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The influence of anaerobic dechlorination on the aerobic degradation of PCBs in e-waste-contaminated soils in an anaerobic-aerobic two-stage treatment

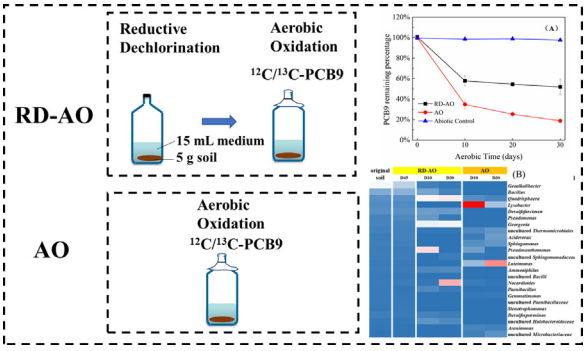
Shuang Wang [a,b,c], Jibing Li [a,b], Longfei Jiang [a,b,*], Shanquan Wang [d], Xuan Zhao [a,b,c], Yeliang Dai [a,b,c], Chunling Luo [a,b,*], Gan Zhang [a,b]

[a] State Key Laboratory of Organic Geochemistry and Guangdong-Hong Kong-Macao Joint Laboratory for Environmental Pollution and Control, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou 510640, China
[b] CAS Center for Excellence in Deep Earth Science, Guangzhou 510640, China
[c] University of Chinese Academy of Sciences, Beijing 100049, China
[d] Environmental Microbiomics Research Center, School of Environmental Science and Engineering, Guangdong Provincial Key Laboratory of Environmental Pollution Control and Remediation Technology, Southern Marine Science and Engineering Guangdong Laboratory (Zhuhai), Sun Yat-Sen University, Guangzhou 510275, China

HIGHLIGHTS

- Anaerobic treatment had a negative impact on aerobic PCB degradation.
- Anaerobic treatment changed the PCB aerobic degrading bacteria.
- Four microbes belonging to different genera were firstly linked with PCB degradation.

GRAPHICAL ABSTRACT



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ABSTRACT

The combination of microbial reductive dechlorination and aerobic oxidation (RD-AO) process was proposed to be a promising strategy for extensive bioremediation of highly chlorinated polychlorinated biphenyls (PCBs). Nonetheless, experimental evidence on the impact of the RD on subsequent AO in anaerobic-aerobic two-stage treatment remains scarce. The present study applied stable-isotope probing (SIP) to explore the RD-AO mediated degradation of PCBs in an e-waste-contaminated soil. The RD-AO treatment resulted in 37.1 % and 48.2 % degradation of PCB180 and PCB9, respectively, while the PCB9 degradation efficiency decreased compared to the sole AO (81.2 %). The inhibition of PCB aerobic degradation might be caused by the alteration of aerobic bacterial community, which was proved by a higher abundance of anaerobic bacteria and a lower abundance of aerobic bacteria being observed in the aerobic stage of RD-AO. Further evidence was obtained using DNA-SIP that the anaerobic stage altered the PCB degraders' community structures and changed three of the five degraders. There were four lineages (*Arenimonas, Steroidobacter, Sulfurifustis*, and *Thermoanaerobacterales*) identified as PCB degraders for the first time. Interestingly, three of them were found in RD-AO microcosm, suggesting that anaerobic-aerobic two-stage treatment can recruit novel bacteria

* Corresponding authors at: State Key Laboratory of Organic Geochemistry and Guangdong-Hong Kong-Macao Joint Laboratory for Environmental Pollution and Control, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou 510640, China.
E-mail address: clluo@gig.ac.cn (C. Luo).

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involved in PCBs aerobic degradation. The present study provided novel insight into the synergistic integration of anaerobic and aerobic processes for extensive degradation of highly chlorinated PCBs.

1. Introduction

Large amounts of electronic waste (e-waste) have been produced by the increasing demand for electronic products and their frequent renewal. *E*-waste recycling can retrieve valuable and reusable materials, such as copper, palladium, gold, and plastics (Zhang et al., 2012). However, the crude dismantling process and improper disposal of solid wastes during e-waste recycling lead to the release of persistent organic pollutants (POPs) and heavy metals to the surrounding environment (Wong et al., 2007; Luo et al., 2011; Wu et al., 2019). Polychlorinated biphenyls (PCBs) are a class of POPs released from e-waste recycling activities that are extremely difficult to be eliminated due to their chemicophysical stability. They therefore pose risks to ecosystems and human health (Bentum et al., 2016; Desborough et al., 2016; Zhao et al., 2018). Much attention has been given to the potential toxicity, transformation, and degradation of PCBs in e-waste-contaminated sites (Jiang et al., 2018; Lu et al., 2020).

Bioremediation has been explored as an approach to remediate PCB-contaminated sites due to its advantages over traditional physical/chemical methods (Ran et al., 2018; Kaur et al., 2021). Bioremediation techniques applied to PCBs include anaerobic dechlorination and aerobic degradation. Highly chlorinated PCBs (≥ 5 chlorine atoms) can be reductively dechlorinated via microbial reductive dechlorination (RD) process, while lightly chlorinated PCBs (< 5 chlorine atoms) can be aerobically degraded into harmless compounds through aerobic oxidation (AO) process (Field and Sierra-Alvarez, 2008; Hughes et al., 2010). Therefore, the combination of RD and AO processes is considered to be a promising strategy to achieve extensive degradation of PCBs, especially highly chlorinated ones (Kaur et al., 2021). For example, Master et al. (2002) found that PCB-contaminated soil exposed to anaerobic treatment 120 days, followed by 28 days of aerobic treatment, resulted in a 67 % reduction in total PCBs. Chen et al. (2014) found that a sequential flooding (180 days)-drying (60 days) treatment produced a higher PCB removal rate in a paddy field microcosm than a sterilized control and constant-drying system. Hence, an anaerobic-aerobic two-stage treatment could improve the degradation of highly chlorinated PCBs compared to anaerobic or aerobic treatment alone (Master et al., 2002; Payne et al., 2013; Chen et al., 2014; Long et al., 2015; Pathiraja et al., 2019a; Pathiraja et al., 2019b). Nonetheless, researchers also reported the negative impact of anaerobic on aerobic bacteria in subsequent aerobic treatment (Zeng et al., 2010; Wang et al., 2018). Moreover, specific changes of functional microorganisms and microbial community composition and diversity in the integrated anaerobic and aerobic PCB-degrading processes remain unclear.

It is difficult to identify functional microorganisms in the environment by traditional cultivation methods, because most microorganisms are unculturable and some cultured microorganisms are inactive in situ. Stable-isotope probing (SIP) is a culture-independent approach that links microbial communities with their functions in complex environments by assimilating stable isotope-labeled substrates (e.g., ^{13}C or ^{15}N) into isotope-enriched cellular components (such as DNA, RNA, and protein) (Radajewski et al., 2000). By obtaining and analyzing the cellular components with molecular biology methods, functional microbes can be identified (Dumont and Murrell, 2005; Li et al., 2019b). This technique has been successfully applied to identify the active microbes involved in aerobic biphenyl/PCB degradation in PCB-contaminated soils (Tillmann et al., 2005; Leigh et al., 2007; Koubek et al., 2013; Leewis et al., 2016; Jiang et al., 2018).

To explore the influence of the anaerobic dechlorination process on aerobic PCB degradation, 2,5- Dichlorobiphenyl (PCB9), which is often used as a model compound for PCB aerobic degradation (Hickey et al., 1992; Koller et al., 2000; Li et al., 2020a; Jiang et al., 2022), was selected as a stable isotope-labeled compound in the present study. The PCB degradation efficiencies, microbial community structures, and functional microbial community compositions and abundances in RD-AO (two stages: 45 days for the RD, and 30 days for the AO) and AO alone (30 days) microcosms of e-waste-contaminated soils were investigated and compared. Results generated in the present study may support bioremediation application of RD-AO at PCBs-contaminated soil or sediment.

2. Materials and methods

2.1. The anaerobic microcosm

E-waste-contaminated soil samples were collected in April 2019 from Qingyuan, Guangdong Province, China (23°0′58"N, 113°33′22″E). Soil samples were air-dried, ground, and sieved (2 mm) to remove impurities, and then homogenized. Finally, all samples were stored at 4 °C. The concentration of total PCBs was measured to be 9780 μg/kg, with a mean value of 4.76 chlorines per biphenyl (Table S1). The calculation of chlorines per biphenyl was referred to a previous study (Huang et al., 2020). The physiochemical properties of the soil are listed in Table S2.

Two treatments were set up, one with the e-waste-contaminated soil (RD-AO) (Fig. S1) and the other with the sterilized soil as an abiotic control. Each experimental set was performed in triplicate. Microcosms were set up according to methods described previously (Wang et al., 2014). Briefly, 15 mL of autoclaved bicarbonate-buffered mineral salt medium with 10 mM of lactic acid amendment was dispensed into 100 mL serum bottles containing 5 g of e-waste-contaminated soil. The serum bottles were sealed with butyl rubber septa (Geo-Microbial Technologies, Ochelata, OK, USA) and secured with aluminum crimp caps. Then, the abiotic control bottles were sterilized daily by autoclaves at 121 °C for 15 min and the sterilized step was repeated three times. Finally, all bottles were homogenized and cultivated in dark at room temperature without shaking. After 45 days of anaerobic incubation, partial bottles were destructively sampled and stored at −20 °C prior to analysis.

2.2. The aerobic SIP microcosm

At the end of the anaerobic stage, an AO treatment was adopted (Fig. S1). The AO treatment remained the addition of 5 g of e-waste contaminated soil and 15 mL of autoclaved bicarbonate-buffered mineral salt medium with 10 mM of lactic acid amendment in 100 mL glass bottles. However, all bottles in the RD-AO, AO, and abiotic control were sealed with a sealing film allowing air to exchange freely in the aerobic stage. Then, to establish SIP microcosms, about 4000 μg/kg of ^{13}C-PCB9 (99 atom % $^{13}C_{12}$; Cambridge Isotope Laboratories, Inc., Tewksbury, MA, USA) or ^{12}C-PCB9 (Dr. Ehrenstorfer Schäfers, Augsburg, Germany) was added to the RD-AO and AO microcosms, which were named RD-AO-C12, RD-AO-C13, AO-C12, and AO-C13, respectively (Table 1). The abiotic control treatment was only supplemented with ^{12}C-PCB9. Each test was performed in triplicate. All samples were cultivated at 25 °C with shaking at

Table 1
Setup of aerobic degradation treatments.

Treatment	Name	Setup
Anaerobic-aerobic two-stage treatment	Abiotic control	Sterile soil amended with unlabeled PCB9
	RD-AO-C12	Soil amended with unlabeled PCB9
	RD-AO-C13	Soil amended with ^{13}C-PCB9
Aerobic treatment alone	AO-C12	Soil amended with unlabeled PCB9
	AO-C13	Soil amended with ^{13}C-PCB9

180 rpm. After 10, 20, and 30 days (D10, D20, and D30, respectively) of incubation, samples from all microcosms were sampled and immediately frozen at −20 °C before chemical and microbial analysis. Because there was no significant difference in the degradation rate of PCB9 between D20 and D30 of the RD-AO microcosms ($p = 0.675$), D10 and D30 were selected as the SIP sampling times.

2.3. Chemical analysis

Total organic carbon was measured using the procedure of previous study (Jiang et al., 2016). Briefly, a 1 g homogeneous soil sample was treated with 10 mL of 1 M HCl solution, then was washed with deionized water to a neutral pH (6–7), and freeze-dried. Subsequently, the soil was analyzed with an elemental analyzer (Vario EL III; Elementar, Hanau, Germany).

Heavy metals were analyzed using a procedure adopted in a previous study, with some modifications (Luo et al., 2005). Briefly, 0.1 g of a homogeneous soil sample was digested in a strong acid (4:1 concentrated HNO_3 and $HClO_4$, *v*/v) and the heavy metals in the solutions were measured by inductively coupled plasma optical emission spectrometry (ICP-OES; 2100DV Optima; Perkin Elmer Waltham, MA, USA). As part of quality assurance and quality control, the standard sample (GBW07410) was used in the digestion and analysis, and its recovery rates were around 90 ± 8 %.

To extract PCBs from soil, 0.5 g of homogenous sample, 20 mL of 1:1 n-hexane/acetone (*v*/v, HPLC), and recovery standards ($^{13}C_{12}$ labeled PCB9, 155, and 206; Dr. Ehrenstorfer Schäfers) were added to a 100 mL glass tube. They were then sonicated for 30 min at 25 °C, and the organic phase was transferred to a flat bottom flask. After three ultrasonic extractions, the organic phase was rotated and evaporated to about 3 mL and then purified in a multilayer column that contained 3 % deactivated neutral alumina, 3 % deactivated neutral silica gel, 50 % H_2SO_4 acid silica gel, and anhydrous Na_2SO_4 (from bottom to top). Finally, the eluate was concentrated to 500 μL under a gentle stream of N_2, with $^{13}C_{12}$ labeled PCB141 added as the internal standard. All 209 PCB congeners were detected by gas chromatography-tandem mass spectrometry (GC–MS/MS; 7890/7000; Agilent, Santa Clara, CA, USA) equipped with a CP-Sil 8 CB column (50 m × 0.25 mm × 0.12 μm) in multiple reaction monitoring mode. The instrumental analysis was conducted according to previous methods (Mao et al., 2019). Nine groups of PCB reference materials (AccuStandard, New Haven, CT, USA) were obtained from commercial company. Each group contains a batch of PCB congeners with different retention times and mass spectrum parameters. The retention time and mass spectrum parameter can be acquired according to the GC–MS/MS detection results (Tables S3 and S4). Finally, the 209 PCB congeners can be identified in the PCB mixture according to the retention time and mass spectrum parameter of peaks on GC–MS/MS chromatograms. However, some PCB congeners shared the same retention time and mass spectrum parameter. Thus, the mixed reference materials containing 209 PCB congeners yielded 168 peaks (co-eluents were counted as multiple congeners).

Finally, the mixture was diluted to produce a standard curve with concentrations of 5, 20, 50, 100, 250, 500, and 1000 μg/L ($R^2 > 0.996$). Quality assurance and quality control were conducted using procedural blanks, instrument blanks, and surrogate recoveries. All PCB congeners were not detected in the procedural blanks and instrument blanks. The surrogate recoveries of $^{13}C_{12}$ labeled 9, 155, and 206 were 58.0 ± 5.97 %, 74.1 ± 5.79 %, and 115 ± 11.0 %, respectively. The PCB concentrations in all samples were corrected by blanks and surrogate recoveries.

2.4. Extraction of DNA, ultracentrifugation, high-throughput sequencing, and bioinformatics analysis

2.4.1. Extraction of DNA and ultracentrifugation

A PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA) was used to extract DNA from soils according to the manufacturer's instructions. The DNA concentration and quality were detected by a NanoDrop 2000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Approximately 5 μg of the DNA from the RD-AO-C12, RD-AO-C13, AO-C12, and AO-C13 microcosms was ultracentrifuged, following a previously reported method (Li et al., 2017). Briefly, DNA was mixed with tris EDTA/CsCl solution at a final buoyancy density of ~1.77 g/mL and was then added to Quick-Seal polyallomer tubes (13 × 51 mm, 5.1 mL; Beckman Coulter, Pasadena, CA, USA) and ultracentrifuged (Optima L-100XP; Beckman Coulter) at 47,500 rpm for 48 h (20 °C). Subsequently, 14 fractions were separated from each tube, and their buoyancy densities were measured by an AR200 digital refractometer according to previous studies. Finally, DNA fractions were purified using a MicroElute DNA-Pure Kit (Omega BioTek, Norcross, GA, USA) according to the manufacturer's instructions. All experiments are performed in triplicate.

2.4.2. Polymerase chain reaction (PCR) and high-throughput sequencing

The PCR was performed using the 515F (5′- GTGYCAGCMGCCGCGGTAA-3′) and 806R (5′- GGACTACNVGGGTWTCTAAT −3′) primers. The 515F primer was labeled with a unique 12-bp barcode to distinguish among the amplification products. The PCR system consisted of 12.5 μL of rTaq premix buffer (TaKaRa, Shiga, Japan), 0.5 μL (100 nM) of each primer, 1 μL of DNA, and 10.5 μL of ddH_2O. The procedure was performed as follows: 95 °C for 3 min; 29 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s; and 72 °C for 10 min. The PCR product was detected by agarose gel electrophoresis (1.0 %) and purified by the MicroElute Cycle-Pure Kit (Omega BioTek). After analysis of concentration and quality, DNA was combined in approximately equimolar amounts and sequenced by Hiseq2500 PE 250 (Illumina, San Diego, CA, USA). All experiments are performed in triplicate.

2.4.3. Bioinformatics analysis

Pear software was used to assemble raw 16S rRNA gene data, which were then analyzed by quantitative insights into microbial ecology (QIIME 2) (Caporaso et al., 2010). Briefly, the sequences were demultiplexed according to the barcode and truncated at the 250 bp position. Then, the sequences were denoised with a dada2 plug-in. Based on the q2-feature-classifier plugin, the taxonomic information of representative sequences was classified using the SILVA database (Callahan et al., 2016). Megan5 software was used to calculate the phylogenetic diversity of functional microorganisms. An alpha diversity analysis, including Shannon_entropy, pielou_evenness, faith_pd, and observed_features, was conducted using QIIME 2. Based on previous studies, the 100 most abundant (%) operational taxonomic units (OTUs) were selected for analysis, to identify which of them represented microbes capable of dissimilating PCB9 (Li et al., 2017; Li et al., 2018). Their genomic DNA was labeled with the ^{13}C isotope and enriched in the heavy DNA fractions, with higher buoyant density values in the ^{13}C-PCB9 than ^{12}C-PCB9 microcosms. In total, 10 OTUs were identified (accession numbers: OL691057-OL691058, OL691060-OL691064, and OL691067-OL691069).

2.5. Quantitative PCR (qPCR)

The primer pair of 515F (5'-GTGYCAGCMGCCGCGGTAA-3′) and 806R (5'-GGACTACHVGGGTWTCTAAT-3′) was used to quantify the microbial 16S rRNA gene. The qPCR mixture contained 10 μL of SYBR green Premix Ex *Taq II* (TaKaRa), 0.4 μL of each primer, 1 μL of the DNA sample, and 8.2 μL of ddH_2O. The standard curve (10^1–10^8, $R^2 = 0.998$) (Fig. S2) was obtained by diluting the recombinant plasmid extracted from *E. coli*. The qPCR procedure was performed using the method described by Jiang et al. (2018). The qPCR experiment is performed in triplicate.

2.6. Statistical analysis

Data are expressed as the mean ± standard deviation. Statistical analyses were performed using Origin 2020 (OriginLab Corporation, Northampton, MA, USA). A parsimony analysis was applied to derive a phylogenetic tree using MEGA 7. The differences between treatments were determined using IBM SPSS statistics (version 24.0; IBM Corp., Armonk, NY, USA).

The least significant difference test was used to determine significant differences at the $p = 0.05$ level.

3. Results and discussion

3.1. Degradation of PCBs

The changes in PCB concentrations in the RD-AO and abiotic control treatments after the sequential 45-day anaerobic and 30-day aerobic incubation, and in AO microcosms after the 30-day aerobic incubation alone, are shown in Figs. 1 and 2. In original soil, there were 32 PCB congeners from dichlorobiphenyl to heptachlorobiphenyl. Among them, heptachlorobiphenyl was only 2,2′,3,4,4′,5,5′-heptachlorobiphenyl (PCB180) with the highest concentration (3470 μg/kg) (Table S1). After the 45-day incubation in RD of the RD-AO process, only PCB 180 decreased significantly, while dichlorobiphenyl to hexachlorobiphenyl increased or unchanged. PCB180 had a removal efficiency of 37.1 %; in contrast to no obvious attenuation in the abiotic control ($p = 0.529$) (Figs. 1 & S3). Meanwhile, the concentration percentage of PCB180 in all PCB congeners declined from 35.5 % to 22.3 %, while other PCBs slightly increased within the range of 0.0629 %–1.77 % (Fig. 1B), indicating a direct dechlorination of PCB180. Among these congeners, 999 μg/kg of low-chlorinated PCBs were produced, while PCB9 did not increase considerably ($p > 0.05$). However, the chlorines per biphenyl in the RD-AO microcosms just declined from 4.76 to 4.34, which seems to have a low dechlorination efficiency. Similar results occurred in previous studies (Table 2). These studies found a significant lag period in PCB dechlorination, leading to a low dechlorination efficiency in the early stage (Fagervold et al., 2011; Chen and He, 2018). Long et al. (2015) found that the chlorines per biphenyl were decreased by only 0.4 in the first 41 days, although reduced by 1.5 during the whole anaerobic stage (70 days). Even reported that dechlorination was not detected in 150 days (Fagervold et al., 2011). Thus, the dechlorination efficiency of 45 days in this research was a normal phenomenon.

In the aerobic stage, the degradation rate of total low-chlorinated PCBs in RD-AO microcosms (18.6 %) was significantly lower than that in AO microcosms (40.8 %) ($p = 0.001$). It showed that the aerobic degradation efficiency of low-chlorinated PCBs decreased by 54.4 %. Among low PCBs, only PCB9 considerably reduced in both RA-AO and AO microcosms ($p < 0.05$) (Fig. 2A). The degradation rate of PCB9 was 48.2 % and 81.2 % in the RD-AO and AO microcosms, respectively, while no degradation was detected in the abiotic control (Fig. 2B). It was found that 65.3 % of the PCB9 degradation occurred within the first 10 days (D10) in the AO treatments, with a further 9.44 % and 6.50 % of PCB9 degraded from days 10 to 20 and days 20 to 30, respectively. Similarly, most PCB9 degradation in the RD-AO microcosms occurred within the first 10 days (Fig. 2B). The higher PCB degradation rate in the AO than RD-AO microcosms demonstrated that the anaerobic dechlorination process negatively affects the aerobic degradation of low-chlorinated PCBs. However, the aerobic degradation efficiency (15 μg PCB/kg/day) in RD-AO in present research was similar to previous studies (Chen et al., 2014; Long et al., 2015) (Table 2). None of these studies focus on the role of anaerobic stage on the subsequent aerobic degradation. The present study firstly proved that the anaerobic dechlorination process negatively affects the aerobic degradation of low-chlorinated PCBs, although the inhibition of aerobic bacterial activity under prolonged anaerobic conditions were widely revealed (Wang et al., 2018). For example, aerobic granular sludge showed a 20 % reduction in ammonia oxidation activity after 4–5 weeks of anaerobic, resulting in the decline of ammonia oxidation efficiency (Pijuan et al., 2009).

Additionally, the RD-AO microcosms could degrade both highly and lightly chlorinated PCBs and the sole AO treatment can only remove the low-chlorinated PCBs (Kaur et al., 2021). Therefore, anaerobic-aerobic two-stage treatment is proved to be a promising strategy for extensive

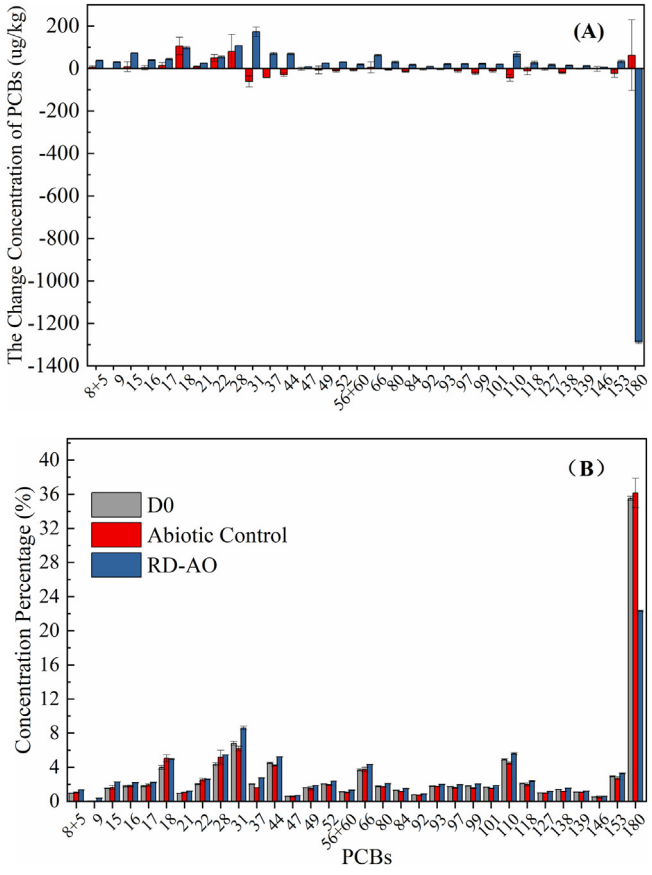

Fig. 1. (A) The change concentrations and (B) the concentration percentage of the PCB congeners in the RD-AO and abiotic control treatments after 45 days of anaerobic incubation. Error bars show the standard deviation of the mean ($n = 3$).

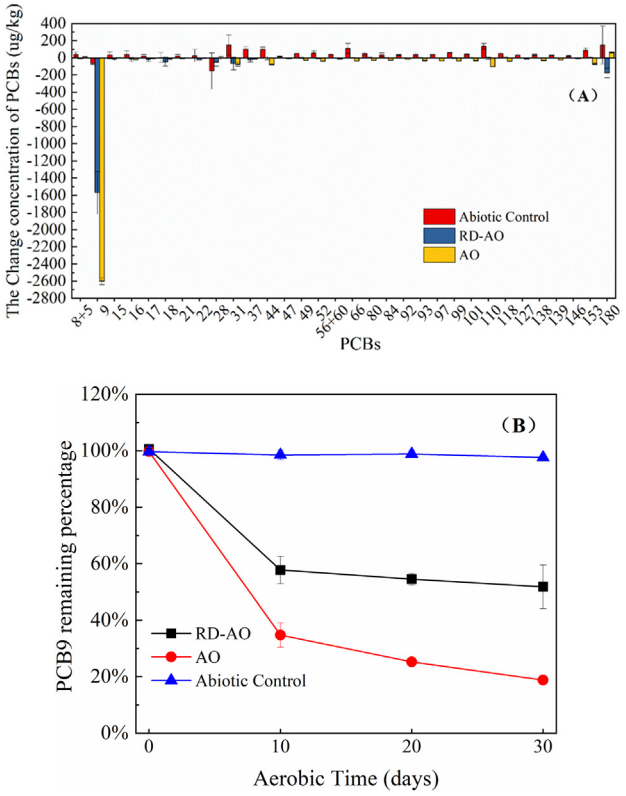

Fig. 2. The total PCB congeners (A) The change concentrations of PCBs and (B) the percentages of PCB9 residue in the abiotic control, RD-AO, and AO treatments after 30 days of aerobic incubation. Error bars show the standard deviation of the mean ($n = 3$).

Table 2
Comparison of the PCB degradation efficiency of the present study with other studies.

Number	PCB congeners	Setup	Anaerobic dechlorination efficiency	Aerobic degradation efficiency	Reference
1	59,000 μg/kg of Aroclor 1260	Bioaugmentation: 120 days of anaerobic stage and 28 days of aerobic stage	CPB decreased from 6.40 to 5.20	66 % or 264 μg/kg/day	(Master et al., 2002)
2	50,000 μg/kg of Aroclor 1260	Bioaugmentation: 28 days of anaerobic stage and 14 days of aerobic stage	Mass fraction of chlorine atom: from 60 % decreased to 36 %	48 % or 571 μg/kg/day	(Pathiraja et al., 2019a)
3	7410 μg/kg PCB mixture: agricultural soil from e-waste recycling areas	Rice cultivation under sequential flooded (180 days)-dry (60 days) condition	–	42 % or 13.0 μg/kg/day	(Chen et al., 2014)
4	1000 μg/kg of Aroclor 1260	Composting: 70 days of anaerobic stage and 28 days of aerobic stage	CPB decreased by 1.50	25 % or 2.55 μg/kg/day	(Long et al., 2015)
5	9780 μg/kg PCB mixture: e-waste-contaminated soil	45 days of anaerobic stage and 30 days of aerobic stage	CPB decreased from 4.73 to 4.34	15.8 % or 19.0 μg/kg/day	The present study

CPB is chlorines per biphenyl.
"–" represents no description of anaerobic dechlorination efficiency in the study.

bioremediation of PCBs. Previous study has shown that highly chlorinated PCBs in sediments were dechlorinated to low chlorinated PCBs only under a long-term anaerobic environment, and exposure of the sediment to oxygen improved the degradation of low chlorinated PCBs (Matturro et al., 2020). Compared to the constant-drying system, the sequential flooding and drying treatment in rice production represents a natural sequential anaerobic-aerobic bioreactor, enhancing the removal of highly chlorinated PCBs in paddy fields surrounding e-waste-contaminated areas (Chen et al., 2014). Consequently, some human activities, such as rice production, boat traffic, and the dredging of nearshore and urban rivers, can inadvertently create sequential anaerobic-aerobic bioreactors and reduce PCBs (Chen et al., 2014; Matturro et al., 2020). Moreover, the inhibition of aerobic degradation reminds us that we need to improve the PCB aerobic degradation efficiency in the anaerobic-aerobic two-stage treatment. Table 2 showed the bioaugmentation of the two-stage treatment had a significantly higher aerobic degradation efficiency than the present study (Master et al., 2002; Pathiraja et al., 2019a, 2019b). It proved the low aerobic degradation efficiency was improved by bioaugmentation.

3.2. Abundance and community structure of bacteria

Figs. 3 and S4 show the microbial abundances and community structures of the RD-AO microcosms in the anaerobic-aerobic two-stage treatment, and AO microcosms in the aerobic treatment. After 45 days of anaerobic incubation, the total 16S rRNA gene copies in the RD-AO (1.08×10^8 copies/g dry soil) treatment were significantly lower than in the original soil (2.76×10^8 copies/g dry soil) ($p = 0.001$); however, the alpha diversity did not change significantly (Fig. S4). The relative abundances of the 16S rRNA gene defined based on the phylum and genus were not different between the microbial community structures of the RD-AO and original soils ($p > 0.05$). At the phylum level, the original soil and RD-AO microcosms were dominated by *Firmicutes* (30.8–34.3 %), *Proteobacteria* (22.4–24.2 %), *Acitinobacteriota* (14.4–14.6 %), and *Desulfobacterota* (12.5–17.3 %) (Fig. 3A). At the genus level, the dominant genera in the original soil and RD-AO microcosms were *Geoalkalibacter* (17.2 % and 12.1 %, respectively) and *Bacillus* (6.93 % and 8.11 %, respectively).

It has been reported that *Dehalococcoides*, *Methanosarcina,* and *Desulfovibrio* could be from a stable microbial community for interspecies carbon and electron transfers, to facilitate the organohalide respiration of *Dehalococcoides mccartyi* (Wang et al., 2019). In addition, *Geoalkalibacter* and *Bacillus* are known to be involved in the reduction of metals (Fe and Mn) and nitrate in the form of extracellular electron transfer (Ye et al., 2000; Greene et al., 2009; Yadav and Patil, 2020). In the present study, *Geoalkalibacter* and *Bacillus* may have worked closely with organohalide-respiring bacteria and played a mediating role in electron sources. However, the reported organohalide-respiring bacteria present in RD-AO microcosms were only *Dehalobacter* and uncultured *Dehalococcoidia*. The relative abundance of *Dehalobacter* just increased from 0.17 % to 0.59 %, while the relative abundance of uncultured *Dehalococcoidia* (0.04–0.06 %) did not change significantly ($p > 0.05$). The organohalide-respiring bacteria need to reach a certain number to achieve reductive dechlorination of PCBs, so there was a dechlorination lag time in the early stage of dechlorination experiments (Fagervold et al., 2011; Chen and He, 2018). When the enrichment of dechlorinating bacteria is promoted by bioaugmentation or biostimulation, the dechlorination efficiency is greatly improved, and the dechlorination lag time is significantly reduced (Master et al., 2002; Chen and He, 2018; Pathiraja et al., 2019a). Therefore, the low relative abundance of organohalide-respiring bacteria might be the main reason for the low dechlorination efficiency in the present research. The low relative abundance of organohalide-respiring bacteria was attributed to their long generation time and the inhibition of other co-existing contaminants in the e-waste contaminated soil, including heavy metals (Xu et al., 2019; Lu et al., 2020; Qiu et al., 2020; Kaur et al., 2021). Heavy metals have been shown to suppress microbial degradation by interacting with enzymes in different ways, such as replacing cations that are physiologically essential to the enzyme, binding DNA bases to break single-stranded DNA, and so on (Sandrin and Maier, 2003; Li and Fang, 2007; Altas, 2009). Moreover, the latest studies found that heavy metals might indirectly inhibit organohalide respiration by affecting non-dechlorinated populations in microcosms containing *Dehalococcoides* (Wu et al., 2019; Lu et al., 2020). Besides, the above results also showed that the in-situ organohalide-respiring bacteria in e-waste-contaminated soils were *Dehalobacter*, not *Dehalococcoidies* which have more attention in previous studies (Wang et al., 2014; Wang et al., 2019; Kaur et al., 2021).

In the aerobic stage, the total 16S rRNA gene copies in the RD-AO and AO treatments were significantly increased ($p < 0.05$) and reached their maximum values on Day 10 (Fig. S4A); meanwhile, their alpha diversities decreased significantly ($p < 0.05$) (Fig. S4B). Compared with the AO microcosms (8.89×10^8 copies/g dry soil, 368 (observed_features)), the RD-AO microcosms had a higher 16S rRNA gene abundance (1.06×10^9 copies/g dry soil) and lower microbial diversity (303 (observed_features)) on Day 10 ($p < 0.01$). Additionally, the changes in microbial community structure differed between the AO and RD-AO microcosms (Fig. 3). In the AO microcosms, *Firmicutes* and *Desulfobacterota* (phylum) significantly decreased from 30.8 % and 17.3 % to 0.78 % and 0.02 %, respectively, and *Proteobacteria* significantly increased from 22.4 % to 65.3 % ($p < 0.01$). The members of the genera, *Lysobacter* and *Luteimonas,* significantly increased from 3.08 % and 1.02 % to 35.5 % and 26.2 % ($p < 0.01$), respectively, and became the predominant bacteria at D10 and D30, respectively. In the RD-AO microcosms, *Desulfobacterota* also significantly declined from 17.3 % to 0.69 %, while *Acitinobacteriota* significantly increased from 14.6 % to 61.6 % ($p < 0.01$). The members of the genera, including *Quadrisphaera*, *Georgenia*, and *Pseudoxanthomonas* (<5 % before aerobic degradation), significantly increased ($p < 0.01$) and became the dominant bacteria (18.8 %, 16.1 %, and 20.6 %, respectively). In summary,

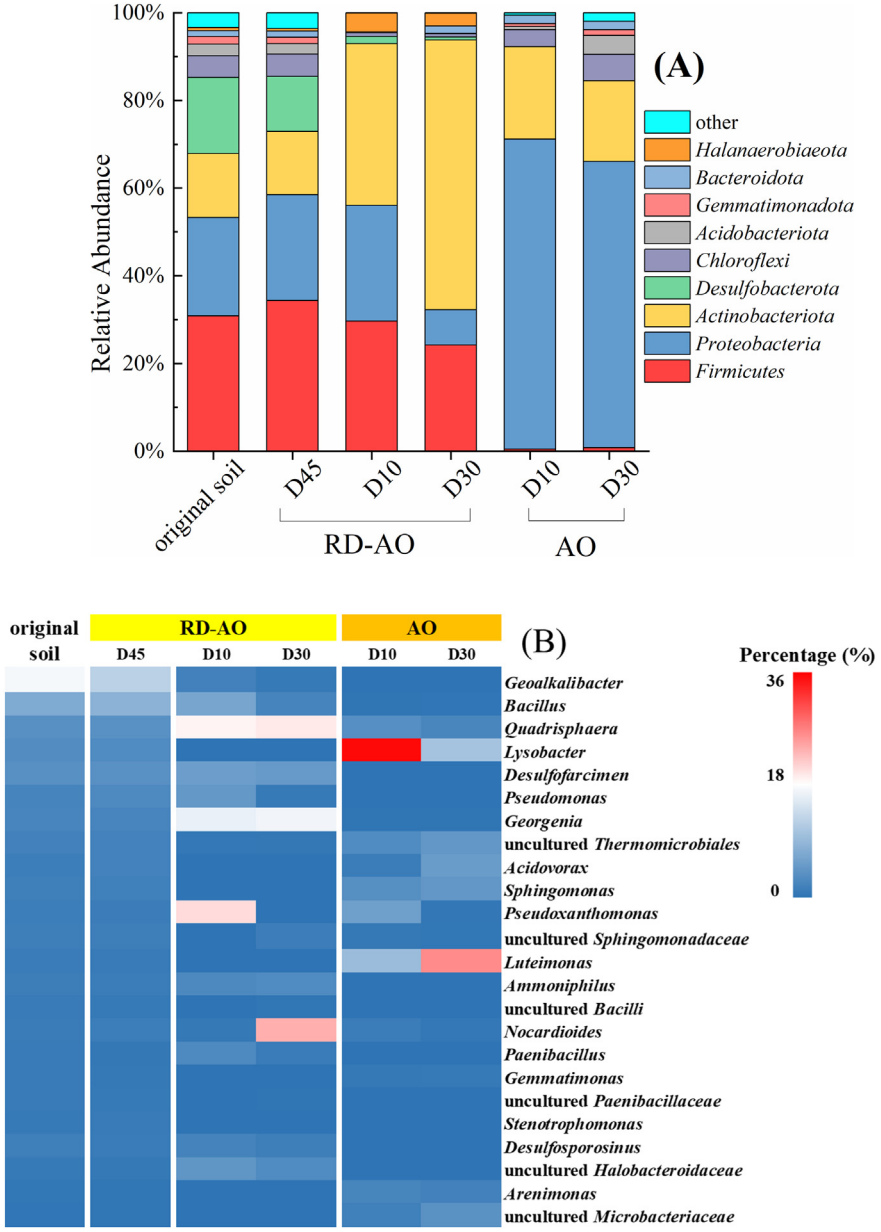

Fig. 3. The relative abundance of dominant microbes in the original soil, RD-AO, and AO microcosms. (A) represents the relative abundance of the top nine bacterial phyla; (B) represents the relative abundance of genera. The selected taxa in all microcosms have a minimal relative abundance >1 %. Original soil represents the initial microbial community in the original soil sample. RD-AO was the microcosm with anaerobic-aerobic two-stage treatment, while AO was the microcosm with aerobic treatment alone. D45, D10, and D30 represent samples collected on days 45 of anaerobic stage, days 10 and 30 of aerobic stage, respectively. The relative abundance of microbes is the average of triplicates.

the number of dominant genera (relative abundance >1 %) in both RD-AO and AO microcosms was 16, and their total relative abundance was 85.4 % and 75.9 %, respectively. Among the dominant genera, the relative abundance of anaerobic and aerobic bacteria (including aerobic and facultative aerobic/anaerobic bacteria) was 15.9 %, 84.1 % in RD-AO microcosm and 3.47 %, 96.5 % in AO microcosm, respectively. Interesting, all dominant genera in RD-AO and AO microcosms were different, except *Quadrisphaera*. The above results showed that anaerobic incubation changed the dominant bacteria, decreased the abundance of aerobic bacteria, and increased the abundance of anaerobic bacteria in RD-AO microcosm compared to the AO microcosm. Accordingly, the differences in 16S rRNA gene copies, microbial alpha diversity, and structure between the AO and RD-AO microcosms in the aerobic stage indicated a noticeable impact of anaerobic incubation on the aerobic microbes. In addition, *Bacillus*, *Pseudomonas*, *Acidovorax*, and *Sphingomonas* have been involved in the aerobic degradation of PCBs (Ohtsubo et al., 2012; Kaur et al., 2021; Su et al., 2021). Among these genera, *Bacillus* and *Pseudomonas* were enriched (2.02 % and 0.77 %, respectively) in RD-AO microcosms, whereas *Acidovorax* and *Sphingomonas* were enriched (5.13 % and 4.45 %, respectively) in the AO microcosms after 30 days.

3.3. Active PCB degraders revealed by DNA-SIP

The microorganisms responsible for ^{13}C-PCB9 assimilation were identified by comparing the relative abundance of specific OTUs in each fraction of the ^{12}C-PCB9 and ^{13}C-PCB9 treatments. As shown in Figs. 4 and S5, totally five OTUs were enriched in the heavy fractions of AO-C13 microcosms, i.e., OTU_108, and OTU_680 with a buoyant density value of 1.7208 g/mL on D10, and OTU_121, OTU_301, and OTU_344 with buoyant density of 1.7295 g/mL on D30. Compared to the relative abundances of

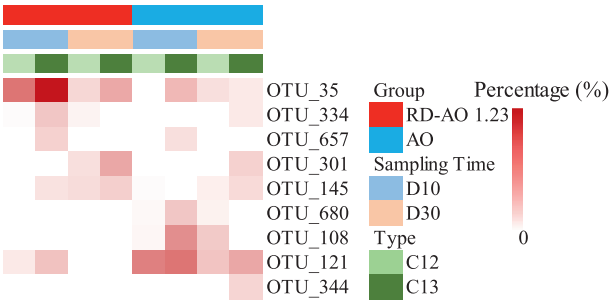

Fig. 4. Heatmap of the SIP-identified OTU in the RD-AO and AO microcosms. RD-AO was the microcosm with anaerobic-aerobic two-stage treatment, while AO was the microcosm with aerobic treatment alone. D10 and D30 represent samples collected on days 10 and 30 at the aerobic stage. C12 and C13 refer to samples amended with ^{12}C-PCB9 and ^{13}C-PCB9, respectively. The value of OTU abundance is the average of triplicates.

the corresponding OTUs in the same fractions of the AO-C12 treatment (0.04 %,0.03 %, 0.25 %, 0 %, and 0 %, respectively), the higher abundances ($p < 0.01$) in the heavy fractions from the AO-C13 treatment (0.53 %, 0.24 %, 0.40 %, 0.19 %, and 0.17 %, respectively) indicated that microorganisms represented by these OTUs played the primary role in degradation of PCB9. According to phylogenetic information, the active PCB degraders (five OTUs) belonged to the three bacterial classes of *Gammaproteobacteria*, *Alphaproteobacteria*, and *Actinobacteria* (Fig. 5). OTU_108, OTU_121, OTU_301, OTU_344, and OTU_680 were identified as *Acidovorax*, *Arenimonas, Sulfurifustis, Sphingomonas,* and *Marmoricola* (Table S5). In the RD-AO-C13 microcosms, OTU_35, OTU_334, and OTU_657 were found in the heavy DNA fractions, with a buoyant density value of 1.7274 g/mL on D10, while OTU_145 and OTU_301 were found (buoyant density: 1.7350 g/mL) on D30. Similarly, the relative abundances of the corresponding OTUs in the heavy fractions of the RD-AO-C13 microcosms (1.23 %, 0.24 %, 0.19 %, 0.20 %, and 0.40 %, respectively) were significantly higher than those of the RD-AO-C12 microcosms (0.68 %, 0.02 %, 0 %, 0.14 %, and 0.13 %, respectively) ($p < 0.05$). The active PCB degraders (five OTUs) in RD-AO belonged to the four bacterial classes of *Gammaproteobacteria*, *Alphaproteobacteria*, *Thermoanaerobacteria*, and *Bacilli* (Fig. 5). OTU_35, OTU_145, OTU_301, OTU_334, and OTU_657 were identified as *Bacillus*, *Steroidobacter*, *Sulfurifutis*, uncultured *Thermoanaerobacterales*, and *Sphingomonas*, respectively (Table S5). Thereinto, OTU_35, which was only detected in the RD-AO microcosms, was identified as a *Bacillus* species (Table S5). It had 100 % similarity with the partial 16S rRNA gene sequence of *Bacillus* sp. FJAT-29792 (MF948294.1) and *Bacillus* sp. ORG7–33 (KU315889.1), and formed a subclade with a high bootstrap value of 100 (Fig. 5). Additionally, OTU_344 and OTU_657 were characterized as the genus *Sphingomonas* (Table S5), and both shared 100 % similarity with the partial 16S rRNA gene sequence of *Sphingomonas* sp. (MF503624.1, GQ369067.1, and HG986669.1) (Fig. 5). OTU_301, which was shared by the RD-AO and AO microcosms, was identified as *Sulfurifustis* (class *Gammaproteobacteria,* order *Acidiferrobacterales,* family *Acidiferrobacteraceae*), and shared 100 % similarity with *S. variabilis* skN76 (NR_137347.1).

Among the active PCB9 degraders, *Sphingomonas, Acidovorax, Marmoricola*, and *Bacillus* have been reported to be involved in the degradation of biphenyl and PCBs (Ohtsubo et al., 2012; Ganesh-Kumar et al., 2013; Li et al., 2020b; Kaur et al., 2021; Ouyang et al., 2021; Su et al., 2021). The genera *Arenimonas* and *Steroidobacter* are Gram-negative, catalase-positive, and oxidase-positive (Sakai et al., 2014; Yuan et al., 2014). *Sulfurifustis* is generally considered to be a sulfur-oxidizing and inorganic carbon-fixing bacteria (Umezawa et al., 2016). Members of *Arenimonas*, *Steroidobacter*, and *Thermoanaerobacterales* can assimilate various organic compounds and sugars, such as acetochlor, polyethylene terephthalate, methoxylated aromatic, polystyrene, and inulin (Lee et al., 2008; Han et al., 2021; Tourova et al., 2021). However, *Arenimonas*, *Steroidobacter*, *Sulfurifustis*, and *Thermoanaerobacterales* have not previously been linked with the degradation of biphenyl/PCBs, and their roles in PCB-contaminated soils remain unclear. SIP results provided compelling evidence that these microbes were directly responsible for PCB degradation. In addition, three of four new PCB degraders were found in RD-AO microcosm, suggesting that anaerobic-aerobic two-stage treatment can recruit novel bacteria involved in PCBs aerobic degradation. In summary, it was found that five bacterial taxa were active PCB degraders in the AO microcosms with the

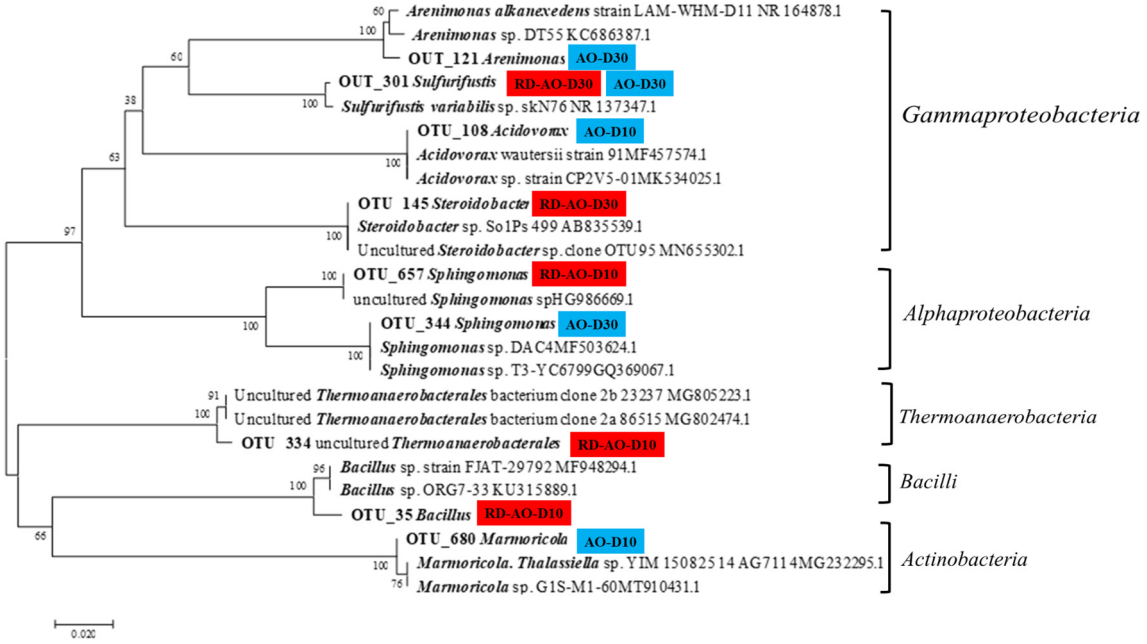

Fig. 5. Phylogenetic tree of OTUs responsible for PCB9 degradation based on the neighbor-joining method using 16S rRNA gene sequences. The bar represents 0.02 substitutions per nucleotide position. The active PCBs degraders identified from the RD-AO and AO microcosms are highlighted in red and blue. RD-AO was the microcosm with anaerobic-aerobic two-stage treatment, while AO was the microcosm with aerobic treatment alone. D10 and D30 represent samples collected on days 10 and 30 at the aerobic stage.

aerobic treatment alone, whereas in addition to *Sulfurifustis* and *Sphingomonas,* another three degraders were detected in the RD-AO microcosms within the anaerobic-aerobic two-stage treatment, indicating a change in the active PCB-degrading microbes during anaerobic incubation.

3.4. Environmental implications

Anaerobic incubation changed the microbial community structures, especially the active aerobic PCB-degrading microbial community of an e-waste-contaminated soil, resulting in a decline in the aerobic degradation rate of PCBs. Changes in the functional microbial communities could lead to differences in biodegradation efficiency (Li et al., 2019a; Li et al., 2019b; Li et al., 2020b; Luo et al., 2021). However, dechlorination during anaerobic processes is indispensable for the complete degradation of highly chlorinated PCBs (Kaur et al., 2021). Therefore, certain measures need to be taken to improve the abundance of indigenous functional microorganisms when applying an anaerobic-aerobic two-stage treatment. Generally, SIP results provide guidance for the improvement of cultivation conditions, benefiting and specifically enriching the target functional PCB degraders. Several DNA-SIP-identified degrading bacteria were obtained by optimized cultivation-based methods in the previous study, and more analyses will be conducted in the future (Li et al., 2019a). For culturable microbes, bioaugmentation is an advantageous approach because indigenous degraders are more competitive than exogenous microorganisms, and there is evidence that they facilitate biphenyl and PCB degradation (Elsa Cervantes-González et al., 2019; Luo et al., 2021). For unculturable microbes, the growth characteristics of targeted microorganisms can be obtained through 16S rRNA or the metagenome, and their preferred nutrition can be supplied for enrichment and to optimize degradation efficiency (Albertsen et al., 2013; Xu et al., 2019; Cai et al., 2021). For example, terpenes (carvone and limonene) are often used to induce the synthesis of the PCB-degrading enzyme system (Winchell and Novak, 2008; Laszlova et al., 2016). In addition, plant growth is an excellent biostimulatory method for enhancing PCB degradation due to root exudates and the sequential flooding-drying environment in the rhizosphere, which has proven to be an efficient technology to regulate functional degraders and improve PCB degradation (Mackova et al., 2009; Kurzawova et al., 2012; Toussaint et al., 2012; Terzaghi et al., 2019).

4. Conclusion

Both highly and lightly chlorinated PCBs can be degraded in an anaerobic-aerobic two-stage treatment (RD-AO), although the low-chlorinated PCB aerobic degradation was inhibited in the AO treatment. An unattractive dechlorination efficiency was found in anaerobic stage owing to the low abundance of organohalide-respiring bacteria. In the aerobic stage, the decreased degradation rate in RD-AO treatment was caused by the changes in microbial community structure and the decrease in the abundance of aerobic bacteria, which was further evidenced by the results from DNA-SIP that anaerobic stage changed the active PCB degrading community, resulting in three of the five PCB degraders in RD-AO and AO microcosms being different. In addition, four (*Arenimonas, Steroidobacter, Sulfurifustis*, and *Thermoanaerobacterales*) were linked with PCB degradation for the first time, three of which were only found in the RD-AO microcosm suggesting that anaerobic-aerobic two-stage treatment can recruit novel bacteria involved in PCBs aerobic degradation. These new PCB degraders may provide an additional alternative for the future application in the field.

CRediT authorship contribution statement

Shuang Wang: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Jibing Li:** Methodology, Software, Formal analysis, Writing – review & editing. **Longfei Jiang:** Data curation, Formal analysis, Methodology, Software. **Shanquan Wang:** Methodology, Resources, Writing – review & editing. **Xuan Zhao:** Methodology. **Yeliang Dai:** Methodology. **Chunling Luo:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing. **Gan Zhang:** Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.157195.

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