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# Evaluation of three activated carbons for combined adsorption and biodegradation of PCBs in aquatic sediment

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ABSTRACT

Three commercial granular activated carbons (GACs) were studied at laboratory scale with a view to the combined adsorption and biodegradation of PCBs in aquatic sediment. The three GACs, with contrasting physico–chemical characteristics, all show a high adsorption of PCBs and are thus capable of reducing aqueous pollutant concentrations. After a one-month incubation with 'Aroclor 1242'-spiked sediment, the three GACs were each colonized by a multispecies biofilm, although with different amounts of attached bacterial biomass and significantly distinct genetic bacterial communities; interestingly, the highest bacterial biomass was attached to the microporous vegetable GAC. The multispecies biofilms developed on the three GACs were all predominantly composed of *Proteobacteria*, especially the β-, γ- and δ- subclasses, *Chloroflexi* and *Acidobacteria*, with genera previously found in environments containing PCBs or biphenyls, or able to perform cometabolic and direct PCB degradation. After an eight-month incubation under aerobic conditions, it was only the vegetable Picabiol GAC, with its low microporous volume, high total surface area and acidic property, that showed a significant (21%) reduction of tri- through penta-CB. Our results suggest that PCB bio-transformation by the bacterial community attached to the GAC is influenced by GAC's physico–chemical characteristics. Thus, a properly selected GAC could effectively be used to a) sequestrate and concentrate PCB from contaminated aquatic sediment and b) act as a support for efficient PCB degradation by an autochthonous bacterial biofilm.



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## 1. Introduction

Polychlorinated biphenyls (PCBs) are listed as toxic and persistent organic pollutants whose production and use are banned worldwide (Reggiani and Bruppacher, 1985; Stockholm Convention on Persistent Organic Pollutants, 2001). However, PCBs are still widely dispersed in aquatic ecosystems, with bioaccumulation from contaminated sediment to the food chain leading to adverse effects on wildlife and human health (Borja et al., 2005; Furukawa and Fujihara, 2008; Pieper and Seeger, 2008). The remediation of PCB-contaminated sediments by dredging, transport and disposal in landfills, or by *in situ* capping using clean sand, can be effective depending on the conditions such as site accessibility, water depth, flow dynamics and nature of the sediment (Murphy et al., 2006; McDonough et al., 2007; Hilber and Bucheli, 2010; Ghosh et al., 2011). Nevertheless, these processes are expensive and can lead to a) deterioration of benthic ecosystems and natural resources, b) suspension of contaminated sediment in the water column, and/or c) contamination of nearby clean sediment (Ghosh et al., 2011; Gomes et al., 2013). Consequently, innovative remediation strategies are required, such as the recent introduction of sorbent amendments (soot carbon, coal, charcoal, coke) into sediment so as to naturally reduce a) the aqueous availability of hydrophobic organic contaminants, b) their bioaccumulation and c) their transport into surface- and groundwater (Song et al., 2002; Jonker and Koelmans, 2002; McDonough et al., 2007, 2008; Cho et al., 2009; Ghosh et al., 2011; Jensen et al., 2011; Paul and Ghosh, 2011). With an extensive internal porous structure giving a high surface area, activated carbon (AC) is able to adsorb a wide range of chemical compounds (Walters and Luthy, 1984; McKay et al., 1985; Daifullah and Girgis, 2003). Thus the application of AC to PCB-contaminated sediment has often proved to be an effective *in situ* PCB stabilization technique for natural long-term sediment remediation, as indicated by the reduction of aqueous PCB concentration and PCB bio-uptake by aquatic organisms such as deposit-feeding polychaetes and filter-feeding organisms (Millward et al., 2005; Cho et al., 2009; Janssen et al., 2010; Hilber and Bucheli, 2010; Beckingham and Ghosh, 2011). Moreover, through monitoring changes in the lipid content and community structure of benthic invertebrates at field application sites and testing the growth effects on submerged aquatic vegetation, Beckingham et al. (2013) recently concluded that negative secondary effects of AC amendment in river sediment were limited, thus confirming the results of Kupryianchyk et al. (2012) on benthic community recolonization. Another attractive aspect of applying AC to sediment is the potential for *in situ* degradation of adsorbed contaminants by microbial processes. AC can act as degradation hotspots due to the development of bacterial biofilms on its surface (McDonough et al., 2008; Mercier et al., 2013) and to the widespread microbial ability to metabolize PCB in sediment (Ghosh et al., 1999; Macedo et al., 2007). However, the influence of the type of AC on PCB biodegradation remains to be explored.

AC can be produced from various precursor substances, such as nutshells, peat, wood, coal and petroleum pitch, which are treated either at high temperature or by chemical activation processes to produce a well-developed porous structure and in some cases a wide range of functional groups. The AC porous structure (pore sizes) as well as the nature of the functional group can influence the PCB adsorption mechanism (Jonker and Koelmans, 2002; Jensen et al., 2011; Amstaetter et al., 2012). In addition, microbial attachment and thus biofilm formation are strongly influenced by the substratum's physico–chemical properties, such as surface charge and hydrophobicity (Liu et al., 2004). Consequently, a proper selection of the type of AC can improve both *in situ* PCB sequestration and biodegradation in contaminated aquatic sediment.

The present study focused on three commercial GACs commonly used in water treatment, but having different physico–chemical properties. The objectives were to test their ability to a) adsorb PCB congeners from a commercial formulation, b) be colonized by bacterial communities brought in by a PCB-contaminated aquatic sediment, and c) support an effective PCB-biotransformation under aerobic conditions.

## 2. Material and methods

### 2.1. *Granular activated carbon (GAC)*

The three commercial GACs selected for this study were Picahydro® S21-W (Jacobi Carbons, Sweden), Picabiol® 2 (Jacobi Carbons, Sweden) and Filtrasorb 400 (cited F400; Chemviron Carbon, USA). All three are commonly used in water treatment and F400 has also been used as an amendment for dichloro-diphenyl-trichloroethane (DDT) and PCB-contaminated sediment (Zimmerman et al., 2005; Tomaszewski et al., 2007; Hilber and Bucheli, 2010). According to the supplier, Picabiol was manufactured specifically with a pore size greater than 50 nm to favour bacterial attachment (Chesneau et al., 2002).

The three GACs were sieved at 1 mm so as to a) remove the fine particles and obtain a common particle size range, and b) enable further GAC separation from the sediment. The size range of the GACs was 1–5 mm after sieving. They were then washed several times with deionized water to stabilize the pH at 8, before being dried and autoclaved twice for 1 h at 105 °C. The BET (Brunauer–Emmett–Teller) surface area was obtained from $N_2$ adsorption isotherms at 77 K on a Micromeritics ASAP® 2050 Xtended Pressure Sorption Analyzer (xPSA) on the washed and not crushed GAC. In addition to giving the total surface area ($S_{BET}$), the isotherms were used to calculate the total porous volume ($V_p$), the microporous volume ($V_{micro}$) and the average pore size with t-plot modelling. The volume of the mesopores ($V_{meso}$) was then estimated by subtracting $V_{micro}$ from $V_p$ as described by Morlay and Joly (2010). The surface acidity and basicity were both determined using titration methods (Guzel, 1996; Moreno-Castilla et al., 1997; Al-Degs et al., 2000). Briefly, 0.5 g of GAC was put into contact with 50 mL of 0.01 N NaOH for surface acidity and with 50 mL of 0.01 N HCl for surface basicity. The samples were shaken at 25 °C for 24 h, following which the mixture was filtered and then back-titrated with 0.01 N HCl or 0.01 N NaOH,

respectively. The concentration of acidic or basic substituent groups on the surface was expressed as mmol/g of GAC. The hydrophilic property was expressed as the sum of the surface basicity and acidity in mmol/g of GAC.

### 2.2. PCB adsorption on GAC

Measurements of each GAC's adsorption for 'Aroclor 1242' (CAS #53469-21-9), a commercial mixture of mono- to penta-CBs (Sigma) containing 12 carbon atoms and 42 wt% chlorine, were performed in batch systems consisting of 100 mL glass flasks sealed with Teflon-coated septa (Fisher Scientific). The 'Aroclor 1242' being formulated as a 400 mg/L acetone stock solution, the acetone was totally evaporated over two days at room temperature before adding a Brunner mineral medium (Deutsche Sammlung für Mikroorganismen, DSMZ, medium no. 457, Braunschweig, Germany) and/or the sieved sediment to the flasks for the experiments. The PCB adsorption experiments were conducted in triplicate for all three sieved commercial GACs at 5 g (dw) in 50 mL of Brunner mineral medium with 'Aroclor 1242' at a final concentration of 40 μg/mL; triplicate control samples without GAC were identically prepared. The flasks were shaken continuously for 160 h at 100 rpm and then incubated in a static condition at 25 °C. The PCB concentration attached to each GAC and the residual PCB in each flask were measured after 300 h and at the end of the eight-month incubation.

### 2.3. Aquatic sediment spiked with Aroclor 1242

In our study, the aquatic sediment historically contaminated with PCB was spiked with 'Aroclor 1242' in order to enhance the growth of aerobic PCB-degrading bacteria and to achieve an optimal observation of interactions between PCB, GAC and bacteria in the aquatic sediment. As described previously by Mercier et al. (2013), surficial sediment (topmost 0–50 cm) was collected from the Lac du Bourget (Savoie, France) at 45°41′38″ N/5°53′26″ E (water depth 1 m) in October 2009. It contained a total of 2.77 wt% organic carbon (NF ISO 10694, 1995), 1.99 mg/kg PAH, 852 mg/kg mineral oils (ISO 16703, 2004) and 1.55 mg/kg of the seven PCB-indicators (dry weight basis) located mainly in the fine fraction (<0.5 mm). The sediment was sieved at 1 mm to remove the coarse particles and access the fine fraction before being spiked with 'Aroclor' 1242 prior to the experiments.

### 2.4. Microcosms

GAC colonization by autochthonous microbial communities from the 'Aroclor 1242'-spiked aquatic sediment was performed in batch systems (100 mL glass flasks closed with Teflon-coated septa) under aerobic conditions. In order to study the colonization of the GAC by the bacteria, three microcosms were prepared for each GAC in 25 mL of Brunner mineral medium with 25 mL (6.4 g [dw]) of aquatic sediment spiked with 40 μg/mL of 'Aroclor 1242'; then, after three days of incubation, 5 g (dw) of washed and sterilized raw GAC were added. Also, three abiotic control microcosms having no contact with any microbial community were similarly set up for each GAC. The flasks were all shaken continuously at 110 rpm (reciprocal agitation) in the dark at 25 °C, with regular weekly flushes of air for 2–3 h under a sterile hood to maintain aerobic conditions. After one month of incubation, the GAC (>1 mm mesh) in the three microcosms was separated from the sediment (<1 mm mesh), using a sterile 1-mm stainless-steel mesh, and washed in five rinsing steps with 50 mM Tris–HCl (pH 7) as described by Mercier et al. (2013). Six sediment microcosms without any GAC amendment and containing 25 mL of Brunner mineral medium and 25 mL of sediment were also prepared; three with and three without 'Aroclor 1242'. After one month of incubation, the sediment, both with and without the 'Aroclor 1242' amendment, was centrifuged at 14,000 *g* for 10 min. The rinsed GACs and collected sediment were stored at −20 °C ready for molecular analysis. In order to evaluate biodegradation of PCBs in presence of GAC, triplicate microcosms of each condition (biotic and abiotic) were prepared as described above then incubated eight months before PCB extraction and analysis.

### 2.5. PCB extraction and analysis

The PCB was extracted and analysed as described by Mercier et al. (2013). Briefly, the liquid and solid phases were first separated by filtration using a funnel lined with quartz wool. The PCB was then extracted from the liquid phase using hexane (HPLC grade, Fisher Scientific) according to NF EN ISO 6468 (1997), and from the solid phase (GAC) according to the method of Sun et al. (2005). After drying at 30 °C for 72 h, the solid was extracted with 100 mL of toluene (Reagent Plus 99%, Sigma Aldrich) during a 45 min heating period (80 °C) and a 90 min rinsing period, and evaporated by rotary evaporation. Two mL were sampled and weighed before a clean up performed with florisil, TBAH sulphite and then sulphuric acid to a final 2 mL volume in hexane. Prior to injection, the dilutions were spiked with PCB 53 as an internal analytical standard (50 μL at 10 ng/μL in hexane for 1 mL). The PCB was then quantified according to NF EN ISO 6468 (1997) using an Agilent Technologies gas chromatograph (GC, 3800 model) equipped with an auto-sampler (3400 model) and coupled to two electron capture detectors (3380 model) equipped with two analytical columns (CPSil 154 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 to 190 °C at 7.5 °C/min and then ramped again to 275 °C at 2 °C/min where it was held for 17 min. A splitless injection system was used with an injection volume of 2 μL, an injector temperature of 270 °C and a detector temperature of 310 °C. The concentration of the seven PCB indicators and four others PCB congeners (CB18, CB31, CB44 and CB149) was monitored.

### 2.6. Biofilm and sediment DNA extraction and quantification

Microbial DNA was extracted directly from 3 × 1 g of rinsed GAC from each microcosm or from 250 mg (dw) of sieved sediment as described by Mercier et al. (2013). Briefly, 1 g (dw) of GAC or 250 mg (dw) of sediment were mixed with 1 mL of a DNA extraction solution containing 100 mM Tris–HCl (pH 8.0), 100 mM EDTA (pH 8.0), 50 mM NaCl and 2% (w/v) sodium dodecyl sulphate (SDS). The samples were then homogenized

at high speed for 10 s with a vortex, incubated twice at 70 °C for 15 min and centrifuged at 10,000 *g* for 15 min. The collected supernatants were incubated for 10 min on ice with 1/10 volume of potassium acetate (pH 5.5) and centrifuged at 14,000 *g* for 10 min. After precipitation with one volume of ice-cold isopropanol, the nucleic acids were washed with 70% ethanol. For DNA extracted from the three GACs, aliquots of crude DNA extract from the three replicates of each microcosm were pooled and purified using a Geneclean Turbo kit (Q Biogene®); the same procedure was followed for DNA extracted from the sediment. DNA quantifications of the crude and purified extracts were performed in triplicate using SYBR Green I dye on a MyiQ iCycler Real Time thermocycler (Bio-Rad). A standard curve (100, 50, 25, 12.5, 6.25 ng) of *Hind*III-digested λ DNA fragments (Promega) was used to estimate the final DNA concentrations. All DNA samples were stored at −20 °C ready for molecular applications.

### 2.7. Bacterial biomass in biofilm and sediment

Quantitative Polymerase Chain Reaction (qPCR) assays of the 16S rRNA genes were carried out in triplicate with a MyiQ iCycler real-time thermocycler (Bio-Rad) using primers 341F (5′ CCTACGGGAGGCAGCAG 3′) and 515R (5′ATTA CCGCGGCTGCTGGCA 3′). The 20 μL reaction mixtures contained 0.4 μM of each primer, 10 μL of iQ™ SYBR® Green Supermix (Bio-Rad), 500 ng of T4gp32 (Q-BIOgene) and 1.25 μL of a 0.5 ng/μL DNA-diluted template. The PCR reactions were performed as follows: 5 min initial activation at 95 °C followed by 35 cycles of 15 s at 95 °C, 30 s at 60 °C, 30 s at 72 °C and 30 s at 80 °C (data acquisition step) with an additional step rising from 60 to 95 °C at 0.5 °C/s. Standard curves were obtained using serial dilutions of a known amount of plasmid DNA containing a fragment of the 16S rRNA gene from *Pseudomonas aeruginosa* Pa44 at a concentration ranging from $10^2$ to $10^8$ copies per μl. Three no-template controls (NTCs) were run for each quantitative PCR assay. The DNA samples had been tested previously for the inhibitory effects of co-extracted substances.

### 2.8. Bacterial community diversity analysis

The diversity of the bacterial communities attached to the GACs and in the sediment was analysed by Capillary Electrophoresis – Polymerase Chain Reaction – Terminal Restriction Fragment Length Polymorphism (CE-PCR-t-RFLP) of the 16S rRNA gene. The bacterial primers 8F (5′-AGAGTTTGATCMTGGCTCAG-3′), labelled at the 5′ end with 6-carboxyfluorescein (6-FAM), and 1492R (5′-TACGGHTACCTTGTTACGACT-3′) were used to amplify an approximate 1500 bp fragment of the 16S rRNA gene (Liu et al., 1997). PCR amplification was performed in a 50 μL reaction mixture containing 1× PCR buffer (Promega), 1.5 mM $MgCl_2$, 0.2 mM of each deoxynucleotide triphosphate, 0.4 pmol of each primer, 1 U of DNA polymerase (GoTaq® Flexi DNA polymerase, Promega) and 1 μL of a 0.5 ng/μL DNA-diluted template. Reaction mixtures were held at 95 °C for 3 min, followed by 35 amplification cycles of 94 °C for 30 s, 56 °C for 30 s and 72 °C for 45 s plus a final extension of 72 °C for 10 min. The PCR products were purified using a PCR clean-up kit (NucleoSpin Extract II Kit, Macherey–Nagel) according to the manufacturer's instructions. Thirty-five nanograms of each fluorescently labelled PCR product were digested with 2.5 U of *Hae*III (Promega) at 37 °C for 3 h in a 12 μL reaction. Fluorescently labelled t-RFs were resolved by capillary electrophoresis in an ABI 310 genetic analyser using an internal size standard (GeneScan™ 600 LIZ, Applied Biosystems). T-RFLP electrophoregrams were analysed using the StatFingerprints Version 2 software (Michelland et al., 2009). All t-RFLP profiles were aligned to the internal standard and to a common

**Table 1 – Textural and chemical characterization of the three GACs considered. The textural characterization was performed from the washed >1 mm GAC fractions.**

| | Granular activated carbon (GAC) | | |
|---|---|---|---|
| | Picahydro S21-W | Picabiol | Filtrasorb 400 (F400) |
| Manufacturer | Jacobi Carbons, Sweden | Jacobi Carbons, Sweden | Chemviron Carbon, USA |
| Precursor material | Coconut | Pine wood | Bituminous coal |
| Activation process | Physical (steam) | Chemical ($H_3PO_4$) | Physical (steam) |
| Initial pH | 8–9 | 1–2 | 8–9 |
| Initial >1 mm fraction | 96% | 96% | 50% |
| $S_{BET}$ ($m^2$/g); $P/P_O$ | 1171; 0.19 | 1827; 0.22 | 882; 0.19 |
| $V_p$ ($cm^3$/g); $P/P_O$ | 0.55; 0.99 | 1.32; 0.99 | 0.52; 0.98 |
| $V_{micro}$ ($cm^3$/g); method | 0.38; *t*-plot | 0.27; *t*-plot | 0.30; *t*-plot |
| Microporosity (vol %) | 69.0% | 20.4% | 57.7% |
| $S_{micro}$ ($m^2$/g); method | 857; *t*-plot | 624; *t*-plot | 661; *t*-plot |
| $V_{meso} = V_p - V_{micro}$ ($cm^3$/g) | 0.17 | 1.05 | 0.22 |
| Mesoporosity (vol %) | 31% | 79.6% | 43.3% |
| Average pore size (nm) | 1.9 | 2.9 | 2.4 |
| Surface acidity (mmol/g) | 0.087 | 0.647 | 0.157 |
| Surface basicity (mmol/g) | 0.497 | 0.051 | 0.328 |
| Hydrophilic property[a] (mmol/g) | 0.584 | 0.698 | 0.485 |

$S_{BET}$ total surface area, $V_p$ total porous volume, $V_{micro}$ microporous volume, $S_{micro}$ microporous surface, $V_{meso}$ mesoporous volume.
[a] Corresponds to the sum of surface acidity and surface basicity.

baseline. Profiles from 60 to 480 pb were normalized with the minimum value equal to 0.

### 2.9. Phylogenetic analysis of the bacterial communities attached to the three GACs

The bacterial 16S rRNA gene sequences for each GAC were amplified from pooled DNA extracted from bacteria attached to the GAC after a one-month incubation with 'Aroclor 1242'-amended sediment. Universal bacterial primers 8F (see Section 2.8) and 1406R (5′-ACGGGCGGTGTGTRC-3′) were used with elongation at 55 °C and 30 cycles. Purified PCR products (Macherey–Nagel) were used to construct three gene libraries with the TOPO-TA Cloning Kit and TOP 10 chemically competent cells (Invitrogen). Positive transformants were screened for correct-length inserts by PCR using primers T3 and T7 targeting the vector, and 50 clones for each GAC sequenced by GATC Biotech. Consensus 16S rRNA gene sequences were constructed and manually verified with the BioEdit program (Hall, 1999). The phylogenetic assignment was based on sequence analysis by the Ribosomal Database Project version 10 Classifier tool (http://rdp.cme.msu.edu/classifier/classifier.jsp; Wang et al., 2007) and by the NCBI Blast tool (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi).

### 2.10. Statistics

DNA-quantification, qPCR and PCB-concentration data were analysed using non-parametric Kruskal–Wallis, and Mann and Whitney tests. Data obtained from the StatFingerprints software were converted into a data frame (bacterial communities as rows and normalized peaks as columns) and subjected to a principal component analysis (PCA) that provided an ordination of the bacterial communities in a factorial map based on the scores of the first two principal components. Statistical ellipses representing 90% confidence were drawn over the replicates. A co-inertia analysis (Dray et al., 2003) was used to study the relationships between the bacterial genetic structure and the physico–chemical characteristics of the GACs. The Monte Carlo randomization test was performed with 999 permutations to assess the statistical significance of the co-structure of the data tables. PCA and co-inertia analyses were performed using ADE-4, an R software (R Development Core Team, 2004; Thioulouse and Dray, 2007).

## 3. Results

### 3.1. Physico–chemical characterization and PCB adsorption/sequestration of the GACs

The three selected commercial GACs derive from mineral (coal) and biomass (wood or coconut) sources and were generated through chemical ($H_3PO_4$) or physical (steam) activation processes. Their main properties, measured on the >1 mm particle fractions, are given in Table 1. The Picabiol GAC provided the highest $S_{BET}$ and the F400 the lowest. The value reported for $V_p$ (total porous volume) was also 2.4-fold higher for the Picabiol than for both the Picahydro S21-W and F400, which were equivalent. The microporous volume ($V_{micro}$) was in the range 0.27–0.38 $cm^3/g$ for all three GACs. However, the physically (steam) activated Picahydro S21-W and F400 GACs showed large fractions of micropores (pore size less than 2 nm) whereas the mesoporosity (pore size from 2 nm to 50 nm) was more developed for the chemically ($H_3PO_4$) activated Picabiol GAC. In addition, surface chemistry analysis revealed that surface basicity is higher than surface acidity for Picahydro S21-W and F400, in contrast to a significantly higher surface acidity for Picabiol. Finally, the Picabiol GAC is the most hydrophilic, and the F400 GAC is the most hydrophobic.

After 300 h of contact with 'Aroclor 1242', the adsorption results showed that 98.3%, 98.6% and 99.8% of the seven PCB-indicators and PCB194 (dry weight basis) were attached to the Picahydro S21-W, F400 and Picabiol GACs, respectively, thus reducing the aqueous contaminant availability. After a prolonged incubation of eight months, the PCB sequestration with the three GACs reached 99.7%, 98.3% and 100%, respectively. These values underline a high adsorption of the PCB congeners included in 'Aroclor 1242' and show that PCB sequestration on the three GACs is rapid and not significantly diminished by prolonged contact time. In addition, considering the adsorption of the seven PCB-indicators onto F400 GAC (Table 2), penta-CBs adsorption (about 90% for PCB 101 and PCB 118) appeared not as high as that of tri- and tetra-CBs (about 99% for PCBs 18, 28, 31, 44 and 52).

### 3.2. Bacterial communities adhering to the GAC surfaces and in the sediment

After a one-month incubation in 'Aroclor 1242'-spiked sediment, the total biomass adhering to the Picahydro S21-W,

**Table 2 – Tri- through penta-CB adsorption (%) of Picahydro S21-W, Picabiol and F400 after an eight-month incubation with Aroclor 1242.**

| PCB congener | Structural name | PCB adsorption (%) | | |
|---|---|---|---|---|
| | | Picahydro S21-W | Picabiol | F400 |
| CB18 | 2,2′,5 Trichlorobiphenyl | 99.9 | 100 | 99.7 |
| CB28 | 2,4,4′ Trichlorobiphenyl | 99.9 | 100 | 99.3 |
| CB31 | 2,4′,5 Trichlorobiphenyl | 99.9 | 100 | 99.3 |
| CB44 | 2,2′,3,5′ Tetrachlorobiphenyl | 99.7 | 100 | 98.8 |
| CB52 | 2,2′,5,5′ Tetrachlorobiphenyl | 99.7 | 100 | 98.6 |
| CB101 | 2,2′,4,5,5′ Pentachlorobiphenyl | 98.5 | 100 | 91.6 |
| CB118 | 2,3′,4,4′,5 Pentachlorobiphenyl | 98.3 | 99.9 | 89.9 |

Picabiol and F400 GAC surfaces, as obtained by total DNA quantification, reached 39.2 (±7.2), 27.2 (±2.6) and 18.3 (±5.9) ng DNA/g of GAC/g of sediment, respectively. The highest total biomass value was obtained for Picahydro S21-W, this being some 1.4 to 2.1 times higher than the DNA amounts estimated for Picabiol and F400. The qPCR results based on the 16S rRNA gene (qPCR efficiency = 97%) confirmed that the Picahydro S21-W activated carbon received the highest GAC colonization by bacterial communities from the 'Aroclor 1242'-spiked sediment with 9.5 (±2.6) × $10^6$ 16S rRNA gene copies/g of GAC/g of sediment (dry weight). The 16S rRNA gene copy numbers for Picabiol and F400 were 6.0 (±1.6) × $10^6$ and 4.4 (±2.4) × $10^6$ copies/g of GAC/g of sediment, respectively.

The native sediment showed a significant 3.3-fold higher bacterial biomass compared to the 'Aroclor 1242'-spiked sediment, with 4.7 (±0.8) × $10^8$ and 1.4 (±0.5) × $10^8$ 16S rRNA gene copies/g of sediment respectively, suggesting a negative impact of 'Aroclor 1242' on sediment bacterial communities in terms of biomass.

### 3.3. *Bacterial diversity*

PCA observation of the t-RFLP profiles (Fig. 1) showed that the ellipses (representing 90% confidence) from both the sediment and the 'Aroclor 1242'-spiked sediment were closely related. There is nevertheless a slight difference between the two ellipses that suggests a PCB impact on the bacterial diversity. A significant discrimination was revealed on the PC1 axis between the bacterial communities of the sediment and those attached to the F400 GAC, on one hand, and the bacterial communities attached to both the Picahydro S21-W and Picabiol GACs, on the other hand, explaining 38.8% of the variability. The bacterial community attached to the mineral GAC (F400) was thus more closely related to the autochthonous bacterial community of the sediment than the bacterial communities attached to the two vegetable GACs (Picahydro S21-W and Picabiol). On the PC2 axis, which explained 19.5% of the variability, a significant discrimination distinguished the bacterial communities attached to the Picahydro S21-W and Picabiol GACs (Fig. 1).

A new factorial map obtained for the t-RFLP data, deduced from co-inertia analysis (simulated $p$-value = 0.006) of the co-structure, also showed a robust discrimination between the three GACs on the co-inertia axes (Fig. 2); the genetic structure of the bacterial community attached to the Picabiol was significantly different from those of the bacterial communities attached to the Picahydro S21-W and F400 on the PC1 co-inertia axis, which explained 82.7% of the variability. The bacterial communities attached to the three GACs each showed a relationship to the relevant GAC's physico–chemical characteristics. Thus the genetic structure of the bacterial community attached to the Picahydro S21-W seems to be explained by the GAC's microporous volume

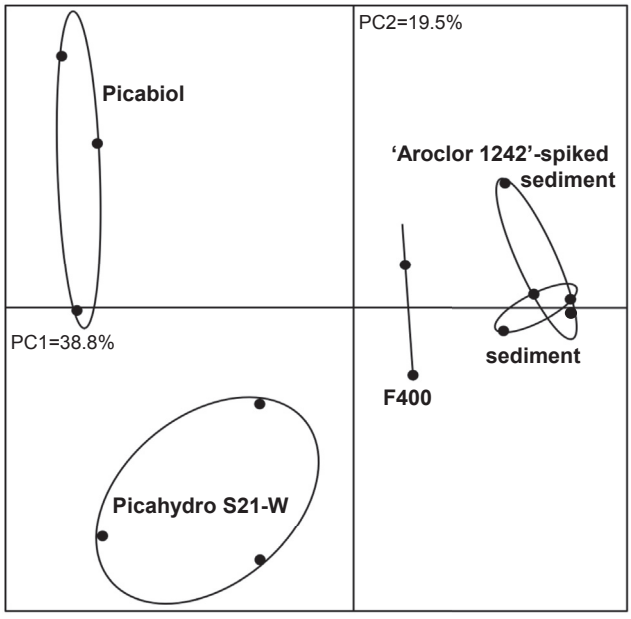


**Fig. 1 – Principal component (PC1 × PC2) factorial map generated from t-RFLP profiles of the 16S rRNA obtained after an incubation of one month at 25 °C from sediment, 'Aroclor 1242'-spiked sediment and biofilm attached to the Picahydro S21-W, Picabiol and F400 GACs after contact with spiked sediment. Statistical ellipses drawn over the plot of the three replicates represent 90% confidence. The percentages of explained variations for the first two axes are indicated within the figure.**

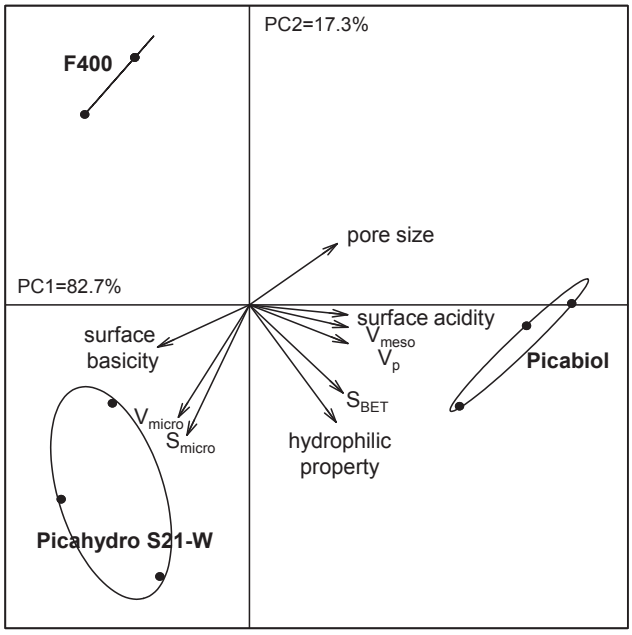


**Fig. 2 – Co-inertia (PC1 × PC2) factorial map generated from t-RFLP data and GAC physico–chemical characteristic correlation matrices obtained for the Picahydro S21-W, Picabiol and F400 GACs after contact with 'Aroclor 1242'-spiked aquatic sediment. Arrows represent the contribution of each physical characteristic to the discrimination of the bacterial diversity onto the GAC. Vectors pointing in the same direction are correlated, with longer vectors contributing more to the co-structure. Statistical ellipses drawn over the plot of the three replicates represent 90% confidence. The percentages of explained variations for the first two axes are indicated within the figure.**

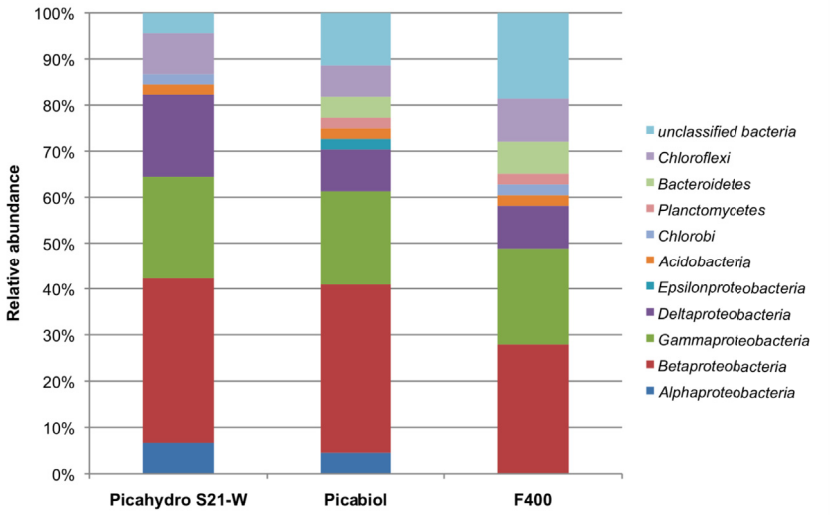


**Fig. 3 – Relative abundance of phylogenetic phyla in each biofilm attached to the Picahydro S21-W, Picabiol and F400 GACs after one-month incubation in 'Aroclor 1242'-spiked sediment.**

and surface, whereas it is the mesoporosity, specific surface and surface chemistry that contributed mainly to the discrimination of the bacterial community attached to the Picabiol, and possibly the hydrophobic property that may explain the genetic structure of the bacterial community attached to the F400.

### 3.4. *Phylogenetic analysis of the bacterial communities in biofilms attached to the GACs*

The taxonomic diversity of the bacterial community colonizing each GAC was accessed by constructing 16S rRNA gene libraries. Despite the fact that the rarefaction curves calculated from each dataset did not raise the plateau (data not shown), suggesting that the number of sampled clones did not reflect the entire diversity, a view of the most abundant bacterial taxon adhering to the GAC was nevertheless provided. The obtained sequences were taxonomically identified at phylum, class, order, family and often genus levels. The phylogenetic analyses revealed very diverse bacterial communities in the biofilms attached to the three GACs with the detection of 6 phyla and more than 20 bacterial orders (Fig. 3 and Table 3). *Proteobacteria* were largely dominant, representing approximately 82%, 73% and 58% of the classified sequences of the biofilms attached to Picahydro S21-W, Picabiol and F400, respectively (Fig. 3). The second-most abundant phyla shared between the three GACs were those belonging to *Chloroflexi* (mainly the *Anaerolineae* class, including strictly anaerobic genera that ferment carbohydrates and amino acids) with 6.8–9.3% of the total clones, and then *Acidobacteria* (2.3%). These three phyla account for about 93%, 82% and 70% of the sequences on Picahydro S21-W, Picabiol and F400, respectively. The *Proteobacteria* were dominated by *Beta-*, *Gamma-* and *Delta-proteobacteria*, with *Epsilon-proteobacteria* (98% sequence similarities with *Sulfuricurvum kujiense*, a chemolithoautotrophic sulphur-oxidizing species that is facultatively anaerobic) being identified only on the Picabiol, and *Alpha-proteobacteria* being detected only on the Picahydro S21-W and Picabiol. Most of the *Beta-proteobacteria* were assigned to the *Burkholderiales*, *Hydrogenophilales* and *Rhodocyclades* orders, whilst the most abundant orders within the *Gammaproteobacteria* were *Chromatiales*, *Pseudomonadales* and *Xanthomonadales* (Table 3). Interestingly, the *Desulfobacterales*, *Desulfuromonadales*, *Myxococcales* and *Synthophobacterales* orders were detected within the *Deltaproteobacteria*, suggesting the presence of strictly anaerobic genera, including sulphate- and sulphur-reducing bacteria. Phyla that were abundant on the Picabiol and F400 GACs included *Bacteroidetes* (mainly the *Sphingobacteriales* order representing 4.5% and 7%, of the respective libraries) and *Planctomycetes* (2.3%), largely observed in the sediment. *Chlorobi* were found only on Picahydro S21-W and F400, representing 2.3% of these libraries. It should be noted that between 4.4% and 18.6% of the clones could not be affiliated with any known group.

### 3.5. *PCB bio-transformation*

After an eight month incubation under aerobic conditions, the residual and GAC-adsorbed PCBs were extracted and the tri- through penta-CB analysed in both the microcosms in contact with the microbial community from the 'Aroclor 1242'-spiked aquatic sediment and in the abiotic control microcosms. Tri- through penta-CB removal was not observed in the biotic microcosms with Picahydro S21-W and F400 (Table SM-1), whereas a 21% total reduction of these congeners, compared with the abiotic control, was observed with Picabiol (Fig. 4), suggesting occurrence of microbial degradation. Other phenomena such as volatilization, especially during the weekly flushes of air, and/or abiotic degradation at the surface of activated carbon may have occurred, however they cannot affect the comparison between the biotic and abiotic microcosms for a given GAC. Similarly, differences were observed in the PCB extraction efficiency from Picahydro S21-W, F400 and Picabiol GACs, reaching 66.7%,

**Table 3 – Taxonomic distribution of bacteria within the biofilm attached to the Picahydro S21-W, Picabiol and F400 GACs. Taxa were identified using the Ribosomal Database Project version 10 Classifier tool (90% confidence threshold).**

| Phylum | Class | Order | % Of clones Or presence (x) on GAC | | | Representative genera in clone libraries |
|---|---|---|---|---|---|---|
| | | | Picahydro | Picabiol | F400 | |
| ***Acidobacteria*** | | | **2.2** | **2.3** | **2.3** | |
| | *Acidobacteria* | *GP6* | x | x | | |
| | *Holophagae* | *Holophagales* | | | x | *Geothrix* |
| ***Bacteroidetes*** | | | **0.0** | **4.5** | **7.0** | |
| | *Bacteroidetes* | | | | x | |
| | *Sphingobacteria* | *Sphingobacteriales* | | x | x | *Sediminibacterium, Ferruginibacter* |
| | Unclassified | | | | x | |
| ***Chlorobi*** | | | **2.2** | **0.0** | **2.3** | |
| | *Ignavibacteria* | *Ignavibacteriales* | x | | x | *Ignavibacterium* |
| ***Chloroflexi*** | | | **8.9** | **6.8** | **9.3** | |
| | *Anaerolineae* | *Anaerolineales* | x | x | x | *Bellilinea* |
| | *Dehalococcoidetes* | *Dehalogenimonas* | | | x | |
| | Unclassified | | | | x | |
| ***Planctomycetes*** | | | **0.0** | **2.3** | **2.3** | |
| | Unclassified | | | x | x | |
| ***Proteobacteria*** | | | **82.3** | **72.8** | **58.1** | |
| | *α-proteobacteria* | | 6.7 | 4.5 | 0.0 | |
| | | *Rhizobiales* | x | | | *Pseudolabrys* |
| | | *Rhodospirillales* | | x | | *Dongia* |
| | *β-proteobacteria* | | 35.6 | 36.4 | 27.9 | |
| | | *Burkholderiales* | x | x | x | *Curvibacter, Albidiferax, Acidovorax, Hydrogenophaga,* |
| | | *Hydrogenophilales* | x | x | | *Sulfuricella, Thiobacillus* |
| | | *Rhodocyclales* | x | x | x | *Azoarcus, Sulfuritalea* |
| | *δ-proteobacteria* | | 17.8 | 9.1 | 9.3 | |
| | | *Desulfobacterales* | | x | | *Desulfobulbus* |
| | | *Desulfuromonadales* | x | | x | *Geobacter* |
| | | *Myxococcales* | | x | x | |
| | | *Syntrophobacterales* | x | | | *Smithella, Syntrophobacter* |
| | | Unclassified | | x | x | |
| | *γ-proteobacteria* | | 22.2 | 20.5 | 20.9 | |
| | | *Chromatiales* | x | | x | |
| | | *Pseudomonadales* | | | x | *Pseudomonas* |
| | | *Xanthomonadales* | x | x | x | *Nevskia* |
| | | Unclassified | x | | | |
| | *ε-proteobacteria* | | 0.0 | 2.3 | 0.0 | |
| | | *Campylobacterales* | | x | | *Sulfuricurvum* |
| **Unclassified** | | | **4.4** | **11.4** | **18.6** | |

Bold percentages are the sum of percentages of individual clones in each phylum.

63.8% and 86.4% respectively based on the quantification of seven PCB-indicator in control flasks with GAC and without GAC, but cannot affect the comparison between the two conditions.

## 4. Discussion

AC has a broad spectrum of applications in environmental remediation, with recent reports of its use as an amendment in sediment to reduce the aqueous concentration of pollutants such as butylins (BT), DDT, polycyclic aromatic hydrocarbons (PAHs) and PCBs (Hilber and Bucheli, 2010; Ghosh et al., 2011). GAC proved to be an efficient substrate for delivering and dispersing PCB-degrading pure strains (Payne et al., 2013), however, the potential PCB-degradation by the autochthonous microbial communities from the polluted sites also needs to be explored in presence of GAC. In this study, three GACs (Picahydro S21-W, Picabiol and F400) with contrasting physico–chemical characteristics were compared for their ability to adsorb PCBs and promote biofilm formation; note that differences in nitrogen physisorption data for F400 and

Picabiol from earlier work (mainly Morlay and Joly, 2010) may originate from differences in the particle size range chosen for the measurements and the methods used to treat raw data from the physisorption isotherms. All three GACs were shown to be very effective in decreasing PCB aqueous concentration after contact with the commercial mixture 'Aroclor 1242'. Interestingly considering the seven PCB-indicators, adsorption onto F400 GAC of the tri- and tetra-CBs seems to be favoured compared to that of penta-CBs; this might be explained by both the higher steric hindrance of the penta-CBs and the marked microporous character of F400 GAC, as shown by its pore size distribution (F400 and Picabiol pore size distribution was determined by Morlay and Joly, 2010). Jensen et al. (2011) suggested also that PCB planar adsorption may be involved in AC selectivity.

In addition to efficient PCB adsorption, the three GACs acted as bacterial immobilization supports, allowing the attachment of sediment bacteria as had been reported by McDonough et al. (2008) and Mercier et al. (2013). Mercier et al. (2013) also showed that the presence of biofilm on Picahydro S21-W particles did not significantly affect PCB adsorption by the GAC. Consequently, during an *in situ* remediation process based on GAC addition, the PCB adsorption on GAC may be coupled with the development of a biofilm having potential bioremediation properties. In the present work, we noted differences in biofilm abundance on each of the three GACs despite being incubated for one month with the same aquatic sediment, that can be related to the intrinsic physico–chemical characteristics of each GAC. In fact, biofilm was more abundant with the Picahydro S21-W GAC; this result may be linked to the mainly microporous character of this GAC, as efficient PCB adsorption in the micropores may limit the inhibition of biofilm formation linked to PCB toxicity. With the F400 GAC, although the microporosity may also have decreased PCB bioavailability, the lower bacterial abundance attached to the particles, compared with Picahydro S21-W, could be due to F400's higher hydrophobic surface. With Picabiol GAC, PCBs, that are supposed to be more bioavailable due to lower microporosity of this GAC, may hence have inhibited bacterial attachment, thus counterbalancing the advantage of the high surface hydrophilicity for biofilm formation.

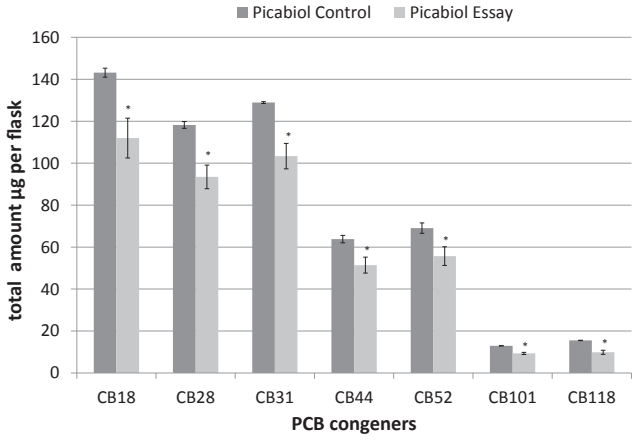


**Fig. 4 – Total amount (residual and GAC-adsorbed) of tri- through penta-CB in the control microcosms and in those in contact with microbial communities from the 'Aroclor 1242'-spiked aquatic sediment amended with Picabiol GAC. Asterisk indicates a significant difference ($P < 0.05$) between the essay and the control for the PCB considered congener.**

Our results are thus in agreement with previous studies (Caldeira et al., 1999; Jonker and Koelmans, 2002; Liu et al., 2004; Carvalho et al., 2007; Jensen et al., 2011; Amstaetter et al., 2012) and suggest that the origin, particle size and/or physico–chemical properties of activated carbons can influence biofilm formation due to relative differences in PCB sorption.

Over the eight months of incubation under aerobic conditions, it was exclusively with the Picabiol GAC that the sediment microcosms showed a significant decline (about 21%) in the tri- through penta-CB concentrations. PCB volatilization (Fairbanks et al., 1987), abiotic degradation (Carvalho et al., 2007) or difference in PCB extraction efficiency from GAC probably do not explain the observed decrease in the extractable PCBs in the presence of Picabiol because those phenomena should occur in both biotic and abiotic conditions. Our result seems to confirm the above hypothesis that the lower microporosity, higher total surface area and hydrophilic character of Picabiol would induce a higher PCB bioavailability, thus favouring bacteria–pollutant contact and accelerating PCB degradation by the bacterial community from the contaminated sediment. However, even with Picabiol, the limited total reduction of PCBs at the end of the eight month incubation suggests that a residual fraction of the GAC-adsorbed PCBs probably escapes degradation by the bacterial community adhering to the GAC surface. This might be explained assuming a stronger adsorption of PCB molecules in the narrowest pores where adsorption forces are the strongest thus hampering PCB molecule mobility on the internal surface of the GAC (Jensen et al., 2011). The bacterial communities in the biofilms developed on the three GACs had diverse compositions although with a dominance of bacterial phyla and genera previously reported in PCB- or biphenyl-contaminated sediment and soil, including *Proteobacteria* (mainly *Beta-*, *Delta-* and *Gamma-proteobacteria*), *Chloroflexi* and *Acidobacteria* (Nogales et al., 2001; Abraham et al., 2002; Macedo et al., 2007; Luo et al., 2008; Correa et al., 2010; Zanaroli et al., 2010; Di Gregorio et al., 2013). Species able to perform cometabolic and direct degradation of PCBs were identified on all three GACs, including *Acidovorax* sp., *Burkholderia* sp., *Comamonas* sp., *Hydrogenophaga* sp., *Pseudomonas* sp., *Geobacter* sp. and *Dehalococcoidetes* (Löffler et al., 2003; Lambo and Patel, 2006; Ohtsubo et al., 2004, 2012). Thus, although the potential to metabolize PCBs was identified in the biofilm from all three GACs, significant PCB degradation was observed only with the Picabiol, again suggesting the influence of each GAC's physico–chemical characteristics. Although it is the less-chlorinated PCB congeners that are preferentially degraded under aerobic conditions in sediment (Furukawa et al., 1978; Bedard et al., 1986; Luo et al., 2008), the PCB removal results with the Picabiol suggest that microorganisms able to degrade congeners with five chlorine atoms may occur on the GAC surface. Nevertheless, anaerobic microniches in the Picabiol macropores with the potential of

reductive dechlorination of PCBs (Bedard, 2008) should not be excluded, even though the microcosms were maintained under aerobic conditions.

## 5. Conclusions

Our results confirm the interest of a PCB-remediation process using AC for combined adsorption and *in situ* biotransformation by natural bacterial biofilms. Although all three commercial GACs were highly efficient in adsorbing PCBs, differences in their porous structure and surface chemistry have implications on PCB sorption and they also clearly influence the biomass and the composition of biofilms that develop naturally on GACs in contact with autochthonous microbial communities of contaminated aquatic sediment. The selection of bacterial communities combined with the specific property of each GAC in terms of bioavailability of adsorbed PCBs will certainly influence the rate and level of biodegradation, which underlines the importance of carefully selecting the type of GAC for optimal efficiency of *in situ* treatment according to the PCB-contaminated environment (i.e. sediment, soil or water).

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2014.04.021.

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