Journal of Hazardous Materials 178 (2010) 417–426

Contents lists available at ScienceDirect

Journal of Hazardous Materials

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

Characterization of the microbial community from the marine sediment of the Venice lagoon capable of reductive dechlorination of coplanar polychlorinated biphenyls (PCBs)

Giulio Zanaroli [a], Annalisa Balloi [b], Andrea Negroni [a], Daniele Daffonchio [b], Lily Y. Young [c], Fabio Fava [a,d,*]

[a] DICASM, Faculty of Engineering, University of Bologna, via Terracini 28, Bologna 40131, Italy
[b] Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Università degli Studi di Milano, via Celoria 2, 20133 Milan, Italy
[c] Biotechnology Center for Agriculture and the Environment, Rutgers University, 59 Dudley Road, New Brunswick, NJ, USA
[d] ENVIREN (The cluster of environmental laboratories of the Emilia Romagna Region), via Gobetti 101, Bologna 40129, Italy

ARTICLE INFO

Article history:
Received 5 November 2009
Received in revised form 15 January 2010
Accepted 19 January 2010
Available online 25 January 2010

Keywords:
Polychlorinated biphenyls
Reductive dechlorination
Marine sediment
T-RFLP
DGGE

ABSTRACT

The native microbial community of a contaminated sediment from Brentella Canal (Venice Lagoon, Italy) was enriched in slurry microcosms consisting of sterile sediment suspended in sterile site water in the presence of 3,3′,4,4′-tetrachlorobiphenyl, 3,3′,4,4′,5- and 2,3′,4,4′,5-pentachlorobiphenyls, 3,3′,4,4′,5,5′- and 2,3,3′,4,4′,5-hexachlorobiphenyls. The enrichment cultures were characterized at each subculturing step by 16S rRNA gene Terminal-Restriction Fragment Length Polymorphism (T-RFLP) and Denaturing Gradient Gel Electrophoresis (DGGE) analysis. About 90% of spiked polychlorinated biphenyls (PCBs) were stoichiometrically converted into di- and tri-chlorinated congeners by each enriched culture via dechlorination of flanked *para* chlorines and *ortho*-flanked *meta* chlorines. A 2-fold increase in PCB-dechlorination rate, the disappearance of lag phase, as well as a remarkable increase of sulfate consumption and a decline of methanogenic activity, were observed throughout subculturing. A reduction of complexity of the archaeal community, which was composed by *Methanomicrobiales* and *Methanosarcinales*, was also observed as a result of culture enrichment. The bacterial community included members of the Alpha, Gamma, Delta and Epsilon divisions of *Proteobacteria*, *Firmicutes* and *Chloroflexi*. Two sequence phylotypes related to the genus *Sulforovum* and the species *Desulfococcus multivorans* and two *Chloroflexi* enriched throughout subculturing, thus suggesting that these bacteria were involved in PCB dechlorination in the marine sediments of Brentella canal.

1. Introduction

Polychlorinated biphenyls (PCBs) are priority pollutants that extensively accumulate in the anoxic reduced subsurface sediments of aquatic ecosystems because of their very low water solubility and high chemical stability [1]. Due to their strong hydrophobicity, PCBs persisting in such sediments enter the food chain through sediment dwelling organisms, thus accumulating in the fatty tissues of animals and humans, where they can exert multiple adverse health effects [2,3].

Despite their recalcitrance to biodegradation, the microbial mediated anaerobic reductive dechlorination of PCBs was reported in a variety of anaerobic sediments [4–6]. The process consists in the sequential reduction of highly chlorinated, toxic and bioaccumulable PCBs into lesser chlorinated congeners commonly having lower toxicity, lower bioaccumulation potential and higher susceptibility to undergo mineralization by aerobic bacteria [6]. If occurring *in situ*, it might therefore mediate a remarkable detoxification of the site and/or reduce of the volume of sediments that need to be removed by expensive and highly impacting dredging operations [7].

PCB dechlorination and PCB-dechlorinating microbial communities have been mainly studied in slurry or sediment-free cultures of a number of freshwater habitats developed in synthetic mineral media supplemented with target PCBs [4–6], where reductive dechlorination has been ascribed to the activity of different indigenous dehalogenating bacteria [5,6].

During the last decade, a number of studies investigated the structure and composition of PCB-dechlorinating microbial communities from freshwater habitats. Several species of the delta subgroup of *Proteobacteria*, low-G + C Gram positive bacteria and

* Corresponding author at: DICASM, Faculty of Engineering, University of Bologna, via Terracini 28, Bologna 40131, Italy. Tel.: +39 0512090330; fax: +39 0512090322.
E-mail addresses: giulio.zanaroli@unibo.it (G. Zanaroli), annalisa.balloi@unimi.it (A. Balloi), andrea.negroni4@unibo.it (A. Negroni), daniele.daffonchio@unimi.it (D. Daffonchio), lyoung@aesop.rutgers.edu (L.Y. Young), fabio.fava@unibo.it (F. Fava).

0304-3894/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2010.01.097

Thermotogales, along with a single species with low sequence similarity to *Dehalococcoides ethenogenes*, were found to be predominant during *ortho*-dechlorination of 2,3,5,6-tetrachlorobiphenyl in a methanogenic enrichment culture of an estuarine sediment collected from the Baltimore Harbor suspended in a salts medium [8]. A *Dehalococcoides*-like bacterium, namely bacterium *o*-17, was later identified as the 2,3,5,6-tetrachlorobiphenyl *ortho*-dechlorinating microbe in sediment-free enrichments obtained from the same culture [9]. Similar results were described for a 2,3,4,5-tetrachlorobiphenyl *para*-dechlorinating sediment-free culture enriched from Charleston Harbor estuarine sediments, where *Deltaproteobacteria*, low-G + C Gram positive bacteria, *Thermotogales* and Green non-sulfur bacteria were detected [10], along with a *Dehalococcoides*-like microorganism, namely bacterium DF-1, later identified as the dechlorinator [11]. Several phylotypes closely related to the *o*-17/DF-1 group as well as sequences highly similar to *Dehalococcoides* spp. were detected in other sediment-free cultures enriched from estuarine sediments of Baltimore Harbor capable of dechlorinating different single PCB congeners [12,13] as well as Aroclor 1260 PCBs [14]. PCB-dechlorinating communities enriched from the Woods Pond section of Housatonic river and Hudson river in sediment-free mineral medium amended with single PCB congeners or Aroclor 1260 were found to contain several different phylotypes, such as *Betaproteobacteria*, *Deltaproteobacteria*, *Gammaproteobacteria*, *Clostridiales*, *Bacteroidetes* and *Dehalococcoides* spp. [15–17], the latter of which were indicated as the dechlorinators. On the contrary, no *Dehalococcoides* spp. or *o*-17/DF-1-like microorganisms were detected in sediment slurry cultures that were inoculated with microorganisms eluted from St. Lawrence river, where the enrichment of sequences highly homologous to *Clostridium pascui* and *Dehalobacter restrictus* was correlated to the dechlorination of spiked Aroclor 1248 PCBs [18].

Much less is known about PCB reductive dechlorination processes and the dehalorespiring microbial communities potentially active in marine sediments [1,19–24], where the occurrence of different biogeochemical conditions, such as higher salinity, availability of sulfate and occurrence of a wide variety of halogenated organic compounds naturally produced by indigenous organisms, might favor the enrichment of dehalogenating microorganisms different from those occurring in freshwater habitats. Furthermore, knowledge on the activity and diversity of the native sediment microbial communities enriched in the presence of water from the site, i.e., under biogeochemical conditions very close to those occurring *in situ*, might provide new insights on PCB dehalogenating strains in sediment and information of special relevance to design a tailored *in situ* biostimulation [7].

PCB dechlorination was recently documented in five contaminated marine sediments of the Brentella Canal (Porto Marghera, Venice Lagoon) when suspended either in a synthetic marine medium or in water coming from the site, i.e., under laboratory geochemical conditions very close to those occurring *in situ*. Interestingly, the dechlorination was more extensive in the presence of the site water than in mineral medium [22], thus suggesting the ability of the PCB-dechlorinating microbial community of the sediment to actively work under the conditions occurring *in situ*. In addition, the process was selective towards *meta* and *para* positions of PCB molecules and was apparently mediated by sulfate-reducing spore forming bacteria [23]. Finally, it was not primed by the addition of exogenous PCBs, including coplanar very toxic PCBs, which however were extensively dechlorinated with rate, extent and selectivity higher than those described so far in the literature [24]. Therefore, it was of special interest to enrich and to characterize the sediment native microbial communities in charge of the dechlorination process. Culturing conditions miming the biogeochemical features of the site were adopted in order to enrich the microflora better representing that occurring at the site. This might provide information on the actual potential of sediments to undergo *in situ* natural restoration.

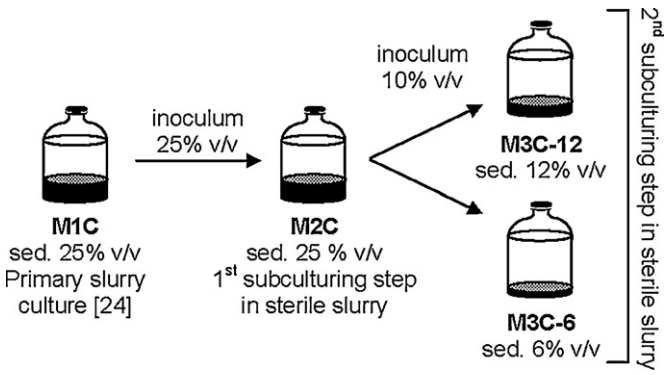

Fig. 1. Flow sheet of the subculturing steps and cultures developed.

2. Materials and methods

2.1. PCB-dechlorinating cultures

The microbial community from a coplanar PCBs dechlorinating primary slurry culture [24] consisting of a PCB contaminated sediment of the Brentella canal (first industrial area of Porto Marghera, Venice Lagoon, Italy) suspended at 25% (v/v) in its own site water, hereinafter designated M1C, was subcultured in sterile slurry microcosms consisting of autoclave-sterilized sediment suspended in filter-sterilized water coming from the same site, in order to simulate the biogeochemical conditions occurring *in situ*. In particular, two transfers of the original culture were made (Fig. 1). For the first transfer, 7.5 mL of the primary slurry culture M1C were added to 22.5 mL of a sterile sediment slurry previously prepared by mixing 5.6 mL of autoclave-sterilized sediment (corresponding to 5.5 g of sterile dry sediment) with 16.9 mL of filter-sterilized site water (final volume: 30 mL; inoculum: 25%, v/v). The culture obtained (25%, v/v sediment slurry; 245 g of dry sediment L^{-1}) was designated M2C. The second subculturing step was performed after 7.5 months of incubation of M2C culture. Two cultures were developed at this step. The first one was obtained by transferring 3.0 mL of M2C culture into 27.0 mL of a sterile sediment slurry consisting of 2.9 mL of autoclave-sterilized sediment (corresponding to 2.8 g of sterile dry sediment) suspended in 24.1 mL of filter-sterilized site water (final volume: 30 mL; inoculum: 10%, v/v). Taking into account the amount of sediment and site water carried by the inoculum (M2C culture), the culture finally consisted of a 12% (v/v) sediment slurry (118 g of dry sediment L^{-1}) and was named M3C-12. The second one was prepared by transferring 3.0 mL of M2C culture into 27.0 mL of a sterile sediment slurry consisting of 1.05 mL of autoclave-sterilized sediment (corresponding to 1.0 g of sterile dry sediment) suspended in 25.95 mL of filter-sterilized site water (final volume: 30 mL; inoculum: 10%, v/v). Taking into account the amount of sediment and site water carried by the inoculum (M2C culture), the culture finally consisted of a 6% (v/v) sediment slurry (59 g of dry sediment L^{-1}) and was named M3C-6. All cultures were developed in the presence of the same 5 coplanar PCB congeners that were originally spiked in the primary slurry culture (M1C), namely 3,3′,4,4′-tetrachlorobiphenyl, 3,3′,4,4′,5- and 2,3′,4,4′,5-pentachlorobiphenyl, 3,3′,4,4′,5,5′- and 2,3,3′,4,4′,5-hexachlorobiphenyl, at the concentration of 100 mg kg^{-1} of dry sediment each.

2.2. Culture preparation and sampling

All slurry cultures were prepared with the same sediment of the Brentella canal and the lagoon water collected at the same site used by Zanaroli et al. [24]. The sediment was contaminated by PCBs partially ascribed to Aroclors 1242 and 1254 (1.6 mg kg^{-1}, on dry wt basis), and had a density, pH and dry weight, of 1663.4 ± 24.3 g L^{-1}, 7.50 ± 0.10 and 59.1 ± 1.8% (w/w), respectively. The water pH and content of Cl^-, NO_2^- and SO_4^{2-} were 7.40 ± 0.05, 20.45 ± 0.49 g L^{-1}, 43.87 ± 0.05 mg L^{-1} and 2.05 ± 0.09 g L^{-1}, respectively.

All cultures had a final volume of 30 mL and were prepared in 50 mL glass serum bottles as follows. The proper amount of sediment was weighted in 50 mL serum bottles equipped with a magnetic bar. Bottles were flushed with a O_2-free N_2:CO_2 (70:30) mixture with a Hungate-similar apparatus for 30 minutes, sealed with a Teflon coated butyl stopper and aluminium crimp sealer and autoclaved for 1 h at 121 °C, on three consecutive days, with incubations at 28 °C between each autoclave treatment. Bottles were then opened under a 0.22 μm filter-sterilized O_2-free N_2:CO_2 (70:30) flow and the proper amount of 0.22 μm filter-sterilized lagoon water was added to the sterile sediment. The resulting sterile slurry was purged with 0.22 μm filter-sterilized O_2-free N_2:CO_2 (70:30) at room temperature for 2 h under vigorous magnetic stirring. While mixing and flushing, the sterile anaerobic slurry was inoculated with the slurry culture grown in the previous subculturing step and spiked with a 10,000 mg L^{-1} stock solution of 3,3′,4,4′-tetrachlorobiphenyl, 3,3′,4,4′,5- and 2,3′,4,4′,5-pentachlorobiphenyl, 3,3′,4,4′,5,5′- and 2,3,3′,4,4′,5-hexachlorobiphenyl in acetone to have a final concentration of 100 mg of each PCB congener per kg of dry sediment. Bottles were then recapped and incubated statically in the dark at 28 °C. Periodic sampling was performed in order to analyze the volume and the composition of the head-space gas, the concentration of SO_4^{2-}, Cl^-, NO_3^-, NO_2^- and that of PCBs. At the end of incubation, an additional slurry aliquot was sampled to extract metagenomic DNA for the analysis of microbial populations. Sampling was performed as described by Fava et al. [23].

2.3. PCBs extraction and analytical procedures

PCBs were batch extracted from duplicate 0.3 mL aliquots of sediment slurry with 3 volumes (0.9 mL) of anhydrous diethyl ether in the presence of octachloronaphthalene (OCN; 0.01 mL of a 40 mg L^{-1} stock solution in hexane) and elemental mercury (0.150 mL), in 1.5 mL vials for gas-chromatography (GC) equipped with Teflon coated screw caps (Hewlett-Packard Co., Palo Alto, CA, USA), as described by Fava et al. [23]. The qualitative and quantitative analysis of the extracted PCBs was performed with a gas chromatograph (5890 series II) equipped with a HP-5 capillary column (30 m by 0.25 mm), a ^{63}Ni electron capture detector and a 6890 series II automatic sampler (Hewlett-Packard Co., Palo Alto, CA, USA) under the analytical conditions described by Fava et al. [23].

Qualitative analysis of the freshly spiked PCBs and their possible dechlorination products was performed by comparing the retention time (relative to OCN) of the CG peaks obtained from the analysis of the sediment organic extracts with those of pure congeners and of PCBs occurring in standard Aroclor 1242 and Aroclor 1254 analyzed under identical conditions. Aroclor PCBs, injected in the presence of OCN, were identified as described by Fava et al. [23]. Quantitative analysis of PCBs was performed by using the GC-ECD response factor of each target PCB obtained through linear calibration curves of Aroclors and pure congeners as described previously [23]. Linear five-points calibration curves (0.5–10.0 mg L^{-1} range) were used to determine the GC-ECD response factor for the freshly spiked 3,3′,4,4′-tetrachlorobiphenyl, 3,3′,4,4′,5- and 2,3′,4,4′,5-pentachlorobiphenyl, 3,3′,4,4′,5,5′- and 2,3,3′,4,4′,5-hexachlorobiphenyl, as well as for their dechlorination products not occurring in the standard Aroclor mixtures and for lower chlorinated PCBs, such as 2,4-/2,5-, 2,4′-/2,3- 3,4-, 3,4′- and 3,3′-dichlorobiphenyl and 2-, 3- and 4-chlorobiphenyl. Response factors were verified monthly. For data calculations, co-eluting congeners and homologues were assumed to be present in equal proportions. The concentrations were expressed as μmol of PCB kg^{-1} of dry sediment. The chlorination degree was reported as average number of Cl per biphenyl, calculated as follows:

$$N = \frac{\sum C_i \cdot n_i}{\sum C_i};$$

where C_i is the concentration of each detected PCB congener (μmol kg^{-1} of dry sediment) and n_i is its number of Cl substituents.

The dechlorination rate was expressed as μmol of Cl released kg^{-1} of dry sediment $week^{-1}$ and calculated as follows:

$$v = \frac{\left(\sum C_i \cdot n_i\right)_{initial} - \left(\sum C_i \cdot n_i\right)_{final}}{t};$$

where C_i is the concentration of each detected PCB congener (μmol kg^{-1} of dry sediment), n_i is its number of Cl substituents and t is the incubation time (weeks).

Gas production in the microcosms was measured with an air-tight syringe while its composition in CH_4, CO_2, N_2 and O_2 was analyzed with a Varian TCD 3300 gas chromatograph equipped with a Carbosieve S-II stainless steel column (3 m by 1/8 in. internal diameter) (Supelco, Inc., Bellefonte, PA, USA) and thermal conductivity detector (Varian Inc., Palo Alto, CA, USA) as described by Fava et al. [23].

The concentration of SO_4^{2-}, Cl^-, NO_3^- and NO_2^- in the water phase of the sediment slurry was determined by using a Dionex DX-120 ion chromatograph equipped with an IonPac AS14 4 mm × 250 mm column, a conductivity detector combined to an ASRS-II Ultra conductivity suppressor system (Dionex, Sunnyvale, CA, USA) as described elsewhere [23]. Linear four-point calibration curves (1.0–20.0 mg L^{-1} range) for SO_4^{2-}, Cl^-, NO_3^- and NO_2^- were obtained by using mixtures of these compounds.

2.4. Metagenomic DNA extraction

Metagenomic DNA was extracted from approximately 250 mg of wet sediment obtained from the slurry centrifugation (0.6 mL for M1C and M2C, 1.25 mL for M3C-12 and 2.5 mL for M3C-6) at 14,000 rpm for 10 minutes. DNA was extracted with the UltraClean Soil DNA kit (Mo Bio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions with some modification. In particular, 4.5 μL of a 100 mg mL^{-1} Proteinase K solution in sterile deionized water plus 8.2 μL of a 100 mg mL^{-1} Lysozyme solution in sterile deionized water were added to the sediment suspension in the bead solution provided with the kit. Sediment suspension was then incubated at 37 °C on a rotary shaker for 30 min prior to chemical (with SDS-containing S1 solution provided with the kit) and mechanical (bead beating on vortex at maximum speed for 10 min) cell lysis. DNA was eluted in 50 μL of TE buffer pH 8.0 and its quality checked on 1.0% (w/v) agarose gel stained with ethidium bromide.

2.5. 16S rRNA gene T-RFLP analysis

For 16S rRNA gene T-RFLP analysis, PCR amplification was performed in 50 μL reaction mixtures containing 1× PCR buffer (Invitrogen, Paisley, UK), 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 0.4 mM each primer, 2.0 U of *Taq* polymerase (Invitrogen, Paisley, UK), and 2.0 μL of template DNA. The primers pairs

used were 5′-end 6-carboxyfluorescein-labelled 6-FAM-27f (5′-AGAGTTTGATCMTGGCTCAG-3′) plus 1525r (5′-AAGGAGGTGWTCCARCC-3′) for Bacteria [25], and 5′-end 6-carboxyfluorescein-labelled 6-FAM-25f (5′-CTGGTTGATCCTGCCAG-3′) [26] plus 1517r (5′-ACGGCTACCTGGTTACGACTT-3′) [27] for Archaea. Reaction mixtures were held at 94 °C for 2 min, followed by 30 cycles of amplification at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s and a final extension of 72 °C for 10 min. Twenty ng of each fluorescently labelled amplicon were digested with MnlI at 37 °C for 2 h in a 20 μL reaction. The digested amplicons were ethanol precipitated and resuspended in a mixture of 14.8 μL of deionized formamide and 0.2 μL of DNA fragment length internal standard (ROX 500; Applied Biosystems, Foster City, CA, USA). Fluorescently labeled T-RFs were separated by capillary electrophoresis in an ABI 310 genetic analyzer. T-RFLP information was analyzed with 310 Genescan version 3.1 software (Applied Biosystems, Foster City, CA, USA).

To determine identity of Terminal-Restriction Fragments (T-RFs) detected, 50 clones randomly selected from bacterial and archaeal 16S rRNA gene clone libraries of M3C-6 culture were individually examined using T-RFLP analysis and representatives of each T-RF were sequenced. 16S rRNA genes from M3C-6 microcosm were PCR amplified with primer pairs 27F-1525r and 25f-1517r as described above, cloned in pCR®4-TOPO® cloning vector (Invitrogen, Paisley, UK) and the resulting plasmids inserted in chemically competent One Shot® TOP10 *E. coli* cells (Invitrogen, Paisley, UK) according to the manufacturer's instructions. Transformed cells were plated on LB agar plates containing 100 μg mL^{-1} ampicillin and incubated overnight at 37 °C. Fifty colonies form each clonal library were randomly tooth-picked and grown overnight in liquid LB medium with 100 μg mL^{-1} ampicillin. Library screening used plasmid extraction, PCR amplification with fluorescent forward primers and T-RFLP analysis of insert. Plasmids extractions were performed with the Qiaprep Spin miniprep kit (Qiagen, Valencia, CA, USA) according the manufacturer's instruction. PCR amplification and T-RFLP analysis of inserts were performed as described above. Inserts producing unique T-RFs were sequenced with 27f (Bacteria) or 25f (Archaea) primers.

2.6. 16S rRNA gene PCR-DGGE analysis

For Bacterial DGGE analysis, PCR amplification was performed with primers GC-357f (5′-CGCCCGCCGCGCCCCGCGCCCGGCCCGCCGCCCCCGCCCCCTACGGGAGGCAGCAG-3′) and 907r (5′-CCGTCAATTCCTTTGAGTTT-3′) [28] in 50 μL reaction mixtures containing 1× PCR buffer (Invitrogen, Paisley, UK), 1.5 mM $MgCl_2$, 0.12 mM each dNTP, 0.3 mM each primer, DMSO 5%, 1.0 U of *Taq* polymerase (Invitrogen, Paisley, UK) and 10.0 ng of template DNA. The reaction began with an initial 94 °C denaturation for 4 min, followed by 10 cycles of 94 °C for 30 s, 61 °C for 1 min, 72 °C for 1 min, 20 cycles of 94 °C for 30 s, 56 °C for 1 min, 72 °C for 1 min and a final extension at 72 °C for 7 min. Archeal 16S rRNA gene DGGE analysis was performed with a hot start polymerase chain reaction (PCR) protocol using primers GC-344f (5′-CGCCCGCCGCGCCCCGCGCCCGTCCCGCCGCCCCCGCCCCACGGGG(C/T)GCAGCAGGCGCGA-3′) [29] and 915r (5′-GTGCTCCCCCGCCAATTCCT-3′) [30] in 50 μL reactions as described above. The thermal cycles used for amplification were as follows: 10 min preincubation at 95 °C, 20 cycles of 94 °C for 1 min, 65 °C for 1 min, 72 °C for 3 min, 20 cycles of 94 °C for 1 min, 55 °C for 1 min, 72 °C for 3 min, and a final extension step at 72 °C for 7 min.

PCR products were resolved with a D-Code apparatus (Bio-Rad, Milan, Italy) on a 7% (w/v) polyacrylamide gel (acrylamide-N,N′-methylenebisacrylamide, 37:1) in 1× TAE with a denaturing gradient from 40% to 60% denaturant for Bacteria and from 40% to 70% for Archaea, where 100% denaturant is 7 M urea and 40% (v/v) formamide. The electrophoresis was run at 90 V for 15 h at 60 °C. The gel was stained in a solution of 1× SYBR-Green (Sigma–Aldrich, Milwaukee, WI) in 1× TAE for 30 min and its image captured in UV transillumination with a digital camera supported by a Gel Doc apparatus (Bio-Rad, Milan, Italy). Bands were cut from the gel with a sterile scalpel and DNA was eluted by incubating the gel fragments for 16 h in 50 μL of sterile deionized water at 4 °C. Eight μL of the solution were then used as template to re-amplify the band fragment using the same primers without the GC-clamp (357f-907r and 344f-915r) and the same PCR conditions described above. The obtained bacterial and archaeal amplicons were then sequenced with primer 907r and 915r, respectively, as described below.

2.7. Sequencing and phylogenetic analysis

Sequencing was performed after amplicon purification with EXOSAP (USB Corporation, Cleveland, Ohio, US) according to the manufacturer's instructions. Sequencing reactions and runs were performed by BMR Genomics (Padova, Italy).

For each 16S rRNA gene sequence, the most closely related sequence and the sequence of the most closely related cultured bacterial strain were retrieved from the GenBank database by using MEGABLAST and from the Ribosomal Database Project-II by using the SEQUENCE MATCH tool. Sequences were submitted to GenBank database under accession numbers GQ423355 to GQ423393.

2.8. Chemicals

3,3′,4,4′-tetrachlorobiphenyl, 3,3′,4,4′,5- and 2,3′,4,4′,5-pentachlorobiphenyl, 3,3′,4,4′,5,5′- and 2,3,3′,4,4′,5-hexachlorobiphenyl, OCN, Aroclor 1242, Aroclor 1254 and pure PCBs used as analytical standards were provided by Ultra-Scientific (North Kingstown, RI, USA). Anhydrous diethyl ether, acetone and hexane (both for pesticide analysis in capillary column GC systems) as well as the ultra-resi analyzed water for ion chromatography were supplied by Mallinckrodt-Baker (Phillipsburg, NJ, USA). Inorganic ions for IC analysis were provided by Aldrich (Strenheim, Germany).

3. Results and discussion

3.1. PCB dechlorination and other microbial activities

Spiked 3,3′,4,4′,5,5′- and 2,3,3′,4,4′,5-hexachlorobiphenyl, 3,3′,4,4′,5- and 2,3′,4,4′,5-pentachlorobiphenyl and 3,3′,4,4′-tetrachlorobiphenyl were initially detected in the slurry culture M2C (i.e., the first subculturing step inoculated with M1C culture) at the concentration of 406 ± 21, 369 ± 19, 405 ± 28, 439 ± 37 and 382 ± 40 μmol kg^{-1} of dry sediment, respectively. Some lower chlorinated PCBs not occurring in the spiked mixture were also detected in the microcosm at the beginning of incubation (Fig. 2); these PCBs were not occurring in the original sediment [24], and derived from M1C culture used as inoculum (25%, v/v), where they markedly accumulated as intermediate or final dechlorination products of coplanar PCBs [24]. Dechlorination activity was detected in M2C culture after the first 1.5 months of incubation, when 3,3′,4,4′,5,5′-CB, 2,3,3′,4,4′,5-CB, 3,3′,4,4′,5-CB, 2,3′,4,4′,5-CB and 3,3′,4,4′-CB were depleted by 7, 28, 32, 27 and 21%, respectively, and bioconverted into medium chlorinated congeners, such as 2,3,3′,4′,5-CB, 3,3′,4,5′-CB, 2,3′,4,4′-CB, 2,3′,4′,5-CB 2,3′,4,5-CB and 3,3′,5-CB (Fig. 2). Such PCBs were then further bioconverted into less chlorinated congeners, mainly 2,3′,5-CB, 2,4-/3,4′-CB and 3,3′-CB (Fig. 2). After 7.5 months of incubation, the spiked 3,3′,4,4′,5,5′-CB, 2,3,3′,4,4′,5-CB, 3,3′,4,4′,5-CB, 2,3′,4,4′,5-CB and 3,3′,4,4′-CB were depleted by 77, 84, 95, 89 and 97%, respectively (Fig. 2), and the average chlorination degree was reduced from the initial 5.2 to 3.0 chlorines per biphenyl molecule (Fig. 3). With the exception of 2-CB, that markedly accumulated only in the primary

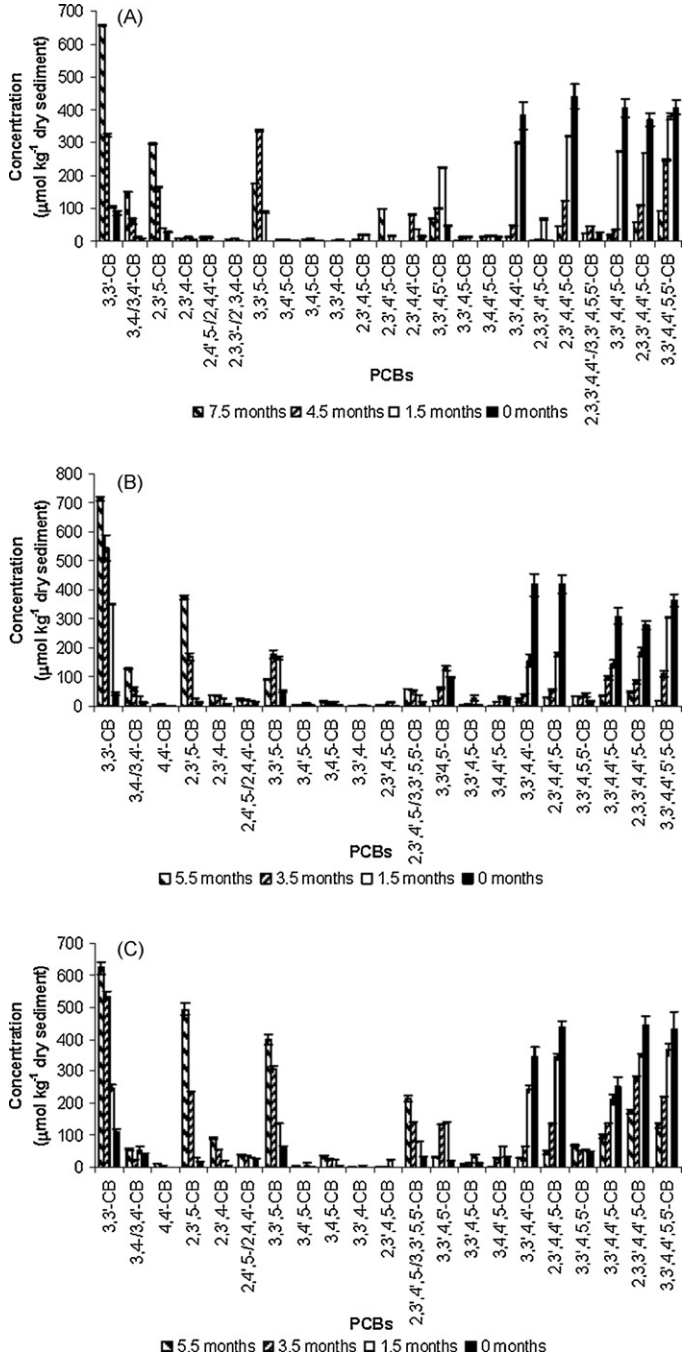

Fig. 2. Concentration (μmol kg^{-1} of dry sediment $\pm$ standard deviation) of spiked coplanar PCBs and of their dechlorination products throughout incubation in M2C (A), M3C-12 (B) and M3C-6 (C) cultures.

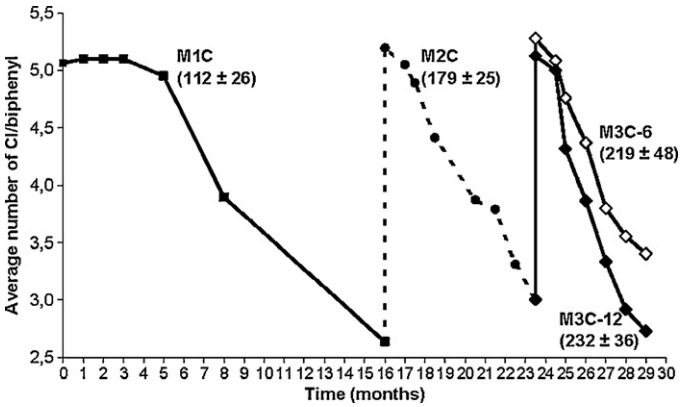

Fig. 3. Average number of Cl per biphenyl in M1C (squares), M2C (circles), M3C-12 (solid diamonds) and M3C-6 (empty diamonds) cultures throughout incubation. PCB-dechlorination rates (μmol Cl removed kg^{-1} dry sediment week^{-1} $\pm$ standard deviation) are given in parenthesis.

culture M1C, the same final dechlorination products were accumulated both in M1C and M2C cultures (24; Fig. 2). This suggests that the main dechlorination activity detected in M1C culture [24], corresponding to pattern H′ described by Bedard and Quensen [4], was maintained after subculturing, whereas the additional *meta* dechlorination activity detected in M1C was partially lost with subculturing. In addition, unlike in M1C culture, where dechlorination activity started after the fifth month of incubation, no lag phase was observed in M2C culture, where the dechlorination started immediately (Fig. 3). Finally, the dechlorination rate observed in M2C culture (179 ± 25 μmol of Cl removed kg^{-1} of dry sediment week^{-1}) was higher than that observed in M1C (112 ± 26 μmol of Cl removed kg^{-1} of dry sediment week^{-1}) (Fig. 3).

A similar dechlorination activity towards spiked coplanar PCBs was observed after further subculturing with the development of M3C-12 and M3C-6 cultures, where no lag phase and the accumulation of the same dechlorination intermediates and final products detected in M2C were observed (Figs. 2 and 3). In M3C-12, the initial average chlorination degree was reduced from 4.9 to 2.8 chlorines per biphenyl molecule within 5.5 months of incubation, leading to depletion of spiked 3,3′,4,4′,5,5′-CB, 2,3,3′,4,4′,5-CB, 3,3′,4,4′,5-CB, 2,3′,4,4′,5-CB and 3,3′,4,4′-CB by 95, 83, 89, 93 and 95%, respectively, with a overall dechlorination rate of 232 ± 36 μmol of Cl removed kg^{-1} of dry sediment week^{-1} (Fig. 3). A slightly lower dechlorination activity was observed in M3C-6 culture, where a reduction from 5.3 to 3.4 chlorines per biphenyl and a dechlorination rate of 219 ± 48 μmol of Cl removed kg^{-1} of dry sediment week^{-1} were measured during 5.5 months of incubation (Fig. 3). A remarkable increase in the dechlorination rate was therefore observed throughout subculturing of M1C culture in sterile sediment slurries developed with site water and exogenous coplanar PCBs. This finding suggests that the marine microbial community of Brentella canal capable of dechlorination pattern H′ was enriched under biogeochemical conditions close to those occurring *in situ*. In addition, the dechlorination of coplanar PCBs by the enriched microbial community of Brentella canal was remarkably higher and broader than that reported previously in river sediments, where PCB dechlorination was slow and limited only to the *meta* or to the *para* positions of the two PCB congeners 3,3′,4,4′-tetrachlorobiphenyl and 3,3′,4,4′,5-pentachlorobiphenyl [31].

Sulfate-reducing and methanogenic activities were also monitored throughout subculturing in order to gain information on the indigenous microbial components involved in PCB dechlorination. A noteworthy sulfate reduction, that completely depleted the initially available 1.9 g L^{-1} of sulfate during the first month of incubation, was detected in M2C culture (Fig. 4). A remarkable biogas production (36.2 mL) was also detected during the first 2.5 months of incubation; however, only 23.7% of produced biogas (corresponding to 7.4 mL) was methane (Fig. 4). A sulfate reduction comparable to that detected in M2C was observed in M3C-12 and M3C-6 cultures, where sulfate was completely depleted after 1.5 months of incubation. On the contrary, a significantly lower amount of biogas (11.5 mL) and methane (2.2 mL) was produced in M3C-12 culture, whereas no methane and only 2.2 mL of biogas were produced in M3C-6 culture (Fig. 4). These data indicate that methanogenic activity was progressively reduced throughout subculturing, thus suggesting that methanogens are not involved in the dechlorination process. On the other hand, the increasing sulfate-reduction activity detected in M2C, M3C-12 and M3C-6 cul-

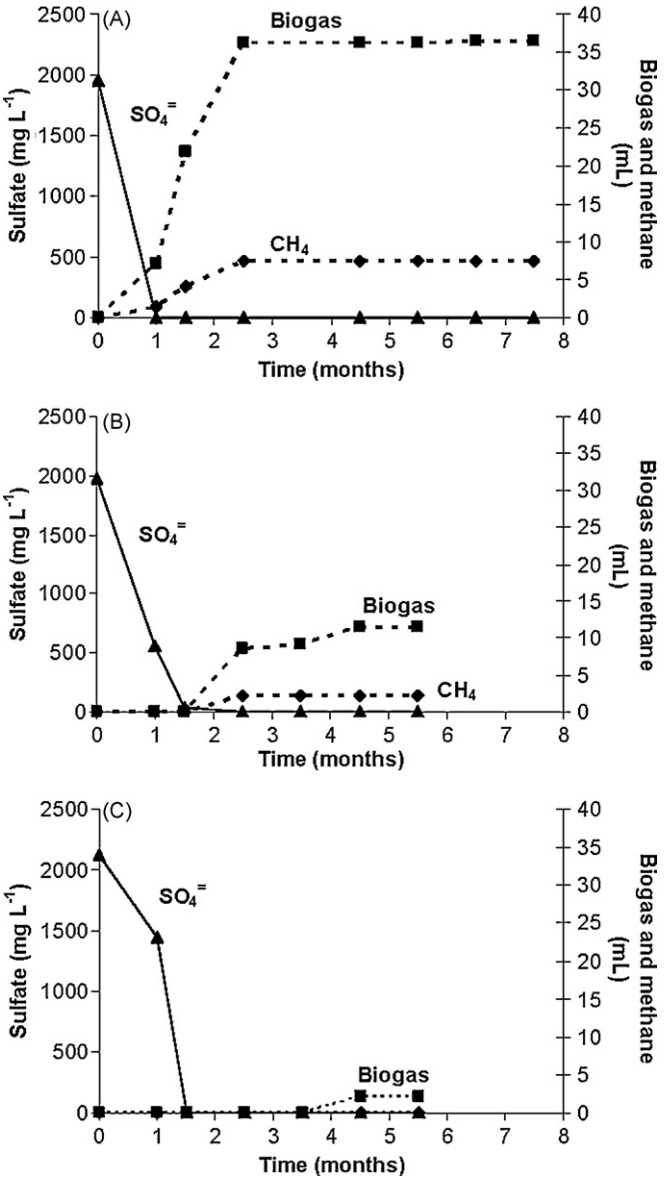

Fig. 4. Sulfate concentration (triangles), total biogas (squares) and methane (diamonds) evolution in M2C (A), M3C-12 (B) and M3C-6 (C) cultures throughout incubation.

tures suggests that sulfate reduction was associated to it. This is in accordance with previous findings observed in marine sediments of the Brentella canal suspended in their own site water [22–24] and in Hudson river [32] and estuarine [9,11] sediments incubated in reduced anaerobic minimal medium. However, while in previous studies PCB dechlorination started only after complete sulfate consumption [24,32], in M2C, M3C-12 and M3C-6 enrichments it occurred both during sulfate reduction and after the complete depletion of sulfate. This seems to indicate that other forms in addition to sulfate-reducing bacteria were involved in PCB dechlorination in the enrichment microcosms developed in this study. This hypothesis is supported by a recent evidence that the growth and activity of PCB-dechlorinating cultures is associated to the co-occurrence of sulfate-reducing bacteria [33].

3.2. Characterization of bacterial communities

According to T-RFLP analysis, remarkable changes in the bacterial community occurred throughout subculturing (Fig. 5A). M1C and M2C shared 8 T-RFs out of 19 and 14 total T-RFs, respectively, corresponding to a Sorensen's similarity coefficient of 0.48; M1C shared 5 T-RFs with M3C-12 (20 T-RFs) and 6 T-RFs with M3C-6 (17 T-RFs), corresponding to Sorensen's similarity coefficients of 0.26 and 0.33, respectively; M2C shared 8 T-RFs with M3C-12 and 6 T-RFs with M3C-6, resulting in Sorensen's similarity coefficients of 0.47 and 0.39, respectively. Finally, the most similar bacterial T-RFLP profiles were those of M3C-12 and M3C-6, i.e., the second transfer cultures having the highest PCB-dechlorinating activity, that shared 12 T-RFs (Sorensen's similarity coefficient of 0.65) (Fig. 5A). Four T-RFs, 119, 196, 206 and 213 bp long, were detected in all subculturing steps (Fig. 5A). T-RF 119, the major T-RF in all cultures with relative abundance of 46, 47, 48 and 53% in M1C, M2C, M3C-12 and M3C-6 profiles, respectively, was generated by M3C-6 clones having the highest sequence similarity to the genus *Sulfurovum* of the Epsilon subgroup of *Proteobacteria* (Table 1). T-RF 206 was identified as a *Chloroflexi* having low similarity (88% identity) with *Dehalococcoides* sp. BHI80-15, whereas no clones representing T-RFs 196 and 213 were found in M3C-6 clone library. In addition, seven T-RFs, namely 145, 152, 208, 212, 214, 217 and 230 bp T-RFs, were detected only or had higher relative abundance both in M3C-12 and M3C-6 (Fig. 5A). With the exception of T-RFs 145 and 230, that were not represented by any clone in the library, all these T-RFs were identified as *Deltaproteobacteria* (Table 1). Clones representing T-RF 152 had 89% identity with the sulfate-reducing bacterium *Desulfosarcina variabilis*, those representing T-RF 208 had 99% identity with the sulfate-reducing bacterium *Desulfococcus multivorans*, whereas those producing T-RFs 210, 212 and 214 had 86–88% identity with *Syntrophus* spp.

Changes in the bacterial community profile throughout subculturing were also observed when comparing the structure of the communities by DGGE (Fig. 6A). The highest similarity was observed between M3C-12 and M3C-6 (Sorensen's similarity coefficient: 0.91), whereas decreasing similarity was detected between M2C and M3C-12 or M3C-6 (Sorensen's similarity coefficient: 0.83), M1C and M2C (Sorensen's similarity coefficient: 0.57) and between M1C and M3C-12 or M3C-6 (Sorensen's similarity coefficient: 0.32 and 0.42, respectively). Three DGGE bands, namely 11E, 3E and 20E, were detected in all subculturing steps (Fig. 6A). Band 11E had high sequence similarity to the genus *Thioalkalispira* of *Gammaproteobacteria*, band 3E to the genus *Anaerovorax* of *Firmicutes*, whereas band 20E showed high sequence similarity with an uncultured *Chloroflexi* bacterium (Table 2). Three additional bands (5E, 7E and 8E) with high sequence similarity to members of the phylum *Firmicutes* (Table 2) were dominant in the primary culture M1C, but they disappeared from band pattern throughout subculturing (Fig. 6A). On the contrary, several bands (10E, 17E, 12E, 13E and 19E) enriched in M2C, M3C-12 and M3C-6 cultures (Fig. 6A). Band 10E had 98% sequence similarity to the genus *Sulfurovum* of *Epsilonproteobacteria* and showed more than 99% identity with the sequence portion that shared with clones of M3C-6 culture representing T-RF 119. Band 17E had high sequence similarity with an uncultured Alphaproteobacterium, whereas bands 12E, 13E and 19E were highly similar to the same uncultured bacterium clone G135 and had low homology to *Firmicutes* (Table 2). In addition, band 14E was the dominant phylotype in M2C culture, with 98% identity to the uncultured *Chloroflexi* bacterium clone SF1, that was previously indicated as the PCB dechlorinator with selectivity for singly flanked chlorines in sediment-free cultures enriched in synthetic medium from estuarine sediments of Baltimore Harbor [12]. Band 14E was also detected in the two most active PCB-dechlorinating cultures M3C-12 and M3C-6; however, it did not clearly represent the dominant phylotype in these enrichment cultures, since it was detected only at low intensities (Fig. 6A). Finally, band 18E was detected only in M3C-12 and M3C-6 cultures and exhibited 98% sequence identity with *D. multivorans* (Fig. 6A, Table 2), i.e., the clos-

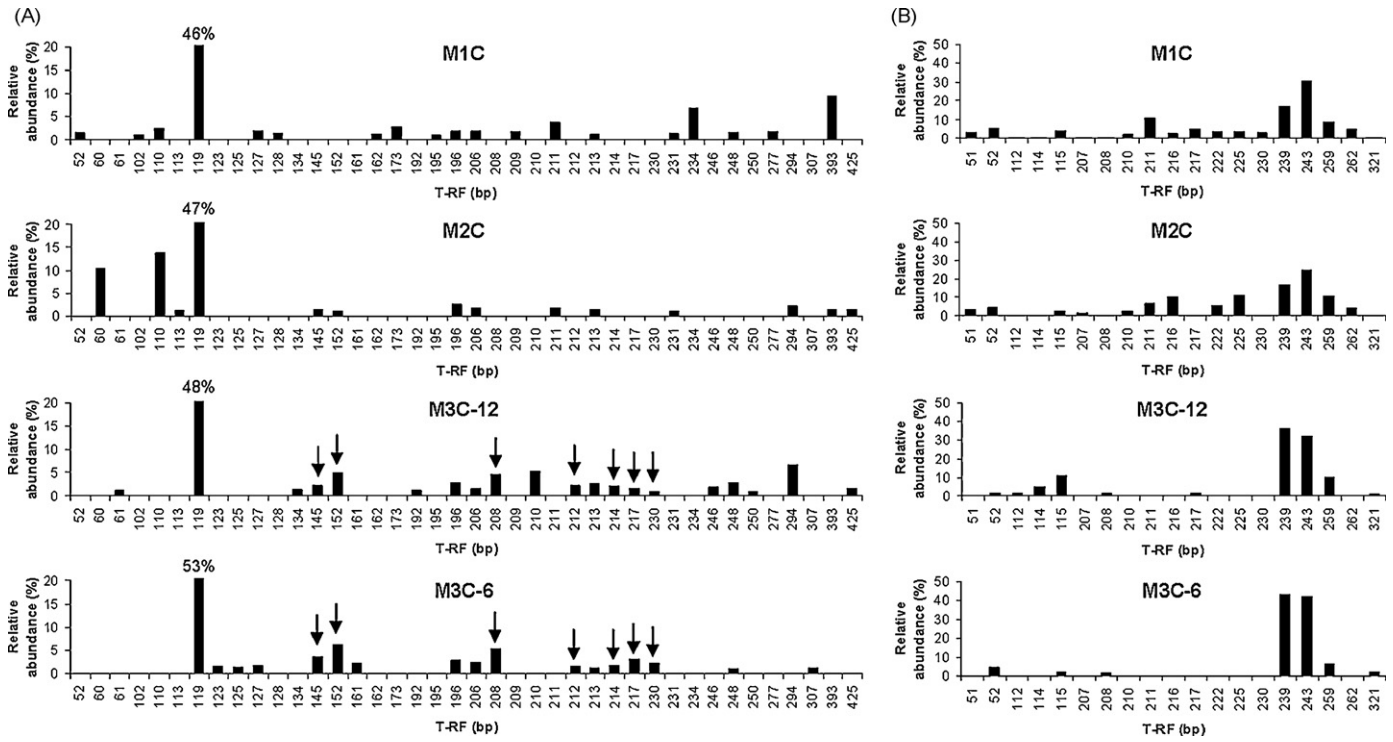

Fig. 5. T-RFLP profiles (percentage relative abundance of each terminal restriction fragment) of bacterial (A) and archaeal (B) populations in each culture at the end of incubation. Arrows indicate T-RFs detected only in or enriched in both M3C-12 and M3C-6 cultures.

est relative of M3C-6 clones representing T-RF 208. Overall, DGGE analysis indicated that *Firmicutes* (bands 5E, 7E, 8E) were lost upon subculturing, whereas several phylotypes, such as an uncultured *Chloroflexi* (band 20E), a *Sulfurovum* sp. (band 10E), a *D. multivorans* (band 18E), an uncultured Alphaproteobacterium (band 17E) and three unidentified bacteria (bands, 12E, 13E, 19E) were enriched in M2C, M3C-16 and M3C-6 throughout subculturing.

Both T-RFLP and DGGE analysis revealed that shifts in the bacterial community occurred throughout subculturing of the coplanar PCB-dechlorinating microbial population from Venice lagoon sediments under laboratory biogeochemical conditions close to those occurring *in situ*. With both fingerprinting methods, the highest level of similarity was found between the two most active PCB-dechlorinating cultures M3C-12 and M3C-6, whereas the lowest similarity was detected when comparing M3C-12 and M3C-6 with the primary culture M1C.

Since PCB-dechlorination activity increased throughout subculturing, phylotypes occurring in all subculturing steps and/or selectively enriched throughout subculturing might represent PCB dehalogenating members of the microbial community. Indeed, according to T-RFLP analysis, an Epsilonproteobacterium phylotype strictly related to the genus *Sulfurovum* (T-RF 119) was dominant in all enriched cultures. The same phylotype was also detected by DGGE (band 10E) in all cultures except M1C,

Table 1
Phylogenetic affiliation of bacterial clones from M3C-6 culture representing Terminal-Restriction Fragments (T-RFs) detected by T-RFLP analysis.

T-RF (bp)	Phylogenetic group	Closest classified relative (% certainty)	Closest match [accession #]	% identity	Closest described bacterium [accession #]	% identity
119	*Epsilonproteobacteria* (100%)	*Sulfurovum* (98%)	Uncultured epsilon proteobacterium clone XME7 [EF061976]	96%	Bacterium 2BP-7 [AF121887]	95%
152	*Deltaproteobacteria* (100%)	*Desulfobacca* (37%)	Uncultured bacterium clone d1-33 [AM409909]	92%	*Desulfosarcina variabilis* [M26632]	89%
206	*Chloroflexi* (74%)	*Caldilinea* (38%)	Uncultured Chloroflexi bacterium clone XME3 [EF061967]	96%	*Dehalococcoides* sp. BHI80-15 [AJ431246]	88%
208	*Deltaproteobacteria* (100%)	*Desulfococcus* (100%)	Uncultured Desulfococcus sp. clone HKT951 [DQ989471]	100%	*Desulfococcus multivorans* DSM 2059 [AF418173]	99%
210	*Deltaproteobacteria* (100%)	*Smithella* (62%)	Uncultured bacterium clone Er-MS-29 [EU542439]	91%	*Syntrophus gentianae* HQgoe1 (T) [X85132]	88%
212	*Deltaproteobacteria* (97%)	*Smithella* (66%)	Uncultured delta proteobacterium clone GoM IDB-33 [AY542232]	92%	*Syntrophus aciditrophicus* SB [CP000252]	88%
214	*Deltaproteobacteria* (100%)	*Smithella* (80%)	Uncultured bacterium clone Er-LLAYS-61 [EU542518]	98%	*Syntrophus aciditrophicus* SB [CP000252]	86%
217	*Deltaproteobacteria* (100%)	*Desulfobacterium* (93%)	Uncultured bacterium clone SSS22A [EU592480]	93%	Bacterium 2BP-6 [AF121886]	91%

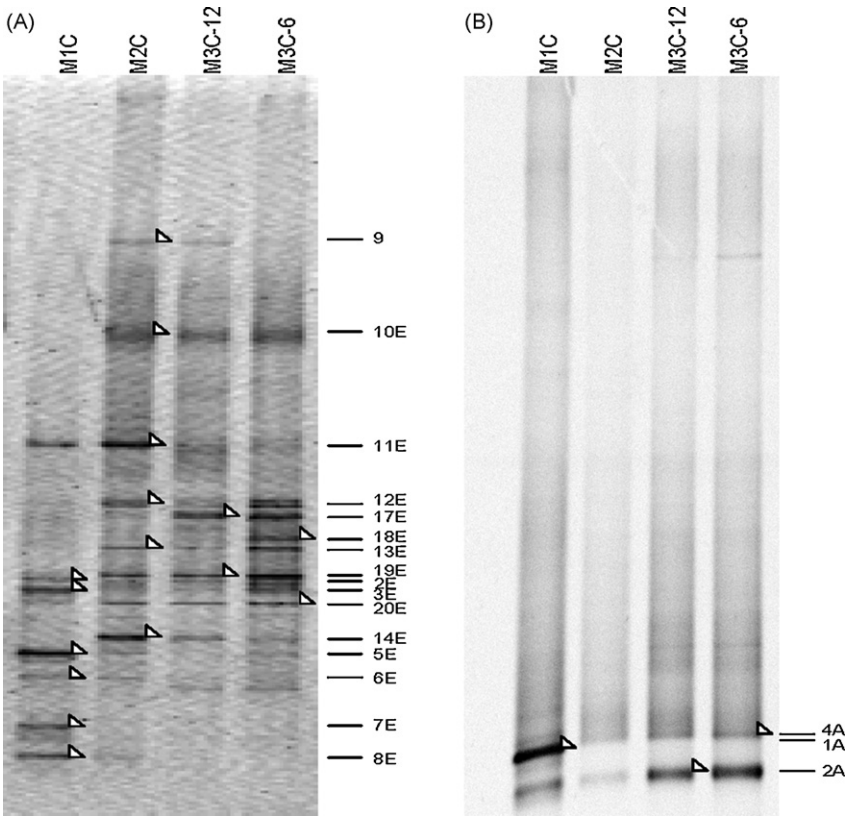

Fig. 6. DGGE profiles of bacterial (A) and archaeal (B) populations in each culture at the end of incubation. Arrows indicate bands that were excised and sequenced. Band numbering is on the right side of each gel.

i.e., the lowest rate PCB-dechlorinating culture. However, to our knowledge, no *Epsilonproteobacteria* were previously detected in PCB-dechlorinating cultures enriched either from freshwater or marine habitats, and a single Epsilonproteobacterium clone (2BP-7 [AF121887]) having high sequence identity (95%) with phylotype representing T-RF 119/band 10E was earlier detected only in a sulfidogenic 2-bromophenol dehalogenating culture enriched from Arthur Kill estuarine sediments [34].

Members of the *Chloroflexi* phylum (T-RF 206, bands 14E and 20E) and a microorganism strictly related to the sulfate-reducing bacterium *D. multivorans* (T-RF 208, band 18E) were also detected or enriched throughout subculturing. Several studies recently reported that a number of *Dehalococcoides* spp. or *Dehalococcoides*-related microorganisms occur in PCB-dechlorinating cultures and suggested they play a key role in PCB dechlorination [9,11,13–17]. In addition, a PCB-dechlorinating *Chloroflexi* ultramicrobacterium was recently grown in pure culture in mineral medium supplemented with cell extracts of sulfate-reducing bacteria [33]. One of the detected *Chloroflexi* had high similarity with bacterium SF1, a clone identified as the PCB dechlorinator in estuarine, sediment-free cultures [12]. However, this microorganism was not enriched in the most active PCB-dechlorinating cultures M3C-12 and M3C-6, where it was detected at low band intensities. None of the two *Chloroflexi* phylotypes that enriched throughout subculturing was found to have high sequence similarity to either *Dehalococcoides* spp. or *Dehalococcoides*-like microorganisms detected so far in PCB-dechlorinating cultures. However, while previous investigations focused on microbial communities enriched from freshwater and estuarine sediments suspended in synthetic mineral media, the PCB-dechlorinating microbial community described here is the first one enriched from an actual site marine sediment, and the first one obtained by subculturing the microflora in sediment and water coming from the site. Thus, it cannot be excluded that different putative PCB-dechlorinating *Chloroflexi* may have been enriched in our cultures due to the different sediment source and/or the different biogeochemical culturing conditions employed.

Some differences in phylotype detection with the two fingerprinting methods were also observed. In particular, the enrichment of 1 Gammaproteobacterium, 1 Alphaproteobacterium and 3 Firmicutes that were not identified in T-RFLP profiles was detected by DGGE analysis. This difference in detection may be a result of different primers used and/or PCR biases [35,36]. In addition, T-RFs identification via T-RFLP analysis of clone library was carried out only on M3C-6 culture, where not all T-RFs were successfully identified, and this has probably limited the information obtained by T-RFLP analysis.

3.3. Characterization of archaeal communities

The same approach was used to investigate the structure of the archaeal community at the end of each subculturing step. According to T-RFLP analysis, 14, 13, 10 and 7 T-RFs were detected in M1C, M2C, M3C-12 and M3C-6 cultures, respectively (Fig. 5B). In particular, phylotypes represented by T-RFs 51, 210, 211, 216, 222, 225, 230 and 262 in M1C culture disappeared throughout subculturing, while T-RFs 239 and 243 became dominant in M3C-12 and M3C-6 cultures. Clones representing the two dominant T-RFs 239 and 243 had the highest identity to two mesophilic aceticlastic methanogens strains of *Methanosaeta harundinacea*, whereas clones representing less abundant T-RFs 52, 259 and 321 had high similarity to *Methanosarcina semesiae* and to unidentified Euryarchaeote clones GoM161 and KZNMV-10-A9, respectively (Table 3). The occurrence of *Methanosaeta* spp. has been detected in previous studies on microbial communities of PCB-dechlorinating slurry cultures of estuarine [8] and river sediments [18]. However, in both cases their enrichment was observed in PCB-free controls

Table 2
Phylogenetic affiliation of bands excised from the bacterial community DGGE gel of M1C, M2C, M3C-12 and M3C-6 cultures.

DGGE band	Phylogenetic group	Closest classified relative (% certainty)	Closest match [accession #]	% identity	Closest described bacterium [accession #]	% identity
17E	*Alphaproteobacteria* (100%)	*Jannaschia* (59%)	Uncultured bacterium clone SSW53Au [EU592324]	97%	*Thalassobius aestuarii* [EU687495]	96%
14E	*Chloroflexi* (10%)	*Caldilinea* (7%)	Uncultured *Chloroflexi* bacterium clone SF1 [DQ021870]	98%		
20E	*Chloroflexi* (100%)	*Levilinea* (60%)	Uncultured *Chloroflexi* bacterium clone Ctrl1-8D [EU522649]	98%		
18E	*Deltaproteobacteria* (100%)	*Desulfococcus* (100%)	*Desulfococcus multivorans* [AF418173]	98%		
10E	*Epsilonproteobacteria* (100%)	*Sulfurovum* (100%)	Uncultured epsilon proteobacterium clone XME34 [EF061978]	99%	Proteobacterium Dex60-82 [AJ431218]	97%
3E	*Firmicutes* (100%)	*Anaerovorax* (99%)	Uncultured *Anaerovorax* clone C14B-1H [EU073780]	97%	*Anaerovorax odorimutans* [AJ251215]	94%
19E	*Firmicutes* (61%)	*Thermacetogenium* (13%)	Uncultured bacterium clone G135 [DQ521095]	95%		
13E	*Firmicutes* (66%)	*Succinispira* (37%)	Uncultured bacterium clone G135 [DQ521095]	98%		
12E	*Firmicutes* (73%)	*Succinispira* (36%)	Uncultured bacterium clone G135 [DQ521095]	97%		
6E	*Firmicutes* (98%)	*Aminomonas* (44%)	Uncultured bacterium clone WLEA-19 [EF117527]	97%	*Thermovirga lienii* strain Cas60314 [DQ071273]	91%
7E	*Firmicutes* (98%)	*Aminomonas* (70%)	Uncultured *Thermovirga* sp. clone TCB169x [DQ647105]	97%	*Thermovirga lienii* strain Cas60314 [DQ071273]	94%
8E	*Firmicutes* (99%)	*Aminomonas* (81%)	Uncultured *Thermovirga* sp. clone TCB169x [DQ647105]	98%	*Thermovirga lienii* strain Cas60314 [DQ071273]	94%
5E	*Firmicutes* (99%)	*Peptococcaceae2* (42%)	Uncultured *Peptococcaceae* bacterium clone A2Dmac [DQ386214]	94%	*Desulfotomaculum geothermicum* [AJ621886]	90%
2E	*Gammaproteobacteria* (100%)	*Alkalispirillum* (53%)	*Ectothiorhodospiraceae* bacterium Su4 [EU837269]	99%	*Thiohalomonas denitrificans* strain HLD 2 [EF117909]	92%
11E	*Gammaproteobacteria* (100%)	*Thioalkalispira* (100%)	Uncultured gamma proteobacterium clone 56S 1B 19 [DQ837265]	99%	*Thioalkalispira microaerophila* [AF481118]	95%
9E	*Gammaproteobacteria* (100%)	*Thioalkalispira* (100%)	*Thiomicrospira* sp. Ch-1 [AF013975]	96%	*Thiomicrospira arctica* [AJ404731]	94%

Table 3
Phylogenetic affiliation of archaeal clones from M3C-6 culture representing Terminal-Restriction Fragments (T-RFs) detected by T-RFLP analysis.

T-RF (bp)	Phylogenetic group	Closest classified relative	Closest match	% identity
52	*Euryarchaeota* (100%)	*Methanosarcina* (100%)	*Methanosarcina semesiae* strain MD1 [AJ012742]	97%
239	*Euryarchaeota* (100%)	*Methanothrix* (100%)	*Methanosaeta harundinacea* strain 6Ac [AY970347]	92–94%
			Methanosaeta harundinacea strain 8Ac [AY817738]	91–93%
243	*Euryarchaeota* (100%)	*Methanothrix* (60%-73%)	*Methanosaeta harundinacea* strain 8Ac [AY817738]	97%
			Methanosaeta harundinacea strain 6Ac [AY970347]	94–97%
			Uncultured archaeon clone WLEC-78 [EF117486]	96–97%
259	*Euryarchaeota* (100%)	*Methanoplanus* (61%)	Uncultured euryarchaeote clone GoM161 Arch1 [AM745169]	92%
321	*Euryarchaeota* (65%)	*Methanosphaera* (12%)	Uncultured archaeon clone KZNMV-10-A9 [FJ712378]	97%

Table 4
Phylogenetic affiliation of bands excised from the archaeal community DGGE gel of M1C, M2C, M3C-12 and M3C-6 cultures.

DGGE band	Phylogenetic group	Closest classified relative	Closest match	% identity
1A	*Euryarchaeota* (100%)	*Methanogenium* (62%)	Uncultured euryarchaeote clone GoM161 [AM745169]	95%
2A	*Euryarchaeota* (96%)	*Methanothermococcus* (42%)	Uncultured archaeon clone 12-PML [AF477923]	99%
4A	*Euryarchaeota* (100%)	*Methanothrix* (84%)	Uncultured archaeon clone MOB7-5 [DQ841240]	100%

rather than in the actively PCB-dechlorinating cultures, thus suggesting that they are not directly involved in PCB dechlorination.

A much lower diversity of the archaeal population was detected in DGGE fingerprinting profiles (Fig. 6B), where only three bands with sequence similarity to uncultured Euryarchaeote clones were detected (Table 4). As suggested above, this might be the consequence of the different primer pairs used and/or PCR biases. In addition, the lower sensitivity of the DGGE technique, as compared to T-RFLP, might have prevented the detection of low abundant phylotypes.

4. Conclusions

The PCB dehalogenating microbial community of a marine sediment from the Brentella canal (Venice lagoon), was enriched in laboratory slurry microcosms consisting of sediment suspended in the water of the site, i.e., under biogeochemical conditions that mimic those occurring *in situ*. The enriched culture dechlorinated five of the most toxic, dioxin-like coplanar PCB congeners with rate and extent remarkably higher than those reported so far in the literature [31]. Similarly to other reports [8,18], the archaeal

population was mainly composed by members of the *Methanomicrobiales* and *Methanosarcinales* subgroups, that apparently were not involved in the dechlorination process. One *Chloroflexi* bacterium having similarity with bacterium SF1, a clone identified as the PCB dechlorinator in estuarine, sediment-free cultures [12] was detected in the developed cultures. However, this clone did not enrich in the most active PCB-dechlorinating cultures M3C-12 and M3C-6, and thus it may not be involved in PCB dechlorination under the biogeochemical culturing conditions employed. No bacterial phylotypes with high sequence homology to already described dehalorespiring bacteria were enriched in the cultures described here. Since the PCB-dechlorinating microbial community described here is the first one enriched from an actual site marine sediment, and the first one obtained by subculturing the microflora in sediment and water coming from the site, this let us speculate that yet unknown PCB dechlorinators may have enriched in our cultures due to the different sediment source and/or the different biogeochemical culturing conditions employed. In particular, an epsilonproteobacterium with high sequence similarity to the genus *Sulforovum*, two *Chloroflexi* with low homology to known or putative dehalorespirers and a bacterium highly homologous to *D. multivorans* were enriched and might thus have a role in PCB dechlorination *in situ* in the marine sediments of Brentella canal.

Acknowledgments

The authors sincerely thank Dr. J.R. Peréz-Jimenéz and Prof. L.J. Kerkhof (Rutgers University, NJ) for their support in the T-RFLP analysis, and Dr. E. Zanotto (Venice Port Authority, Italy) for having supplied the sediment and water samples employed in the study. This research was funded by the Italian MIUR and the ENVIREN laboratories cluster of the Emilia Romagna Region (Italy).

References

[1] J.F. Brown Jr., R.E. Wagner, PCB movement, dechlorination and detoxification in the Acushnet estuarine, Environ. Toxicol. Chem. 9 (1990) 1215–1233.
[2] S. Safe, K. Connor, K. Ramamoorthy, K. Gaido, S. Maness, Human exposure to endocrine-active chemicals: hazard assessment problems, Regul. Toxicol. Pharmacol. 26 (1997) 52–58.
[3] D.O. Carpenter, Polychlorinated biphenyls (PCBs): routes of exposure and effects on human health, Rev. Environ. Health 21 (2006) 1–23.
[4] D.L. Bedard, J.F. Quensen III, Microbial reductive dechlorination of polychlorinated biphenyls, in: L.Y. Young, C.E. Cerniglia (Eds.), Microbial Transformation and Degradation of Toxic Organic Chemicals, Wiley-Liss Division Wiley, New York, 1995, pp. 127–216.
[5] J. Wiegel, Q. Wu, Microbial reductive dehalogenation of polychlorinated biphenyls, FEMS Microbiol. Ecol. 32 (2000) 1–15.
[6] D.L. Bedard, Polychlorinated biphenyls in aquatic sediments: environmental fate and outlook for biological treatment, in: H.M. Häggblom, I. Bossert (Eds.), Dehalogenation: Microbial Processes and Environmental Applications, Kluwer Press, Dordrecht, The Netherlands, 2003, pp. 443–465.
[7] F. Fava, S.N. Agathos, Uncertainty and research needs in the area of the biological restoration of contaminated sediments, in: D. Reible, T. Lanczos (Eds.), Biological Assessment and Remediation of Contaminated Sediments. Assessment and Remediation of Contaminated Sediments, NATO Science Series, IV Earth and Environmental Sciences, Vol. 73, Springer, The Netherlands, 2006, pp. 239–246.
[8] T.R.P. Pulliam Holoman, M.A. Elberson, L.A. Cutter, H.D. May, K.R. Sowers, Characterisation of a defined 2,3,5,6-tetrachlorobiphenyl-ortho-dechlorinating microbial community by comparative sequence analysis of gene coding for 16S rDNA, Appl. Environ. Microbiol. 64 (1998) 3359–3367.
[9] L.A. Cutter, J.E.M. Watts, K.R. Sowers, H.D. May, Identification of a microorganism that links its growth to the reductive dechlorination of 2,3,5,6-chlorobiphenyl, Environ. Microbiol. 3 (2001) 699–709.
[10] J.E.M. Watts, Q. Wu, S.B. Schreier, H.D. May, K.R. Sowers, Comparative analysis of polychlorinated biphenyl-dechlorinating communities in enrichment cultures using three different molecular screening techniques, Environ. Microbiol. 3 (2001) 710–719.
[11] Q. Wu, J.E.M. Watts, K.R. Sowers, H.D. May, Identification of a bacterium that specifically catalyzes the reductive dechlorination of polychlorinated biphenyls with doubly flanked chlorines, Appl. Environ. Microbiol. 68 (2002) 807–812.
[12] S.K. Fagervold, J.E.M. Watts, H.D. May, K.R. Sowers, Sequential reductive dechlorination of *meta*-chlorinated polychlorinated biphenyl congeners in sediment microcosms by two different *Chloroflexi* phylotypes, Appl. Environ. Microbiol. 71 (2005) 8085–8090.
[13] J.E.M. Watts, S.K. Fagervold, H.D. May, K.R. Sowers, A PCR-based specific assay reveals a population of bacteria within the *Chloroflexi* associated with the reductive dehalogenation of polychlorinated biphenyls, Microbiology 181 (2005) 2039–2046.
[14] S.K. Fagervold, H.D. May, K.R. Sowers, Microbial reductive dechlorination of Aroclor 1260 in Baltimore Harbor sediment microcosms is catalyzed by three phylotypes within the phylum *Chloroflexi*, Appl. Environ. Microbiol. 73 (2007) 3009–3018.
[15] D.L. Bedard, J.J. Bailey, B.L. Reiss, G. Van Slyke Jerzak, Development and characterization of stable sediment-free anaerobic bacterial enrichment cultures that dechlorinate Aroclor 1260, Appl. Environ. Microbiol. 72 (2006) 2460–2470.
[16] D.L. Bedard, K.M. Ritalahti, L.E. Löffler, The *Dehalococcoides* population in sediment-free mixed cultures metabolically dechlorinates the commercial polychlorinated biphenyl mixture Aroclor 1260, Appl. Environ. Microbiol. 73 (2007) 2513–2521.
[17] T. Yan, T.M. LaPara, P.J. Novak, The reductive dechlorination of 2,3,4,5-tetrachlorobiphenyl in three different sediment cultures: evidence for the involvement of phylogenetically similar Dehalococcoides-like bacterial populations, FEMS Microbiol. Ecol. 55 (2006) 248–261.
[18] K.-H. Oh, E.B. Ostrofsky, Y.-C. Cho, Molecular characterization of polychlorinated biphenyl-dechlorinating populations in contaminated sediments, J. Microbiol. 46 (2008) 165–173.
[19] J.L. Lake, R.J. Pruell, F.A. Osterman, An examination of dechlorination process and pathways in new Bedford Harbor sediments, Mar. Environ. Res. 33 (1992) 31–47.
[20] A.C. Alder, M.M. Häggblom, S. Oppenheimer, L.Y. Young, Reductive dechlorination of polychlorinated biphenyls in anaerobic sediments, Environ. Sci. Technol. 27 (1993) 530–538.
[21] G.D. Øfjord, J.A. Puhakka, J.F. Ferguson, Reductive dechlorination of Aroclor 1254 by marine sediment cultures, Environ. Sci. Technol. 28 (1994) 2286–2294.
[22] F. Fava, S. Gentilucci, G. Zanaroli, Anaerobic biodegradation of weathered polychlorinated biphenyls (PCBs) in contaminated sediments of Porto Marghera (Venice Lagoon, Italy), Chemosphere 53 (2003) 101–109.
[23] F. Fava, G. Zanaroli, L.Y. Young, Microbial reductive dechlorination of pre-existing PCBs and spiked 2,3,4,5,6-pentachlorobiphenyl in anaerobic slurries of a contaminated sediment of Venice Lagoon (Italy), FEMS Microbiol. Ecol. 44 (2003) 309–318.
[24] G. Zanaroli, J.R. Perez-Jimenez, L.Y. Young, L. Marchetti, F. Fava, Microbial reductive dechlorination of weathered and exogenous co-planar polychlorinated biphenyls (PCBs) in an anaerobic sediment of Venice Lagoon, Biodegradation 17 (2006) 19–27.
[25] W.G. Weisburg, S.M. Barns, D.A. Pelletier, D.J. Lane, 16S ribosomal DNA amplification for phylogenetic study, J. Bacteriol. 173 (1991) 697–703.
[26] D.J. Lane, 16S/23S rRNA sequencing, in: E. Stackebrandt, M. Goodfellow (Eds.), Nucleic Acid Techniques in Bacterial Systematics, John Wiley & Sons, New York, 1991, pp. 115–147.
[27] F.T. Robb, K.R. Sowers, S. DasSharma, A.R. Place, H.J. Schreier, Thermophiles, in: E.M. Fleischmann (Ed.), Archaea: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor, NY, 1995, pp. 202–203.
[28] A.M. Sass, H. Sass, M.J. Coolen, H. Cypionka, J. Overmann, Microbial communities in the chemocline of a hypersaline deep-sea basin (Urania basin, Mediterranean Sea), Appl. Environ. Microbiol. 67 (2001) 5392–5402.
[29] L. Raskin, J.M. Stromley, B.E. Rittmann, D.A. Stahl, Group-specific 16S rRNA hybridization probes to describe natural communities of methanogens, Appl. Environ. Microbiol. 60 (1994) 1232–1240.
[30] D.A. Stahl, R.I. Amann, Development and application of nucleic acid probes, in: E. Stackebrandt, M. Goodfellow (Eds.), Nucleic Acid Techniques in Bacterial Systematics, Wiley, New York, 1991, pp. 205–248.
[31] C.-E. Kuo, S.-M. Liu, C. Liu, Biodegradation of coplanar polychlorinated biphenyls by anaerobic microorganisms from estuarine sediments, Chemosphere 39 (1999) 1445–1458.
[32] M.J. Zwiernik, J.F. Quensen III, S.A. Boyd, $FeSO_4$ amendments stimulate extensive anaerobic PCB dechlorination, Environ. Sci. Technol. 32 (1998) 3360–3365.
[33] H.D. May, G.S. Miller, B.V. Kjellerup, K.R. Sowers, Dehalorespiration with polychlorinated biphenyls by an anaerobic ultramicrobacterium, Appl. Environ. Microbiol. 74 (2008) 2089–2094.
[34] V.K. Knight, L.J. Kerkhof, M.M. Häggblom, Community analyses of sulfidogenic 2-bromophenol-dehalogenating and phenol-degrading microbial consortia, FEMS Microbiol. Ecol. 29 (1999) 137–147.
[35] M.T. Suzuki, S.J. Giovannoni, Bias caused by template annealing in the amplification of mixtures of 16S rRNA genes by PCR, Appl. Environ. Microbiol. 62 (1996) 625–630.
[36] F. Von Wintzingerode, U.B. Gobel, E. Stackebrandt, Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis, FEMS Microbiol. Rev. 21 (1997) 213–229.