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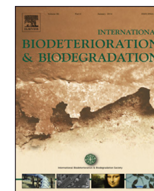

Decrease of the level of extractable polychlorinated biphenyls in soil microcosms: Influence of granular activated carbon and inoculation by natural microbial consortia

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Two bacterial consortia prepared from a polychlorinated biphenyl (PCB)-contaminated soil enrichment were used as inocula, for aerobic PCB-degradation experiments in soil microcosms. The consortia were prepared either as a planktonic culture, or as a biofilm attached to granular activated carbon (GAC). Both consortia were mainly composed of members of the α-, β- and γ-subclasses of the phylum Proteobacteria. The most abundant bacteria, belonged to the genera *Pseudomonas*, *Achromobacter*, *Ochrobactrum* and *Halomonas* which are commonly associated with soil contaminated with biphenyl or PCBs. The decrease of the level of extractable PCB congeners was assessed in microcosms containing the same PCB-polluted soil from which the consortia were prepared and it was spiked or not with Aroclor 1242. When Aroclor 1242 was added to soil, mainly low-chlorinated congeners were removed, whereas in non-spiked soil, decreases of extractable PCBs levels were observed for a broader range of congeners. The biofilm-coated GAC was less efficient than the planktonic cells to decrease the total amount of extractable PCBs. This limitation was possibly due to the differences in the bacterial composition of the two inocula and to the reduced bioavailability the GAC-adsorbed PCBs. Nevertheless, the biofilm-coated GAC accelerated the aerobic removal of the extractable PCBs during the first three months of incubation, albeit limited in terms of total PCB-removal.

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1. Introduction

Due to their high toxicity and persistence, along with their potential accumulation in the food web, polychlorinated biphenyls (PCBs) are listed among the most hazardous environmental contaminants (Reggiani and Bruppacher, 1985; Stockholm Convention, 2001). This family of 209 molecules, consisting of a biphenyl core with one to nine chlorine atoms, was used in numerous applications such as insulation, lubrication, pesticide extension, adhesion, heat transfer, sealing, painting, and carbonless copy paper during the 20th century. Although its applications were severely regulated, contaminated soils are still found near old transformers and industrial sites (Adebusoye et al., 2008; Vasilyeva et al., 2010) and PCBs represent an ubiquitous source of pollution.

Several physical and chemical methods, such as soil-washing, soil burial, incineration and thermal desorption, have been proposed to remediate PCB-contaminated soils (US Environmental Protection Agency, 1999, 2001; Svab et al., 2009), but they are often expensive and disrupt soil (bio)processes. Technologies using activated carbon (AC) have also been proposed for treating PCB-contaminated sites (Hilber and Bucheli, 2010; Jensen et al., 2011; Ghosh et al., 2011; Gomes et al., 2013), taking advantage of the high capacity of AC to adsorb PCB congeners. The introduction of AC directly into PCB-polluted soils has proved an effective *in situ* stabilization technology for reducing PCB bioavailability (Vasilyeva et al., 2006, 2010; Hilber and Bucheli, 2010; Gomes et al., 2013). AC is also an efficient support material for microorganisms attachment and for the formation of multi-species biofilms (Olmstead and Weber, 1991; Mercier et al., 2013, 2014) including

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PCB-degrading microorganisms. In fact, although PCBs are not easily mineralized by microorganisms, several bacterial strains and mixed communities are able to metabolize at least partly, several PCB congeners under aerobic condition (Bedard et al., 1984, 1986; Liz et al., 2009); they degrade PCBs through the 2,3-dioxygenase pathway, converting them to chlorobenzoic acids and then to CO_2, Cl^- and biomass for the less chlorinated ones (Abramowicz, 1995; Abraham et al., 2002). A broad range of microorganisms from diverse genera carry this pathway (Pieper, 2005; Adebusoye et al., 2008; Liz et al., 2009; Xu et al., 2011). Although the less-chlorinated PCB congeners are preferentially degraded under aerobic conditions (Furukawa et al., 1978; Bedard et al., 1986), microorganisms capable of biotransforming congeners with seven or eight chlorine atoms have also been described (Liz et al., 2009). However, low concentrations of both PCB congeners and naturally active PCB-degrading bacteria may limit their *in situ* biodegradation. Thus, co-localizing PCBs and a PCB-degrading biofilm onto the surface of AC might represent an efficient and cost-effective strategy for treating PCB-polluted sites; the AC acting simultaneously as an adsorbent for PCBs and as a material to immobilize the degrading microorganisms, thus forming *in situ* biodegradation hotspots. Until now, granular activated carbon (GAC) proved to be an efficient substrate material for delivering and dispersing PCB-degrading pure strains (Payne et al., 2013). However, the PCB-degrading abilities of the autochthonous microbial communities from polluted soils need to be explored, avoiding the introduction of exogenous microorganisms whose survival may be affected by the environmental conditions and/or that may impact the microbial natural diversity (Thompson et al., 2005; Tyagi et al., 2011).

The formation of biofilms of indigenous bacteria from aquatic sediment onto GAC was previously described (Mercier et al., 2013), and it was shown that the diversity of the bacterial communities developed as biofilms depends on the properties of the GAC (Mercier et al., 2014). In this context, the objective of this study was to investigate the effect of inoculating PCB-contaminated soil microcosms with bacterial consortia prepared by enrichment from the same contaminated soil. The consortia were prepared as a PCB-degrading planktonic-cell suspension or GAC-attached biofilm. Soil microcosms were inoculated with either one of the planktonic-cell or the biofilm consortium. Then, the profiles of the level of extractable PCB congeners were followed over a period of 6 months by comparing the remaining concentration of PCBs in the inoculated versus sterile soil microcosms. The microcosms were prepared using the polluted backfill soil, either native or spiked with Aroclor 1242, in order to access the effect of the pollution level on biodegradation.

2. Materials and methods

2.1. Polluted soil (P-soil)

A polluted backfill soil (here named "P-soil") was kindly supplied by SITA France (Suez Environment, France), a society specialized in the treatment of polluted waste and material. It was composed mainly of raw fine sandy silt (46 wt% < 0.04 mm and 26 wt% > 0.5 mm). The P-soil was sieved at 2 mm to remove the coarse particles before use. This fraction (<2 mm) contained 2129 μg of 13 PCBs per kg of dry soil (608 $\mu g\ kg^{-1}$ of soil slurry); the majority of congeners having more than 5 chlorine atoms (Table 1).

2.2. Chemicals and cultural conditions

Aroclor 1260 (CAS #11096-82-5; Promochem, GMBH, GB) or Aroclor 1242 (CAS #53469-21-9; Supelco Analytical, USA), two commercial mixtures of penta-to hepta-CBs and mono-to penta-CBs respectively, were formulated as 500 $mg\ l^{-1}$ acetone stock solutions. Biphenyl (CAS #92-52-4; Supelco Analytical, USA) was formulated as a 10 $g\ l^{-1}$ stock solution in ethanol. All cultures were performed in 100 ml glass vials closed with Teflon-coated septa (Fisher Scientific, USA). The acetone and ethanol solvents from Aroclor and biphenyl respectively, were totally evaporated for two days at room temperature before adding the Brünner mineral medium (Deutsche Sammlung fur Mikroorganismen, DSMZ, medium no. 457, Braunschweig, Germany; hereafter termed the "medium") and/or the sieved soil to the flasks for the experiments (see below). The cultures were incubated at 25 °C at 110 rpm, with aeration performed once a week for one hour by piercing the septum with a sterile needle mounted on a 0.22 μm filter.

The commercial Picahydro S21–W GAC (Jacobi Carbons, Sweden) with grain size ranging from 1 to 5 mm (Mercier et al., 2013, 2014) was used for the experiments. The GAC was washed several times with deionized water until the pH of the water was stabilized to 8.0, using a sieve to remove the fine carbon particles, then dried and autoclaved twice for 1 h at 105 °C.

2.3. Enrichment of PCB-degrading microbial community and preparation of the inocula

A microbial community was enriched from the P-soil spiked with Aroclor 1260 in order to increase the selection pressure toward bacteria degrading both the lower and highly chlorinated PCBs under aerobic conditions. An initial culture was prepared by mixing 50 ml of the medium amended with Aroclor 1260 at a final concentration of 50 $\mu g\ ml^{-1}$, 8 g of GAC and 10 g of P-soil, and incubating for 5 weeks. This enrichment was sub-cultured during one year under the same conditions (Supplementary material SM1). The enriched bacterial community, named PopSIT, was used to prepare the microbial consortia used as inocula for the PCB degradation experiments.

PopSIT was inoculated at 5% in a medium amended with biphenyl at a final concentration of 0.3 $\mu g\ ml^{-1}$. This PopSIT culture was sub-cultured in a medium amended with biphenyl (0.3 $\mu g\ ml^{-1}$ final concentration) to obtain a planktonic-cell community, subsequently named B1C1. Simultaneously, the PopSIT culture was inoculated in the same medium supplemented with GAC (90 g of GAC in 140 ml medium) to obtain an inoculum named B2C2, composed of both planktonic cells (B2C2-P) and GAC coated with a biofilm (B2C2-G). Both cultures were incubated for 4 weeks before PCBs degradation experiments. B1C1, B2C2-P and B2C2-G (rinsed biofilm-coated GAC), were sampled in triplicate to analyze their bacterial composition the day of inoculation of the microcosms (T0). Moreover, biofilm formation on GAC was confirmed by microscopic observations (data not shown) and by the recovery of DNA from rinsed GAC (Supplementary material 1 SM1, Table SM1-1) as previously described in Mercier et al. (2013).

2.4. Microcosm experiments

Three conditions were applied (Table 2) using 10 g of native P-soil or P-soil spiked with Aroclor 1242 (final concentration of 40 $\mu g\ ml^{-1}$), both with and without GAC amendment (5 g), 25 mL of medium, and with the addition of inocula: planktonic-cell B1C1 (5 ml), or B2C2, i.e. biofilm-coated GAC B2C2-G (5 g) together with 5 ml of B2C2-P.

All microcosm experiments were performed, in triplicate, in 100 ml glass vials closed with Teflon-coated septa (Fisher Scientific, USA) with air in the headspace. Nine flasks were prepared for each condition. Similarly, abiotic control microcosms without GAC were prepared in triplicate in the two following conditions: native P-soil and P-soil spiked with Aroclor 1242. The soil of abiotic microcosms

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