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Insights into the biodegradation process of 2,4,5-trichlorophenoxyacetic acid under anaerobic condition

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Abstract

Background Chlorophenoxy compounds represent a group of selective herbicides widely used around the world. Chlorophenoxy herbicides are toxic, chemically stable, and can migrate into groundwater through soil leaching, posing a significant threat to drinking water safety and human health. Chlorophenoxy herbicides in groundwater aquifers are subject to anaerobic processes; however, the pathway and microbiology involved in the attenuation of chlorophenoxy herbicides under anaerobic condition are largely unknown. Here, the anaerobic degradation process of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), a typical chlorophenoxy herbicide, was investigated.

Results The initial 52.5 ± 2.3 μM 2,4,5-T was completely degraded by a sediment-derived microbial consortium, with 3,4-dichlorophenol, 2,5-dichlorophenol, 3-chlorophenol (3-CP) and phenol being identified as the intermediate products. Reductive dechlorination of 3-CP to phenol and the subsequent elimination of phenol were the key transformation steps in the overall degradation process of 2,4,5-T. Amplicon sequencing suggested that *Dehalobacter*, *Sulfuricurvum*, *Bacteroides*, *Acetobacterium*, and *Clostridium* sensu stricto 7 might contribute to the transformation of 2,4,5-T to phenol, and *Smithella*, *Syntrophorhabdus*, *Methanofollis* and *Methanosaeta* likely cooperated to accomplish the complete mineralization of phenol.

Conclusions This study reported the anaerobic degradation of 2,4,5-T via reductive dechlorination and the subsequent syntrophic metabolization of phenol, an intermediate product transformed from 2,4,5-T. *Dehalobacter* was identified as the organohalide-respiring population catalyzing the reductive dechlorination reaction. *Syntrophorhabdus* and methanogenic populations were likely involved in anaerobic phenol oxidation and facilitated the complete mineralization of 2,4,5-T.

Keywords 2,4,5-Trichlorophenoxyacetic acid, Anaerobic degradation, Reductive dechlorination, *Dehalobacter*, Syntrophic metabolism

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Introduction

2,4,5-Trichlorophenoxyacetic acid (2,4,5-T), a herbicide developed in the late 1940s, has been extensively used in agriculture for the selective control of broadleaf weeds (Maharana et al. 2015). Concentrations of residue 2,4,5-T typically range from 10 to 80 μg/kg in agricultural areas where 2,4,5-T was heavily applied (Fox et al. 2012). The half-life of 2,4,5-T in soils has been reported to vary between 12 and 59 days, depending on environmental conditions such as soil type, temperature, moisture, and microbial activity (Smith 1978). Due to the carcinogenic and mutagenic potentials to human beings (Hoffman and Eastin 1982; Bukowska 2004; Michalowicz 2010; Waggoner et al. 2013), the production and use of 2,4,5-T was restricted by the 1998 Rotterdam Convention (Hough 2000). Despite 2,4,5-T has been banned in many countries for years, it remains as one of the contaminants most frequently detected in soils, surface water and groundwater after a long period of usage (Nakamura et al. 2008; Brodsky et al. 2009; Xiong et al. 2014; Zazou et al. 2017). The natural attenuation of 2,4,5-T in various environmental matrices has been reported, and this observation has attracted a lot of attentions to study the microbiology and transformation mechanisms involved in 2,4,5-T degradation under different biogeochemical conditions (Itoh et al. 2013; Itoh 2014; Lechner et al. 2018).

The aerobic biodegradation of 2,4,5-T had been intensively studied in the last 40 years (Cho et al. 2002; Hayashi et al. 2016). The overall transformation process starts with ether bond cleavage followed by oxidative dechlorination and ring breakage, of which the latter reaction breaks the benzene ring into some metabolites common to the tricarboxylic acid (TCA) cycle such as acetyl CoA, pyruvate, succinate and oxaloacetate (Harwood and Parales 1996; Fuchs et al. 2011; Chenprakhon et al. 2019). A diverse array of bacterial strains, which belong to the genera *Cellulosimicrobium*, *Burkholderia*, *Bradyrhizobium*, *Sphingomonas*, *Nocardioides*, and *Raoultella*, have been identified and characterized for their abilities to utilize 2,4,5-T as carbon substrate and/or energy source (Kitagawa et al. 2002; Huong et al. 2007; Korobov et al. 2018).

The anaerobic biodegradation of 2,4,5-T has been explored using a variety of environmental samples, including soils, sediments, municipal sewage sludge and aquifer materials (Suflita et al. 1984; Mikesell 1985; Gibson and Suflita 1986, 1990; Al-Fathi et al. 2019). The transformation pathway involved in the degradation of 2,4,5-T varies, depending on the initialization steps and the sequence in which the chlorine substituents are removed from the benzene ring (Gibson and Suflita 1990). The degradation of 2,4,5-T can be initiated with two types of reactions, ether bond cleavage and reductive dechlorination. In some studies, ether bond cleavage is observed as the primary initiation reaction, whereas other studies have reported that reductive dechlorination takes place before ether cleavage. Nevertheless, reductive dechlorination of the monochlorophenol isomers (e.g., 3-chlorophenol, 4-chlorophenol), which are common intermediates during the transformation of 2,4,5-T, is often recognized as the rate-limiting step in the overall degradation process (Suflita et al. 1984). Subsequent conversion of a monochlorophenol to phenol and the mineralization of phenol to CO_2 and/or CH_4 were barely observed. Several populations, such as *Desulfitobacterium*, *Dehalobacter*, *Dehalococcoides*, have been implicated in the reductive dechlorination of various chlorophenols, but their participations in the degradation of 2,4,5-T remain unclear (Wang et al. 2014; Lechner et al. 2018). To date, the microbiology involved in the 2,4,5-T degradation is largely unknown, and no pure culture able to thrive on 2,4,5-T under anaerobic condition has been reported.

Here, we investigated the biodegradability of 2,4,5-T in an anaerobic consortium originated from a freshwater sediment microbiota. This study is aiming to (i) identify the intermediates produced during the anaerobic metabolism of 2,4,5-T and elucidate the pathway leading to the decomposition of this compound, (ii) unravel the microbial community responsible for the anaerobic degradation of 2,4,5-T, (iii) reveal the microbial populations mediating the key steps in the degradation process of 2,4,5-T.

Materials and methods

Chemicals

2,4,5-T (>97.0%) was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). 2,4,5-Trichlorophenol (2,4,5-TCP), 3,4-dichlorophenol (3,4-DCP), 2,5-dichlorophenol (2,5-DCP), 2,4-dichlorophenol (2,4-DCP), 3-chlorophenol (3-CP), 2-chlorophenol (2-CP), 4-chlorophenol (4-CP) and phenol (all >98.0%) were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals used in this study were analytical or higher grade.

Construction of the 2,4,5-T-degrading microbial consortium

Freshwater sediment sample was collected from a location (latitude 41° 39′ 46″, longitude 123° 6′ 20″) at the Xi River (Shenyang, Liaoning, China) as described (Li et al. 2019). Inside an anaerobic chamber (Coy Laboratory, Ann Arbor, MI, USA), approximately 2 mL of homogenized sediment slurry was dispensed into 160 mL serum bottles each containing 100 mL bicarbonate-buffered (pH 7.2) mineral salts medium and a headspace of N_2/

CO_2 (80:20, v/v) (Löffler et al. 2005). Lactate (5 mM) and hydrogen (10 mL, *ca.* 413 µmol) were provided as carbon source and electron donor, respectively. Filter-sterilized (0.22 µm pore size) 2,4,5-T stock solution (19 mM dissolved in 20 mM NaOH) was prepared and amended to each vessel to reach an initial concentration of 50 µM. Following the complete consumption of 2,4,5-T and its intermediate products (e.g., 3-CP, phenol), the sediment microcosm was repeatedly transferred (3%, v/v) under the same cultivation conditions to obtain the solid-free, 2,4,5-T-degrading consortium designated as XR/245T. The first and second generations of the XR/245T consortium were abbreviated as the 1st XR/245T and 2nd XR/245T, respectively. Inoculated from the 2nd XR/245T consortium, a 3-CP-degradating subculture designated as the XR/3CP was established and transferred with H_2 (10 mL) as electron donor and 3-CP (50 µM) as carbon substrate/electron accepter. Extra sediment microcosm vessels were established as described above, autoclaved and amended with the same amount of 2,4,5-T to serve as abiotic controls. All vessels were incubated in the dark at 30 °C without shaking.

Analytical methods

Culture supernatants (0.5 mL each sample) were filtrated (0.22 µm pore size) and stored at 4 °C prior to analysis. 2,4,5-T, chlorophenols and phenol were analyzed using an Agilent 1260 high performance liquid chromatography (HPLC) system equipped with an Agilent Hypersil reverse-phase C18 column (4.6 mm in diameter × 250 mm in length, 5 µm particle size) and a diode-array-detector set at 280 nm. Samples were separated at a flow rate of 1.0 mL min^{-1} at 35 °C using 2% acetic acid in deionized water (eluent A) and 100% acetonitrile (eluent B) as mobile phases. The initial mobile phase composition of 45% A and 55% B was held for 9 min, linearly changed to 80% A and 20% B over 3 min, and changed back to 60% A and 40% B over 1 min followed by a 2 min hold for column re-equilibration. Structures of the degradation products were confirmed using UPLC-Q-Exactive Orbitrap mass spectrometry (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a C18 column (2.1 mm in diameter × 100 mm in length, 1.9 µm particle size, Thermo Fisher Scientific). The analysis was performed using a Dionex UltiMate™ 3000 RSLC system (Thermo Fisher Scientific) in negative electrospray ionization mode. The mobile phase was 0.1% (v/v) acetic acid and 100% acetonitrile (40:60, v/v; isocratic, 8.0 min) at a flow rate of 0.3 mL min^{-1}. The column temperature was 25 °C and the injection volume was 3 µL. For the MS analysis, the Q-Orbitrap mass spectrometer (Thermo Fisher Scientific, USA) was coupled with the heat electrospray ionization (HESI). The auxiliary gas, sheath gas and sweep gas were set to flow rates of 22, 2 and 0 (arbitrary units), respectively. The spray voltage was set at 2.8 kV under negative mode and the S-lens RF level was set at 50 V. The auxiliary gas heater temperature and capillary temperature were both set to 300 °C. Nitrogen was used for spray stabilization and the damping gas in the C-trap. The analysis was performed in the full scan mode with a negative ion swing. The resolution was 70,000 (FWHM at 200 m/z). The automatic gain control target was set at 3.0×10^6 with a maximum injection time of 200 ms. The full MS scan ranges were set from 100 to 500 m/z. The acquired mass spectrometric data were processed using Thermo Xcalibur 3.0 software (Thermo Fisher Scientific).

DNA isolation and 16S rRNA gene amplicon sequencing

Samples collected from the sediment microcosm, the 1st and 2nd XR/245T consortium were used for 16S rRNA gene amplicon sequencing. Cells were harvested from 1 mL culture suspensions by vacuum filtration onto 0.22 µm membrane filters. Genomic DNA was isolated from the filters using the TIANamp Soil DNA Kit (TIANGEN Biotech, Beijing, China) following the manufacturer's instructions. DNA concentration was estimated using a Qubit 4 fluorometer (Thermo Fisher Scientific), and DNA quality was assessed using 0.8% agarose gel electrophoresis. Amplification of the hypervariable V3-V4 region of the bacterial 16S rRNA gene was performed using barcoded-primers U431F (5′-ACT CCTACGGGAGGCAGCAG-3′) and 806R (5′-GGA CTACHVGGGTWTCTAAT-3′) (Teng et al. 2018). Amplicon sequencing and analysis were performed by GENEWIZ Inc. (Suzhou, Jiangsu, China). Briefly, the purified amplicons (10 nM) were pooled in equimolar and paired-end sequencing was performed using an Illumina MiSeq PE250 platform (Illumina, San Diego, CA, USA) as described (Kozich et al. 2013). High-quality sequences were clustered into operational taxonomic units (OTUs) at a 97% identity threshold using Vsearch clustering (1.9.6) (Rognes et al. 2016). The classification of OTUs was performed using the SILVAngs server with default settings (Quast et al. 2013). Ace, Chao1, Shannon and Simpson indices were calculated using QIIME (1.9.1) pipeline as described (Bolger et al. 2014). The relative abundances of the OTUs were calculated and clustered at the genus-level using the complete group clustering method embedded in R (package vegan).

Results

Degradation of 2,4,5-T in the sediment microcosm and the XR/245T consortium

Four compounds (designated as M1, M2, M3 and M4) were detected as the potential products transformed from 2,4,5-T. Compounds M1 and M2, which exhibited HPLC

retention times of 11.83 min and 11.68 min, respectively, were produced at the early stage of the entire incubation period. At day 20, compound M3 was detected at a HPLC retention time of 7.70 min. Compound M4 with a HPLC retention time of 4.74 min emerged at day 60. The accumulation of compound M4 was accompanied by a significant decrease in the peak area of compound M3 (Fig. S1). By matching retention times with authentic standards, compounds M1, M2, M3 and M4 were preliminarily identified as 3,4-DCP, 2,5-DCP, 3-CP and phenol, respectively (Fig. S1).

The initial 52.5 ± 2.3 μM 2,4,5-T was depleted within 14 days in the sediment microcosm (Fig. 1A). In contrast, 2,4,5-T concentrations remained essentially unchanged in the abiotic controls (Fig. S2), indicating that the observed 2,4,5-T-degrading activity was mediated by the sediment microbiota. It took an incubation period of 48 days for 2,4,5-T and its transformation products (e.g., 3-CP, phenol) to disappear in the sediment microcosm. In the 1st XR/245T consortium, the amended 51.0 ± 1.1 μM 2,4,5-T decreased to 18.5 ± 1.6 μM over a period of 10 days. Meanwhile, 3,4-DCP reached to a maximum concentration of 21.3 ± 2.1 μM, and a minor amount of 3-CP (3.2 ± 0.2 μM) was produced. Subsequently, 3-CP was accumulated to 48.6 ± 3.2 μM by day 22 and then dechlorinated to phenol, which was slowly consumed and eventually disappeared at day 66 (Fig. 1B). In the 2nd XR/245T consortium, 2,5-DCP and 3,4-DCP were the first intermediates produced from 2,4,5-T and both compounds were subsequently dechlorinated to 3-CP, which reached a maximum concentration of 49.5 ± 1.2 μM at day 31. After a lag period of 18 days, the accumulated 3-CP underwent further transformation to phenol and the generated phenol gradually disappeared in the incubation system within the next 50 days (Fig. 1C). The XR/245T consortium maintained the 2,4,5-T-degrading ability, but the incubation times required for the complete elimination of 2,4,5-T and its transformation products (e.g., 3-CP, phenol) extended to 70 and 90 days in the 1st and 2nd XR/245T consortium, respectively. 3-CP and phenol accumulated to concentrations nearly equal to the initially amended 2,4,5-T and their transformations occurred at rates much slower than those of 3,4-DCP and 2,5-DCP (Fig. 1B, C). These observations suggest that the reductive dechlorination of 3-CP to phenol and the subsequent transformation of phenol are the key steps in the overall degradation process of 2,4,5-T. Notably, accumulation of a transformation product beyond phenol was not observed in the aqueous phase. We speculated that the disappeared phenol was eventually mineralized to CO_2 and/or CH_4 (Fig. 2), a degradation mechanism that had been proposed in previous studies (Qiu et al. 2008; Nobu et al. 2015; Harindintwali et al. 2022).

Mass spectrometric identification of the intermediate products

Mass spectrometric analysis was performed to confirm the identities of compounds M1, M2 and M3. Compound M1 was eluted at 2.82 min as shown in the extracted ion chromatogram (EIC) (Fig. 3A) and the deprotonated M1 ion was represented by two adducts at *m*/*z* 160.96 and *m*/*z* 162.95 (Fig. 3B). The retention time and primary mass spectrum of compound M1 matched with those of the 3,4-DCP standard (Fig. 3A, B, S3). Similarly, compound M2 exhibited identical retention time (i.e., 2.75 min) and mass characteristics to the 2,5-DCP standard (Fig. 3C, D, S3). Despite 3,4-DCP and 2,5-DCP displayed highly similar mass spectrometric patterns, these two compounds could be distinguished from each other based on their EIC retention times (i.e., 2.82 min for 3,4-DCP and 2.75 min for 2,5-DCP). The retention time of compound M3 was identical (i.e., 1.50 min) as that of the 3-CP standard (Fig. 3E, S3). In addition, the deprotonated

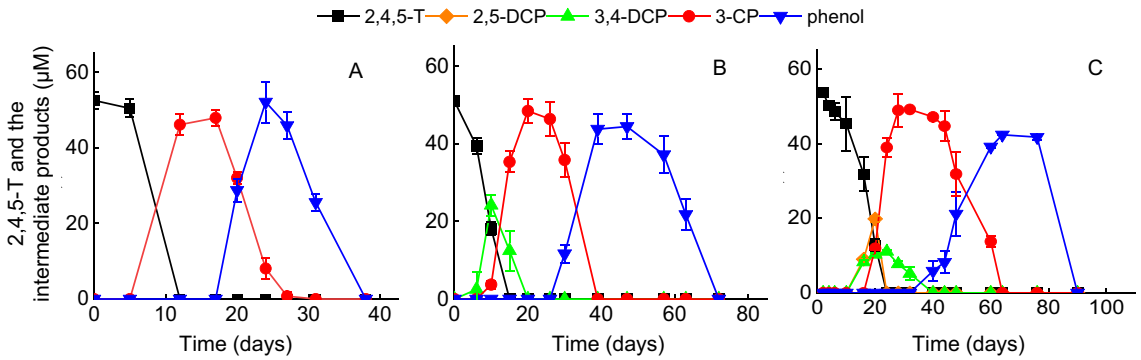

Fig. 1 Anaerobic biotransformation of 2,4,5-T in the sediment microcosm (**A**), the 1st XR/245T consortium (**B**) and the 2nd XR/245T consortium (**C**). The error bars represent the standard deviations of triplicate samples and are not shown when smaller than the symbol

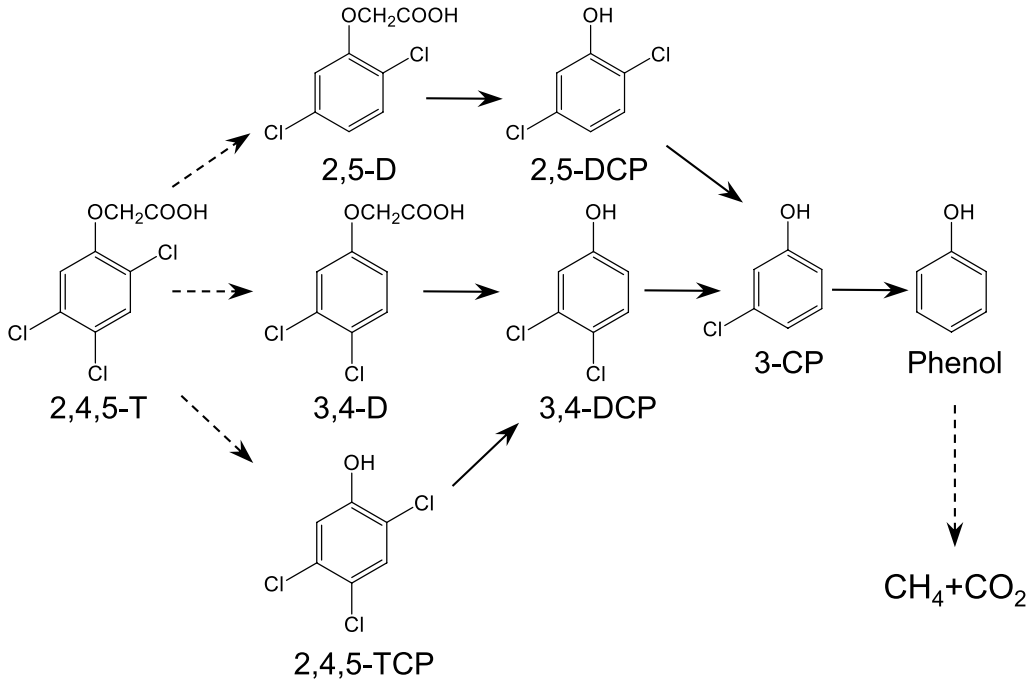

Fig. 2 The proposed biodegradation pathway of 2,4,5-T under anaerobic condition. Solid lines indicate the processes observed in this study, and dashed lines indicate potential transformation process in which the speculated products were not analyzed. Note, a potential degradation product of phenol was not detected under the HPLC settings used in this study

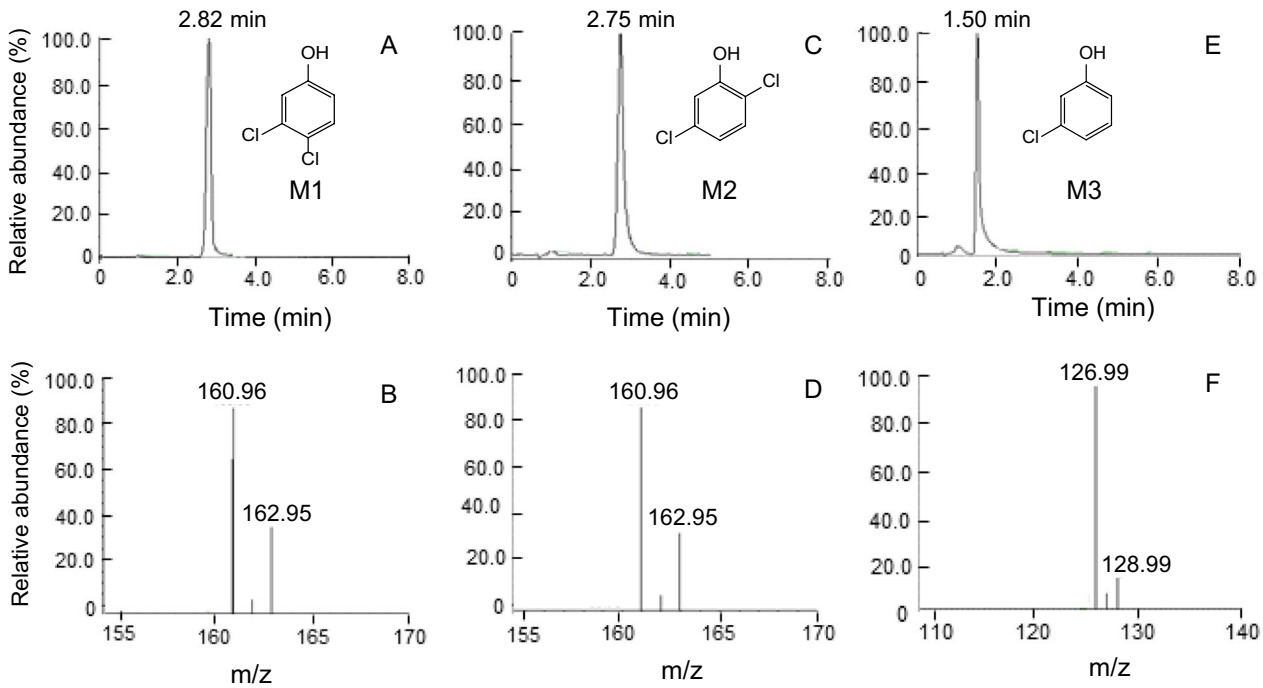

Fig. 3 Mass spectrometric analysis of the intermediate products transformed from 2,4,5-T. The total ion chromatogram (**A**) and primary mass spectrum (**B**) of compound M1; the total ion chromatogram (**C**) and primary mass spectrum (**D**) of compound M2; the total ion chromatogram (**E**) and primary mass spectrum (**F**) of compound M3

ions generated from compound M3 and 3-CP were identical and represented by two adducts at *m*/*z* 126.99 and *m*/*z* 128.99 (Fig. 3F, S3). Collectively, mass spectrometric analysis provided additional evidence to confirm the identities of the intermediate products transformed from 2,4,5-T. These results corroborate that anaerobic degradation of 2,4,5-T proceeded via sequential reductive dechlorination. The overall degradation pathway observed in this study was illustrated in Fig. 2.

Biodiversity and microbial composition of the 2,4,5-T-degrading community

The rarefaction curves of the total sequenced amplicons reached the plateau (Fig. S4), indicating the sequencing depth was sufficient to unravel the composition of the 2,4,5-T-degrading community. The sediment microcosm contained the highest OTUs number (i.e., 594). Following consecutive transfer events, the total OTUs numbers decreased to 391 and 100 in the 1st and 2nd XR/245T consortium, respectively (Fig. S4). As listed in Table S1, the Ace and Chao1 indices were calculated as 660.8 and 674.0 for the sediment microcosm and decreased to 101.6 and 100.8 in the 2nd XR/245T consortium. The Shannon and Simpson indices, which reflect the abundance and diversity of species, were the highest (6.9 and 1.0) in the sediment microcosm. After two transfers, the Shannon and Simpson indices decreased to 4.6 and 0.9, respectively, in the 2nd XR/245T consortium.

The OTUs obtained from the 2,4,5-T-degrading community could be assigned to 31 phyla, 103 families, and 131 genera. The dominant phyla in the sediment microcosm were Firmicutes (29.8%), Bacteroidetes (28.4%) and Proteobacteria (15.3%) (Fig. S5A). Firmicutes remained as the most abundant one (33.9%) in the 1st XR/245T consortium, but its abundance decreased to 19.6% in the 2nd XR/245T consortium. The relative abundances of Bacteroidetes and Proteobacteria decreased to 19.1% and 10.7%, respectively, in the 2nd XR/245T consortium. Sequences affiliated with Epsilonbacteraeota only accounted for 1.6% of the total sequenced amplicons in the sediment microcosm. The relative abundance of Epsilonbacteraeota significantly increased to 25.8% in the 2nd XR/245T consortium. At the family level, Bacteroidetes_vadinHA17 (20.2%), Peptococcaceae (13.9%), Syntrophaceae (9.6%), Clostridiaceae (7.7%) and Rikenellaceae (5.5%) were the most abundant ones in the sediment microcosm (Fig. S5B). In the 2nd XR/245T consortium, sequences belonging to Bacteroidetes_vadinHA17 nearly disappeared and the relative abundances of Syntrophaceae and Clostridiaceae also dropped to 1.2% and 2.4%, respectively. Thiovulaceae only represented a minor fraction (1.2%) in the sediment microcosm, but its relative abundance increased to 25.6% in the 2nd XR/245T consortium. Rikenellaceae and Peptococcaceae were consistently present in the sediment microcosm and the 1st and 2nd XR/245T consortium, with relative abundances ranging from 5.5 to 6.8% and 9.8 to 13.9%, respectively.

The top 30 most abundant OTUs in the 2,4,5-T-degrading community at the genus level were shown in the clustering heatmap (Fig. 4). The sediment microcosm was dominated by the unclassified "vadinHA17" within the phylum Bacteroidetes, as well as the genera *Smithella*, *Clostridium* sensu stricto 7, *Cryptanaerobacter* and *Dehalobacter*; however, the relative abundances of *Smithella* and "vadinHA17" significantly decreased in the 2nd XR/245T consortium. Following two consecutive transfers, the dominant OTUs in the 2nd XR/245T consortium were changed to *Sulfuricurvum*, *Dehalobacter*, *Lentimicrobium*, *Syntrophorhabdus* and *Methanosaeta*.

Identification of the key microbial populations involved in the degradation of 3-CP

Degradation of 3-CP is the critical step for the complete decomposition of 2,4,5-T. To investigate the microbial populations responsible for 3-CP degradation, a subculture (i.e., XR/3CP consortium) was established and supplied with 3-CP as carbon source/electron acceptor (Fig. 5). Following a lag period of 15 days, the initial 40.5 ± 2.2 μM 3-CP was completely degraded within the next 25 days. Phenol was accumulated to a maximum concentration of 30.5 ± 3.1 μM at day 40 and gradually disappeared in the next 23 days. The composition and abundant genera (i.e., with a relative abundance ≥ 1%) in the XR/3CP consortium was shown in Fig. 6. The most abundant bacterial genera belonged to *Sulfuricurvum* (29.3%), *Acetobacterium* (13.7%), *Smithella* (5.1%), *Lentimicrobium* (4.0%), *Dehalobacter* (3.8%), *Bacteroides* (3.6%), *Syntrophorhabdus* (3.5%) and *Clostridium* sensu stricto *7* (3.2%). The methanogenic genera *Methanofollis* (3.9%) and *Methanosaeta* (2.3%) were also detected in the XR/3CP consortium, suggesting that methanogenesis occurred concurrently during the degradation of 3-CP.

Discussion

The degradation pathway of 2,4,5-T

Here, we investigated the degradation process and environmental fate of 2,4,5-T using a microbial consortium established from freshwater river sediment. Ether cleavage and reductive dechlorination are the main reactions driving the transformation and degradation of 2,4,5-T under anaerobic condition. The complete removal of the generated phenol suggested that further transformation process took place in the incubation system, likely the mineralization reactions that converted phenol to CO_2 and/or CH_4 (Chenprakhon

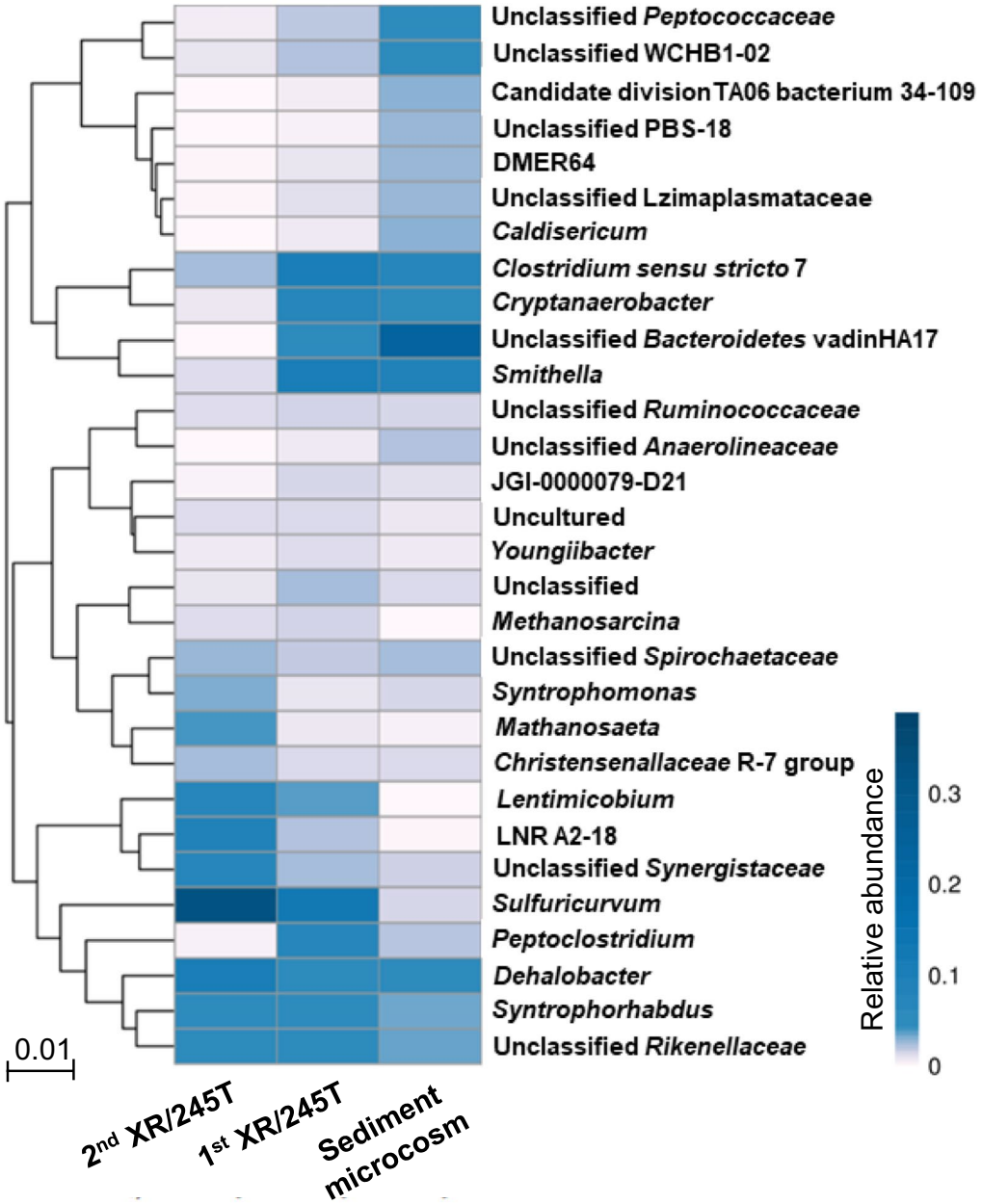

Fig. 4 Heatmap of the top 30 most abundant genera in the sediment microcosm and the XR/245T consortium. The color code represents the relative abundance value of each taxon displayed in the heatmap, ranging from light blue (low abundance) to dark blue (high abundance). The tree scale bar indicates nucleotide substitutions per site

et al. 2019). Ether bond cleavage as the initialization reaction for the degradation of 2,4,5-T has been reported in several anaerobic cultures derived from soils (Chang et al. 1998a, b; Lechner et al. 2018), sediments (Gibson and Suflita 1990; Bryant 1992; Al-Fathi et al. 2019), sewage sludge (Suflita et al. 1984; Mikesell 1985). In this reaction, the acetyl group was removed from the aromatic ring and 2,4,5-TCP was formed as the first degradation product of 2,4,5-T. In addition, the initialization of 2,4,5-T degradation via reductive dechlorination also has been described in cultures derived from methanogenic aquifer slurries (Gibson and Suflita 1990), soil (Chang et al. 1998a, b) and sediment (Al-Fathi et al. 2019). The removal of the *para-*, *meta-* or *ortho*-chlorine substituent in the benzene ring of 2,4,5-T can produce a variety of

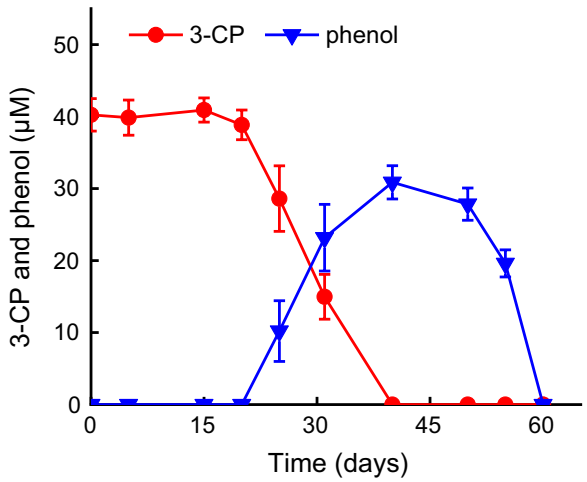

Fig. 5 Anaerobic transformation of 3-CP in the XR/3CP consortium. The error bars represent the standard deviations of triplicate samples and are not shown when smaller than the symbol. Note, a potential degradation product of phenol was not detected under the HPLC settings used in this study

dichlorophenoxyacetic acids such as 2,5-dichlorophenoxyacetic acid (2,5-D), 2,4-dichlorophenoxyacetic acid (2,4-D) and 3,4-dichlorophenoxyacetic acid (3,4-D) as the initial products. In the follow-up reactions, 2,5-D, 2,4-D and 3,4-D are subject to ether cleavage and can further undergo reductive dechlorination reaction to form 3-CP or 4-CP. Neither a dichlorophenoxyacetic acid nor 2,4,5-trichlorophenol was detected during the entire incubation period; therefore, it can be concluded that the anaerobic degradation of 2,4,5-T primarily involves reductive dechlorination reaction followed by the further degradation of phenol (e.g., mineralization).

Microbial diversity is essential for the rapid degradation of 2,4,5-T

The major microbial components in the 2,4,5-T-degrading community were largely the same between the sediment microcosm and the XR/245T consortium. Alpha diversity indices showed that a dramatic reduction in microbial richness and diversity, which might result in the prolonged incubation time to accomplish the complete breakdown of 2,4,5-T following the transfer events. The reduction in biodiversity might be due to the inability of certain taxa to utilize 2,4,5-T as carbon substrate and/or energy source or their susceptibility to the toxicity of 2,4,5-T. It has been reported that the function and activity of a community largely depend on the succession of the microbial diversity in the original sample (Ahmad et al. 2019). Transferring cultures using a small inoculum size may lead to the loss of minor taxa with a low relative abundance (Jung et al. 2016). Overall, microbial diversity is a crucial factor in shaping community structure and is essential for ecosystem functioning, particularly in the utilization and degradation of complex substances such as 2,4,5-T (Xu et al. 2024). Understanding the dynamics of the 2,4,5-T-degrading community is essential for optimizing bioremediation strategies and leveraging microbial communities for environmental cleanup efforts.

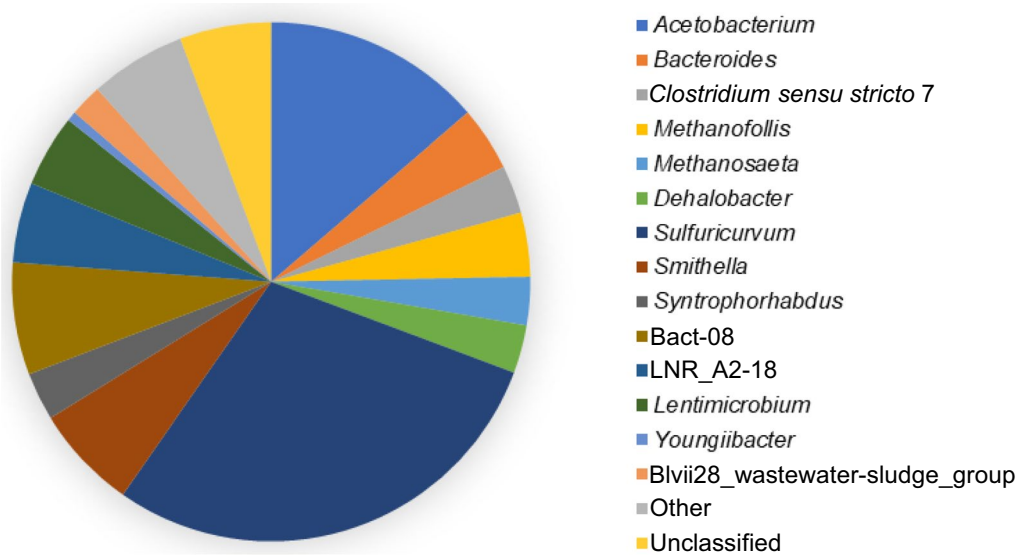

Fig. 6 Microbial community structures at the genus level in the XR/3CP consortium. Note, "Other" refers to the OTUs detected at low abundances (i.e., ≤ 1% of the total sequenced amplicons)

The involvement of reductive dechlorination in the degradation process

The formation and subsequent elimination of 3,4-DCP, 2,5-DCP and 3-CP evidence that reductive dechlorination is a key reaction facilitating the overall degradation process of 2,4,5-T. Reductive dechlorination is the signature biochemical reaction catalyzed by organohalide-respiring bacteria (OHRB), which utilize organohalogens as electron acceptors for energy conservation (Li et al. 2022). The ability to grow with chlorophenols had been demonstrated in a variety of OHRB phylotypes, including *Dehalobacter*, *Dehalococcoides*, and *Desulfitobacterium* (Tront et al. 2006; Adrian et al. 2007; Wang et al. 2014). Among them, *Desulfitobacterium* is the only known OHRB phylotype capable of reductive dechlorination of 2,4,5-T (Lechner et al. 2018). In this study, *Dehalobacter* was the only OHRB phylotype present in the 2,4,5-T-degrading community. Some members of this genus are capable of metabolizing certain chlorinated aromatic compounds, including chlorobenzenes and chlorophenols (Wang et al. 2014; Al-Fathi et al. 2019). For example, *Dehalobacter* sp. strain TCP1, strain 12DCB1 and strain 13DCB1 have been implicated in the reductive dechlorination of 2,4,6-trichlorophenol, 1,2-dichlorobenzene or 1,3-dichlorobenzene (Nelson et al. 2011; Wang et al. 2014). A *Dehalobacter*-containing enrichment culture, which was obtained from contaminated sandy soil, was able to dechlorinate β-hexachlorocyclohexane (van Doesburg et al. 2005). In addition to *Dehalobacter*, *Sulfuricurvum* and *Clostridium* sensu stricto 7 are also likely involved in the reductive dechlorination of 2,4,5-T. Although *Sulfuricurvum* has not yet to be described as a chlorophenol-dechlorinating phylotype, several studies have documented the increase of *Sulfuricurvum*-like sequences during the reductive dechlorination of polychlorinated biphenyls and in situ biostimulation for the remediation of chlorinated compounds (Yu et al. 2017). Previous studies indicated that *Clostridium* sensu stricto 7 was frequently present in microbial communities involved in the degradation of chlorophenols. Certain *Clostridium* spp. strains have the potential to utilize acetate or lactate, generating H_2 that can serve as electron donor for reductive dechlorination reactions (Freeborn et al. 2005; Behrens et al. 2008). The uncharacterized *Clostridium* sensu stricto 7 may play a supporting role in the anaerobic biodegradation of 2,4,5-T (Li et al. 2010; Xu et al. 2018).

Elimination of 3-CP is the key process for the anaerobic degradation of 2,4,5-T

3-CP has been identified as an intermediate product during the degradation of 2,4,5-T, but there is limited research demonstrating its complete degradation under anaerobic condition. The degradation of 3-CP observed in this study can be divided into two phases: the removal of the *meta*-chlorine substituent via reductive dechlorination and the further degradation of phenol. The microbial composition in the 3-CP-degrdading community was similar to that of the 2,4,5-T-degrdading community. In addition to potential dechlorinators (e.g., *Dehalobacter*), syntrophic fermenters such as *Syntrophorhabdus*, *Syntrophobacter* and *Syntrophomonas*, as well as methanogens such as *Methanofollis* and *Methanosaeta* were also present in the XR/3CP consortium. Previous studies showed that cooperation of *Syntrophorhabdus* with hydrogenotrophic or acetoclastic methanogens facilitate the anaerobic mineralization of phenol via the benzoate degradation pathway (Qiu et al. 2008). *Syntrophobacter* and *Syntrophomonas* are also hydrogen- and acetate-producing bacteria that can metabolize aromatic compounds into small molecules for methanogens to utilize and therefore sustain the methanogenesis process in a syntrophic manner (Chellapandi et al. 2018). The microbial composition of the 3-CP-degrading community suggests that phenol generated from 3-CP can undergo decomposition through syntrophic metabolism. In summary, our results demonstrate that dechlorinators, syntrophic fermenters and methanogens work cooperatively to accomplish the complete breakdown of 2,4,5-T and its degradation intermediates under anaerobic condition.

Conclusion

We show that, under anaerobic condition, 2,4,5-T is primarily transformed through sequential reductive dechlorination, followed by the subsequent degradation of phenol. *Dehalobacter* is identified as the OHRB population responsible for the removal of chlorine substituents from 2,4,5-T. In addition, *Syntrophorhabdus* and methenogens such as *Methanofollis* and *Methanosaeta* likely play syntrophic roles in the anaerobic phenol oxidation. This study explores the anaerobic degradation process of chlorophenoxy herbicides and provides insights into microbial determinants controlling the environmental fate of these toxins.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13717-024-00545-1.

Additional file 1: Table S1. Alpha diversity indices of the 2,4,5-T-degrading sediment microcosm and the XR/245T consortium; Fig. S1: HPLC chromatograms of the standards for 2,4,5-T, 2,4,5-TCP, 3,4-DCP, 2,5-DCP, 2,4-DCP, 3-CP, 4-CP, 2-CP, and phenol, and the intermediate products identified in the 2nd XR/245T consortium at day 20 and at day 60. Fig. S2: Steady 2,4,5-T concentrations in the heat-killed sediment microcosm. Fig. S3: The total ion chromatograms and the primary mass spectra of the authentic

standards for 3,4-DCP, 2,5-DCP and 3-CP. Fig. S4: Rarefaction curves of 16S rRNA gene amplicon sequences obtained from the sediment microcosm and the XR/245T consortium. Fig. S5: Community structure at the phylum level and family level in the sediment microcosm, the 1st XR/245T and 2nd XR/245T consortium.

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Author contributions
JY and XYL initiated the study and led development of the manuscript. YL, YZW, ZPZ, JJW and HJJ conducted the experiments. TYZ, YRC and YY collected the data and developed figures. All authors contributed critically to the drafts.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable in this section.

Consent for publication
Not applicable in this section.

Competing interests
The authors declare that they have no competing interests.

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