International Biodeterioration & Biodegradation 105 (2015) 127–136

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

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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ARTICLE INFO

Article history:
Received 13 March 2015
Received in revised form
31 August 2015
Accepted 31 August 2015
Available online 11 September 2015

Keywords:
PCBs
Aerobic microcosms
Bacterial community
Soil remediation
Granular activated carbon (GAC)
Biofilm-coated GAC

ABSTRACT

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.

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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http://dx.doi.org/10.1016/j.ibiod.2015.08.024
0964-8305/

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 μg 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 μg 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 μg ml^{-1}. This PopSIT culture was sub-cultured in a medium amended with biphenyl (0.3 μg 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 μg 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

Table 1
Quantification of the PCB congeners in the native P-soil and the P-soil spiked with Aroclor 1242. Values are given in μg kg^{-1} of soil slurry (10 g of soil in 25 ml of liquid medium).

PCB congener	Structural name	PCBs concentration (μg kg^{-1})	
(UIPAC number)	(-chlorobiphenyl)	Native P-soil	P-soil spiked with Aroclor 1242
CB 18	2,2′,5	4.6	1799.9
CB 28	2,4,4′	4.9	1576.2
CB 31	2,4′,5	6.3	1752.4
CB 44	2,2′,3,5′	7.4	927.3
CB 52	2,2′,5,5′	16.8	957.6
CB 101	2,2′,4,5,5′	33.7	183.0
CB 118	2,3′,4,4′,5	16.6	193.1
CB 138	2,2′,3,4,4′,5′	114.3	119.2
CB 149	2,2′,3,4′,5′,6	102.6	84.8
CB 153	2,2′,4,4′,5,5′	121.1	115.8
CB 170	2,2′,3,3′,4,4′,5	35.1	47.5
CB 180	2,2′,3,4,4′,5,5′	114.4	101.5
CB 194	2,2′,3,3′,4,4′,5,5′	30.5	38.7

Table 2
Estimation of the total removal of the 13 quantified CB congeners (sum of concentrations) after 6 months of incubation.

Condition	Material	Treatment	Absolute PCB removal at 6 months[a] μg/kg of slurry	Relative PCB removal at 6 months[b] μg/kg of slurry	Relative PCB removal at 6 months[b] %
A	P-soil	A1 no amendment	100 ± 29 (a)	52 ± 29 (a)	8 ± 5 (a)
		A2 GAC	**189 ± 27 (b)**	69 ± 27 (a)	14 ± 5 (a)
B	P-soil	B1 free-cell suspension	**182 ± 24 (b)**	**136 ± 24 (b)**	**22 ± 4 (b)**
		B2 culture with GAC	**192 ± 31 (b)**	90 ± 31 (a)	16 ± 5 (a)
C	P-soil + Aroclor 1242	C1 free-cell suspension	1377 ± 601 (A)	1431 ± 601 (A)	18 ± 8 (A)
		C2 culture with GAC	1731 ± 343 (A)	819 ± 343 (A)	12 ± 5 (A)

Significant differences between conditions were searched applying ANOVA test. Distinct tests were applied for condition without Aroclor (groups a and b) and with Aroclor (group A). (±) indicates the standard deviation calculated for 3 values obtained in the same condition.
Significantly different results are given in bold.
[a] Absolute PCB removal is the difference, after 6 months of incubation, between the sum in the biotic slurries (3 replicates) and the sum in the corresponding blank (average value 3 replicates).
[b] Relative PCB removal is the difference between sum at T0 (average value 3 replicates) and the sum after 6 months of incubation (3 replicates), from which the same difference for the corresponding blank (average value 3 replicates) was subtracted.

was sterilized three times (before addition of Aroclor 1242) by autoclaving at 120 °C for 40 min at 24 h intervals. The microcosms were incubated at 25 °C under reciprocal agitation (110 rpm). Until the end of experiment, aeration was performed once a week for one hour by piercing the septum with a sterile needle mounted on a 0.22 μm filter. Triplicate flasks corresponding to each condition (biotic and abiotic) were sacrificed after 0, 3 and 6 months of incubation for quantifying the PCB congeners. Thus, without Aroclor 1242 addition, for conditions A and B (Table 2), the native soil contained 608 μg kg^{-1} of total analyzed PCB (see Section 2.5 for details), with higher concentrations of highly chlorinated congeners than less chlorinated congeners (Table 1). After Aroclor 1242 addition, for condition C, the total analyzed PCB concentration was 7897 μg kg^{-1}, with higher amounts of less-chlorinated congeners (Table 1). Thus, the addition of Aroclor 1242 greatly increased the total PCB concentration and the proportion of the less-chlorinated congeners.

2.5. PCB extraction and analysis

Liquid and solid phases were separated by filtration using a funnel lined with quartz wool. Extraction from the liquid phase was performed using hexane (HPLC grade, Fisher Scientific) according to NF EN ISO 6468 (1997). Extraction from the solid phase (GAC) was performed according to the method of Sun et al. (2005) with a fully automated Soxtec™ extractor (Soxtec™ 2050, Foss France). After drying (30 °C, 72 h) the solid was extracted with 100 ml toluene (Reagent Plus 99% Sigma Aldrich) during a 45 min heating period (80 °C) and a 90 min rinsing period. The extract was evaporated by rotary evaporation. Preliminary tests showed that the efficiency of this method for PCB extraction from Picahydro S21-W reached 80% (data not shown). The solid- and liquid-phase extracts were mixed and a volume of about 30 ml was obtained; the actual volume was precisely determined by weighing. Two mL were sampled and weighed before cleaning up with florisil, TBAH-sulfite and then sulfuric acid to a final volume of 2 ml in hexane. The dilutions were adapted to fit the calibration range (2–100 μg l^{-1}). Prior to injection, the dilutions were spiked with PCB 53 as an analytical internal standard (50 μl at 10 ng μl^{-1} in hexane for 1 ml). The PCBs were quantified according to the EN ISO 6468 standard using gas chromatography (GC) (3800 model) equipped with an auto-sampler (3400 model) and coupled to two electron capture detectors (3380 model) from Agilent Technologies (Paris, France) equipped with two analytical columns (CPSil 8CB, 50 m × 0 × 25 mm × 0.25 μm and CPSil 19CB, 50 m × 0 × 25 mm × 0.20 μm Supelco). The GC oven program was 125 °C held for 2 min before being ramped at 7.5 °C min^{-1} to 190 °C and then ramped again at 2 °C min^{-1} to 275 °C where it was held for 17 min. A splitless injection system was used with an injection volume of 2 μl and an injector temperature of 270 °C. The detector temperature was 310 °C. The limit of quantification was 35 ng g^{-1} for each PCB congener. The set of seven PCB congeners, called “indicator PCBs” (NF EN 15308, 2008), currently used in Europe to estimate “total PCBs” was completed with 6 other PCB congeners (Table 1). Hence, 13 PCB congeners were used as indicators due to their relatively high concentration in the Aroclor mixtures, their occurrence in the P-soil and their wide chlorination range (three through eight chlorine atoms per molecule).

2.6. Composition of the inocula

A volume of 500 μL of the planktonic-cell B1C1 and B2C2-P

inocula were recovered in triplicate immediately before the microcosms inoculation (see Section 2.4). Moreover, 3 × 1 g of wet biofilm-coated GAC (B2C2-G, >1 mm mesh) were separated from the planktonic-cell population B2C2-P, using a sterile 1-mm stainless steel mesh, and washed through five rinsing steps with 50 mM Tris–HCl (pH 7) in order to eliminate unattached bacteria and to access to the biofilm, as described by Mercier et al. (2013). The DNA was extracted from samples of the culture suspension and GAC biofilm as described by Mercier et al. (2013, 2014). Aliquots of 0.5 ng μl^{-1} diluted DNA were stored at −20 °C ready for molecular applications. Triplicate extracted DNA were pooled for each condition before the sequencing of the barcoded libraries of the 16S rRNA V3–V4 rRNA gene fragments, amplified with the primers F479 (5′-CAGCMGCYGCNGTAANAC-3′) and R888 (5′-CCGYCAATTCMTTTRAGT-3′) as described by Terrat et al. (2015). Pyrosequencing was carried out using a GS Junior 454 Sequencer (Roche Applied Science) according to the manufacturer's instructions. Pyrosequencing data were analyzed using the GnS-PIPE (version 1.1.11) pipeline and the bioinformatics parameters described in SM2 Table SM2-1. Briefly, the raw reads were sorted according to the base pair multiplex identifiers added during the PCR amplification. All reads with mismatches in the primer sequence and ambiguities in sequence, sequence length less than 300 bp and without presence of exact primer sequences were discarded. Rigorous dereplication (i.e. clustering of strictly identical sequences) was performed using a PERL program. The retained reads were then aligned using INFERNAL alignment. Normalization was carried out to obtain the same number of reads for each sample. Operational Taxonomic Units (OTUs) were clustered with a 95% similarity cutoff at the genus level to obtain reliable representation of bacterial communities through taxonomic classification of short 16S rRNA gene sequences, taking into account error rate from high throughput sequencing and also the variability of the 16S rRNA gene in bacterial genomes. All the reads were taxonomically assigned according to the Silva r114 reference database (Quast et al., 2013). During the analysis, all singletons corresponding to reads detected only once and not clustered (that might be artefacts such as PCR chimeras and large sequencing errors produced by the PCR and the pyrosequencing) were checked on the basis of the quality of their taxonomic assignments (Terrat et al., 2015). The dataset is available on the EBI database system in the Sequence-Read Archive (SRA), under study accession number PRJEB9579 (http://www.ebi.ac.uk/ena/data/view/PRJEB9579).

2.7. Data analyses and statistics

Mean and respective standard deviations were calculated from triplicate flasks for each PCB congener and the total analyzed congeners. Changes in PCB congeners concentrations were expressed as the ratio between the concentration in the biotic microcosms and that in the corresponding abiotic controls at 0, 3 and 6 months incubation. Thus, the ratio (R) for a given time of incubation (T) and a given PCB congener (CBn) is expressed by Equation (1):

$$RCBn_T = [CBn]biotic_T / [CBn]abiotic_T \quad (1)$$

Values of $[CBn]abiotic_T$ are those obtained from the triplicate non-spike sterile soil microcosms for conditions A and B, and from the triplicate Aroclor 1242-spike sterile soil microcosms for condition C.

The CB concentration ratio (%) presented in Figs. 2 and 3 were calculated according to Equation (2):

$$RCBn_T\% = 100 \times RCBn_T \quad (2)$$

This way of presenting the results of PCBs biodegradation is commonly used (Murínová et al., 2014). Three ratios were calculated for each condition, corresponding to the three values of the biotic microcosms; the $[CBn]abiotic_T$ value being the average of the three replicates.

Significant differences between conditions presented in Figs. 2 and 3 were searched applying the Kruskal–Wallis non parametric test as the data did not constantly fit a normal distribution.

The abatement rates of PCB congeners concentration (CBAR%) after 6 months incubation shown in Fig. 1 were calculated according to Equation (3). Fig. 1 shows exclusively the CBAR% values for the congeners that according to the Kruskal–Wallis non parametric test, for those exhibiting a significant difference between the biotic and the abiotic microcosms after 6 months incubation:

$$CBAR\% = 100 \times (RCBn_{T0} - RCBn_{T6months})/RCBn_{T0} \quad (3)$$

Three abatement rates were calculated for each condition, corresponding to the three values of $RCBn_{T6months}$; the $RCBn_{T0}$ being the average of the three replicates.

Significant differences between conditions presented in Fig. 1 were searched applying the Kruskal–Wallis non parametric test as the data did not constantly fit a normal distribution.

The total removal of the 13 PCB congeners (sum of concentrations) after 6 months of incubation shown in Table 2 was expressed as absolute and relative PCB removal. Absolute PCB removal (A-PCBr) was calculated as the difference, after 6 months of incubation, between the sum in the biotic slurries (3 replicates) and the sum in the corresponding blank (average value of 3 replicates), according to Equation (4):

$$A - PCBr = \sum [CBn]biotic_{T6months} - \sum [CBn]abiotic_{T6months} \quad (4)$$

The relative PCB removal (R-PCBr) was calculated as the difference between the sum at T0 (average value of 3 replicates) and the sum after 6 months of incubation (3 replicates), from which the same difference for the corresponding blank (average value 3 replicates) was subtracted according to Equation (5):

$$R - PCBr = \left(\sum [CBn]biotic_{T0} - \sum [CBn]biotic_{T6months}\right) - \left(\sum [CBn]abiotic_{T0} - \sum [CBn]abiotic_{T6months}\right) \quad (5)$$

In Table 2, significant differences between conditions were searched applying ANOVA test as the data followed a normal distribution and displayed homogeneous variances. Distinct tests were applied for the microcosms without Aroclor and with Aroclor 1242.

All statistics were performed using XLSTAT version 2014.2.01.

3. Results

3.1. Composition of the inocula

A total of 3715, 3627 and 4113 pyrosequences with an average length of 371 pb were obtained from the B1C1 inoculum, B2C2–P and B2C2-G respectively. A low richness and evenness were observed in both inocula B1C1 and B2C2 (Shannon index: 1.71 and 1.26, and Evenness: 0.42 and 0.37, respectively). Although the communities had a similar bacterial composition at the phylum level, with prevalence of *Proteobacteria* (principally α-, β- and γ-subclasses), they remarkably differed at the genus level (Table 3). For the B1C1 consortium, 58.5% of the sequences were associated with *Pseudomonas*, 10.4% with *Achromobacter*, 9.7% with

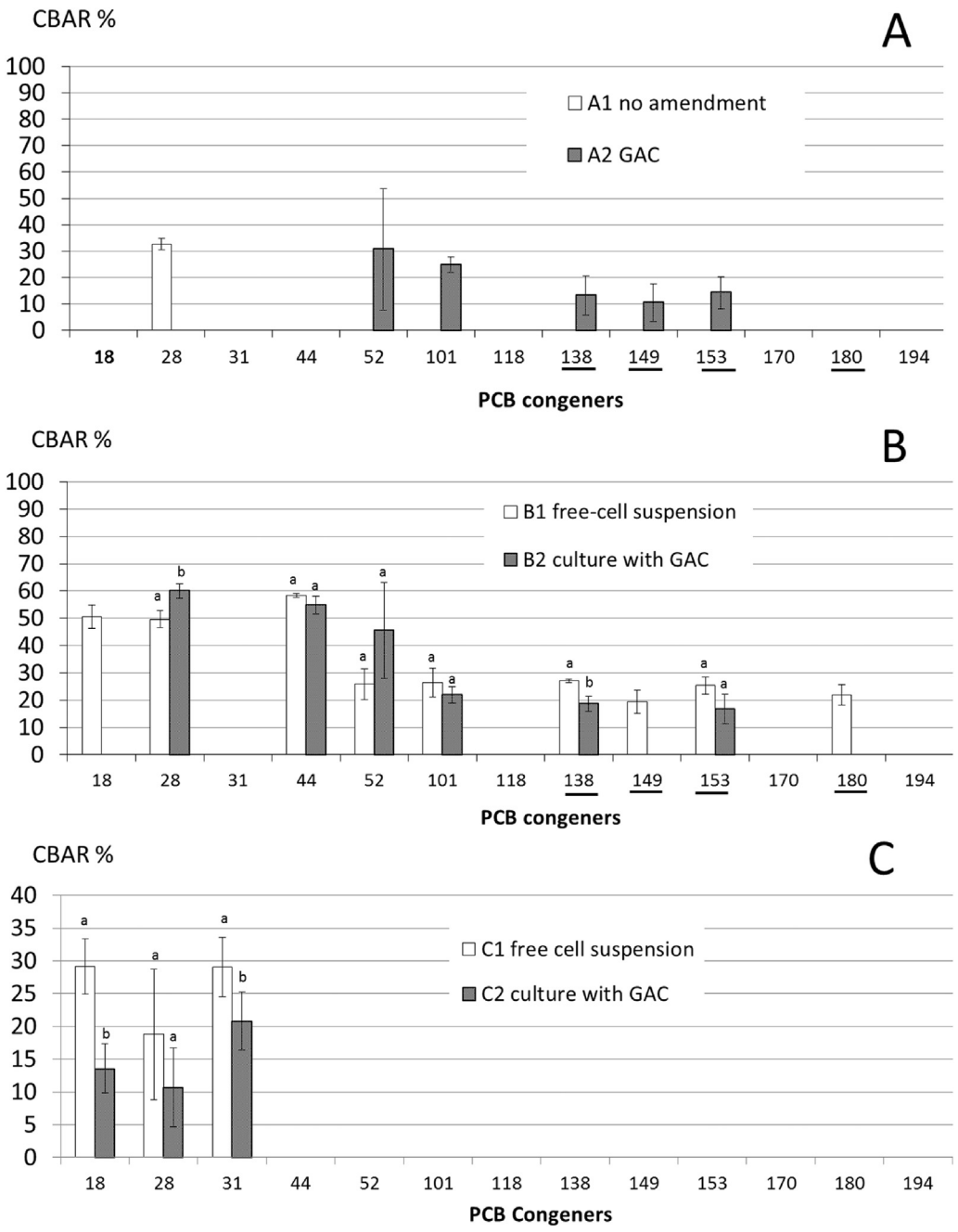

Fig. 1. Abatement rate of PCB congeners concentration (CBAR%) after 6 months incubation, calculated for the congeners presenting a significant difference between the biotic and the abiotic conditions at 6 months (according to the Kruskal–Wallis non parametric test): in the native P-soil (A and B) and in the P-soil spiked with Aroclor 1242 (C). CBAR values are calculated from Equation (3) described in the Materials and Methods section. The error bars represent the standard deviation for three abatement rates corresponding to the three values of RCBn at 6 months; the RCBn at T0 being the average of the three replicates. Significant differences between conditions were searched applying the Kruskal–Wallis test. The congeners presenting the highest concentrations in the raw P-soils are underlined in A and B.

Ochrobactrum, 7.6% with *Halomonas* and 13.7% with others genus each representing between 0.1 and 3.9% of the total sequences. The B2C2–P planktonic community mainly contained bacteria affiliated to *Pseudomonas* (63.1% of the total sequences), *Ochrobactrum* (19.8%), *Bordetella* (5.4%), *Achromobacter* (5.0%). In GAC-attached B2C2-G biofilm, the *Pseudomonas* genus was largely prevalent, representing 91.5% of the total sequences. *Ochrobactrum* and *Achromobacter* were also detected in the B2C2-G biofilm but sequences of these genera were less represented than in the planktonic community of the corresponding culture (B2C2-P).

3.2. Time-course profiling of non inoculated microcosms

Results presented in Table 2 and thorough the study are based on comparison between biotic microcosms and abiotic controls, thus it is reasonable to consider that microbial degradation is the main cause for PCB depletion in the microcosms without GAC. Abiotic degradation at the surface of AC, and/or volatilization especially during the weekly flushes of air, may also have occurred, however these processes cannot affect the comparison between the biotic and abiotic microcosms. In the presence of GAC, PCB

Table 3
Relative contribution (%) of each taxonomic genus to the composition of the enriched natural consortia grown in the absence (conditions B1 and C1, named B1C1) or presence of granular activated carbon (GAC, conditions B2 and C2, named B2C2) and used for microcosm experiments. For conditions B2 and C2, the inoculum included the planktonic community B2C2–P and the GAC-attached community B2C2-G.

Phylum	Class	Genus	Planktonic culture conditions B1 and C1 (B1C1) (%)	Culture in presence of GAC conditions B2 and C2 (B2C2)	
				Planktonic community (B2C2–P) (%)	GAC-attached community (B2C2-G) (%)
Firmicutes		*Cohnella*	1.54	0.59	0.12
Proteobacteria	*α-Proteobacteria*	*Ochrobactrum*	9.69	19.79	5.30
		Holospora	3.92	1.86	0.16
		Candidatus_Odyssella	0.28	0.16	0.00
		Novosphingobium	0.20	0.08	0.00
		Sphingopyxis	0.16	0.00	0.00
		Sphingomonas	0.12	0.00	0.00
		Oceanibaculum	0.08	0.00	0.08
		Rhodovibrio	0.08	0.00	0.00
	β-Proteobacteria	*Achromobacter*	10.45	4.99	1.74
		Bordetella	2.22	5.42	0.43
		Pusillimonas	0.36	2.41	0.00
	γ-Proteobacteria	*Halomonas*	7.64	1.19	0.32
		Luteimonas	0.16	0.00	0.00
		Pseudoxanthomonas	0.08	0.00	0.00
		Pseudomonas	58.49	63.14	91.49
Unclassified			0.36	0.04	0.00
Unknown			3.68	0.24	0.28
Less than 0.1%			0.51	0.08	0.08

depletion may be the result of both biodegradation and decrease of PCB extractability. After 6 months incubation, the total concentration of the analyzed PCBs in microcosms without GAC (condition A1) differed significantly from that of the corresponding abiotic control (Supplementary Material Sm3, Table Sm3-1). This suggests the occurrence of naturally PCB- degrading organisms in the raw P-soil.

The absolute PCB removal (Table 2) was significantly higher in the condition A2 (GAC) than in the condition A1 (without GAC). Furthermore, at the congener level, in the native P-soil, the relative extractable PCBs (E-PCBs) concentration decreased after 6 months of incubation solely for CB 28, with 33% removal (Fig. 1A) whereas after the addition of GAC, the general CB-degradation pattern was different with a decrease observed for CB 52, CB 101, CB 138, CB 149 and CB 153 (from 10 to 31%).

3.3. *Influence of microcosms inoculation*

Experiments were performed in order to evaluate the respective effects of microcosms inoculation with and without biofilm-coated GAC, in the native PCB-contaminated P-soil (low pollution level) and in the P-soil spiked with Aroclor 1242 (high pollution level).

The absolute PCB removal (Table 2) was significantly higher in both B1 and B2 conditions than in the A1 (no amendment) condition, however efficiency did not differ significantly between conditions B1 and B2.

Considering the relative PCB removal after 6 months incubation (Table 2), only the condition B1 microcosms (free cell suspension) exhibited a significantly higher PCBs depletion.

When Aroclor 1242 was added to the soil, a decrease of the total analyzed PCBs concentration was observed after 6 months of incubation (Table SM3-1), however neither total, nor absolute removal were significantly different between the two treatments (Table 2, conditions C1 and C2).

Bioaugmentation with the planktonic-cell suspension (B1C1) decreased the relative E-PCBs in soil, with 22% and 18% PCB removal in the native PCB-contaminated P-soil and in the Aroclor 1242-spiked P-soil, respectively after 6 months incubation (Table 2). The relative E-PCBs in the microcosms amended with B2C2-G plus B2C2–P was lower than those amended with the planktonic-cell suspension B1C1 with 14% and 12% relative E-PCBs abatement after 6 months in the native P-soil and in the Aroclor 1242-spiked P-soil, respectively (Table 2). However, the difference between the results obtained with the planktonic cells alone and those obtained with the GAC-attached biofilm was only significant in absence of added Aroclor. The percentage of PCBs abatement in the two treatments was comparable whatever the initial PCB concentration in the soil. Nevertheless, the bacterial communities degraded a greater mass of PCBs in the microcosms containing the Aroclor 1242-spiked P-soil than in those without Aroclor (Table 2, comparison of conditions B and C).

At the congener level, when the B1C1 planktonic-cell inoculum was applied to the native P-soil, a significant decrease for the concentration of congeners with three to seven chlorine atoms was observed, with 20%–27% decrease for CB 138, CB 149, CB 153 and CB 180 (Fig. 1B) which were prevalent in the native P-soil (Table 1). The same type of pattern was obtained with B2C2 (culture with GAC), whereas E-CB concentration did not decrease for CB 18, CB 149 and CB 180 (Fig. 1B).

When the P-soil was spiked with Aroclor 1242, i.e. when tri- and tetra chlorinated congeners prevailed, a relative concentration decrease after 6 months of incubation was observed solely for the tri-chlorinated congeners (CB 18, CB 28 and CB 31, Fig. 1C) with both inocula B1C1 and B2C2.

For the same experiment, the evolution of abatement levels between 0, 3 and 6 months of incubation was examined. Taking into account the congeners degraded with both B1C1 and B2C2 inocula, the abatement profiles differed for the two treatments. Under condition B (raw P-soil), E-CB concentrations decreased all along the 6 months incubation period (Fig. 2A) when the B1C1 planktonic-cell suspension was used, whereas with the B2C2 inoculum, E-CB depletion dropped rapidly during the first 3 months of incubation and then stabilized (Fig. 2B). A similar tendency was observed when the P-soil was spiked with Aroclor 1242 (condition C, Fig. 3), although for the condition C2 (culture with GAC, Fig. 3B) the high variability of the concentration ratio values at 3 months makes the results difficult for interpretation.

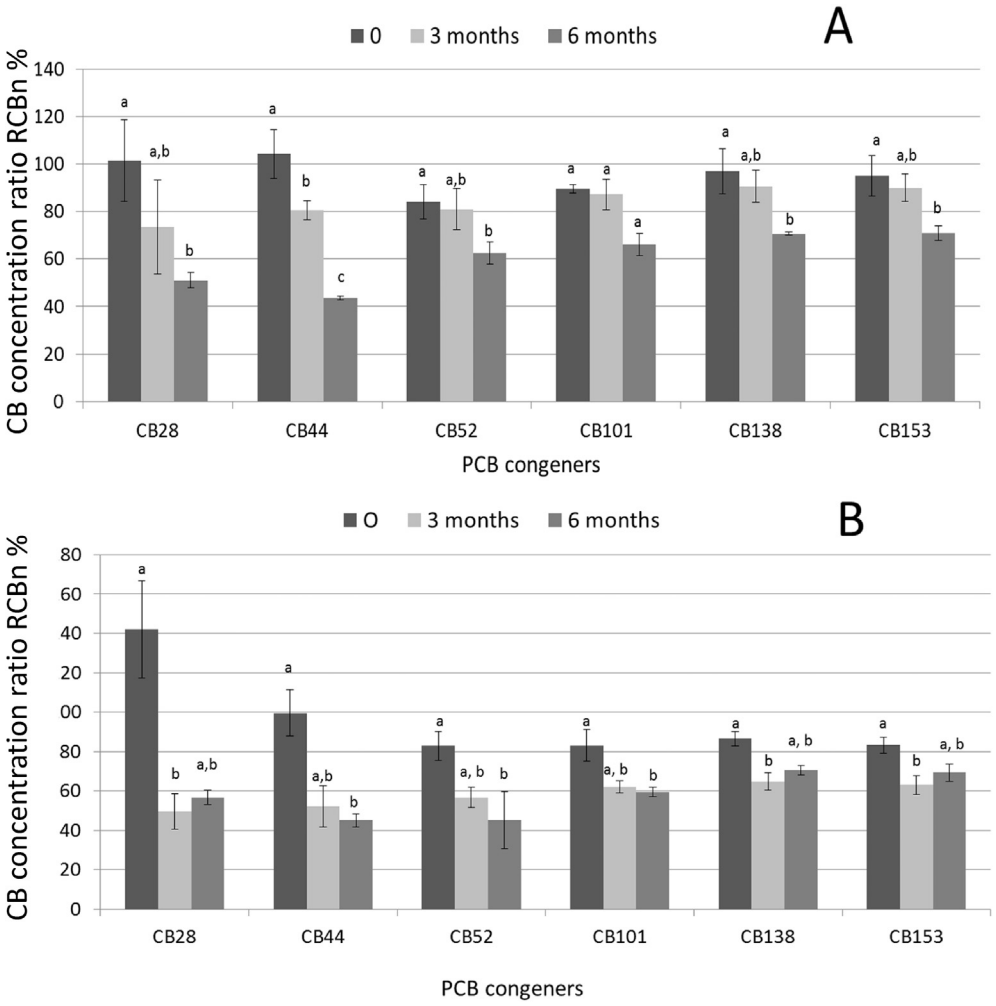

Fig. 2. Degradation of PCB congeners in microcosm containing the native contaminated soil at 0, 3 and 6 months of incubation. CBs degradation is represented as the ratio between the CB concentration in the biotic slurries and that in the corresponding abiotic control. A: free-cell suspension B1C1 inoculum; B: culture with GAC B2C2 inoculum. The CB concentration ratios (%) were calculated according Equation (2) described in the Materials and Methods section. Only the congeners showing a significant difference between the biotic and the abiotic conditions at 6 months are shown. All microcosms were performed in triplicate, three ratio were calculated for each condition, corresponding to the three values of the biotic microcosms; the $[CBn]_{abioticT}$ being the average of the three replicates in abiotic conditions. The error bar represents the standard deviation for the three ratio values. Significant differences between values at different incubation times were searched applying the Kruskal–Wallis non parametric test.

4. Discussion

The bacteria detected in the enriched population belonged mostly to the γ- but also to the α- and β-subclasses of the *Proteobacteria*. The prevalence of these groups had already been reported in PCB-contaminated soils (Nogales et al., 2001; Abraham et al., 2002, 2003; Macedo et al., 2007; Correa et al., 2010). Thus B1C1 and B2C2 communities are composed of bacteria previously detected at PCB- or biphenyl-contaminated sites and even with a direct implication in PCB biodegradation (Pérez-Pantoja et al., 2012). The prevalent organisms were phylogenetically related to the genera *Pseudomonas, Achromobacter* and *Ochrobactrum* among which are known PCB-degrading strains (Ahmed and Focht, 1973; Bopp, 1986; Tandlich et al., 2001; Dercová et al., 2008; Adebusoye et al., 2008; Liz et al., 2009; Murínová et al., 2014). Organisms belonging to the genus *Bordetella* were previously detected in a bioreactor treating a PCB-polluted soil (Fedi et al., 2005). Also, members of the moderately halophilic *Halomonas* genus were shown to degrade a wide range of aromatic compounds including phenol, benzoate or biphenyl (Fathepure, 2014). The composition of the two planktonic communities (B1C1 and B2C2-P) exhibited common features, whereas the bacterial community of the biofilm attached to the GAC strongly differed from the others and was characterized by a strong prevalence of the genus *Pseudomonas*. Obviously, the presence of GAC in the culture medium impacted on the bacterial diversity. Mercier et al. (2014) already showed that the abundance and community structure of biofilms are influenced by GAC properties, such as pore size and hydrophobicity.

In the microcosms without GAC, the inocula prepared with the PopSIT microbial community, pre-selected from a soil contaminated with highly chlorinated congeners, proved to enhance the aerobic biodegradation of PCBs in the same soil. Our results are in agreement with previous works showing that the less-chlorinated PCB congeners are preferentially degraded under aerobic conditions (Furukawa et al., 1978; Bedard et al., 1986). PCB volatilization (Fairbanks et al., 1987) and abiotic degradation (Carvalho et al., 2007) alone are not likely to explain the observed decrease in the extractable PCB because these processes should occur under both biotic and abiotic conditions. In presence of GAC, PCB extraction was less efficient, as shown by the lower initial PCB concentration ratios (RCBn) at T0 (Fig. 3B compared to 3A). However, when no Aroclor 1242 was added and taking account of both biodegradation

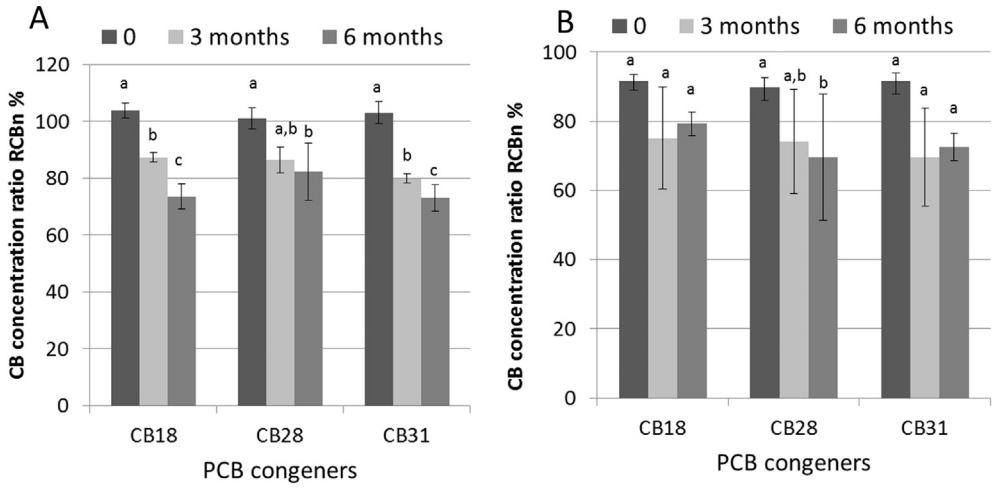

Fig. 3. Degradation of PCB congeners in Aroclor 1242-spiked soil microcosms at 0, 3 and 6 months of incubation. A: free cell suspension B1C1 inoculum; B: culture with GAC B2C2 inoculum. CBs degradation is represented as the ratio between the CBs concentration in the biotic slurries and that in the corresponding abiotic controls $RCBn_T$, according to Equation (2) described in the Materials and Methods section. Only PCB-congeners showing a significant difference between the biotic and the abiotic conditions at 6 months are shown. All microcosms were performed in triplicate, three ratio were calculated for each condition, corresponding to the three values of the biotic microcosms; the $[CBn]_{abioticT}$ being the average of the three replicates in abiotic conditions. The error bar represents the standard deviation for the three ratio values. Significant differences between values at different incubation times were searched applying the Kruskal–Wallis non parametric test.

and extraction efficiency, the relative abatement between 0 and 6 months, was lower in the biofilm-inoculated microcosms than those inoculated with the free-cells suspension. In all conditions, the inoculation of soil with biofilm-coated GAC did not help decrease the E-PCB concentration levels more efficiently than free cells suspensions.

The decrease of E-PCB level varied among the PCB congeners and depended on the experimental conditions. When low-chlorinated congeners were present in abundance due to Aroclor 1242 addition, they seemed to be degraded preferentially: among the molecules that were quantified, congeners with more than three chlorine atoms were poorly metabolized. Conversely, in the native P-soil containing mainly high-chlorinated congeners, decreases of E-PCB levels were observed for congeners containing up to seven chlorine atoms. Whereas aerobic biodegradation processes were probably the main ones occurring in all microcosms, we cannot exclude that dechlorination might have occurred in anaerobic microenvironments inside the biofilm. These results are consistent with previous reports showing that the number and position of the chlorine atoms influence significantly the congeners' susceptibility to oxidative degradation (Macedo et al., 2007; Furukawa and Fujihara, 2008). Congener's susceptibilities during PCBs degradation also depend on the composition of the mixture of PCB congeners (Xu et al., 2011), and the presence of co-substrates (Murínová et al., 2014). Moreover, addition of Aroclor 1242 in the spiked microcosms may have affected the composition and activity level of the inoculated microorganisms. As a matter of fact, a slight impact of Aroclor 1242 addition was previously observed on the bacterial diversity of aerobic sediment microcosms (Mercier et al., 2014). Although soil inoculation clearly enhanced PCBs degradation in the microcosms containing no GAC, the biodegradation level was limited in all the experiments, reaching a maximal of about 20% removal of the initial concentration of the analyzed PCB congeners, when B1C1 planktonic-cells suspension was added. This level is close to those previously reported for oxidative PCB-remediating processes that are not coupled to anaerobic dechlorination. After 6 months of incubation, Fedi et al. (2005) obtained a maximal of 30% PCB removal when cyclodextrin, which increases PCB availability was added to their process; without cyclodextrin, the maximal removal did not exceed 15%. Recently, Lehtinen et al. (2014) observed that biostimulation with pine needles under aerobic conditions resulted in 38% removal of total PCB after 2 months of incubation at the bench scale with aged PCB-polluted soils. Incomplete degradation of PCBs has frequently been associated to the formation of toxic degradation products (Camara et al., 2004).

Aside from confirming the positive effect of bioaugmentation on the PCBs biodegradation rate and level, our study also explored the combined effects of amendment with GACs and PCB-degrading bacteria. The use of AC for the *in situ* stabilization of PCBs contaminants in soils and the reduction of PCBs bioavailability to soil invertebrates and plants has previously been reported (Hilber and Bucheli, 2010; Ghosh et al., 2011; Gomes et al., 2013). AC might also be used to gather PCB-degrading bacteria into a biofilm for *in situ* bioremediation. This provides a novel dual approach to treat PCBs pollution by sequestrating PCBs and enhancing its degradation, which may provide an alternative approach to bioaugmentation (Rojas-Avelizapa et al., 2003; Di Toro et al., 2006; Payne et al., 2013) or biostimulation (Master et al., 2002; Di Gregorio et al., 2013). In our study, the addition of raw GAC alone, without inoculation, resulted in a decrease of E-PCB level in the native P-soil microcosms. This decrease is most likely due to the combined effect of the high affinity and adsorption capacity of Picahydro S21-W GAC toward PCBs, allowing pollutant concentration onto its surface, and the PCB-degrading abilities of the indigenous microbial communities that may colonize the GAC surfaces (Mercier et al., 2013, 2014). Recently, Payne et al. (2013) obtained 80% decrease of total PCB concentration in sediment mesocosms, using two pure bacterial cultures performing combined anaerobic and aerobic PCB degradation. These bacteria (*Dehaloccocoides chlorocoercia* DF1 and *Burkholderia xenovorans* LB400) were only immobilized onto GAC, not grown as biofilms. In contrast, to avoid the introduction of exogenous microorganisms, in our microcosms experiments, we introduced a mature GAC-attached biofilm produced by using a consortium selected from the same soil that was used in the microcosms experiments. Interestingly, bioaugmentation with biofilm-coated GAC was less efficient to decrease total E-PCBs level during the 6 months experiment than bioaugmentation with planktonic cells, in absence of added Aroclor 1242. However, the CB abatement pattern was significantly different between the 2 soil treatments, with a faster depletion for

those containing the biofilm-coated GAC. The maximum CB depletion was reached after 3 months of incubation, but it was also limited as no additional decrease was observed between 3 and 6 months. This apparent paradox might be due to the fact that the presence of GAC induces two phenomena with contradictory effects: (i) it locally increases PCBs concentration, thus favoring degradation of the low-concentrated compounds, and (ii) it decreases PCBs availability for the degrading bacteria. As previously reported (Caldeira et al., 1999; Carvalho et al., 2007), a residual amount of pollutant might not be bioavailable for the bacteria, suggesting that part of the adsorbed PCBs was strongly bound to the GAC and/or that the GAC porous network was not entirely accessible to the bacteria. Previous PCBs adsorption capacity studies using Picahydro S21-W GAC showed that, after 300 h of contact with Aroclor 1260 or Aroclor 1242, more than 99% of the 7 PCB-indicators and CB 194 (dry weight basis) were adsorbed by the GAC, thus reducing contaminant availability (Mercier et al., 2013). Other results suggested that the bioavailabitily of adsorbed PCBs depends on GAC properties (Mercier et al., 2014). Moreover, the selective enrichment of only few genera in the biofilm community attached to GAC, dominated by *Pseudomonas*-related organisms, may have affected the global efficiency of the biodegradation process. For example, the bacteria exhibiting the best abilities to generate a biofilm onto Picahydro S21-W may have not been the most efficient PCB-degraders. The relative abundance of each genus in the consortium might be important for efficient PCBs biodegradation.

Finally, results suggest that the GAC acted both as a support material favoring contact between PCBs and bacteria, but influencing the structure of the bacterial community, and as a strong PCB-sequestering material, decreasing pollutant bioavailability for bacteria. This dual antagonist role of GAC could thus exert a variable relative influence on the global E-PCBs level decrease, depending on the type of AC, and the profiles and concentrations of PCB congeners.

5. Conclusion

A bacterial community selected from a PCB-polluted soil was successfully used to stimulate the biodegradation of the PCBs in this soil. The potential for microbial processes to degrade PCBs was evaluated for the first time using a bacterial consortium, selected from a polluted soil, which was used to generate a biofilm onto the GAC and applied as an inoculum. The total decrease of E-PCBs level was not more efficient in microcosms inoculated with a biofilm-coated GAC than with a planktonic-cell suspension. However, considering that GAC amendments decrease the toxicity of PCBs in polluted soils and sediments, application of biofilm-coated GAC amendment should result in an overall decrease both in toxicity and net E-PCBs concentration. Further experiments using GACs with contrasting physical and chemical characteristics should allow finding a suitable compromise between decrease of PCBs bioavailability and biodegradation efficiency.

Acknowledgments

The present research, funded by BRGM and the FUI (French Inter Ministerial Funding), was performed in the framework of the PCB-AXELERA program, action ACPCB. Authors gratefully acknowledge technical support by Stéphanie Coulon. Authors sincerely thank the GenoSol platform (INRA, Dijon, France, www2.dijon.inra.fr/plateforme_genosol/) for the analysis of the 454 pyrosequencing raw data.

Appendix A. Supplementary material

Supplementary material related to this article can be found at http://dx.doi.org/10.1016/j.ibiod.2015.08.024.

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