Effect of polyhydroxyalkanoates on the microbial reductive dechlorination of polychlorinated biphenyls and competing anaerobic respirations in a marine microbial culture

EFFECT OF POLYHYDROXYALKANOATES ON THE MICROBIAL REDUCTIVE DECHLORINATION OF POLYCHLORINATED BIPHENYLS AND COMPETING ANAEROBIC RESPIRATIONS IN A MARINE MICROBIAL CULTURE

Alberto Botti, Elena Biagi, Eliana Musmeci, Alessia Breglia, Micaela Degli Esposti, Fabio Fava, Giulio Zanaroli*

Dept. of Civil, Chemical, Environmental and Material Engineering (DICAM), Alma Mater Studiorum University of Bologna, Via Terracini 28, 40131 Bologna, Italy

*corresponding author (giulio.zanaroli@unibo.it)

Abstract

The effect of polyhydroxyalkanoates (PHAs) with different composition on the reductive dechlorination activity of a polychlorinated biphenyls (PCBs) dechlorinating marine microbial community and on the activity of sulfate-reducing (SRB) and methanogenic bacteria (MB), were investigated in marine sediment microcosms and compared with the main monomer, 3-hydroxybutyric acid (3HB). Despite PHAs were fermented more slowly than 3HB, all electron donors stimulated constantly sulfate-reduction, methanogenesis and, only transiently, PCB reductive dechlorination. No relevant differences were observed with different compositions of PHAs. According to electron balances, the majority of the supplied electrons (50%) were consumed by SRB and to less extent by MB (9-31%), while a small percentage (0.01%) was delivered to OHRB. In the studied conditions PHAs were confirmed as potential slow-hydrogen releasing compounds in marine environment but their fermentation rate was sufficiently high to mainly stimulate the competitors of organohalide respring bacteria for electron donors.

Keywords

Polychlorinated biphenyls (PCBs); Polyhydroxyalkanoates (PHAs); Reductive dehalogenation; Sulfate-reduction; Methanogenesis; Marine sediments

Introduction

Polychlorinated biphenyls (PCBs) are widespread persistent organic pollutants in marine sediments, for which a sustainable remediation approach is still lacking (Šrédlová and Cajthaml, 2022). It is known that PCBs may undergo microbial reductive dechlorination processes in marine sediments. Microbial reductive dechlorination is an anaerobic process that converts highly chlorinated PCB congeners into less chlorinated ones, thus promoting a partial sediment detoxification (Häggblom and Bossert, 2003; Yu et al., 2016). Bacteria involved in PCB reductive dehalogenation are organohalide respiring bacteria (OHRB) using PCBs as terminal electron acceptors of the respiratory chain and hydrogen and/or acetate as electron donors (Häggblom and Bossert, 2003). However, the time scale of microbial reductive dechlorination is usually of months/years (Payne et al., 2019; Zanaroli et al., 2010). The process can be primed via the addition of organic substrates that upon fermentation release electron donors for OHRB (Chang et al., 2006). One of the key points of biostimulation is to attain a long lasting release of reducing equivalents in time, so as to lower the application costs reducing the frequency of the amendments' replenishment (Koenigsberg et al., 2006). Starting from these

considerations, the use of biodegradable polymers is a promising approach for the stimulation of OHRB. Indeed, the hydrolysis step can slow down the release of readily fermentable organic matter over time, thus ensuring a prolonged, slow release of the electron donors used by OHRB (Koenigsberg and Sandefur, 1999). An additional important aspect of biostimulation is the presence of other anaerobic bacteria respiring different electron acceptors and competing with OHRB for hydrogen, such as sulfate-reducing (SRB), methanogenic (MB) and acetogenic bacteria (AB) (Häggblom and Bossert, 2003; Wiegel and Wu, 2000; Zanaroli et al., 2012). From a kinetic point of view, OHRB appear to be favored thanks to their higher affinity for hydrogen compared to their competitors (Häggblom and Bossert, 2003). Yet, the abundance of electron acceptors used by competitors can favor the latter when high concentrations of reducing equivalents are supplied. Hence, thanks to the hydrolysis step, biodegradable biopolymers could release the necessary amount of reducing equivalents in a longer time, resulting in lower hydrogen concentration (Aulenta et al., 2006). In this regard, PLA (polylactic acid) and sorbitol polylactate esters were successfully used to remediate groundwater sites polluted by tetrachloroethylene (PCE) (Koenigsberg et al., 2006; Koenigsberg and Sandefur, 1999). More recently, attention has been paid to polyhydroxyalkanoates (PHAs), a family of microbial biopolyesters that can be produced with pure and mixed cultures using a wide range of organic waste streams as feedstock. For instance, PHAs production was obtained from municipal wastewaters (Morgan-Sagastume et al., 2014) and from agro-industrial by-products such as grape pomace (Martinez et al., 2022). As for bioremediation purposes, the commercial homopolymer poly-3-hydroxybutyrate (PHB) and PHAs heteropolymers (poly(3-hydroxybutyrate-co-3-hydrolyvalerate)), have been proven effective in enhancing the microbial reductive dechlorination of chlorinated aliphatic hydrocarbons in polluted groundwater in laboratory and pilot-scale tests (Baric et al., 2014; Pierro et al., 2017). Conversely, no information is available on the effect of PHAs on microbial reductive dechlorination processes in marine environments, where the biodegradation of PHAs has been reported to be faster than in freshwater ecosystems (Kasuya et al., 1998; Mergaert et al., 1994) and may thus provide electron donors at higher concentrations to OHRB and their competitors. In addition, the anaerobic microbial community of marine sediments profoundly differs from freshwater environments. While in the latter MB and AB are the mostly active anaerobic microbes (Aulenta et al., 2008), in marine sediments the large amount of sulfates favors the growth of SRB, which are potentially stronger competitors of OHRB due their high affinity for hydrogen (Isa et al., 1986; Lovley et al., 1982; Lovley and Klug, 1983). Finally, limited information is available on the effect of PHAs composition on their biodegradation rate in marine environments. Indeed, it is not clear if the polymer composition can affect the hydrolysis rate, resulting in faster or slower fermentation rate. For example, a faster hydrolysis has been reported for

PHAs with higher content in the monomer 3-hydroxyvalerate (Kasuya et al., 1998; Mergaert et al., 1994). Instead, studies in real seawater (Doi et al., 1992; Volova et al., 2010) as well as aquarium tests mimicking real dynamic conditions (Thellen et al., 2008) did not show significant differences in the hydrolysis rate of films of heteropolymers with different compositions.

The objectives of this study were: i) to assess the suitability of PHAs as long-term, slow releasing electron donors to stimulate the microbial reductive dechlorination of PCBs in marine sediments; ii) to evaluate the effects of the supplemented PHAs on the main anaerobic competitors of OHRB (SRB, MB); and iii) to identify the influence of the polymer composition on the metabolic activities. To this aim, we investigated the effects of two PHAs with different composition (ratio of 3-hydroxybutyrate to 3-hydroxyvalerate 75:25 and 88:12 mol %, respectively), and of their main monomer 3-hydroxybutyrate as a rapidly fermentable control, on the reductive dechlorination activity of a PCB-dechlorinating marine microbial culture inoculated in marine sediment microcosms. Since in closed microcosm systems the consumption of natural electron acceptors may reduce their concentration to levels much lower than those occurring in natural open environments, thus altering the natural competition for electron donors between different terminal-electron accepting processes, consumed natural electron acceptors, namely sulfate, were periodically replenished during the study to maintain microcosms under actual site biogeochemical conditions.

Materials and methods

PCB dechlorinating culture, microcosms preparation, sampling and maintenance

A marine culture previously enriched with OHRB able to reductively dechlorinate PCBs (Nuzzo et al., 2017a) was cultivated in anaerobic slurry microcosms prepared with sediment and marine water collected in the Pialassa della Baiona, Ravenna, Italy. Microcosms were prepared in 100 mL glass serum bottles with 70 mL of sediment slurry (20% w/v of sediment) under anaerobic conditions (nitrogen gas in the headspace) and sealed with butyl rubber stopper and aluminum crimp. Under stirring and nitrogen flow, the anaerobic slurry was spiked with a 20'000 $mg \cdot L^{-1}$ stock solution of Aroclor 1254 in acetone to a final PCBs concentration of 100 $mg \cdot kg_{dry\ sediment}^{-1}$ and inoculated (5% v/v) with the PCB dechlorinating culture. Microcosms were then amended with one of the following organic electron donors (final concentration 20 mM): 3-hydroxybutyric acid (3HB) (from a 2.3 M stock solution in sterile distilled water), poly-3-hydroxybutyrate-co-3-hydroxyvalerate having a 3-hydroxybutyrate:3-hydroxyvalerate molar ratio 75:25 (PHBHV75) and poly-3-hydroxybutyrate-co-3-hydroxyvalerate having a 3-hydroxybutyrate:3-hydroxyvalerate molar ratio 88:12 (PHBHV88)

(both added as powder). Microcosms with no electron donors were set up as control. Each condition was prepared in triplicates. Microcosms were incubated statically in the dark at 30°C for 89 days. Periodic sampling (after 0, 30, 61, 75 and 89 days of incubation) was performed to analyze the volume and the composition of the head-space gas, the concentration of SO_4^{2-} in the water phase and the concentration of PCBs in the sediment. Each electron donor was supplied at the beginning of the incubation (day 0) and then monthly (i.e., on days 30 and 61), since 1 month was the estimated time required to completely ferment the added PHAs (Fig. S1). In addition, consumed SO_4^{2-} was replenished periodically to bring its concentration to the initial one (2.5 $g \cdot L^{-1}$) by adding a 2.1 M stock solution of Na_2SO_4, in particular on days 30, 61 and 75.

Preliminary test to estimate the time required to ferment the PHAs

The CO_2 production was identified as index to evaluate the fermentation activities stimulated by the PHAs. To do so, lab-scale microcosms were set-up as previously described. The stimulation effect of the PHAs was compared to a microcosm with no amendments, labeled as control. PHAs were added to the microcosm at the beginning of the experiment. Head-space gas was measured to monitor the CO_2 production. PHAs were considered to be completely degraded when the carbon dioxide production rate became equal to the one of the unamended control (Fig. S1) (Harrison et al., 2018).

Extraction and analysis of PCBs

PCBs in the sediment were extracted following a modified method from Rosato *et al.* (2020). Batch extraction was performed overnight at 30°C and 150 rpm from 1 mL of sediment slurry, with 3 mL of a hexane:acetone (9:1) mixture and octachloronaphtalene (OCN) (0.04 $mg \cdot L^{-1}$) as internal standard. The recovered organic phase was filtered on an Extrabond® Slica column (Scharlab, Barcelona, Spain) and added with 10 mg of elemental copper (Sigma Aldrich, St. Luis, Missouri, USA) as described in Riis and Babel (1999). An aliquot of the sample was placed in 1.5 mL vials for gas-chromatography (GC) equipped with Teflon coated screw caps (LLG-Labware, Meckenheim, Germany). The qualitative and quantitative analysis of the extracted PCBs was performed with a gas chromatograph (6890 series II) equipped with a HP-5 capillary column (30 m by 0.25 mm), a ^{63}Ni electron capture detector (μECD) and a 6890 series II automatic sampler (Agilent Technologies, Santa Clara, CA, USA). The column was operated at the following conditions: initial temperature 60°C; isothermal for 1 min; initial temperature rate 40°C/min; final temperature 140°C; isothermal for 2 min; initial temperature rate 1.5°C/min; final temperature 185°C; initial temperature rate 4.5°C/min; final temperature 275°C; isothermal for 5 min; injector (splitless mode), 250°C; detector ECD, 320°C; carrier gas flow rate (N_2) 1.5 mL/min; sample volume 1 μl. Aroclor PCBs, injected in the presence of OCN, were identified as described in Fava *et al.* (2003) by matching the detected

peaks with the chromatographic profiles of the standard PCB mixtures Aroclor 1254 and Aroclor 1242 previously characterized (Frame et al., 1996) and comparing the retention time (relative to OCN) of each peak with those of PCBs of the same standard Aroclors analyzed under identical conditions. Quantitative analysis of the freshly spiked PCBs and their possible dechlorination products was performed by using the GC-ECD response factor of each target PCB congener or group of co-eluting congeners obtained from six-points calibration curves (0.5-50 $mg \cdot L^{-1}$) of Aroclors 1254 and 1242 and the weight percentage of each congener occurring in the same Aroclors reported elsewhere (Frame, 1997). PCB concentrations were expressed as µmol of PCBs·$kg_{dry\ sediment}^{-1}$. The chlorination degree was calculated as average number of chlorines per biphenyl molecule, as showed in equation 1.

(1) $$Chlorination\ degree = \frac{\mu mol\ of\ organic\ chlorine}{\mu mol\ of\ total\ PCBs} = \frac{\sum C_i \times n_i}{\sum C_i}$$

Where C_i is the molar concentration of each detected PCB congener (µmol·$kg_{dry\ sediment}^{-1}$) and n_i is the number of its Cl substituents.

Analysis of sulfates, head-space gas and 3HB

Gas production in the microcosms was measured with an airtight syringe while its composition in CH_4, CO_2, N_2 and O_2 was analysed with a µGC (model 3000 A – Agilent Technologies, Milano, Italy) under the following conditions: injector temperature 90 °C; column temperature 60 °C; sampling time 20 s; injection time 50 ms; column pressure 25 psi; run time is 45 s and the carrier gas was nitrogen. The concentration of SO_4^{2-} in the water phase of the sediment slurry was determined using a Dionex ICS-1000 ion chromatograph equipped with an IonPac AS14 4 mm × 250 mm column, a conductivity detector combined to an AERS-500 suppressor system (Dionex, Sunnyvale, CA, USA). Quantitative analysis were performed by using the conductivity detector response factor obtained from a five points calibration curve (0.5-50 $mg \cdot L^{-1}$) of Na_2SO_4. 3HB was determined by HPLC-RID equipped with a Varian Hi-Plex H column (300 x 7.7 mm), under the following conditions: mobile phase, sulfuric acid 5 mM; flow rate, 0.6 mL/min; operating temperature, 65°C. For statistical analysis of headspace gas data and reduction of the chlorination degree, normality of the data distribution was tested using Shapiro-Wilk's test and significant differences were tested using two tailed t-test and 0.05 as significance threshold. R statistical software (https://www.r-project.org/) was used to perform statistics.

Electron and mass balances

The electrons equivalents supplied by each electron donor were calculated considering the moles of electron donor supplemented and its composition (i.e., the molar ratio of the monomeric units present in the two PHBHV polymers) and the stoichiometry of the chemical equations reported below (eq. 2, 3 and 4), assuming the complete oxidation of electron donors to CO_2 by fermenting communities including short-chain fatty acids oxidizing syntrophic bacteria (Leeson et al., 2004):

(2) 3-hydroxibutyric acid $C_4H_8O_3 + 5\,H_2O \rightarrow 4\,CO_2 + 9\,H_2$

(3) 3-hydroxyvaleric acid $C_5H_{10}O_3 + 7\,H_2O \rightarrow 5\,CO_2 + 12\,H_2$

(4) Hydrogen $H_2 \rightarrow 2\,H^+ + 2\,e^-$

The moles of electrons consumed by each metabolism were calculated considering the moles of electron acceptor reduced (i.e., moles of SO_4^{2-} reduced to H_2S for SRB; moles of organic Cl removed from the PCB mixture for OHRB) or of reduction product generated (i.e., moles of CH_4 produced from CO_2 reduction for MB), and the corresponding electrons required for the reduction of one mole of electron acceptor (8 electrons for SO_4^{2-} reduction, 2 electrons for PCB reduction and 8 electrons for methanogenesis). It has to be considered that PHAs are not directly used as reducing agents, but they are initially hydrolyzed and subsequently fermented to acetate and hydrogen, which act as the main electron donors for anaerobic respiring metabolisms (Amanat et al., 2021; Aulenta et al., 2008). The two main pathways, using H_2 or acetate, supply the same reducing power. In particular, CH_4 can be produced: i) by acetoclastic methanogens, which directly convert acetate to CH_4 and CO_2 (eq. 5); ii) via syntrophic acetate oxidation to CO_2 and H_2 (eq. 6), subsequently converted to methane (eq. 7) (Conrad, 2020). In both routes, one mole of methane is produced from one mole of acetate, which supplies 8 electrons.

(5) acetoclastic methanogenesis $C_2H_4O_2 \rightarrow CH_4 + CO_2$

(6) syntrophic acetate oxidation $C_2H_4O_2 + 2\,H_2O \rightarrow 4\,H_2 + 2\,CO_2$

(7) methanogenic conversion $CO_2 + 4\,H_2 \rightarrow CH_4 + 2\,H_2O$

Similarly, sulfate reducing bacteria can use hydrogen (eq. 8), as the product of syntrophic acetate oxidation (eq. 6), or directly acetate (eq. 9) as electron donors, requiring 8 electrons in the reduction process (Liamleam and Annachhatre, 2007).

(8) sulfate reduction via hydrogen $4\,H_2 + SO_4^{2-} + 2\,H^+ \rightarrow H_2S + 4\,H_2O$

(9) sulfate reduction via acetate $C_2H_4O_2 + SO_4^{2-} + 2\,H^+ \rightarrow H_2S + 2\,CO_2 + 2\,H_2O$

The stimulation yield (%) was calculated as the ratio between the moles of electrons consumed by each metabolism and the moles of electrons provided by the electron donor, multiplied times 100. It has to be noted that reducing equivalents can accumulate in the form of acetate, not being furtherly metabolized by anaerobic respiring bacteria (Aulenta et al., 2008; Liamleam and Annachhatre, 2007).

Chemicals

Aroclor 1242, Aroclor 1254 and octachloronaphtalene were provided by Ultra-Scientific. Inorganic ions for IC analysis, 3-hydroxybutyric acid and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) with 25 mol% 3 HV units (PHBHV75, powder, custom grade) and with 12 mol% 3 HV units(PHBHV88, powder, custom grade) were supplied by Sigma Aldrich. 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.

Bacterial DNA extraction, 16S rRNA gene amplification and sequencing

Approximately 300 mg of sediment samples taken from the inoculated sediment at the beginning of the experiment (0 day) and from all the microcosms at the end of the experiment (89 days) were used for characterization of the microbial community. The marine culture previously enriched with OHRB able to reductively dechlorinate PCBs used as inoculum was also analyzed. DNA extraction was performed using DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Extracted DNA samples were quantified using Qubit 3.0 fluorimeter (Invitrogen, Waltham, MA, USA) and stored at –20 °C until further processing. The V3-V4 hypervariable region of the 16S rRNA gene was PCR amplified in 50 uL final volume containing 25 ng of microbial DNA, 2X KAPA HiFi HotStart ReadyMix (Roche, Basel, Switzerland), and 200 nmol/L of microbial 341F and 785R primers carrying Illumina overhang adapter sequences (Klindworth et al., 2013). Thermal cycle was set as follows: 3 min at 95°C, 25 cycles of 30 s at 95°C, 30 s at 55°C, and 30 s at 72°C, and a final 5-min step at 72°C (Palladino et al., 2022). PCR products were purified using Agencourt AMPure XP magnetic beads (Beckman Coulter, Brea, CA, United States). Indexed libraries were prepared by limited-cycle PCR with Nextera technology and cleaned-up with the same magnetic beads protocol. Libraries were then normalized to 4 nM and pooled, prior to denaturation with 0.2 N NaOH. Sequencing was performed on Illumina MiSeq platform using a 2 × 250 bp paired-end protocol, following the manufacturer's instructions (Illumina, San Diego, CA, United States).

Bioinformatics and statistics

Paired-end sequenced reads were merged using the VSEARCH algorithm (v2.15.2) (Rognes et al., 2016) and analyzed using QIIME2 (version 2022.8) (Bolyen et al., 2019). Briefly, the DADA2 (Divisive Amplicon Denoising Algorithm 2) (Hall and Beiko, 2018) plugin was used to remove noise, chimeras, and to generate Amplicon Sequence Variants (ASVs). ASVs were taxonomically assigned using the SILVA reference database version 138 (Yilmaz et al., 2014). Normalization by rarefaction to the number of sequences in the sample with the least coverage was performed. Microbial community relative abundance profiles at different phylogenetic level were obtained. Statistical analysis was performed using the R statistical software (www.r-project.org), v. 4.0.4. Kruskal-Wallis rank-sum test, followed by Tukey's post hoc statistics, was used to assess significance of differences in microbial community profiles among groups of samples. Sequence reads were deposited in the National Center for Biotechnology Information Sequence Read Archive (NCBI SRA; BioProject ID PRJ PRJNA884891). Dataset was integrated with sequencing data from the pristine sediment, previously obtained (NCBI SRA; BioProject ID PRJNA841561, Biosample SAMN2859734).

Results and discussion

The effect of polyhydroxyalkanoates on the microbial activities in a marine PCB dechlorinating culture was assessed by monitoring the main terminal electron accepting processes, i.e., methanogenesis, sulfate-reduction, and PCB reductive dechlorination. Methane production and sulfate reduction were used as indicators to quantify the stimulation of OHRB competitors, while changes in the composition of the spiked PCB mixture over time was used to assess the enhancement of reductive dechlorination processes, i.e., OHRB. Additionally, the microbial community structure was analyzed to assess the changes in the community composition induced by the amendments and complement the information on the effects on metabolic activities.

Methanogenic activity

Methanogenic activity is reported in Fig. 1 as cumulative amount of methane (mM) produced over time. In the control microcosms, the final CH_4 concentration was 0.29 ± 0.06 mM. A remarkably higher CH_4 production was observed when stimulating with 3HB (53.1 ± 4.5 mM). It is known that methanogenic activities are favored in presence of high amount of electron donors, especially in marine environments, where MB can outcompete SRB when high concentrations of reducing equivalents are available (Isa et al., 1986). Thus, the large production of CH_4 observed when feeding with 3HB indicates a fast fermentation rate, and therefore a high production rate and concentration of hydrogen. An additional sampling was performed in the middle of the third month of incubation

(day 75) to better assess any changes in the microbial metabolisms rate in the month following the periodic supplementation of electron donors. No methanogenic activities were observed in the cultures amended with 3HB after day 75, indicating a complete depletion of the replenished fatty acid during the first two weeks of incubation (day 75). In the microcosms amended with PHAs, a lower stimulation of the methanogenic activity was detected (cumulative concentration 13.9 ± 3.5 and 16.4 ± 2.2 mM for PHBHV88 and PHBHV75, respectively) compared to those amended with the monomer. Moreover, a constant CH_4 production was observed during the third month of incubation (when the intermediate sampling was performed, at day 75). These data indicate a slower and prolonged fermentation of the two polymers compared to 3HB, and thus a hydrogen release control performed by the hydrolysis step. Furthermore, methane production did not significantly differ among the cultures amended with the two PHAs (p-value = 0.6), indicating a similar degradation rate and thus the lack of a significant effect of their composition on the hydrolysis step.

Sulfate reduction activity

Sulfate depletion was detected in the control microcosms only during the first month (Fig. S2) with an average depletion rate of 0.046 ± 0.001 $g \cdot L^{-1} \cdot day^{-1}$. In the following months the sulfate reduction activity was negligible in the absence of external electron donors, possibly due to the consumption of the majority of the indigenous electron donors. In parallel, a complete removal of sulfates was observed in all the amended microcosms after 30 days, (Fig. S2) corresponding to an apparent average depletion rate of at least 0.086 $g \cdot L^{-1} \cdot day^{-1}$. The complete consumption of sulfate (replenished to its original concentration at day 30, along with the electron donors) within one month of incubation in all the amended microcosms was confirmed at day 61 as well. The sulfate depletion rate in the microcosms amended with the different electron donors was thus assessed more accurately during the third month of experiment, when microcosms were sampled both two weeks (on day 75) and one month (on day 89) after sulfate replenishment to its original concentration at day 61 (along with electron donors). During the interval of time 61-75 days, 3HB remarkably primed sulfate reduction, that depleted sulfate with a rate of 0.22 ± 0.01 $g \cdot L^{-1} \cdot day^{-1}$ (Fig. 2). After further replenishment of the original sulfate concentration only (day 75), negligible sulfate depletion was detected in the period 75-89 days. The absence of stimulation of sulfate reducers after day 75 is in line with the lack of CH_4 production observed in the same period of incubation (Fig. 1), indicating a complete fermentation of the monomer within 14 days. Conversely, both PHAs stimulated sulfate reduction to a lesser extent (lower depletion rate) throughout the whole month (Fig. 2). In particular, sulfate depletion rates of 0.12 ± 0.01 $g \cdot L^{-1} \cdot day^{-1}$ and 0.09 ± 0.04 $g \cdot L^{-1} \cdot day^{-1}$ were observed in the incubation period 61-75 days

and after sulfate replenishment in the incubation period 75-89 days, respectively, in the presence of PHBHV75. Similar sulfate depletion rates were detected in the presence of PHBHV88 (0.13 ± 0.04 $g \cdot L^{-1} \cdot day^{-1}$ and 0.12 ± 0.04 $g \cdot L^{-1} \cdot day^{-1}$ in the incubation periods 61-75 days and 75-89 days, respectively). The more constant and prolonged stimulation of SRB exerted by PHAs compared to the monomer is in agreement with the constant methanogenic activity observed in the microcosms amended with PHAs during the third month of incubation (Fig. 1), and further supports the conclusion on a controlled, prolonged release of organic fermentable matter from PHAs due a rate-limiting hydrolysis step.

PCB Reductive dechlorination

Reductive dechlorination of PCBs occurred in the unamended control, leading at the end of the incubation (day 89) to the depletion of hexa- and penta-chlorinated congeners by 43 ± 11 and 49 ± 2 %, respectively, and to the concomitant increase of concentration of tetra-, tri- and di-chlorinated congeners (Fig. 3) with a dechlorination pattern similar to the one reported by Rosato *et al.* (2020) with the same marine dechlorinating culture. In particular, PCB dechlorination in the unamended control was negligible during the first month of incubation (0.9 ± 0.3 % reduction of the chlorination degree of the PCB mixture on day 30), starting from month 2 and progressively leading to a 14.4 ± 1.7 % reduction of the chlorination degree of the PCB mixture at the end of the incubation (Fig. 4).

The supplementation of 3HB, PHBHV75 and PHBHV88 stimulated the onset of PCB dechlorination, that started more rapidly during the first month leading to a significantly higher (p-values*:* 3HB, 0.0005; PHBHV75, 0.0002; PHBHV88, 0.04) decrease of the chlorination degree after 30 days of incubation compared to the unamended microcosms (7.4 ± 0.6 %, 5.3 ± 1.7 % and 7.0 ± 0.5 %, in the microcosms supplemented with 3HB, PHBHV88 and PHBHV75, respectively) (Fig. 4). However, a similar PCB dechlorination activity, both in terms of dechlorination pattern (data not shown) and final extent, was detected in all microcosms amended with electron donors at the end of the incubation, when the reduction of the chlorination degree (3HB, 14.0 ± 1.4%; PHBHV88 12.4 ± 1.1%; PHBHV75 15.3 ± 2.3%) did not significantly differ (p-values*:* 3HB, 0.8; PHBHV75, 0.6; PHBHV88, 0.2) from that of the unamended control (Fig. 4). The stimulation effect of electron donors was thus limited to the early growth stage of the OHRB of the inoculated marine community and was not affected by the use of PHAs (compared to the monomer 3HB) or their composition.

Electron balances and stimulation yield

The monitoring of anaerobic metabolisms competing with organohalide respiration for hydrogen suggested a different stimulation effect performed by the electron donors tested: the monomer appeared to be fermented faster than the polymers, enhancing methanogenic and sulfate reducing activities more than PHAs. Yet, no differences were identified in the stimulation of PCB reductive dehalogenation between the different electron donors. To better understand and to compare the effects of electron donors on the different terminal electron accepting processes, the balance between the electrons supplied by each amendment and the electrons consumed by each metabolism was calculated. Acetoclastic or hydrogenotrophic metabolisms were considered to consume the same amount of reducing equivalents as reported in the Materials and Methods section. The electrons provided by the complete oxidation to CO_2 of the electron donors were calculated under the following assumptions: i) the monomer was depleted within two weeks; ii) the heteropolymers were depleted in one month, with a constant degradation rate. Regarding the monomer, the complete consumption within two weeks was supported by the absence of sulfate reduction and methanogenic activities in the interval 75-89 days, i.e., 15 days after the amendment with 3HB. To confirm the assumption, at the end of the experiment, 3HB was re-supplied to the microcosms and the concentration of 3HB in time was monitored, confirming its complete degradation within 8 days (Fig. S3). As for the polymers, the degradation time was estimated considering the results of the preliminary fermentation test (Fig. S1). Given the similar methanogenic and sulfate-reduction rates detected during the first 2 weeks (days 61-75) after PHAs supplementation and the following 2 weeks (days 75-89), PHAs were assumed to be consumed constantly during the month, thus supplying half of the amended moles of electron equivalents every two weeks. The stimulation yield for each metabolism was then calculated considering the first 2 weeks after the supplementation of the amendments on day 61 (days 61-75) and the following 2 weeks (days 75-89). The majority of electron equivalents was consumed by sulfate reducers, with no remarkable differences between the electron donors (Fig. 5). SRB consumed 50 ± 2 % of the electrons provided by 3HB, 47 ± 4 % of the electrons provided by PHBHV75 and 57 ± 10 % of the electrons provided by PHBHV88. Conversely, MB consumed a higher fraction of the electron equivalents provided by the monomer (31.0 ± 0.9%) compared to those provided by the polymers (13 ± 3 % and 9 ± 7 % of electron equivalents provided by PHBHV75 and PHBHV88, respectively). Considering that MB typically have a lower affinity for hydrogen than SRB and OHRB (Häggblom and Bossert, 2003), the lower stimulation of MB performed by PHAs compared to the monomer is in agreement with a slower and more constant fermentation of the polymers due to the hydrolysis step, and thus a slower release of reducing equivalents. Reductive dehalogenation accounted for a negligible consumption of the reducing equivalents supplied by both the polymers

and the monomer (0.01-0.02%). The supplementation of the organic matter amended to the sediment reduced the lag-phase of dehalorespiring bacteria, possibly due to a stimulation of the indigenous fermentative microbes. Later on, the fermentation of the indigenous organic matter started to provide reducing equivalents in sufficient amount to meet the demand of OHRB. Such a low efficiency in priming the reductive dechlorination of PCBs by the electron donors might be due to the relative low amount of PCBs available as electron acceptors to OHRB (approximately $7 \cdot 10^{-3}$ mmol) and their lower bioavailability compared to other electron acceptors, in particular sulfate (approximately 2 mmol). Another factor that might have decreased the stimulation efficiency could be the low concentration of the inoculated OHRB in the marine culture in respect to the typical concentration of SRB in surface marine sediments (Leloup et al., 2007; Nuzzo et al., 2017b). Low priming efficiencies on PCB reductive dechlorination were reported previously also with other organic electron donors. For instance, Chang *et al.* (2006) used lactate (20 mM) in river sediments as amendment for PCB reductive dechlorination. From the data reported, stimulation yields on single PCB congeners were in the order of magnitude of 10^{-4} %. Moreover, low efficiencies were observed also when using PHAs to stimulate the reductive dechlorination of other chlorinated compounds, such as PCE. Aulenta *et al.* (2008) reported stimulation yields of ~ 2% on the reductive dechlorination of chlorinated solvents with concentration of PHAs 10 times lower than in this experiment (1.5 - 2 mM). Possibly, the higher yield obtained by Aulenta *et al.* (2008) compared to the one observed in this study could be due to the higher amount of halogenated electron acceptor available ($8 \cdot 10^{-2}$ mmol) and the absence of sulfates that prevented the strong competition of SRB. Moreover, the higher solubility and the higher Gibbs free energy of the reduction reaction of PCE make it a more bioavailable and more effective electron acceptor than PCBs (Chen and He, 2018; Holmes et al., 1993; Lombard et al., 2014), sustaining higher growth rates and biomass yields (Wang et al., 2014) that might explain the higher stimulation yields reported. Overall, the low stimulation yields obtained with electron donors in this study suggest that the supplemented amendments provided an excess of electron equivalents, and thus that PHAs were fermented by the marine microbial community too rapidly to stimulate efficiently and in a selective way OHRB, leading to the stimulation of competitors with lower affinity for the electron donor. This might be due to a very high intrinsic biodegradability of PHAs under marine conditions, and/or to the very high surface area available to microbes of the PHAs polymers, that were provided as fine powder.

Microbial community characterization

The composition of the bacterial communities in the original sediment, the microbial culture used as inoculum, the inoculated sediment at the beginning of the experiment, and all the microcosms at the end of the experiment (89 days), was analyzed by sequencing of the V3-V4 hypervariable region of the 16S rRNA gene. The sequencing yield per sample ranged between 90038 and 153802 high quality reads. Family-level community profile showed that amending with 3HB and PHBHV resulted in a pervasive modification of the community composition (Fig. S4). Indeed, after 89 days of incubation with 3HB, the sediment microbial community featured a significant dominance of *Dethiosulfatibacteraceae* (32 ± 8 %) with respect to day 0 (0.5 ± 0.8 %, p-value = 0.02). This group of bacteria is known to ferment hydroxyl-fatty acids, e.g. lactic acid (An et al., 2017; Matturro et al., 2017), and to use thiosulfate or sulfur as electron acceptor (Takii et al., 2007). Moreover, 3HB amended microcosms were the only ones in which sequences assigned to the acetoclastic methanogenic Archaea genus *Methanosaeta* were detectable (0.7 ± 0.6 %), supporting the higher methane production observed when adding the monomer. For what concern both PHBHV75 and PHBHV88, at day 89 a microbial community significantly dominated by *Spirochaetaceae* (22 ± 6 % in PHBHV75, 19 ± 10 % in PHBHV88, 0.6 ± 0.5 in day 0 samples; p = 0.03) was found, in line with previous reports which observed an enrichment of this group when amending microbial communities with polyhydroxyalkanoates (Matturro et al., 2018; Yang et al., 2020). *Spirochaetes* were addressed as possible acetogens in an enriched microbial community able to reductively dechlorinate TCE (Ziv-El et al., 2011) and have been reported among lineages encoding PHA depolymerases (Viljakainen and Hug, 2021). Conversely, the control microcosms maintained a community similar to the one of the inoculated sediments at day 0, without evident dominance at family level. For what concerns dehalogenation, the commonly recognized group of organohalide respiring bacteria are Dehalococcoidia, which were abundant in the inoculum (approximately 8%) and present to a lower percentage in the inoculated sediment (0.7 ± 0.5 %). After 89 days of incubation, sequences assigned to Dehalococcoidia were found to a similar amount in the unamended control (1.3 ± 0.4 %), whereas relative abundances equal to 0.1 %, 0.2 % and 0.2 % were identified respectively when amending with 3HB, PHBHV75 and PHBHV88. Considering that reductive dechlorination proceeded to a similar extent in all the studied conditions, the amendments clearly did not stimulate directly OHRB, but they caused a hyper proliferation of other bacterial species (namely *Dethiosulfatibacteraceae* and *Methanosaeta* in the case of 3HB or *Spirochaetaceae* when amending with PHAs), thus reducing the relative quantification of organohalide respiring bacteria.

Aspects possibly affecting PHA biostimulation efficiency

In this experiment, PHAs were investigated as potential biostimulating agents for PCB reductive dehalogenation in marine sediments, assessing their priming effect both on OHRB as well as on their main competitors for electron donors. Although the majority of the electrons equivalent provided by the heteropolymers primed the competing bacteria, the amendments partially stimulated PCB reductive dehalogenation, specifically during the early phase of incubation. Such a limited stimulation of dehalorespiration is in contrast with what reported in previous studies on the reductive dehalogenation of chlorinated solvents in freshwater systems, which indicated PHAs as effective stimulating agents. PHB was found to be an effective source of reducing equivalents in a pilot-scale plant treating real groundwater contaminated by PCE (Matturro et al., 2018; Pierro et al., 2017). A similar set-up was applied on a synthetic water contaminated by PCE, revealing a higher efficacy of the heteropolymer PHBHV in stimulating the reductive dechlorination process compared to PHB (Amanat et al., 2022). A partial stimulation of methanogenic bacteria was observed also in this case, but it did not negatively affect the reductive dechlorination process. The lower biostimulation efficacy of PHA on OHRB that was observed in this study compared to data reported in the literature is possibly due to a stronger competition by sulfate-reducing bacteria in the marine environment compared to freshwater systems, given the much higher concentration of sulfates available and consequently of actively growing SRB in anoxic marine sediments. As a matter of fact, sulfate-reduction was the main metabolism stimulated by the amendments and sulfate-reducing taxa represented an important fraction of the microbial community under all conditions tested (Fig. S4). Considering that OHRB can outcompete SRB at low hydrogen concentrations (Hoelen and Reinhard, 2004), a higher selectivity of PHA in biostimulating OHRB may be necessary to attain biostimulation of microbial reductive dehalogenation processes in marine environments. For instance, reducing the fermentation rate of PHA might avoid the hyperproliferation of competitors. Fermentation rate could be tuned by adjusting the specific surface area, being polymers degradation a surface process (Chinaglia et al., 2018). For example, a reduction in PHA degradation rate of up to 10 times was reported using thin films instead of polymer powder (Modelli et al., 1999). While in this study PHA were added to the sediment as powder, resulting in a too quick fermentation and fast release of reducing equivalents, the effects of granulometry, shape, and size (Colwell et al., 2017) on PHA fermentation rate and ability to stimulate more selectively OHRB in the marine environment should be further investigated. Moreover, the polymer composition could play an important role in defining the degradation rate. Although in this study the polymer's composition did not affect significantly its biostimulation effects, previous studies reported a slower fermentation of PHAs according to an increasing content of 3-hydroxyvalerate (Amanat *et al.*, 2022; Kaplan *et al*, 1994). Conversely, other biodegradation tests reported faster weight losses for heteropolymers with higher content of 3HV

(Kasuya et al., 1998; Mergaert et al., 1994). Considering the contradictory results reported in the literature, the fermentation rate of PHAs (including the homopolymer poly-3-hydroxybutyrate) might be dependent on the studied environment and further studies would be required to deepen the relationship between the polymer's composition and its degradation rate in different biogeochemical contexts.

Conclusions

Polyhydroxyalkanotes were found to be fermented more slowly than the monomer 3HB by a marine microbial community, and may thus act as potential slow hydrogen release compounds in marine environments. The fermentation rate of poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) was apparently not dependent on its composition, in the range 12-25% mol% 3 HV. PHAs stimulated the reductive dechlorination of PCBs only during the early phases of the incubation, while constantly stimulated the main competitors of OHRB for electron donors, i.e., SRB and to less extent MB. This was probably due to a high fermentation rate, resulting in high hydrogen concentrations. Hence, the use of PHAs as slow hydrogen release compounds for the stimulation of reductive dechlorination processes in marine environments might be promising, but further studies should optimize their use to increase the selectivity of the stimulating agents towards OHRB.

Acknowledgments

The research leading to these results has been conceived under the International PhD Program "Innovative Technologies and Sustainable Use of Mediterranean Sea Fishery and Biological Resources (www.FishMed-PhD.org). This study represents partial fulfilment of the requirements for the Ph.D. thesis of Alberto Botti.

Funding

This study was carried out under project TARANTO. TARANTO has received funding from the Italian MUR through the National Operational Programme on Research and Innovation 2014-2020, under grant agreement number ARS01_00637.

Bibliography

Amanat, N., Matturro, B., Rossi, M.M., Valentino, F., Villano, M., Papini, M.P., 2021. Assessment

of long-term fermentability of pha-based materials from pure and mixed microbial cultures for potential environmental applications. Water (Switzerland) 13, 897. https://doi.org/10.3390/w13070897

Amanat, N., Matturro, B., Villano, M., Lorini, L., Rossi, M.M., Zeppilli, M., Rossetti, S., Petrangeli Papini, M., 2022. Enhancing the biological reductive dechlorination of trichloroethylene with PHA from mixed microbial cultures (MMC). J. Environ. Chem. Eng. 10, 107047. https://doi.org/10.1016/j.jece.2021.107047

An, B.A., Shen, Y., Voordouw, G., 2017. Control of sulfide production in high salinity Bakken shale oil reservoirs by halophilic bacteria reducing nitrate to nitrite. Front. Microbiol. 8. https://doi.org/10.3389/fmicb.2017.01164

Aulenta, F., Fuoco, M., Canosa, A., Papini, M.P., Majone, M., 2008. Use of poly-β-hydroxy-butyrate as a slow-release electron donor for the microbial reductive dechlorination of TCE. Water Sci. Technol. 57, 921–925. https://doi.org/10.2166/wst.2008.073

Aulenta, F., Majone, M., Tandoi, V., 2006. Enhanced anaerobic bioremediation of chlorinated solvents: environmental factors influencing microbial activity and their relevance under field conditions. J. Chem. Technol. Biotechnol. 81, 1463–1474. https://doi.org/10.1002/jctb

Baric, M., Pierro, L., Pietrangeli, B., Papini, M.P., 2014. Polyhydroxyalkanoate (PHB) as a slow-release electron donor for advanced in situ bioremediation of chlorinated solvent-contaminated aquifers. N. Biotechnol. 31, 377–382. https://doi.org/10.1016/j.nbt.2013.10.008

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J.E., Bittinger, K., Brejnrod, A., Brislawn, C.J., Brown, C.T., Callahan, B.J., Caraballo-Rodríguez, A.M., Chase, J., Cope, E.K., Da Silva, R., Diener, C., Dorrestein, P.C., Douglas, G.M., Durall, D.M., Duvallet, C., Edwardson, C.F., Ernst, M., Estaki, M., Fouquier, J., Gauglitz, J.M., Gibbons, S.M., Gibson, D.L., Gonzalez, A., Gorlick, K., Guo, J., Hillmann, B., Holmes, S., Holste, H., Huttenhower, C., Huttley, G.A., Janssen, S., Jarmusch, A.K., Jiang, L., Kaehler, B.D., Kang, K. Bin, Keefe, C.R., Keim, P., Kelley, S.T., Knights, D., Koester, I., Kosciolek, T., Kreps, J., Langille, M.G.I., Lee, J., Ley, R., Liu, Y.X., Loftfield, E., Lozupone, C., Maher, M., Marotz, C., Martin, B.D., McDonald, D., McIver, L.J., Melnik, A. V., Metcalf, J.L., Morgan, S.C., Morton, J.T., Naimey, A.T., Navas-Molina, J.A., Nothias, L.F., Orchanian, S.B., Pearson, T., Peoples, S.L., Petras, D., Preuss, M.L., Pruesse, E., Rasmussen, L.B., Rivers, A., Robeson, M.S., Rosenthal, P., Segata, N., Shaffer, M., Shiffer, A., Sinha, R., Song, S.J., Spear, J.R., Swafford, A.D.,

Thompson, L.R., Torres, P.J., Trinh, P., Tripathi, A., Turnbaugh, P.J., Ul-Hasan, S., van der Hooft, J.J.J., Vargas, F., Vázquez-Baeza, Y., Vogtmann, E., von Hippel, M., Walters, W., Wan, Y., Wang, M., Warren, J., Weber, K.C., Williamson, C.H.D., Willis, A.D., Xu, Z.Z., Zaneveld, J.R., Zhang, Y., Zhu, Q., Knight, R., Caporaso, J.G., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37, 852–857. https://doi.org/10.1038/s41587-019-0209-9

Chang, B.-V., Chiu, T.-C., Yuan, S.-Y., 2006. Dechlorination of Polychlorinated Biphenyl Congeners by Anaerobic Microorganisms From River Sediment. Water Environ. Res. 78, 764–769. https://doi.org/10.2175/106143006x107380

Chen, C., He, J., 2018. Strategy for the Rapid Dechlorination of Polychlorinated Biphenyls (PCBs) by Dehalococcoides mccartyi Strains. Environ. Sci. Technol. 52, 13854–13862. https://doi.org/10.1021/acs.est.8b03198

Chinaglia, S., Tosin, M., Degli-Innocenti, F., 2018. Biodegradation rate of biodegradable plastics at molecular level. Polym. Degrad. Stab. 147, 237–244. https://doi.org/10.1016/j.polymdegradstab.2017.12.011

Colwell, J.M., Gauthier, E., Halley, P., Laycock, B., Nikoli, M., Bottle, S., George, G., 2017. Lifetime prediction of biodegradable polymers. Prog. Polym. Sci. 71, 144–189. https://doi.org/10.1016/j.progpolymsci.2017.02.004

Conrad, R., 2020. Importance of hydrogenotrophic, aceticlastic and methylotrophic methanogenesis for methane production in terrestrial, aquatic and other anoxic environments: A mini review. Pedosphere 30, 25–39. https://doi.org/10.1016/S1002-0160(18)60052-9

Doi, Y., Kanesawa, Y., Tanahashi, N., Kumagai, Y., 1992. Biodegradation of microbial polyesters in the marine environment. Polym. Degrad. Stab. 36, 173–177. https://doi.org/10.1016/0141-3910(92)90154-W

Fava, F., Zanaroli, G., Young, L.Y., 2003. 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, 309–318. https://doi.org/10.1016/S0168-6496(03)00069-2

Frame, G.M., 1997. A collaborative study of 209 PCB congeners and 6 Aroclors on 20 different HRGC columns: 1. Retention and coelution database. Fresenius. J. Anal. Chem. 357, 701–713. https://doi.org/10.1007/s002160050237

Häggblom, M.M., Bossert, I.D., 2003. Dehalogenation, Microbial Processes and Environmental Applications. Kluwer Academic Publishers, Rutgers University, USA.

Hall, M., Beiko, R.G., 2018. 16S rRNA Gene Analysis with QIIME2, in: Methods in Molecular Biology. Humana Press Inc., pp. 113–129.

Harrison, J.P., Boardman, C., O'Callaghan, K., Delort, A.M., Song, J., 2018. Biodegradability standards for carrier bags and plastic films in aquatic environments: A critical review. R. Soc. Open Sci. 5. https://doi.org/10.1098/rsos.171792

Hoelen, T.P., Reinhard, M., 2004. Complete biological dehalogenation of chlorinated ethylenes in sulfate containing groundwater. Biodegradation 15, 395–403. https://doi.org/10.1023/B:BIOD.0000044592.33729.d6

Holmes, D.A., Harrison, B.K., Dolfing, J., 1993. Estimation of Gibbs Free Energies of Formation for Polychlorinated Biphenyls. Environ. Sci. Technol. 27, 725–731. https://doi.org/10.1021/es00041a017

Isa, Z., Grusenmeyer, S., Verstraete, W., 1986. Sulfate Reduction Relative to Methane Production in High-Rate Anaerobic Digestion : Microbiological Aspects. Appl. Environ. Microbiol. 51, 580–587.

Kaplan, D.L., Mayer, J.M., Greenberger, M., Gross, R., McCarthy, S., 1994. Degradation methods and degradation kinetics of polymer films. Polym. Degrad. Stab. 45, 165–172. https://doi.org/10.1016/0141-3910(94)90133-3

Kasuya, K.I., Takagi, K.I., Ishiwatari, S.I., Yoshida, Y., Doi, Y., 1998. Biodegradabilities of various aliphatic polyesters in natural waters. Polym. Degrad. Stab. 59, 327–332. https://doi.org/10.1016/s0141-3910(97)00155-9

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., Glöckner, F.O., 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res. 41, 1–11. https://doi.org/10.1093/nar/gks808

Koenigsberg, S., Willett, A., Sutherland, M., 2006. Controlled release electron donors: Hydrogen release compound (HRC)-an overview of a decade of case studies. Bioremediat. J. 10, 45–57. https://doi.org/10.1080/10889860600842837

Koenigsberg, S.S., Sandefur, A.C., 1999. The Use of Hydrogen Release Compound for the

Accelerated Bioremediation of Anaerobically Degradable Contaminants : The Advent of The-Release Electron Donors. Remediation 10, 31–53.

Leeson, A., Beevar, E., Bruce, H., Fortenberry, J., Coyle, C., 2004. Principles and Practices of Enhanced Anaerobic Bioremediation of Chlorinated Solvents.

Leloup, J., Loy, A., Knab, N.J., Borowski, C., Wagner, M., Jørgensen, B.B., 2007. Diversity and abundance of sulfate-reducing microorganisms in the sulfate and methane zones of a marine sediment, Black Sea. Environ. Microbiol. 9, 131–142. https://doi.org/10.1111/j.1462-2920.2006.01122.x

Liamleam, W., Annachhatre, A.P., 2007. Electron donors for biological sulfate reduction. Biotechnol. Adv. 25, 452–463. https://doi.org/10.1016/j.biotechadv.2007.05.002

Lombard, N.J., Ghosh, U., Kjellerup, B. V., Sowers, K.R., 2014. Kinetics and threshold level of 2,3,4,5-tetrachlorobiphenyl dechlorination by an organohalide respiring bacterium. Environ. Sci. Technol. 48, 4353–4360. https://doi.org/10.1021/es404265d

Lovley, D.R., Dwyer, D.F., Klug, M.J., 1982. Kinetic Analysis of Competition Between Sulfate Reducers and Methanogens for Hydrogen in Sedimentst. Appl. Environ. Microbiol. 43, 1373–1379.

Lovley, D.R., Klug, M.J., 1983. Sulfate Reducers Can Outcompete Methanogens Sulfate Concentrationst at Freshwater. Appl. Environ. Microbiol. 45, 187–192.

Martinez, G.A., Puccio, S., Domingos, J.M.B., Morselli, E., Gioia, C., Marchese, P., Raspolli Galletti, A.M., Celli, A., Fava, F., Bertin, L., 2022. Upgrading grape pomace contained ethanol into hexanoic acid, fuel additives and a sticky polyhydroxyalkanoate: an effective alternative to ethanol distillation. Green Chem. 24, 2882–2892. https://doi.org/10.1039/d2gc00044j

Matturro, B., Frascadore, E., Rossetti, S., 2017. High-throughput sequencing revealed novel Dehalococcoidia in dechlorinating microbial enrichments from PCB-contaminated marine sediments. FEMS Microbiol. Ecol. 93, 1–10. https://doi.org/10.1093/femsec/fix134

Matturro, B., Pierro, L., Frascadore, E., Papini, M.P., Rossetti, S., 2018. Microbial community changes in a chlorinated solvents polluted aquifer over the field scale treatment with poly-3-hydroxybutyrate as amendment. Front. Microbiol. 9, 1–11. https://doi.org/10.3389/fmicb.2018.01664

Mergaert, J., Wouters, A., Anderson, C., Swings, J., 1994. In situ biodegradation of poly(3-

hydroxybutyrate) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) in natural waters. Can. J. Microbiol. 41, 154–159. https://doi.org/10.1139/m95-182

Modelli, A., Calcagno, B., Scandola, M., 1999. Kinetics of aerobic polymer degradation in soil by means of the ASTM D 5988-96 standard method. J. Environ. Polym. Degrad. 7, 109–116.

Morgan-Sagastume, F., Valentino, F., Hjort, M., Cirne, D., Karabegovic, L., Gerardin, F., Johansson, P., Karlsson, A., Magnusson, P., Alexandersson, T., Bengtsson, S., Majone, M., Werker, A., 2014. Polyhydroxyalkanoate (PHA) production from sludge and municipal wastewater treatment. Water Sci. Technol. 69, 177–184. https://doi.org/10.2166/wst.2013.643

Nuzzo, A., Hosseinkhani, B., Boon, N., Zanaroli, G., Fava, F., 2017a. Impact of bio-palladium nanoparticles (bio-Pd NPs) on the activity and structure of a marine microbial community. Environ. Pollut. 220, 1068–1078. https://doi.org/10.1016/j.envpol.2016.11.036

Nuzzo, A., Negroni, A., Zanaroli, G., Fava, F., 2017b. Identification of two organohalide-respiring Dehalococcoidia associated to different dechlorination activities in PCB-impacted marine sediments. Microb. Cell Fact. 16, 1–13. https://doi.org/10.1186/s12934-017-0743-4

Palladino, G., Caroselli, E., Tavella, T., D'Amico, F., Prada, F., Mancuso, A., Franzellitti, S., Rampelli, S., Candela, M., Goffredo, S., Biagi, E., 2022. Correction to: Metagenomic shifts in mucus, tissue and skeleton of the coral Balanophyllia europaea living along a natural CO2 gradient. ISME Commun. 2, 2022. https://doi.org/10.1038/s43705-022-00165-w

Payne, R.B., Ghosh, U., May, H.D., Marshall, C.W., Sowers, K.R., 2019. A Pilot-Scale Field Study: In Situ Treatment of PCB-Impacted Sediments with Bioamended Activated Carbon. Environ. Sci. Technol. 53, 2626–2634. https://doi.org/10.1021/acs.est.8b05019

Pierro, L., Matturro, B., Rossetti, S., Sagliaschi, M., Sucato, S., Alesi, E., Bartsch, E., Arjmand, F., Papini, M.P., 2017. Polyhydroxyalkanoate as a slow-release carbon source for in situ bioremediation of contaminated aquifers: From laboratory investigation to pilot-scale testing in the field. N. Biotechnol. 37, 60–68. https://doi.org/10.1016/j.nbt.2016.11.004

Riis, V., Babel, W., 1999. Removal of sulfur interfering in the analysis of organochlorines by GC-ECD. Analyst 124, 1771–1773.

Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahé, F., 2016. VSEARCH: A versatile open source tool for metagenomics. PeerJ 2016, 1–22. https://doi.org/10.7717/peerj.2584

Rosato, A., Barone, M., Negroni, A., Brigidi, P., Fava, F., Xu, P., Candela, M., Zanaroli, G., 2020.

Microbial colonization of different microplastic types and biotransformation of sorbed PCBs by a marine anaerobic bacterial community. Sci. Total Environ. 705, 135790. https://doi.org/10.1016/j.scitotenv.2019.135790

Šrédlová, K., Cajthaml, T., 2022. Recent advances in PCB removal from historically contaminated environmental matrices. Chemosphere 287, 132096. https://doi.org/10.1016/j.chemosphere.2021.132096

Takii, S., Hanada, S., Tamaki, H., Ueno, Y., Sekiguchi, Y., Ibe, A., Matsuura, K., 2007. Dethiosulfatibacter aminovorans gen. nov., sp. nov., a novel thiosulfate-reducing bacterium isolated from coastal marine sediment via sulfate-reducing enrichment with Casamino acids. Int. J. Syst. Evol. Microbiol. 57, 2320–2326. https://doi.org/10.1099/ijs.0.64882-0

Thellen, C., Coyne, M., Froio, D., Auerbach, M., Wirsen, C., Ratto, J.A., 2008. A processing, characterization and marine biodegradation study of melt-extruded polyhydroxyalkanoate (PHA) films. J. Polym. Environ. 16, 1–11. https://doi.org/10.1007/s10924-008-0079-6

Viljakainen, V.R., Hug, L.A., 2021. The phylogenetic and global distribution of bacterial polyhydroxyalkanoate bioplastic-degrading genes. Environ. Microbiol. 23, 1717–1731. https://doi.org/10.1111/1462-2920.15409

Volova, T.G., Boyandin, A.N., Vasiliev, A.D., Karpov, V.A., Prudnikova, S. V., Mishukova, O. V., Boyarskikh, U.A., Filipenko, M.L., Rudnev, V.P., Bá Xuân, B., Vit Dũng, V., Gitelson, I.I., 2010. Biodegradation of polyhydroxyalkanoates (PHAs) in tropical coastal waters and identification of PHA-degrading bacteria. Polym. Degrad. Stab. 95, 2350–2359. https://doi.org/10.1016/j.polymdegradstab.2010.08.023

Wang, S., Chng, K.R., Wilm, A., Zhao, S., Yang, K.L., Nagarajan, N., He, J., 2014. Genomic characterization of three unique Dehalococcoides that respire on persistent polychlorinated biphenyls. Proc. Natl. Acad. Sci. 111, 12103–12108. https://doi.org/10.1073/pnas.1404845111

Wiegel, J., Wu, Q., 2000. Microbial reductive dehalogenation of polychlorinated biphenyls. Fems Microbiol. Ecol. 32, 1–15.

Yang, Z., Sun, H., Zhou, Q., Zhao, L., Wu, W., 2020. Nitrogen removal performance in pilot-scale solid-phase denitrification systems using novel biodegradable blends for treatment of waste water treatment plants effluent. Bioresour. Technol. 305, 122994. https://doi.org/10.1016/j.biortech.2020.122994

Yilmaz, P., Parfrey, L.W., Yarza, P., Gerken, J., Pruesse, E., Quast, C., Schweer, T., Peplies, J.,

Ludwig, W., Glöckner, F.O., 2014. The SILVA and "all-species Living Tree Project (LTP)" taxonomic frameworks. Nucleic Acids Res. 42, 643–648. https://doi.org/10.1093/nar/gkt1209

Yu, H., Feng, C., Liu, X., Yi, X., Ren, Y., Wei, C., 2016. Enhanced anaerobic dechlorination of polychlorinated biphenyl in sediments by bioanode stimulation. Environ. Pollut. 211, 81–89. https://doi.org/10.1016/j.envpol.2015.12.039

Zanaroli, G., Balloi, A., Negroni, A., Daffonchio, D., Young, L.Y., Fava, F., 2010. Characterization of the microbial community from the marine sediment of the Venice lagoon capable of reductive dechlorination of coplanar polychlorinated biphenyls (PCBs). J. Hazard. Mater. 178, 417–426. https://doi.org/10.1016/j.jhazmat.2010.01.097

Zanaroli, G., Negroni, A., Vignola, M., Nuzzo, A., Shu, H.Y., Fava, F., 2012. Enhancement of microbial reductive dechlorination of polychlorinated biphenyls (PCBs) in a marine sediment by nanoscale zerovalent iron (NZVI) particles. J. Chem. Technol. Biotechnol. 87, 1246–1253. https://doi.org/10.1002/jctb.3835

Ziv-El, M., Delgado, A.G., Yao, Y., Kang, D.W., Nelson, K.G., Halden, R.U., Krajmalnik-Brown, R., 2011. Development and characterization of DehaloR 2, a novel anaerobic microbial consortium performing rapid dechlorination of TCE to ethene. Appl. Microbiol. Biotechnol. 92, 1063–1071. https://doi.org/10.1007/s00253-011-3388-y

Figure captions

Figure 1. Cumulative methane production in the control, unamended microcosms (CTR), and in those amended with the monomer (3HB) and PHA (PHBHV75 and PHBHV88). Electron donors were supplemented at the beginning of the incubation (day 0) and on days 30 and 61.

Figure 2. Sulfate depletion rate detected in the first two weeks (days 61-75) and in the following two weeks (days 75-89) of the third month of experiment (days 61-89). Sulfate consumed during the first two weeks was replenished to its original concentration on day 75.

Figure 3. Concentration of PCB congeners detected in the unamended control microcosm at the beginning and at the end of the incubation (day 89).

Figure 4. Percentage reduction of the chlorination degree of the PCB mixture during incubation

Figure 5. Stimulation yields of SRB, MB and OHRB for each electron donor in the 15-day period immediately after replenishment of electron donors (61-75 days, green rectangle) and in the following one (75-89 days, yellow rectangle).

Figure 1

Click here to access/download;Figure(s);Fig1.tif

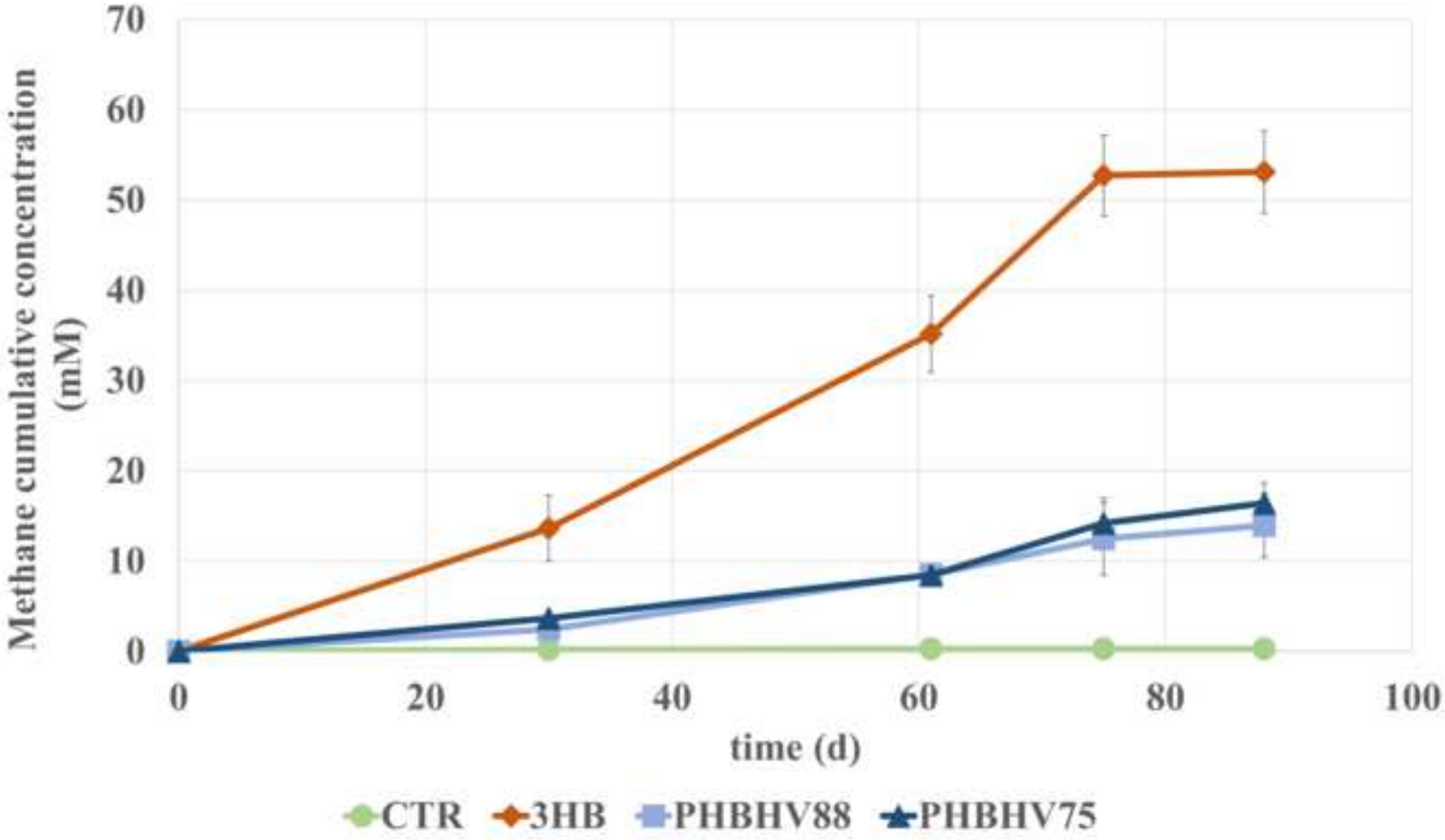


Figure 2

Click here to access/download;Figure(s);Fig2.tif

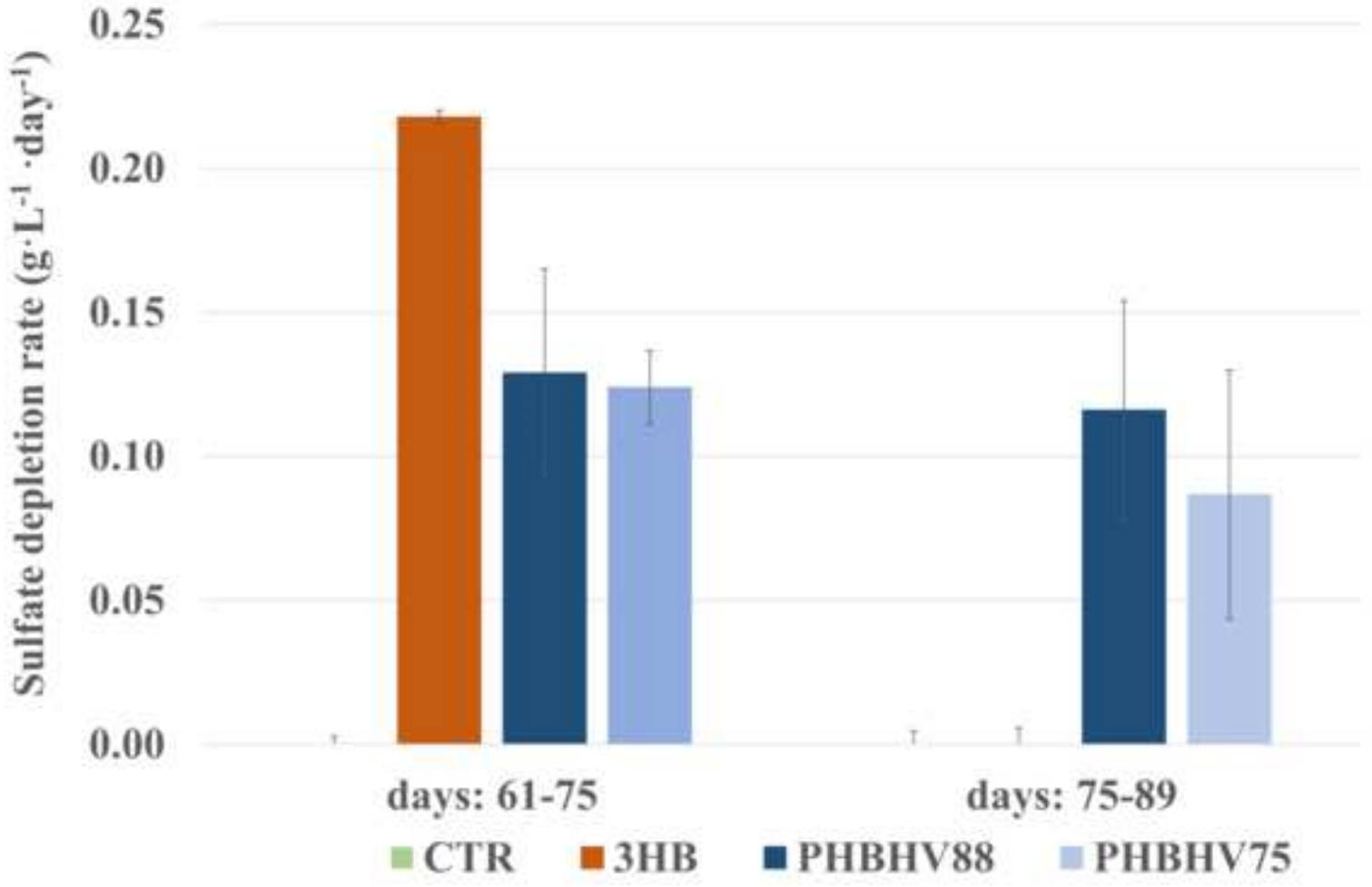


Figure 3

Click here to access/download;Figure(s);Fig3.tif

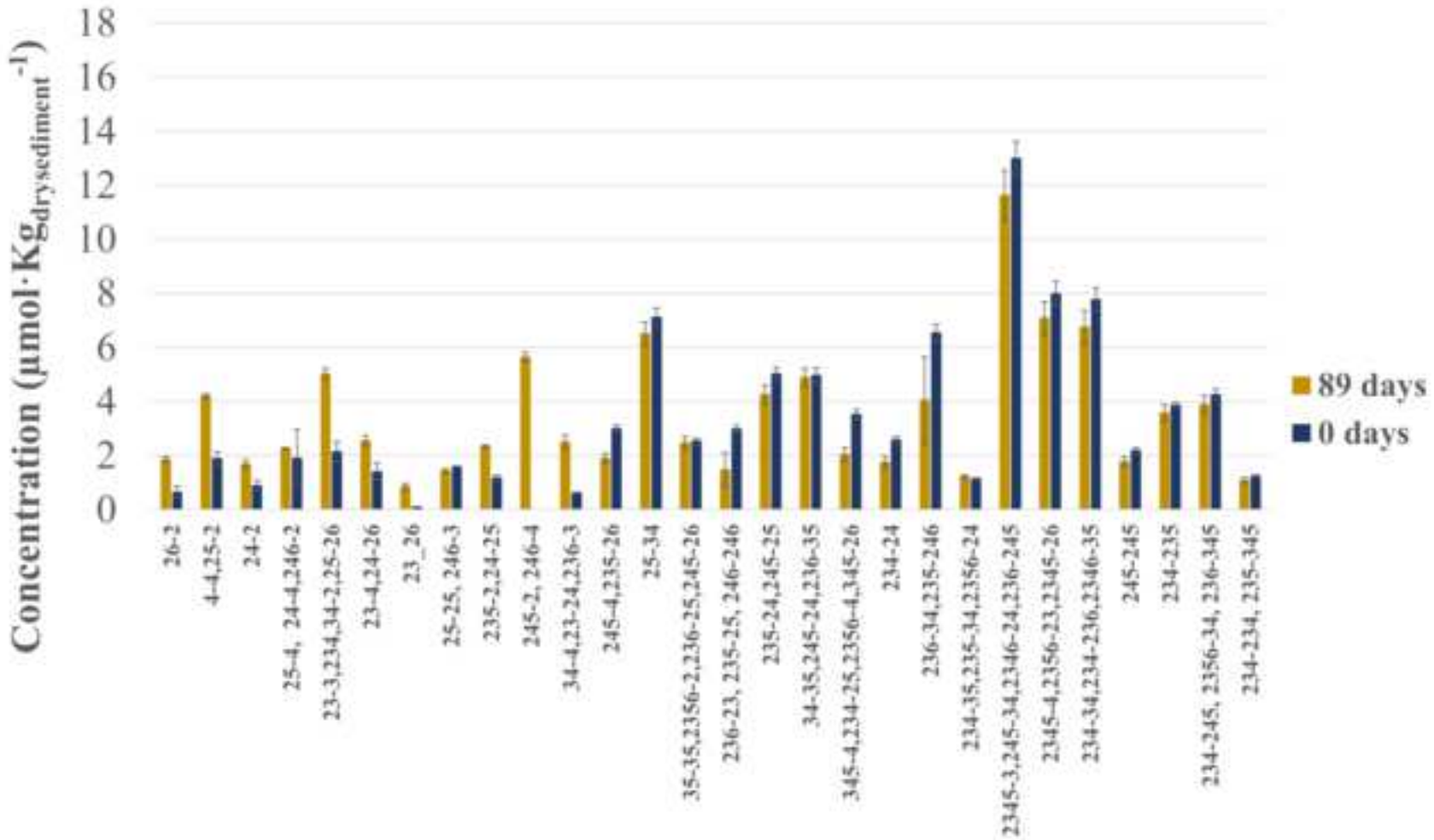


Figure 4

Click here to access/download;Figure(s);Fig4.tif

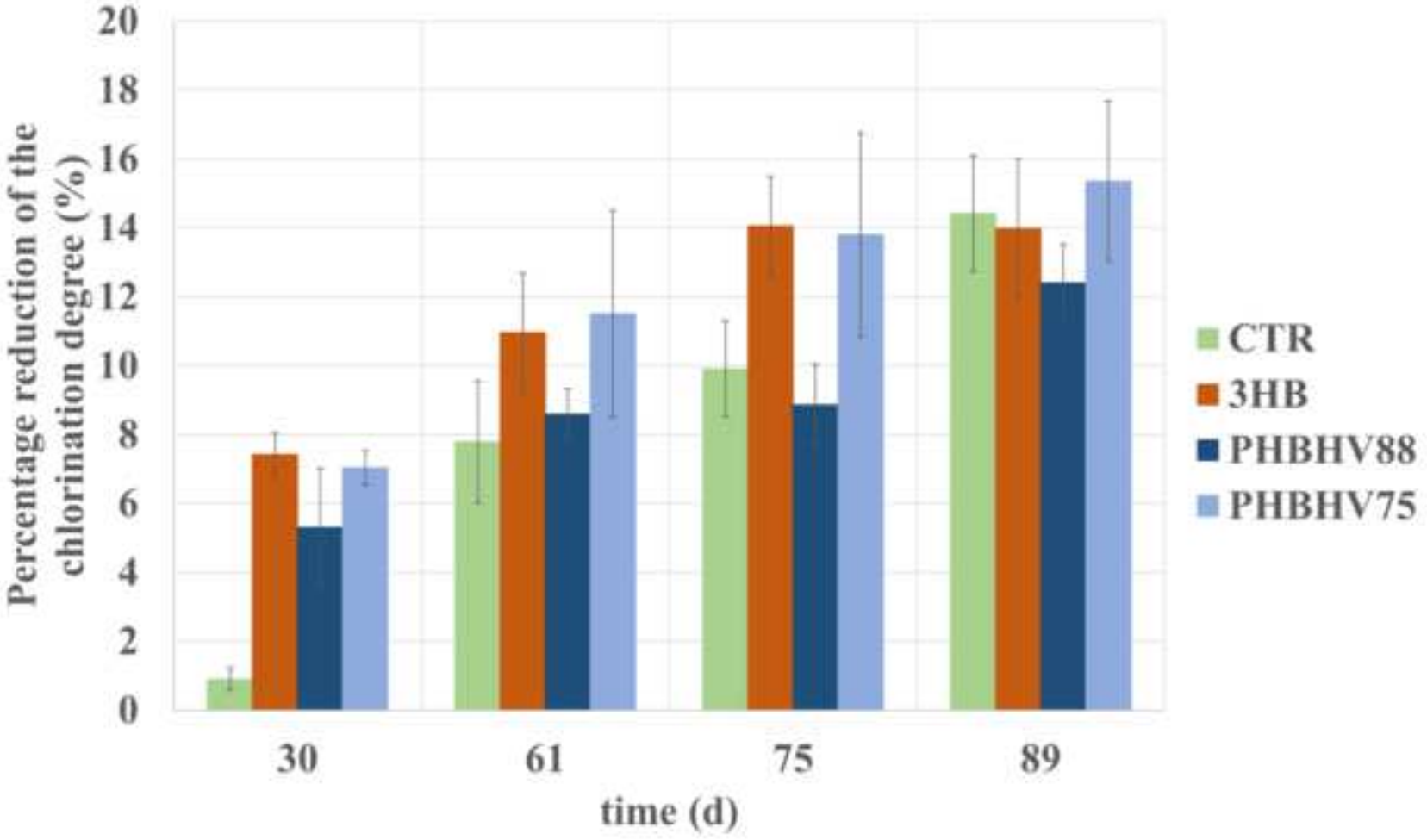
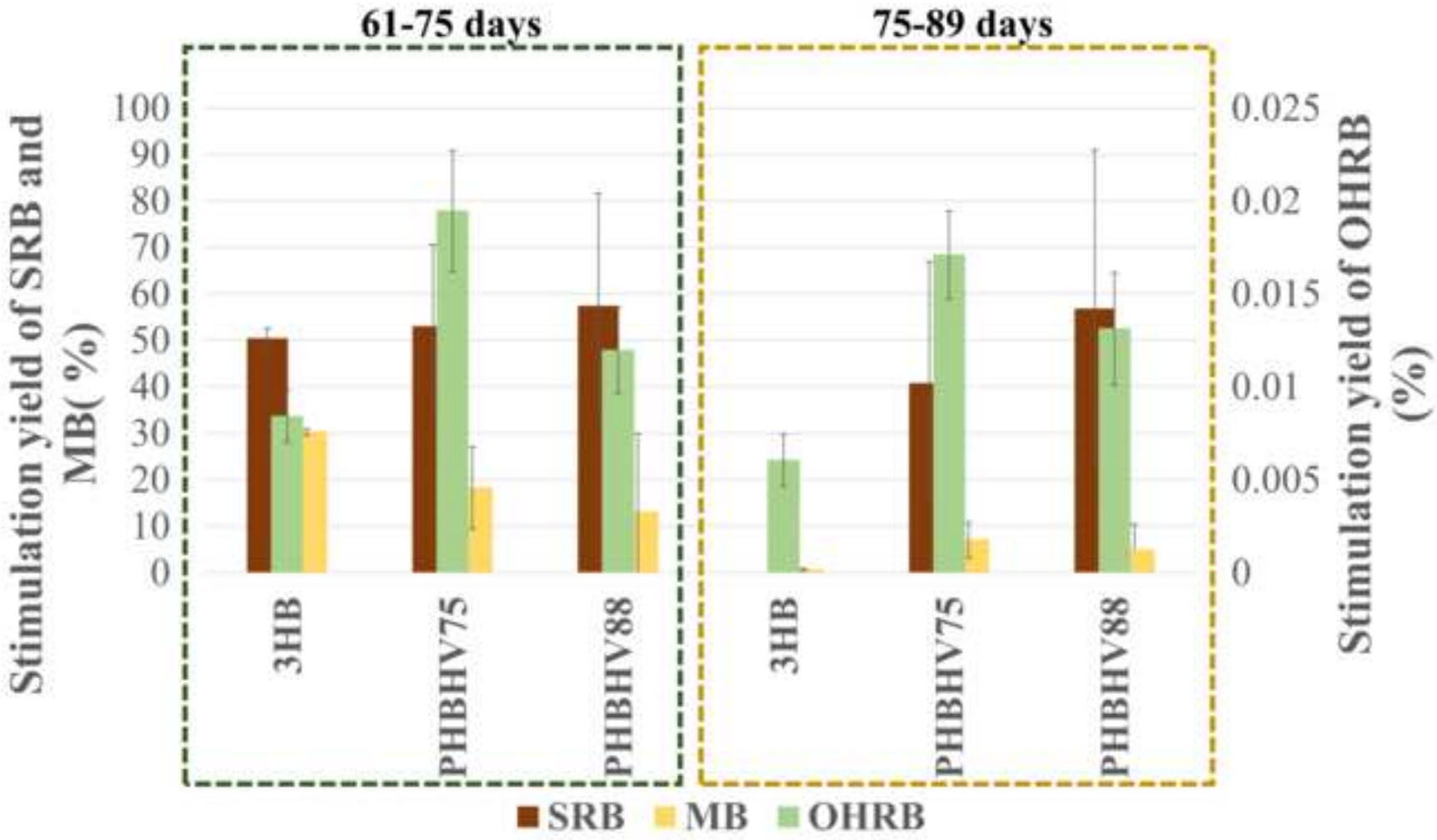


Figure 5



Supplementary information for the manuscript:

EFFECT OF POLYHYDROXYALKANOATES ON THE MICROBIAL REDUCTIVE DECHLORINATION OF POLYCHLORINATED BIPHENYLS AND COMPETING ANAEROBIC RESPIRATIONS IN A MARINE MICROBIAL CULTURE

Alberto Botti, Elena Biagi, Eliana Musmeci, Alessia Breglia, Micaela Degli Esposti, Fabio Fava, Giulio Zanaroli*

Dept. of Civil, Chemical, Environmental and Material Engineering (DICAM), Alma Mater Studiorum University of Bologna, Via Terracini 28, 40131 Bologna, Italy

*corresponding author (giulio.zanaroli@unibo.it)

Summary:

S1

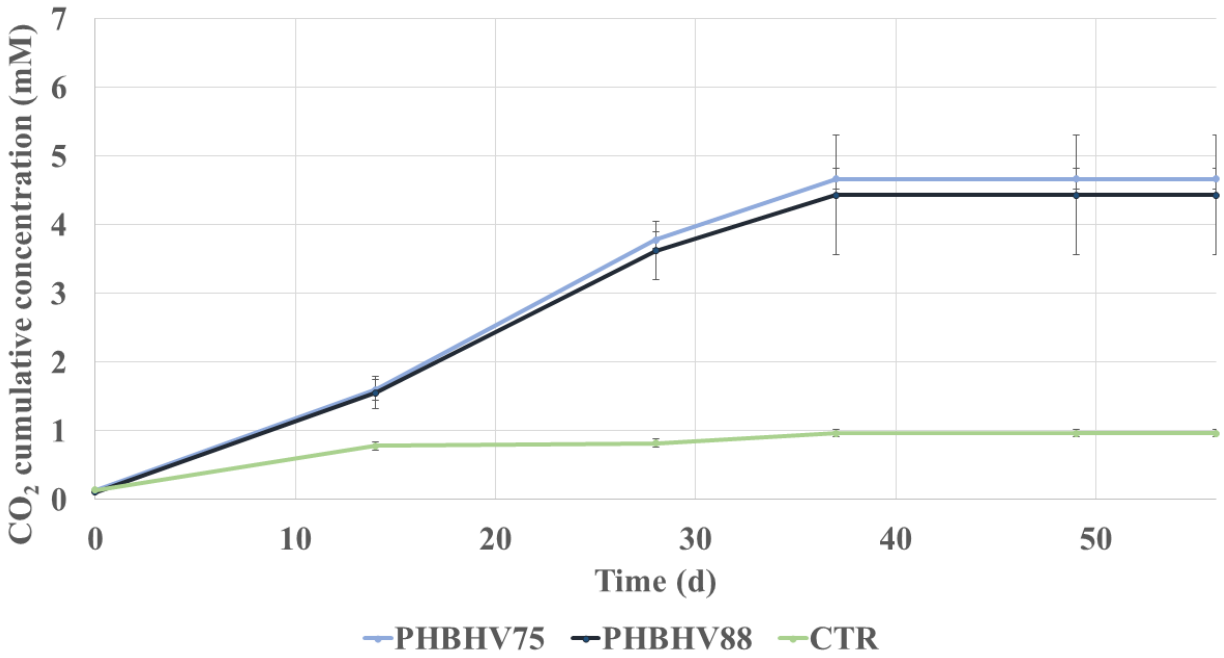


Figure S1. Cumulative CO_2 production in the control, unamended microcosms (CTR), and in those amended with PHAs (PHBHV75 and PHBHV88).

Fig. S1 illustrates the CO_2 cumulative production observed in lab-scale microcosms of a previous experiment. The incubation time required to complete degrade the heteropolymers was estimated from the gas profile. On day 37, the CO_2 cumulative concentrations were 4.7 ± 0.1 for PHBHV75, 4.4 ± 0.9 mM or PHBHV88 and 0.96 ± 0.06 mM for the control. A plateau in the gas production was observed after 37 days from the addition of the heteropolymers. Thus it was speculated that in the studied condition the polymers were depleted in less than 37 days.

S2

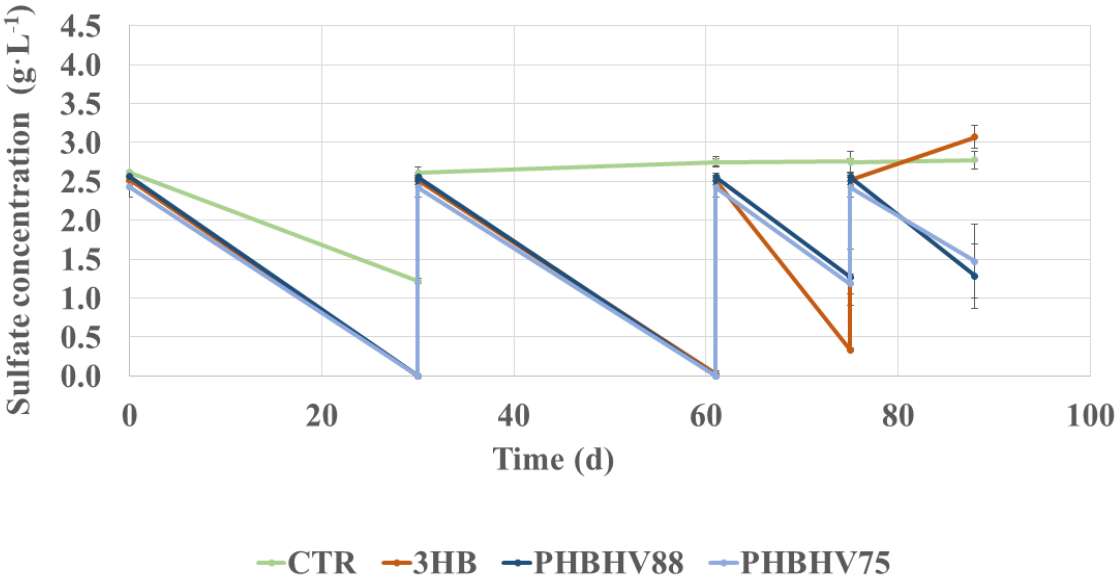


Figure S2. Profile in time of the sulfate concentrations of the aqueous phase in the control, unamended microcosms (CTR), and in those amended with the monomer (3HB) and PHAs (PHBHV75 and PHBHV88). Sulfates were replenished on days 30 and 61.

S3

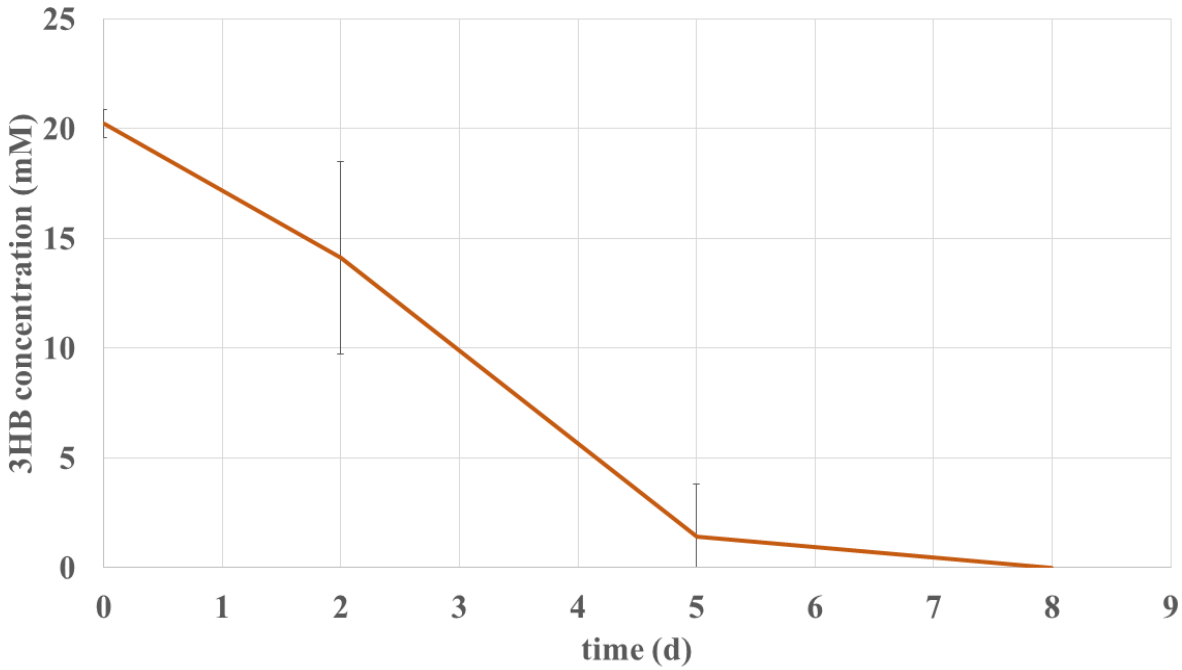


Figure S3. Profile in time of 3HB concentration in the aqueous phase in the lab-scale microcosms

S4

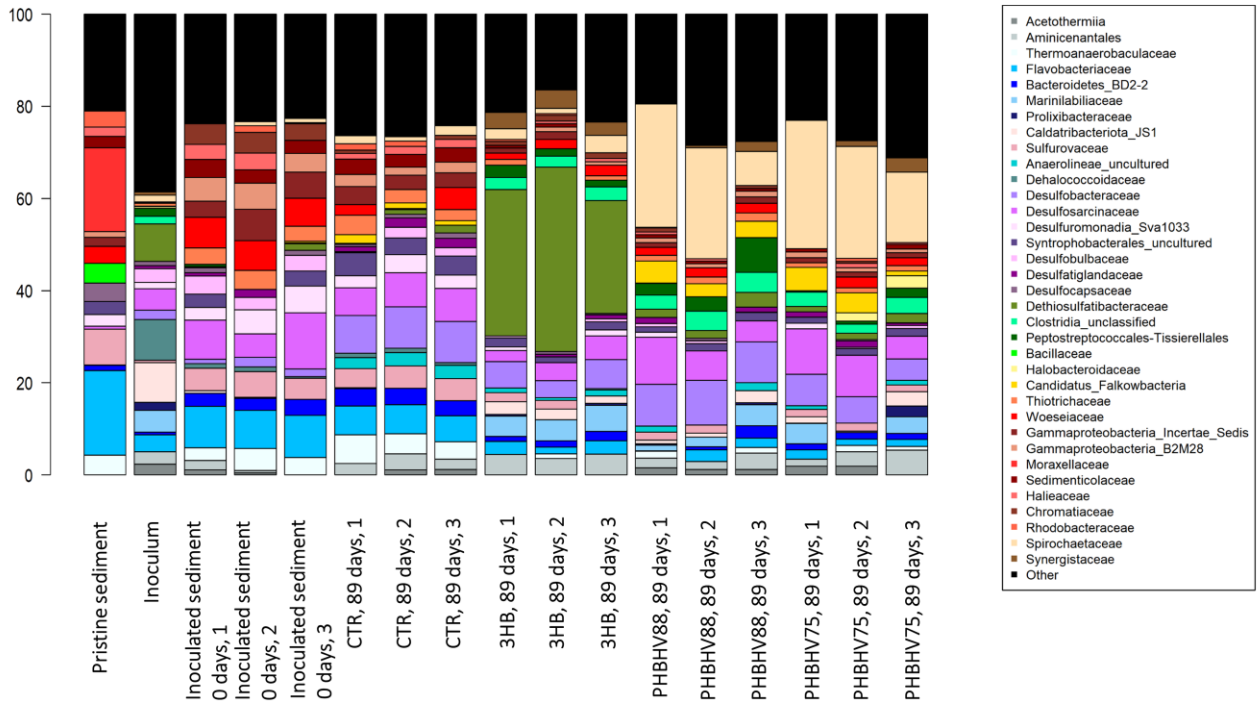


Figure S4. Microbial community phylogenetic profiles at the family level of the pristine sediment, inoculum, inoculated sediment at the beginning of the experiment (0 day) and of all the studied microcosms at the end of the experiment (89 days). Bacterial families having relative abundance >2% in at least 1 sample are depicted. Color legend is shown in the right panel. Black color is used to indicate the percentage of "Other" reads, including unassigned sequences and families with a relative abundance which did not pass the mentioned threshold.