination pattern of PCE for the duplicate active cultures is shown in Fig. S5. In the active cultures (I) and (II) amended with PCE, a fast dechlorination of approximately 40 µmol/L PCE to 40 µmol/L cDCE occurred. From week 15 to week 36, a complete dechlorination from 40 µmol/L cDCE over VC to ethene was observed. The dechlorination potential of the Yangtze River culture XX01 for PCE was already investigated in the study of Kranzioch et al. (2013) and confirmed for comparison purposes in this study. Approximately 180 µmol/L of chloride formation corresponded to the complete dechlorination of PCE to ethene. Because ethene is volatile (Kranzioch et al. 2013), only low concentrations were found in the aqueous phase. The chloride balance was only possible in the case of PCE, which shows a high water solubility compared to HCB,

HCHs, PCP or PCB 180 (Table S6). Because the bioavailable dissolved concentrations and therefore the transformation rates of the more hydrophobic chlorinated pollutants were low, increases in chloride concentrations could not be detected.

The halo-respiring bacteria *Dehalococcoides* spp., *Desulfitobacterium* spp., *Dehalobacter* spp., *Desulfomonile* spp. and *Desulfuromonas* spp. were analysed by quantitative PCR at the end of the incubation period of 56 weeks (Table 1). The results confirmed previous reports (Kranzioch et al. 2013, 2015) that PCE dechlorination in the Yangtze River cultures resulted predominantly in increased gene copy numbers of *Dehalococcoides* spp. and *Desulfitobacterium* spp. In this study, growth of *Dehalococcoides* spp. was also observed with PCP and HCB. Several previous studies (Adrian and Görisch 2002; Adrian et al. 2007; Duan and Adrian 2013; Kaufhold et al. 2013) have shown that *Dehalococcoides* spp. hold the ability to dechlorinate PCP and HCB.

Thus far, only transformation of CPs has been demonstrated for pure cultures of *Desulfitobacterium* spp. (Utkin et al. 1994; Gerritse et al. 1996). In this study, an increase in *Desulfitobacterium* spp. was observed with all tested compounds. The most pronounced increase in *Desulfitobacterium* spp. copy numbers was found with PCP. *Desulfitobacterium* spp. also showed the highest increase in gene copy numbers with PCB180, thus indicating a capability to dehalogenate PCBs. However, *Desulfitobacterium* spp. can use a variety of electron acceptors for growth (Maphosa et al. 2010), and *Desulfitobacterium* dehalogenans can grow fermentatively on pyruvate (van de Pas et al. 2001). Therefore, additional experiments are required to provide clear evidence for specific dechlorination capabilities.

An increase in the bacterial gene copies of *Dehalobacter* spp. was found with HCH, PCP and PCB180 (Table 1). Lal et al. 2010 reported that *Dehalobacter* spp. is capable of HCH dechlorination. Additionally, a correlation between the dechlorination of the PCB mixture Aroclor 1260 and the increase in *Dehalobacter* spp. gene copies was reported (Wang and He 2013).

Slightly increased gene copy numbers of *Desulfuromonas* spp. were observed with HCB, HCH, PCB180 and PCE. Similar to *Desulfitobacterium* spp., *Desulfuromonas* spp. are known for their versatile metabolism and as having the capability to use a wide range of electron acceptors such as sulphate, sulphite or nitrate or to grow by fermentation of pyruvate (Maphosa et al. 2010).

In summary, for HCB, PCP and PCE, a removal of up to four chlorine substituents was demonstrated for the Yangtze River enrichment culture (Table S1). In all cultures, the oxidative reductive potential was between +92 and −150 mV, which is a suitable environment for reductive dechlorination (Figure S7). For PCB180 carrying 7 chlorine substituents, a transformation to metabolites with 6 substituents was shown. In case of the HCHs, a preferential transformation of α- and γ-HCH was observed. The detected dechlorination products of PeCB, PCB138 and PCB153 have already been analysed and detected in the Yangtze River water column (Wang et al. 2009). The main PCB180 transformation product of the Yangtze River culture, i.e. PCB146, has not been studied in the field. However, this study suggests the inclusion of PCB146 in future monitoring programmes.

## Conclusions

The Yangtze enrichment culture XX01, enriched from TGR sediment, was able to dechlorinate HCB, PCP, PCB180, α-, β-, γ- and δ-HCH and PCE. The formation of specific metabolites, such as 1,3- and 1,4-DCBs, 3,4- and 3,5-DCPs and PCB146, was demonstrated. The results suggest that indigenous Yangtze TGR microorganisms play an important role in the transformation of critical environmental compounds in the field and can transform a variety of different chlorinated pollutants.

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