Dechlorination of *p,p'*-DDTs coupled with sulfate reduction by novel sulfate-reducing bacterium *Clostridium* sp. BXM

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ABSTRACT

A novel non-*dsrAB* (without dissimilatory sulfite reductase genes) sulfate-reducing bacterium (SRB) *Clostridium* sp. BXM was isolated from a paddy soil. Incubation experiments were then performed to investigate the formation of reduced sulfur compounds (RSC) by *Clostridium* sp. BXM, and RSC-induced dechlorination of *p,p'*-DDT in culture medium and soil solution. The RSCs produced were 5.8 mM and 4.5 mM in 28 mM sulfate amended medium and soil solution respectively after 28-day cultivation. The *p,p'*-DDT dechlorination ratios were 74% and 45.8% for 5.8 mM and 4.5 mM RSCs respectively at 6 h. The metabolites of *p,p'*-DDT found in the two reaction systems were identified as *p,p'*-DDD and *p,p'*-DDE. The dechlorination pathways of *p,p'*-DDT to *p,p'*-DDD and *p,p'*-DDE were proposed, based on mass balance and dechlorination time-courses. The results indicated that RSC-induced natural dechlorination may play an important role in the fate of organochlorines.

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1. Introduction

Organochlorines, especially persistent organic pollutants (POPs), are of great concern as environmental pollutants due to their toxicity, persistence and bioaccumulation. They include polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane and related compounds (DDTs), and hexachlorocyclohexane (HCHs) isomers, etc. These POPs were manufactured and used all over the world during the 1970s, but are now banned. Nonetheless, DDTs are allowed by the Stockholm Convention on Persistent Organic Pollutants (POPs) of 2001 to use in some developing countries for public health purposes and are still an environmental threat (Buser, 1995; Thomas et al., 2008; Behrooz et al., 2009; Wang et al., 2010; Zhou et al., 2011).

Microorganisms play an important role in the fate of organochlorines in natural and controlled environments (El Fantroussi et al., 1998). Sulfate-reducing bacteria (SRB) are widely distributed in the environment, including paddy soils, sea water, thermal springs, oil field water and sediments. They are involved in numerous reactions under anaerobic environments (e.g., oxidation of organic matter, dechlorination of organochlorines, and sulfur cycling) (Jorgensen, 1982; Habicht and Canfield, 1996; Gilmour et al., 1998). SRB

constitute a diverse group of prokaryotes with the common ability to metabolize oxidized sulfur compounds (OSCs, e.g. sulfate, sulfur) to RSCs (e.g., sulfide, hydrosulfide), and drive the production of metabolic ATP (Shen and Buick, 2004). Several studies have reported the dechlorination of organochlorines by SRB. Trichloroethylene (TCE) was reductively dechlorinated to *cis*-1, 2-dichloroethylene (cDCE) by sulfate-reducing cultures enriched from a contaminated subsurface soil (Pavlostathis and Zhuang, 1991). Carbon isotope fractionation factors were determined with the dichloro elimination of γ -hexachlorocyclohexane (γ -HCH) by *Desulfococcus multivorans* DSM 2059 and *Desulfovibrio gigas* DSM 1382 (Badea et al., 2009). Extracts of *Desulfomonile tiedjei* cells dechlorinated 3, 5-dichlorobenzoate almost completely to 3-chlorobenzoate before the later metabolite was consumed (Deweerd and Suflita, 1990). The co-metabolism, fermentation and respiratory dechlorination mechanisms of SRB have been well defined (Holliger and Schraa, 1994; El Fantroussi et al., 1998). However, the understanding of RSC-induced abiotic dechlorination related to sulfate-reducing process is deficient. We hypothesized that there may be a relation between dechlorination of organochlorines and RSCs produced by SRB, in view of the chemical dechlorination potential of RSCs (S^{2-} , HS^-) which has been investigated (Perlinger et al., 1996; Hassan, 2000; Gan et al., 2006; Zheng et al., 2006; Ba et al., 2011).

The primary purpose of this study was therefore to isolate SRB from paddy soil and investigate the generation of RSCs and

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RSC-induced dechlorination of *p,p'*-DDTs under sulfate-reducing conditions, and furthermore to reveal the coupling mechanism between SRB-mediated sulfur redox reactions and the dechlorination of organochlorines.

2. Materials and methods

2.1. Reagents and solvents

Standard samples of *p,p'*-DDT (*p,p'*-dichlorodiphenyltrichloroethane) (98.5% pure), *p,p'*-DDD (1,1-dichloro-2,2-bis(4-chlorophenyl)-ethane) (99.0% pure) and *p,p'*-DDE (1-Chloro-2-[2,2-dichloro-1-(4-chlorophenyl)ethenyl]-benzene) (98.5% pure) were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Anhydrous sodium sulfate (Na_2SO_4) and other chemical reagents were of analytical grade made in China. Chromatographically pure hexane and methanol were obtained from J&K Chemical LTD (Utah, USA). Serum bottles were purchased from Glasgerätebau OCHS GmbH (Göttingen, Germany).

2.2. Soil sample collection, enrichment and isolation of SRB

Paddy soil mud for enrichment of SRB in the upper section of a paddy field was collected from Yunnan Dehong region (24°41'29"N, 97°58'37"E), in the southwest of China. For soil solution incubation experiment, the soil materials were air-dried, ground with a mortar and crushed to pass through a 2.0 mm sieve. Soil organic carbon (2.32%) was determined by potassium dichromate oxidation titration, and soil total Fe (28.4 g kg⁻¹) was determined by ICP-OES. Plant-available sulfur (14.4 mg kg⁻¹) was extracted by $\text{Ca}(\text{H}_2\text{PO}_4)_2$ (0.01 mol l⁻¹) and determined by turbidimetry. Soil-available Fe (36.5 mg kg⁻¹) was extracted by DTPA- CaCl_2 -TEA and determined by ICP-OES. No DDTs were detected by GC-ECD in the tested soil.

An anoxic freshwater medium was used for enrichment of SRB from the fresh paddy soil. The medium contained (g l⁻¹): K_2HPO_4 0.2, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.2, $(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ 0.2, Na_2SO_4 (depending on different treatments), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0.5, yeast extract 1.0, vitamin C 0.2, sodium thioglycolate 0.1, sodium lactate 3.5. The pH of the medium was adjusted to 7.2 with 6 M NaOH solution (Azabou et al., 2007). The medium was autoclaved and cooled to room temperature under an atmosphere of N_2/CO_2 (90/10, V/V) (Widdel and Bak, 1992). Fresh paddy soil samples (5.0 g) were put into 100 ml anoxic water and mixed homogeneously, then 5 ml of suspension was added to serum bottles containing 50 ml medium and incubated at 30 °C in the dark. Standard anaerobic culturing techniques were used throughout the experiments (Widdel and Bak, 1992). During incubation, the enrichment was briefly shaken once a day. New cultures were inoculated with 10% inoculum (v/v), and after 15 transfers a culture with a stable microbial community was obtained (Selesi and Meckenstock, 2009).

For the isolation of SRB, liquid medium was used via serial dilutions. The method was analogous to the dilution technique for determination of most probable numbers (MPN technique). The best proof of the positive growth of SRB is the production of RSCs. This can be detected by a simple chemical test with 5 mM CuSO_4 and 50 mM HCl. The intensity of the brown color of the colloidal CuS gives a good estimate of RSC production (Bak and Widdel, 1986). The cell morphology was observed under a phase contrast microscope (Nikon E2100) and scanning electron microscope (Hitachi S-2000N).

Soil solution preparation was performed in the serum bottle. Air-dried soil (20 g) mixed with deionized deoxygenated water (60 ml) in serum bottle, and then the bottle was purged with nitrogen gas for 5 min to create an anaerobic environment. Each serum bottle was sealed with a butyl rubber stopper, covered with aluminum foil, and then autoclaved. Exponential phase BXM culture (1.0 ml) and/or sulfate were added into bottle according to different treatments. Then, the bottle was incubated at 30 °C in the dark for 28 days. The supernatant would be withdrawn for soil solution experiment.

2.3. DNA extraction, PCR amplification, sequencing and phylogenetic analyses

Cell material for sequencing of the 16S rRNA genes was taken from 4 ml of well-grown liquid cultures. All general molecular techniques were performed according to described standard methods (Sambrook et al., 1989). Extraction of genomic DNA from the cells was performed with a FastDNA Spin Kit (Takara) for medium according to the manufacturer's protocol.

Amplification of the 16S rRNA gene was performed using the primer set Ba27F-Ba1492R (Weisburg et al., 1991), resulting in a full-length PCR product. The identities of the 16S rRNA gene sequences were confirmed by searching the international sequence databases using the BLAST programs (<http://www.ncbi.nlm.nih.gov/BLAST>). The best matching sequences were retrieved from the database and analyzed with CLUSTAL 2.0 (Thompson et al., 1994). The phylogenetic tree was constructed using neighbor-joining approach implemented in MEGA 4.1 (Tamura et al., 2007). The topologies of the resultant tree were calculated by bootstrap analysis (Felsenstein, 1985) based on 1000 replications.

2.4. Utilization of sulfur compounds by SRB

Growth experiments were performed in triplicate with different sulfur compounds. Sulfate, sulfite, elemental sulfur and thiosulfate were added to the medium at initial concentrations of 28 mM, 28 mM, 28 mM and 14 mM, respectively. Growth was monitored by following changes in optical density of the medium at 600 nm (OD 600) using spectrophotometer (Pgeneral T6) (Qiu et al., 2009) at selected 3, 6, 8, 10, 13, 16 and 28 days.

2.5. Determination of RSCs in filter-sterilized and heat-treated SRB cultures (medium and soil solution) by ion chromatography

RSCs in 0.1 M sodium hydroxide solution were easily oxidized to sulfate by hydrogen peroxide, which could be determined by ion chromatography (Ubuka et al., 2001). The 28-day cultivated SRB medium/soil solution was filter-sterilized and heat-treated, then 10 ml solution was added into 500 µl 0.2 M sodium hydroxide solution and placed in a test tube with a Teflon-lined screw cap. After addition of 30 ml 30% hydrogen peroxide and 460 µl double distilled water, the mixture was incubated at 50 °C for 60 min. After cooling, ion chromatography was applied to determine the RSCs in the mixture. The ion chromatography system consisted of the following parts: GP50 gradient pump, a column oven LC25, and an electrochemical detector ED50. The ion chromatography column system used was a Dionex Ionpac AS14 column (4.0 × 250 mm).

2.6. Dechlorination of *p,p'*-DDT in filter-sterilized and heat-treated SRB cultures (medium and soil solution)

Four culture medium treatments in triplicate were performed: (i) 28 mM Na_2SO_4 without SRB (ii) 28 mM Na_2SO_4 + SRB, (iii) 14 mM Na_2SO_4 without SRB (iv) 14 mM Na_2SO_4 + SRB, and incubated in the dark for 28 days in 50 ml serum bottles. For the *p,p'*-DDT dechlorination test, all the decline phase cultures were filtered firstly and transferred into the other 50 ml serum bottles in an anaerobic chamber, then heat-treated at 100 °C for 30 min for three times to eliminate the biological influence before addition of *p,p'*-DDT. The *p,p'*-DDT was dissolved in methanol to 1.0 mg ml⁻¹ and filter sterilized as stock solution. The *p,p'*-DDT methanol solution (50 µl) was added into serum bottle with microsyringes, and 2.0 ml methanol was added into serum bottles for complete dissolution of *p,p'*-DDT. The initial concentration of *p,p'*-DDT was 1.0 µg ml⁻¹ medium. At selected times of 6, 30, 54, 78 and 102 h, 1.0 ml solution was withdrawn with injectors to investigate amounts of *p,p'*-DDT and dechlorination metabolites as described in the following section 2.7.

For soil solution experiments, four paddy soil/water mixtures (20 g/60 ml) treatments were performed: (i) control (without SRB and/or sulfate amendment), (ii) SRB, (iii) 28 mM Na_2SO_4 , (iv) 28 mM Na_2SO_4 + SRB. Each treatment included 3 replicates and all the treatments were incubated at 30 °C in the dark for 28 days in 100 ml serum bottles. Then, 50 ml supernatant soil solution was withdrawn with microsyringes and filtered into 50 ml serum bottles in an anaerobic chamber. Soil solution was heat-treated at 100 °C (30 min) for three times before addition of *p,p'*-DDT. The *p,p'*-DDT stock solution was prepared as above. The initial concentration of *p,p'*-DDT was 1.0 µg ml⁻¹ soil solution. The amounts of *p,p'*-DDT and dechlorination metabolites were determined as described in section 2.7.

2.7. Determination of *p,p'*-DDT and its metabolites by GC-MSD

Ultrasonic extraction was used for extraction of *p,p'*-DDT and its metabolites in medium and soil solution. Hexane of analytical grade was used as extractive solvent. The sample (1.0 µl) was drawn from serum bottles and quickly ultrasonically extracted, 5.0 min for every ultrasonic extraction for three times with a total of 10 ml hexane (Tor et al., 2006). The ultrasonic extracts were passed through a funnel filled with 2.0 g anhydrous sodium sulfate to eliminate residual water (Hussen et al., 2006). Then the samples were evaporated to 1.0 ml with nitrogen gas.

The remaining amounts of *p,p'*-DDT and metabolites were analyzed with an Agilent 7890A gas chromatograph equipped with an Agilent 5975C mass selective detector (GC-MSD). The column used was a HP-5MS, 30 m × 0.25 mm id with a 0.25 µm film thickness. The injector temperature was 250 °C and the flow rate of helium gas was 1.0 ml min⁻¹ (Manirakiza et al., 2000).

3. Results

3.1. Morphological, phylogenetic and physiological characteristics of strain BXM

Cells of strain BXM were non-motile, spore-forming, non-flagellar, rod-shaped, and Gram-positive. Individual cells were 2.0–3.0 µm long and 0.3–0.5 µm wide (Fig. 1). Phylogenetic analysis (Fig. 2) showed that the closest relatives of strain BXM was *Clostridium sporogenes* (X68189) with 99.8% 16S rRNA gene sequence similarity.

Fig. 1. Phase contrast (a) and transmission electron micrograph (b) of *Clostridium* sp. BXM. Bar (a) 5.0 µm, (b) 3.0 µm.

The phylogenetic parallel discussed above suggests that the strain represents a novel species of genus *Clostridium* within the family *Clostridiaceae* in the phylum *Firmicutes*, for which the name *Clostridium* sp. BXM is proposed. The nucleotide sequence data were deposited in GenBank under accession number: JN092128. The *Clostridium* sp. BXM was able to utilize the following sulfur compounds: sulfate, thiosulfate and sulfur coupled with oxidation of lactate. Sulfate was the best electron acceptor, while sulfite could not be utilized (data not shown).

3.2. Differences of RSC concentration

Differences of RSCs concentration between with SRB and without SRB were determined in dependence on sulfate level and medium (Figs. 3 and 5). In culture medium, concentrations of RSCs were significantly greater in treatments with SRB than in those without SRB: 5.8 mM versus 0.7 mM with 28 mM sulfate in the medium, and 1.3 mM versus 0.3 mM with 14 mM sulfate in the medium (Fig. 3). The ratio of RSC formation increased with increasing sulfate concentration.

In the soil solution, the higher concentration of RSCs (4.45 mM) was obtained with 28 mM sulfate plus SRB, while the lowest value (0.18 mM) was obtained in the control group (without sulfate and SRB) (Fig. 5).

3.3. Time-courses of *p,p'*-DDT and its metabolites dechlorination by RSCs

The residual concentration of *p,p'*-DDT in culture medium showed no significantly decline in the controls (without SRB) during reaction for 102 h, given that the *p,p'*-DDT concentration fluctuated

Fig. 2. Phylogenetic tree constructed by the neighbor-joining method, using 1475 nucleotides from 16S rDNA sequences, showing the position of strain BXM in relation to members of the genus *Clostridium*. The bar represents 0.05 changes per nucleotide position.

between 1058 and 926 ng ml⁻¹, 1085 and 989 ng ml⁻¹ in treatments with 14 and 28 mM sulfate (Fig. 4a and c). By contrast, the residual concentration of *p,p'*-DDT decreased by 74% with 28 mM sulfate + SRB, and by 50% with 14 mM sulfate + SRB during reaction of 6 h. All of the original *p,p'*-DDT disappeared in treatments with addition of SRB during 54 h (Fig. 4b and d).

The total ion current chromatograph for metabolites of *p,p'*-DDT shown that the compounds were *p,p'*-DDD and *p,p'*-DDE, based on the unique fragmentation peaks (data not shown). The concentration of *p,p'*-DDD < 80 ng ml⁻¹ was detected in medium with sulfate, but no *p,p'*-DDE (Fig. 4a and c). With the decrease of *p,p'*-DDT, the concentration of *p,p'*-DDD gradually increased to 672 and 693 ng ml⁻¹ and subsequently decreased in treatments with SRB + 28 mM sulfate, and SRB + 14 mM sulfate. The concentration of *p,p'*-DDE gradually increased to 2.0 and 96 ng ml⁻¹ and subsequently declined (Fig. 4b and d). The results suggested that SRB + sulfate resulted in dechlorination of *p,p'*-DDT into *p,p'*-DDD/*p,p'*-DDE, with even further dechlorination of *p,p'*-DDD and *p,p'*-DDE. The dechlorination of *p,p'*-DDT, *p,p'*-DDD and *p,p'*-DDE in culture medium were all RSC dependent.

In the soil solution, there was little variation of the *p,p'*-DDT concentration in the control group, and in treatments with addition of 28 mM sulfate alone (Fig. 6a and c). By contrast, the residual

Fig. 3. Impacts of SRB and additional sulfate levels (14, 28 mM) on RSC production in 28-day cultivation medium.

Fig. 4. Impact of RSCs on *p,p'*-DDT transformation and production of metabolites *p,p'*-DDD and *p,p'*-DDE in culture medium. Treatments including: (a) 14 mM sulfate (containing 0.30 mM RSCs). (b) 14 mM sulfate + SRB (containing 1.30 mM RSCs). (c) 28 mM sulfate (containing 0.70 mM RSCs). (d) 28 mM sulfate + SRB (containing 5.80 mM RSCs). (Bars on symbols represent standard deviations; where invisible, bars fall within symbols).

concentration of *p,p'*-DDT decreased by 20% with SRB alone, and by 46% with SRB + sulfate during reaction for 6 h (Fig. 6b and d), and continuously decreased by 67% with SRB alone, and by 82% with SRB + sulfate over 54 h (Fig. 6b and d).

The *p,p'*-DDD and *p,p'*-DDE were also identified as metabolites of *p,p'*-DDT in soil solution (data not shown). Approximately 386 ng ml⁻¹ of *p,p'*-DDD, and 77 ng ml⁻¹ of *p,p'*-DDE accumulated in soil solution with SRB + sulfate, and the corresponding concentrations with SRB alone were 492 ng ml⁻¹ of *p,p'*-DDD, and 21 ng ml⁻¹ of

p,p'-DDE (Fig. 6b and d). By contrast, the accumulation of *p,p'*-DDD and *p,p'*-DDE was much lower in treatments without SRB, 87 ng ml⁻¹ and 42 ng ml⁻¹, respectively, with sulfate alone, and 91 ng ml⁻¹ and 7 ng ml⁻¹ in the control group (without SRB and sulfate) (Fig. 6a and c). The results showed that SRB + sulfate or SRB alone resulted in dechlorination of *p,p'*-DDT into *p,p'*-DDD/*p,p'*-DDE in soil solution, while SRB + sulfate resulted in more accumulation of DDE, but less accumulation of DDD than SRB alone. The dechlorination of *p,p'*-DDT, *p,p'*-DDD and *p,p'*-DDE that occurred in soil solution was also RSC dependent.

4. Discussion

4.1. Description of *Clostridium* sp. BXM and environmental implications

Phylogenetic trees were constructed with parsimony and distance functions using various combinations of algorithms. Clearly, strain BXM clusters within the group consisting of the *Clostridium sporogenes* (X68189), *Clostridium botulinum* A (L37585) and *Eubacterium combesii* (AY533380). The closest sulfate-reducing bacterium is *Pelosinus fermentans* (DQ145536) with 97% 16S rRNA gene similarity. Phylogenetically, the strain BXM represents a novel species of genus *Clostridium* and is clearly distinct from all other known SRB (Castro et al., 2000).

Members of the genus *Clostridium* are ubiquitous chemo-organotrophic microorganisms. Most of them can use carbohydrates and/or proteinaceous compounds as energy sources. In general, the *Clostridium* species produce sulfide when grown on peptone/yeast extract/glucose medium, or from the fermentation of cysteine. Most of the *Clostridium* species, for instance, *Clostridium*

Fig. 5. Impacts of SRB and additional sulfate levels (0, 28 mM) on RSCs production in 28-day cultivation soil solution.

Fig. 6. Impact of RSCs on *p,p'*-DDT transformation and production of metabolites *p,p'*-DDD and *p,p'*-DDE in soil solution. Treatments including: (a) CK (without SRB and sulfate) containing 0.18 mM RSCs. (b) SRB (containing 1.52 mM RSCs). (c) Sulfate (containing 0.83 mM RSCs). (d) SRB + sulfate (containing 4.45 mM RSCs). (Bars on symbols represent standard deviations of three replicates; where invisible, bars fall within symbols).

thermohydrosulfuricum, *Clostridium thermosaccharolyticum* and *Clostridium thiosulfatireducens* produce sulfide by thiosulfate or sulfur reduction (Hernandez-Eugenio et al., 2002), whereas strain BXM reduced sulfate, thiosulfate and sulfur to sulfide when grown on medium amended with yeast extract and lactate. Here, we proposed that *Clostridium* sp. BXM is classified as a novel non-*dsrAB* (without dissimilatory sulfite reductase genes) sulfate-reducing bacterium (Leloup et al., 2004). The present results also imply that *Clostridium* sp. BXM may be significant for organochlorines remediation, because the genus *Clostridium* is widely distributed in marine, estuarine sediments, paddy soils, freshwater ponds or anoxic sewage sludge (Drake, 1994; Wang et al., 2009; Li et al., 2011).

4.2. Dechlorination mechanism of *p,p'*-DDTs by *Clostridium* sp. BXM produced RSCs

The previous studies about SRB biotic dechlorination mechanism mainly focused on three aspects (El Fantroussi et al., 1998; Häggblom and Bossert, 2003). The first is transformation of chlorinated compounds by SRB using a co-metabolism process. Egli et al. (1988) showed that when carbon tetrachloride was added to heterotrophic cultures of *Desulfobacterium autotrophicum* the compound disappeared and gave rise to chloroform and dichloromethane. The dechlorination was fortuitous because this transformation was not energetically useful for the bacterium (another carbon source and/or electron acceptor is used for this purpose) (Holliger and Schraa, 1994). The second aspect studied previously is utilization of organochlorines by SRB as sole source of carbon and energy (fermentation). Formation of acetate from dichloromethane was observed in *Dehalobacterium formicoaceticum* strain DMC culture, suggesting that this organism was responsible for both

dichloromethane degradation and acetate formation (Mägli et al., 1996). The result could be explained by use of organochlorines as carbon and energy sources by SRB that utilize chlorinated C1 and C2 hydrocarbons. The third aspect studied previously is utilization of the chlorinated compounds as electron acceptors (respiration). *Desulfomonile tiedjei* DCB-1 was isolated from a stable methanogenic consortium growing on 3-chlorobenzoate (3CB) as the sole source of carbon and energy (Shelton and Tiedje, 1984). The organochlorines were used as electron acceptors and energy for growth of microorganisms was generated from exergonic dechlorination reactions (Holliger and Schraa, 1994).

In the present study, reductive dechlorination of *p,p'*-DDTs occurred in filter-sterilized/heat-treated *Clostridium* sp. BXM decline phase medium and soil solution. The dechlorination of *p,p'*-DDTs were also RSC dependent. In this study, all living cells and enzymes which were potentially responsible for dechlorination in culture medium and soil solution were eliminated by filter-sterilization and heat treatment. Therefore, the RSCs were the only cause of DDTs dechlorination which formed during sulfate reduction. Chemical dechlorination of S²⁻ and HS⁻ (RSCs) has been well documented (Perlinger et al., 1996; Hassan, 2000; Zheng et al., 2006; Choi et al., 2009; Ba et al., 2011). The addition of 20 mM HS⁻ increased the dechlorination rate constants of 1,1,1-TCA by FeS by one order of magnitude. SEM analysis results showed that the addition of transition metal Ni (II) and HS⁻ caused a noticeable morphological change of FeS surface which enhanced the reductive dechlorination area (Choi et al., 2009). The reaction of chlorinated fumigants chloropicrin (trichloronitromethane) and 1, 3-dichloropropene (1, 3-D) with H₂S and HS⁻ was examined in well-defined anoxic aqueous solutions by Zheng et al. (2006). Analyses of the transformation kinetics and products revealed that the reaction between 1, 3-D and hydrogen sulfide species is S_N2 nucleophilic substitution and/or electron-

Scheme 1. Diagram of RSC-induced dechlorination mediated by SRB.

transfer process (Zheng et al., 2006). Miller et al. (1998) reported that more than 20% of the observed hexachloroethane dechlorination in filter-sterilized samples can be attributed to reaction with H_2S and S_n^{2-} species. These reductants could promote reductive dechlorination through dissociative electron-transfer processes involving a free radical intermediate; alternatively, they may serve as “nucleophilic reductants”. The above evidence demonstrated that RSCs (S^{2-} and HS^-) have dechlorination potential in view of ability to transform sulfide into hydrosulfide and hydrogen sulfide in aquatic systems (Eq. (1)).

Our results suggest a novel abiotic dechlorination mechanism mediated by RSCs formed by SRB (Scheme 1). During this reductive dechlorination process, RSCs served as nucleophilic reductants and/or electron donor, and ultimately transformed into oxidized sulfur compounds (OSCs) (Zheng et al., 2006).

Besides the level of sulfate, the amended organic material which serves as both electron donor and carbon source (Savage et al., 2010) was another limiting factor for the generation of RSCs. SRB can utilize an impressive array of organic matter to support their metabolic activity (Zhang and Young, 1997; Aeckersberg et al., 1998). The conversion of ^{35}S sulfate to ^{35}S sulfide was enhanced by amendment with 10 mM lactate up to 7-fold in laboratory sediment incubations (Harris et al., 2006). In the present study, the additional sodium lactate was 3.5 g l^{-1} in culture medium which enhanced the generation of RSCs according to Eq. (2) (Sani et al., 2010).

The DDT dechlorination ratio of a few strains has been documented, such as 21% by *Cladosporium* sp. AJR³ 18501 within 12 days (Juhász and Naidu, 1999), 51% by *Bacillus* sp. BHD-4 within 7 days (Kantachote et al., 2003), and 90.3% by bacteria strain DDT-6 within 21 days (Fang et al., 2010). In this study, the dechlorination efficiency was nearly 100% within 102 h which was much higher than the mentions above. On the other hand, the biological RSCs production processes in non-*dsrAB* SRB are fundamentally dependent on the presence of hydrogenases. The [FeFe] class of hydrogenases is especially abundant in *Clostridium* spp (Calusinska et al., 2011). It gives the possibility that *Clostridium* mediated RSCs-dechlorination has a promising application prospect.

4.3. Dechlorination pathways of *p,p'*-DDTs by RSC

Several previous studies have confirmed that DDD and DDE are the dechlorination metabolites of DDT (Azeredo et al., 2008; Wrobel et al., 2009; Xing et al., 2009). In this study, the accumulated concentration of *p,p'*-DDD was higher than *p,p'*-DDE, showing that *p,p'*-DDD was the predominant metabolite of *p,p'*-DDT in the two reaction systems (Figs. 4b and d, 6b and d). Although the three aliphatic chlorines in DDT may be reductively removed, DDD is the major dechlorination product of DDT degradation (Li et al., 2010). Based on the present results, putative dechlorination pathways are summarized. The *p,p'*-DDT was dechlorinated to *p,p'*-DDD coupled with proton acceptance, and simultaneously dehydrochlorinated to *p,p'*-DDE by accepting protons and electrons. Furthermore, part of *p,p'*-DDD and *p,p'*-DDE may be continuously dechlorinated because the total amount of *p,p'*-DDT, *p,p'*-DDD and *p,p'*-DDE were not equivalent to the initial amount of *p,p'*-DDT. No attempt was made to clarify the further dechlorination products of *p,p'*-DDD and *p,p'*-DDE, in view of DDTs reductive dechlorination pathway and metabolism have been well defined. The present results were in accordance with the common biotic reductive dechlorination pathways (Wedemeyer, 1967), and support previous finding that DDD and DDE were the two main metabolites of DDT in anaerobic environments (Ssebugere et al., 2010; Yang et al., 2010; Zhang et al., 2011). Therefore, the results implicated that RSC-induced natural dechlorination may play an important role in the fate of organochlorines.

5. Conclusions

The overall importance of RSC-induced dechlorination depends on abundance of RSCs in the environment. Sulfide/hydrosulfide is naturally present in the top layers of marine and freshwater sediments. Marine water contains relatively high sulfate concentration (28 mM). Thus, sulfate reduction by SRB is the dominant process producing large amounts of sulfide/hydrosulfide via the decomposition of organic matter in marine sediment. Although sulfate concentrations in freshwater (0.12 mM in typical river water) are much lower than in marine water, high levels of organic matter, particularly in lakes and paddy fields, are sufficient to produce measurable RSC. Therefore, the RSC-induced dechlorination could be ubiquitous in organochlorine contaminated sulfate-reducing environment. Here we have provided an example of RSC production by SRB and RSC-induced abiotic dechlorination in an experimental medium and soil solution, thus significantly extending knowledge of the dechlorination mechanism of SRB.

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