# Reductive dechlorination of weathered PCBs in the marine sediments of Brentella canal of Venice Lagoon

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The occurrence microbial-mediated reductive dechlorination processes towards polychlorinated biphenyls (PCBs) has been documented in several subsurface sediments of contaminated freshwater and, less frequently, marine systems, spiked with exogenous PCBs and suspended in synthetic media. This work was aimed at detecting and characterizing the occurrence of reductive dechlorination processes towards weathered PCBs in contaminated marine sediments of Porto Marghera area (Venice lagoon, Italy) within slurry microcosms of sediments suspended in the water coming from the same contaminated site. Reductive dechlorination of weathered PCBs occurred in both sediments. The detected processes exhibited *meta*- and *para*-specificity and were not significantly "primed" after spiking with exogenous PCBs, that were rapidly and extensively dechlorinated. PCB dechlorination seemed to be mediated by sulfate-reducing bacteria, that probably started to use PCBs as electron acceptors during the sulfate reduction but in particular when their native electron acceptor was completely depleted. Such activities were detected under geochemical conditions that closely mimic those occurring in situ, and this allows to speculate that similar processes might also be in progress in situ.

## 1. Introduction

PCBs are poorly biodegradable and highly toxic contaminants. Due to their high hydrophobicity, PCBs released into aquatic systems tend to strongly accumulate in anoxic freshwater, estuarine and marine sub-surface sediments, where they can persist for several months up to many years, and through which they enter the food chain (Brown and Wagner 1990; Bedard and Quensen, 1995; Wiegel and Wu, 2000).
Several studies have documented that highly chlorinated PCBs occurring in anaerobic sediments can undergo a progressive reductive dechlorination mainly directed to the *meta* and *para* position of the biphenyl ring through which they are bio-converted into low-chlorinated, mainly *ortho*-substituted congeners, generally less toxic and prone to bioaccumulate than parent compounds (Bedard and Quensen, 1995; Wiegel and Wu, 2000). The process has been often ascribed to methanogenic bacteria and less frequently to $SO_4^=$-reducing bacteria, even though recent studies on highly enriched cultures ascribed PCB dechlorination to members of the phylum *Chloroflexi*, able to use organic acids and/or $H_2$ as electron donors and PCBs as electron acceptors (Haggblom and

Bossert, 2003; Bedard et al., 2006; Bedard et al., 2007; Fagervoldet al., 2005; Fagervold et al., 2007; Yan et al., 2006a; Yan et al., 2006b).
On the contrary, a little is known about the occurrence of PCB reductive dehalogenation processes and of PCB dehalogenating microbial populations in marine sediments (Alder et al., 1993; Berkaw et al., 1996; Lake et al., 1992; Øfjord et al., 1994; Palekar et al., 2003), where in general sulfidogenic conditions prevail on methanogenesis (Ward and Wrinfey, 1985; Kafkewitz and Togna, 1998). Thus, more information on the potential fate of aged PCBs in marine contaminated sediments are required. In particular, evidences for these processes under geochemical conditions that closely mimic those occurring in situ are highly required, as these might provide information on the occurrence of the same processes in situ (Bedard and Quensen, 1995; Wiegel and Wu, 2000; Apitz et al., 2004).
Therefore, a long term study was undertaken to detect and characterize the microbial reductive dechlorination processes *vs.* weathered PCBs in sediments of the Brentella Canal of the Porto Marghera area (Venice lagoon, Italy) suspended in water coming from the same site, i.e. under geobiochemical conditions close to those of the site, to determine the potential of the site to undergo in situ microbial decontamination.

## 2. Experimental Approach

Two sets of 30 ml slurry-phase anaerobic microcosms consisting of sediment suspended at 25% (v/v) in its own site water were developed with two PCB contaminated sediments (referred to as sediment 1 and sediment 2, respectively) collected from different locations in the Brentella canal, Porto Marghera (Venice lagoon, Italy).
The first set, developed with sediment 1, consisted of untreated microcosms as well as of pasteurized microcosms (15 minutes treatment at 90°C), molybdate-amended (20 mM) and 2-bromoethanesulfonate (BES)-amended (30 mM) microcosms, in order to select for spore-forming bacteria, to inhibit sulfate-reducing bacteria and to inhibit methanogenic bacteria, respectively. In addition, sterile microcosms were set up under each condition. A parallel group of identical microcosms was also spiked with 2,3,4,5,6-pentachlorobiphenyl (20 mg/kg dry wt sediment) in order to study the possibility of "priming" (i.e. stimulating) the dechlorination of the sediment aged PCBs and the mechanism through which they were dechlorinated under each of the experimental conditions.
The second set of microcosms, developed with sediment 2, consisted of 4 untreated microcosms (2 biologically active and 2 autoclave-sterilized) and 4 microcosms (2 biologically active and 2 autoclave-sterilized) spiked with 3,3',4,4'-tetrachlorobiphenyl, 3,3',4,4',5-pentachlorobiphenyl, 2,3',4,4',5-pentachlorobiphenyl, 3,3',4,4',5,5'-hexachlorobiphenyl and 2,3,3',4,4',5-hexachlorobiphenyl at 100 mg/kg of dry sediment each, in order to investigate: a) the possibility of "priming" the dechlorination of PCBs pre-existing in the sediment, and b) the potential biological fate of target co-planar dioxin-like PCBs under the geochemical conditions created in the microcosms.
Sterile microcosms were prepared through autoclave-sterilization performed at 121°C for 1 h in three consecutive days; in the case of spiked control microcosms, exogenous PCBs were added after autoclave sterilization. All the developed microcosms were then incubated stationary at 25 ± 1°C in the dark and periodically sampled and analyzed to

determine the volume and composition of the head-space gas as well as the concentration of PCBs and inorganic anions ($SO_4^{=}$ and $Br^-$)(Fava et al., 2003a,b; Zanaroli et al., 2006).

## 3. Results

A total PCB concentration of 0.784 ± 0.351 $\mu$mol (kg dry wt sediment)$^{-1}$ was detected in the biologically-active and sterile microcosms of sediment 1 at the 7$^{th}$ day of incubation. Significant changes of the initial PCB distribution profile were unequivocally observed in the untreated microcosms at the 20$^{th}$ week, where an extensive depletion of highly chlorinated biphenyls together with a stoichiometric accumulation of tri- and di-chlorinated, *ortho*-substituted biphenyls were observed (Figure 1A).
A less extensive but significant transformation of endogenous PCBs was also observed in the pasteurized microcosms, where the accumulation of 2-chlorobiphenyl was also observed (Figure 1B), whereas only a poor PCB transformation was detected in the microcosms supplemented with BES or molybdate (Figures 1, C and D, respectively).

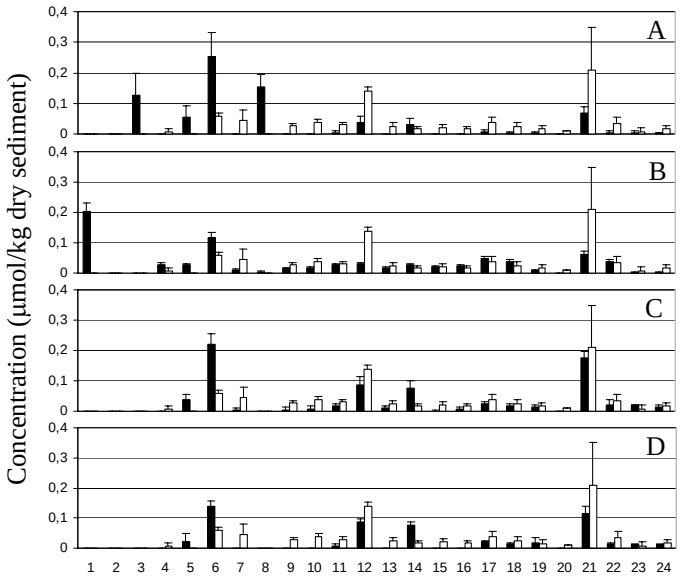


**Figure 1.** Bioconversion of sediment-carried PCBs after 20 weeks of incubation. White bar: sterile microcosms; Black bar: biologically active microcosms. A: sediment and water; B: pasteurized microcosms; C: BES; D: Molybdate (After Fava et al. 2003b).
(1):2-CB; (2):4-CB; (3):2,6-/2,2'-CB; (4):2,4-/2,5-CB; (5):2,4'-/2,3-CB; (6):2,4,6-CB; (7):2,2',5-/2,2',4-/4,4'-CB; (8):2,3,6-/2,3',6-CB; (9):2,3,3'-/2',3,4-/2,2',5,6'-CB; (10):2,2',4,6'-/2,3,4'-CB; (11):2,2',5,5'-CB; (12):3,3',4-CB; (13):2,2',3,5-CB; (14):3,4,4'-/2,3,3',6-/2,2',3,4'-CB; (15):2,2',3,4-/2,3,4',6-CB; (16):2,3',4',5-CB; (17):2,3',4,4'-/2,2',3,5',6-CB; (18):2,2',3,4',5-/2,2',4,5,5'-CB; (19):2',3,4,4',5-/2,2',3,4',5',6-/2,3',4,4',5-CB; (20):2,2',3,4',5,5'-CB; (21):2,2',3,3',4,6'-/2,2',4,4',5,5'-/2,3,3',4,4'-CB; (22):2,3,3',4,5,6-/2,2',3,4,4',5-/2,3,3',4,4',6-CB; (23):2,2',3,3',4,5,6-/2,3,3',4,4',5'-/2,2',3,3',4,5',6,6'-CB; (24):2,2',3,4,4',5,5'-CB.

**Table 1**. Microbial activities detected in the microcosms after 20 weeks of incubation.

| Microcosms | Sulfate depletion (%) | Produced Gas (ml) | Produced $CH_4$ (ml) | Released $Br^-$ (mg/l) |
|---|---|---|---|---|
| S + W | 29.9 ± 7.8 | 1.25 ± 0.21 | 0 | 0 |
| Pasteurized | 41.2 ± 14.2 | 0.65 ± 0.21 | 0 | 0 |
| with molybdate | -12.4 ± 2.7 | 2.40 ± 1.00 | 0.17 ± 0.01 | 0 |
| with BES | 47.3 ± 35.0 | 1.55 ± 0.64 | 0 | 6.01 ± 0.88 |

Comparable changes in the endogenous PCBs distribution profiles were observed in the 2,3,4,5,6-pentachlorobiphenyl-spiked microcosms (Fava et al. 2003b).
A detectable production of gas was observed in all the biologically active microcosms during the 20 weeks of incubation (Table 1). In the molybdate-amended microcosms a significant methane production and no consumption of the initial 2.1±0.1 g $l^{-1}$ of $SO_4^=$ were observed (Table 1). On the contrary, a consumption of about 30-50 % of the initial $SO_4^=$ was observed in the untreated, pasteurized, and BES-amended microcosms, where no methane production was detected. In addition, in the BES-amended microcosms a release of about 6 mg $l^{-1}$ of $Br^-$ was measured (Table 1). The same changes were observed in the PCB-spiked set of microcosms, except for sulfate consumption in the untreated microcosms, where it was slightly faster and more extensive than in the corresponding non-spiked microcosms (data not shown) (Fava et al. 2003b).
Taken together, these results indicate that indigenous sulfate-reducing bacteria were responsible for the detected PCB-reductive dechlorination. A low dechlorination activity was detected in the microcosms supplemented with BES, where a marked consumption of $SO_4^=$ was observed. A significant release of $Br^-$ was also observed in these microcosms; this suggests that BES acted as the preferential electron acceptor for the dehalogenating bacteria, thus inhibiting PCB dechlorination. The detection of both reductive dechlorination activity and sulfidogenic activity in the pasteurized microcosms, indicates that spore-forming, sulfate-reducing bacteria were involved in the process. The exogenous 2,3,4,5,6-pentachlorobiphenyl was markedly bioconverted into its tetra-, tri- and di- *ortho*-chlorinated daughter products in the spiked untreated and pasteurized microcosms, showing that the process had a higher selectivity towards *meta*- and *para*- positions. Finally, the dechlorination of the spiked PCB did not significantly affect the onset of the pre-existing PCB dechlorination, probably because of the low concentration (≈ 1 mg/kg) of the weathered PCBs in the sediment and/or the inhibition by high concentrations of $SO_4^=$ and salt occurring in these microcosms.

A total amount of sediment carried PCBs corresponding to 1.60 ± 0.13 mg/kg of dry sediment was found to occur in the non-spiked sterile and biologically active microcosms of sediment 2 after 7 days of microcosm incubation. The total amount of pre-existing PCBs in the spiked microcosms was expected to be the same; however, we could not determine it, as the added PCBs interfered with GC-ECD congeners estimation.

No transformation of sediment-carried PCBs was detected in the sterile microcosms until the end of the experiment. On the contrary, a significant change in PCB profile was found to occur in the corresponding non-spiked biologically active microcosms, as compared to the sterile ones, starting from the 5$^{th}$ month of incubation. At the end of the experiment (after 16 months), several hexa-, penta- and tetra-chlorinated congeners were found to be bioconverted on a molar basis into less chlorinated PCBs, such as 2,2',5/2,2',4/4,4'-chlorobiphenyl and 2,4/2,5-chlorobiphenyl (Figure 3). 4-monochlorobiphenyl also accumulated in the biologically active microcosms at the end of the experiment, whereas 2-monochlorobiphenyl was depleted.

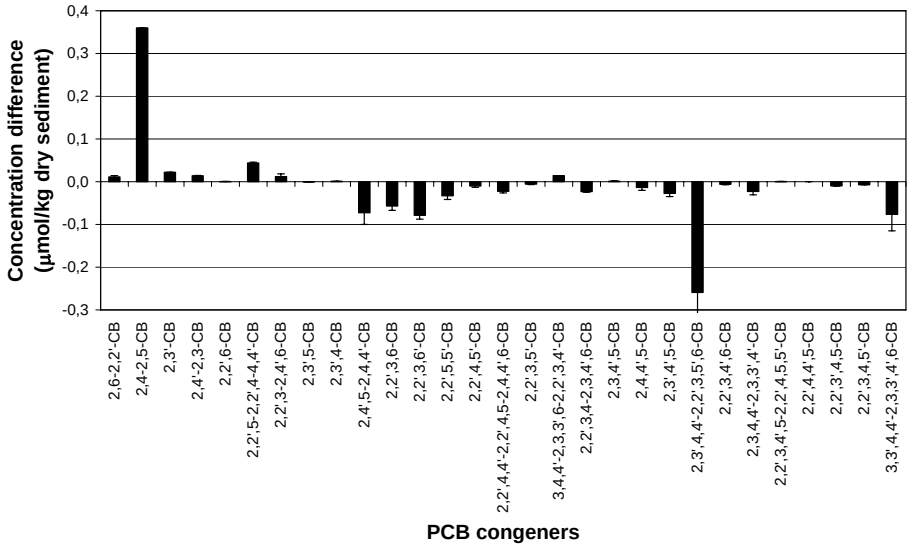


**Figure 3**. Difference in the concentration of each sediment-carried PCB congener in the non spiked biologically active microcosms as compared to the corresponding sterile ones after 16 months of incubation.

A little sulfate consumption was observed in the active microcosms since the first month of incubation. Sulfate was then quickly depleted, becoming 8.4% of the initial concentration (1.95± 0.03 g/l) after 2 months of incubation and undetectable at the end of the 3$^{rd}$ month of experiment (Table 2). No significant biogas production was observed in the active microcosms until sulfate was not completely depleted. A large amount of biogas (27.5 ± 16.5 ml) consisting of more than 47% of methane was detected in the same microcosms between the 3$^{rd}$ and the 5$^{th}$ month of incubation, i.e. immediately after complete sulfate depletion and before PCB dechlorination started. Methane production was detected at a lower rate all over the experiment (up to the 16$^{th}$ month) (Table 2).

**Table 2**. Overall sulfate consumption, gas and methane production (± standard deviation) in the biologically active microcosms after 16 months of incubation. Initial sulfate concentration in all microcosms was 1.95± 0.03 g/l.

| | Non spiked microcosms | Spiked microcosms |
|---|---|---|
| $SO_4^=$ consumption (%) | 100 | 100 |
| Biogas production (ml) | 34.7 ± 20.4 | 20.4 ± 4.9 |
| $CH_4$ production (ml) | 15.0 ± 8.5 | 8.6 ± 2.9 |

Very similar trends, both in terms of sulfate consumption and methane production, were observed in the parallel biologically active spiked microcosms, where the overall amount of produced methane was about 60% of that detected in the non spiked ones (Table 2). The biotransformation of several sediment-carried PCBs could not be quantified in the spiked microcosms, as some of them were produced from the spiked congeners dechlorination. However, the fate of about 30% of the GC-ECD peaks ascribed to pre-existing PCBs could be monitored and compared with that observed in the non spiked microcosms (data not shown). A significant transformation of such pre-existing PCBs and of the exogenous PCBs was detected starting from the 5$^{th}$ month of incubation. However, the dechlorination of the exogenous PCBs only slightly influenced the bioconversion extent and pattern of pre-existing PCBs), suggesting that the dechlorination of the latter was not significantly primed by the addition of the 5 exogenous coplanar congeners.

The exogenous PCBs were markedly bioconverted into less chlorinated congeners, such as 3,3',5,5'-/2,3',4,4'-, 2,3',4',5- and 2,4,4',5-tetrachlorobiphenyl, 2,4,4'-, 2,3',4- and 2,3'5-trichlorobiphenyl and 3,4- and 3,4'-dichlorobiphenyl between the 5$^{th}$ and the 8$^{th}$ month of incubation, and were found to be depleted by more than 80% at the end of the experiment (data not shown). This finding is of great relevance, as indicates that indigenous microbial consortia selected in the spiked primary microcosms were able to rapidly and extensively dechlorinate some dioxin-like coplanar PCBs that are regarded as some of the most toxic PCBs reported in the literature (Kimbrough 1995). The extensive dechlorination of the spiked PCBs only slightly intensify rate and extent of the dechlorination of the PCBs pre-existing in the sediment.

## 4. Conclusions

The occurrence of microbial-mediated, reductive dechlorination processes towards weathered PCBs and spiked high chlorinated and co-planar PCBs has been demonstrated in 2 contaminated sediment of the Brentelle Canal of the Porto Marghera area (Venice lagoon, Italy). The detected processes exhibited *meta*- and *para*-specificity (apparently they proceed through dechlorination pattern H' and M) and were not significantly "primed" by the dechlorination of exogenous PCBs, that were rapidly and extensively dechlorinated. PCB dechlorination seemed to be mediated by sulfate-reducing bacteria, that probably started to use PCBs as electron acceptors during the sulfate reduction but in particular when their native electron acceptor was completely depleted.

Such activities were detected under geochemical conditions that closely mimic those occurring *in situ*, and this allows to speculate that similar processes are also in progress *in situ*. However, the detected PCB dechlorination processes were slow and partial. In general microbial processes occurring *in situ* are very constrained temporally and spatially. Further, natural sediment systems are complex, heterogeneous, and subjected to (bio)turbation phenomena and low and variable temperatures. Thus, the findings described above do not necessarily prove that biodegradation will provide sufficient natural *in situ* decontamination of the site but for sure, if properly combined with other lines of microbial and biogeochemical evidence coming from the same site (Apitz et al., 2004), a strong indication of biodegradation *in situ*, and justify further investigations.

## 5. References


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