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Enhanced Biotransformation of DDTs by an Iron- and Humic-Reducing Bacteria *Aeromonas hydrophila* HS01 upon Addition of Goethite and Anthraquinone-2,6-Disulphonic Disodium Salt (AQDS)

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Supporting Information

ABSTRACT: A fermentative facultative anaerobe, strain HS01 isolated from subterranean sediment, was identified as *Aeromonas hydrophila* by 16S rRNA sequence analysis. The biotransformation of 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane (DDT), 1,1-dichloro-2,2-bis(4-chlorophenyl) ethylene (DDD), and 1,1-dichloro-2,2-bis (4-chlorophenyl) ethane (DDE) by HS01 was investigated in the presence of goethite and anthraquinone-2,6-disulphonic disodium salt (AQDS). The results demonstrated that HS01 was capable of reducing DDTs, goethite and AQDS. And goethite can significantly enhance the reduction of DDT, DDD and DDE to some extent, while the addition of AQDS can further accelerate the reduction of Fe(III) and DDTs. The products of DDT transformation were identified as a large amount of dominant DDD, and small amounts of 1-chloro-2,2-bis-(*p*-chlorophenyl)ethane (DDMU), unsym-bis(*p*-chlorophenyl)-ethylene (DDNU), and 4,4'-dichlorobenzophenone (DBP). The results of cyclic voltammetry suggested that AQDS could increase the amounts of reactive biogenic Fe(II), resulting in the enhanced transformation of DDTs. This investigation gives some new insight in the fate of DDTs related to iron- and humic-reducing bacteria.

KEYWORDS: *Aeromonas hydrophila HS01, DDT transformation, reductive dechlorination, microbial iron reduction, AQDS*

INTRODUCTION

DDTs, including 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane (DDT) with its metabolites 1,1-dichloro-2,2-bis(4-chlorophenyl) ethylene (DDD) and 1,1-dichloro-2,2-bis (4-chlorophenyl) ethane (DDE), were classified as priority pollutants by United States Environmental Protection Agency, due to their toxicity, long biological half-life and high lipophilicity.[1] Although it has been banned as an insecticide in many countries for over 40 years, DDT contamination of soil and groundwater remains a widespread environmental concern because of its persistence and stability. Therefore, it is necessary to understand in-depth the natural elimination of DDTs and further develop remediation methods for the cleanup of residues of DDTs.

Microorganisms play an important role in the fate of DDT in natural environments. A range of dehalorespiring bacteria, such as *Aeromonas hydrophila*, *Alcaligenes eutrophus*, *Boletus edulis*, and *Eubacterium limosum*, have been demonstrated to degrade DDT in both pure culture and natural soil.[2−5] And some non-halorespiring bacteria, such as *Shewanella decolorationis*,[6] showed the ability of DDT transformation under anaerobic conditions. However, in natural environments, there are limited amounts of dehalorespiring bacteria, and the dehalogenation ability of some other non-halorespiring bacteria were usually too weak,[2] so direct microbial DDT reduction only represented a small fraction of the total DDT attenuation.[7] Castro[8] found that the disappearance of DDT in flooded soil was a consequence of biodegradation rather than chemical processes.

Recently, the biogeochemical cycles of major and trace elementals and their impacts on the contaminant dynamics have attracted increasing attention.[9] Many studies demonstrated that mineral associated Fe(II) or some Fe(II)-ligand complex can serve as a powerful reductant for dechlorination of chlorinated organic compounds (COCs). Glass et al.[10] demonstrated the important role of ferrous iron for DDT transformation under waterlogged soil. In addition, many studies reported that iron-reducing bacteria can reduce iron-minerals into biogenic Fe(II), which can effectively enhance the reduction of carbon tetrachloride,[11] trichloroethylene[12] and DDT[6] under anoxic conditions. Hence, the biogenic Fe(II) can mediate the electron transfer from iron reducing bacteria to COCs, which may also be an important way of natural attenuation of COCs, expanding the possibilities for halogenated-organics bioremediation.

Humic substances are the major organic content in anoxic environments such as flooded soils, aquatic sediments, and aquifers. Many studies[13,14] have demonstrated that, based on the cycles of quinone to hydroquinone, humics can serve as an

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electron shuttle between Fe(III)-reducing microorganisms and insoluble Fe(III) oxides resulting in acceleration of microbial Fe(III) oxides reduction. Anthraquinone-2,6-disulphonic disodium salt (AQDS), as a model compound of humic acid, can be reduced by reductants to anthrahydroquinone-2,6-disulfonate (AH_2DS), while AH_2DS could abiotically transfer electrons to Fe(III) or other reducible substances with the regeneration of AQDS.[15] The presence of quinone can also improve the abiotic reduction of nitro aromatic pesticides.[13] However, to our best knowledge, only a few studies have examined the effect of humics substance on microbial degradation of COCs.[16]

In the anaerobic iron-bearing environments, many fermentative microorganisms were reported with weak ability of Fe(III) reduction,[2,16] but the abundance of fermentative microorganisms is usually 1 or 2 orders of magnitude higher than the iron-reducing bacteria,[17] so the contribution of fermentative bacteria to the natural substrate transformations (e.g., Fe(III) oxides, humic substance, and COCs) cannot be ignored. *A. hydrophila*, as a fermentative bacteria, has been reported as capable of Fe(III) reduction and DDT transformation under anaerobic conditions,[2,18,19] but the effect of iron oxides and AQDS on the transformation of DDTs by *A. hydrophila* remains unclear.

In this study, we isolated a fermentative facultative anaerobe *A. hydrophila* strain HS01 from subterranean sediment. The objectives of this study were to (i) characterize strain HS01, (ii) determine the rate and extent of DDT, DDD and DDE biotransformation by HS01 in the presence of α-FeOOH and AQDS under anoxic conditions, and (iii) investigate the role of α-FeOOH and AQDS and evaluate their reactivity. Since all the iron minerals, humics, and iron-/humic-reducing bacteria exist widely in the natural subsurface environments, this study will be helpful for understanding natural attenuation of chlorinated compounds and may have the potential for developing in situ remediation strategies of chlorinated compound-contaminated sites.

MATERIALS AND METHODS

Experimental Materials. DDE (99%) and DDD (99.3%) were purchased from Supelco (USA). DDT (100%), DDMU (1-chloro-2,2-bis-(*p*-chlorophenyl)ethane, 100%), DDNU (unsym-bis(*p*-chlorophenyl)-ethylene, 100%), DBP (4,4′-dichlorobenzophenone, 100%), decachlorobiphenyl (1,1′-biphenyl,2,2′,3,3′,4,4′,5,5′,6,6′-decachloro, PCB209, 100%) and 4,4′-dichlorobiphenyl (4,4′-dichloro-1,1′-biphenyl, PCB15, 100%) were purchased from Accustandard (USA). High-performance liquid chromatography (HPLC)-grade acetone and *n*-hexane (Acros Organics, USA) were used without further purification. Goethite (α-FeOOH) was synthesized using hydrated ferric nitrate and potassium hydroxide as previously described,[16] and the X-ray diffraction (XRD) pattern of α-FeOOH is shown in Figure S1. All solutions were prepared in deionized water. Anhydrous sodium sulfate was purified by drying at 450 °C for 4.5 h.

Enrichment and Isolation of the Strain. Standard anaerobic techniques were used for all experiments in this study. All anaerobic media were boiled and cooled under a constant stream of 80%N_2−20%CO_2, dispensed into aluminum-sealed culture bottles under the same gas phase, capped with butyl rubber stoppers, and sterilized by autoclaving (115 °C, 20 min). Besides the sterile media, inoculation and sampling were conducted by using sterile syringes and needles. All vials were incubated in a Bactron Anaerobic/Environmental Chamber II (Shellab, Sheldon Manufacturing Inc., Cornelius, OR) at 30 °C in dark. A sample of subterranean forest sediment (Sihui, China) was extruded into glass tubes, stoppered, and immediately transported back to the laboratory and stored in the anaerobic chamber, serving as an inoculum for enrichment. The basal medium, modified from ATCC 1768 medium, contained the following components (in grams per liter of deionized H_2O): $NaHCO_3$, 2.5; NH_4Cl, 0.25; $NaH_2PO_4{\cdot}2H_2O$, 0.68; KCl, 0.10. In addition, the trace mineral solution and vitamin solution were added at 1% (v/v).[20] For enrichment, glucose (5 mM) and AQDS (50 mM) were added as organic matter and electron acceptor, respectively. The enrichment was initiated by adding 5.0 g (wet weight) of anaerobic sediment to sterile serum bottles containing the glucose-AQDS basal medium (20 mL) in the anaerobic chamber. The headspaces (5.2 mL) of serum bottles were evacuated and replaced with 80%N_2−20%CO_2. Medium without inoculum was incubated under the same conditions as the controls. By regular subculturing (10−15 days intervals), 10% (v/v) inoculum was transferred to a fresh glucose-AQDS basal medium three times. To obtain pure-culture isolates, the enriched populations were serially diluted and plated onto glucose Fe(III) basal medium agar plates for single colony isolation. Distinct colonies were picked and streaked three times on glucose-AQDS basal medium agar slants before further characterization.

Identification of the Strain. The obtained bacterial strain was characterized by using standard physiological−biochemical procedures.[21] Additional phenotypic characteristics were determined by the Biolog microbial identification system. Extraction of genomic DNA and amplification of 16S rRNA gene were conducted as described by Li et al.[22] A preliminary sequence similarity search was performed against known sequences available in the GenBank using Blast.[23] Multiple alignments with corresponding nucleotide sequences of representatives of the genus *Aeromonas* retrieved from GenBank were carried out using clustal x program.[24] The neighbor joining phylogenetic tree was performed by using the software package mega version 4,[25] and it was evaluated using the bootstrap values[26] based on 1000 replicates.

DDTs Transformation Experimental Procedures. Strain HS01 was aerobically inoculated in a nutrient broth for 18 h in a shaker at 150 rpm and 30 °C. The bacteria were harvested by centrifugation (8000*g* at 5 °C for 10 min) and washed twice with 20 mL of sterile buffer solution. The buffer solution contained the following components (in grams per liter of deionized H_2O): NaCl 5.88, KCl 0.11, NH_4Cl 0.25, and PIPES 15.1185. Trace mineral and vitamin solutions were added at 1% (v/v).[20] The resting cell suspension of HS01 was resuspended in sterile buffer solution to an optical density of 2.1−2.3 (λ = 600 nm). A density of 2.2 corresponded to approximately 8.1×10^8 cells mL^{-1} based on preliminary experiments that correlated culture optical density with viable cell counts determined by serial dilution and plating. We used 100 mL serum bottles with Teflon-coated butyl rubber stoppers and crimp seals as test reactors. α-FeOOH (25 mM) was preweighed in the test reactors. The reactors were then filled with 20 mL PIPES buffer (50 mM) solution to keep the pH value at 7.0. Eight batch experiments, including controls, were conducted in this study: (1) control; (2) α-FeOOH; (3) α-FeOOH + Fe(II)(3 mM); (4) killed cells; (5) HS01 (1 mL resting cells); (6) HS01 (1 mL resting cells) + AQDS (0.1 mM); (7) HS01 (1 mL resting cells) + α-FeOOH (25 mM); (8) HS01 (1 mL resting cells) + α-FeOOH (25 mM) + AQDS (0.1 mM). Subsequently, 19 mL of the experimental medium and 50 μL of acetone-based stock solution of DDT, DDD or DDE were added to each vial to provide an initial concentration of 14 μM, 15.6 μM and 15.7 μM. Besides, 10 μM glucose was injected into the HS01-contained systems as the electron donor. The mixture was then purged with O_2-free N_2 gas for 30 min and sealed with Teflon-coated butyl rubber stoppers and crimp seals. All vials were conducted in triplicate and incubated in a BACTRON Anaerobic/Environmental Chamber II at 30 °C in dark. Prior to use, all materials such as serum bottles, butyl rubber stoppers, pipet tips, and solutions were sterilized in an autoclave at 121 °C for 15 min. The batch experiments were finished for several times, and the sampling intervals were dependent on the degradation rates and not always stable in the experiment. At the beginning, the sampling intervals were arranged for day 7, 14, and 21. And then some supplementary batch experiments were also carried out to improve the kinetic curves.

Analytical Methods. According to the Hazardous Substances Data Bank (HSDB), the solubility of DDT, DDD and DDE in water is 5.5, 90, and 40 μg L^{-1} at 25 °C, respectively. And the octanol/water partition coefficient (log Kow) of DDT, DDD and DDE is 6.91, 6.02 and 6.51, respectively. More than 99.5% of the added DDT, DDD or DDE was extracted from the solid phase in this HS01 + α-FeOOH system.The extraction of DDT, DDD and DDE from the samples were performed by the ultrasonic extraction method as described in Cao et al.[2] The analysis of DDTs was carried out using a gas chromatograph (Thermo Fisher Trace) equipped with a Thermo Fisher DSQ mass selective detector and Trace TR-5MS silica fused capillary column (Thermo Fisher Scientific, USA, 30 m × 0.25 mm i.d., 0.25 μm film thickness). The injector temperature was 200 °C and the flow rate of helium was 1.0 mL min^{-1}. The column temperature was set at 100 °C for 2 min and increased at a rate of 15 °C min^{-1} to 160 °C, and then switched to a rate of 5 °C min^{-1}. The temperature was finally increased to 270 °C and maintained isothermally for 10 min. The ion used for quantification of DDT, DDD and DDE was m/z 235, 235 and 246, respectively. External standards of DDT, DDD and DDE were prepared in hexane and standard curve-fit linear line. The concentration reported is the average of triplicate measurements. Triplicate concentrations are consistent with relative percent differences, typically less than 15%. To measure dissolved Fe(II) and Fe(III), the vials containing iron were centrifuged at 1000g, and the supernatant was filtered through a 0.22 μm syringe filter. Dissolved Fe(II) and Fe(III) were measured using the ferrozine method.[27] The total Fe(II) concentration, known as HCl-extractable Fe(II), was determined by extracting Fe(II) from the suspensions using 0.5 M HCl for 1.5 h and assaying the extract using 1,10-phenanthroline colorimetric assay.[6] The difference between the total and dissolved Fe(II) was defined as sorbed Fe(II). Cyclic voltammetry (CV) was carried out in a conventional three-electrode electrochemical cell using a CHI 660C potentiostat. A glass carbon electrode was used as the working electrode, with a saturated calomel electrode (SCE) and Pt wire as the reference and counter electrode, respectively.[6] Unless mentioned otherwise, all the reported voltages refer to SCE. The CV measurements were performed with the suspended solutions taken from the batch kinetic studies and conducted under nitrogen atmosphere at 25 °C at a scan rate of 20 mV s^{-1}.

RESULTS

Identification of the Strain. The strain HS01 was a fermentative facultative anaerobe, which formed white rounded colorless, transparent colonies on aerobic LB agar plates and yellow rounded opaque colonies on anaerobic glucose-AQDS basal medium agar plates. From the microscopy and SEM images in Figure S2, cells were straight rods, of 0.3−0.5 μm in width and 1.2−1.8 μm in length with a flagellum. Strain HS01 was Gram-negative, and other physiological−biochemical properties and carbon sources utilization are shown in Table S1.

The 16S rRNA gene sequence of strain HS01 was submitted to the GenBank database with the accession number FJ562211. On the basis of 16S rRNA gene sequences analysis, strain HS01 showed a close relationship with members of the genus *Aeromonas* in the phylogenetic tree, having the highest similarity of 99% with *Aeromonas hydrophila* CCM 7232 and *Aeromonas hydrophila* WAB 1943. Its position in the 16S rRNA phylogenetic tree is shown in Figure 1. The strain isolated in this study was consequently identified as *Aeromonas hydrophila* strain HS01 and has been deposited in China Center for Type Culture Collection (CCTCC AB 209165).

DDT Transformation. The kinetics of DDT transformation in different reaction conditions were given in Figure 2. It showed that more than 50% of the initial DDT was removed within 21 days in the culture of HS01, indicating that strain HS01 is capable of reducing DDT. The presence of α-FeOOH

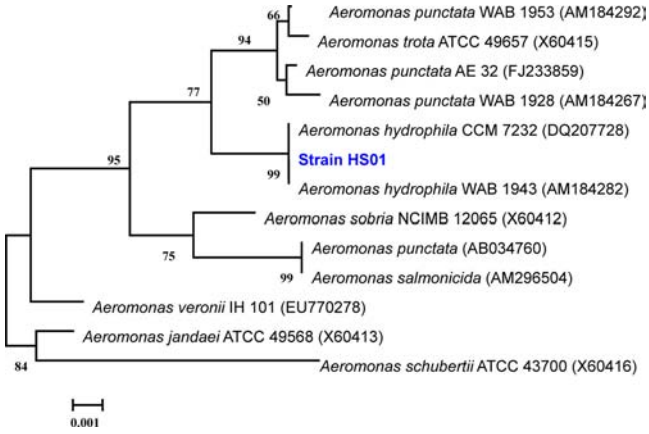

Figure 1. Phylogenetic dendrogram obtained by neighbor-joining analysis based on 16S rRNA gene sequences, showing the position of strain HS01 among phylogenetic neighbors.

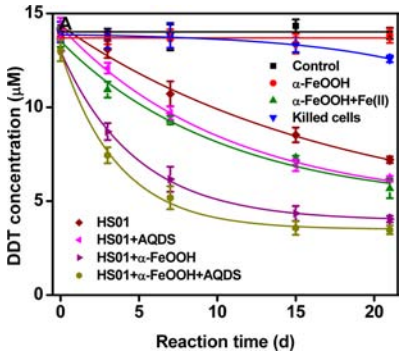

Figure 2. Kinetics of DDT transformation in eight treatments under anoxic condition at 30 °C and pH 7.0 (PIPES): control, α-FeOOH, α-FeOOH + Fe(II), killed cells, HS01, HS01 + AQDS, HS01 + α-FeOOH and HS01 + α-FeOOH + AQDS. Error bars show standard deviation from triplicates.

obviously improved DDT reduction by HS01, causing a 69% removal of DDT within 21 days. Moreover, approximately 56% and 73% of DDT were reduced after a 21-day incubation in the treatments of HS01 + AQDS and HS01 + α-FeOOH + AQDS, suggesting that AQDS also has a positive impact on the transformation rate of DDT in the culture with strain HS01 or HS01 + α-FeOOH. Control experiments with α-FeOOH or killed cells of HS01 were also conducted, and the results showed there was no significant change in the DDT concentration in the control treatment of α-FeOOH after a 21-day experimental period, and less than 5% of DDT was removed by the killed cells of HS01, indicating an inability of HS01 for DDT transformation after inactivation treatment. At the same time, approximately 60% of DDT had been transformed in the abiotic solution with α-FeOOH + Fe(II). These results demonstrated that sorbed Fe(II) on α-FeOOH was an important factor in transforming DDT under anoxic conditions, which had also been reported.[6] The pseudo-first-order rate constants (K_{obs}) were calculated and shown in Table 1. The values of K_{obs} for all the treatments were ranked as the order: HS01 + α-FeOOH + AQDS > HS01 + α-FeOOH > α-FeOOH + Fe(II) ≈ HS01 + AQDS > HS01.

Products Formation. To explore other possible transformation products of DDT in this anaerobic system, the metabolites of DDT in the system of HS01 + α-FeOOH + AQDS was detected by GC-MS/MS after one year's

Table 1. Kinetics Rate Constants (k_{obs}) and the Half-Life Time ($t_{1/2}$) of DDT, DDD and DDE in Five Different Treatments under Anoxic Condition at 30°C and pH 7.0 (PIPES): (a) α-FeOOH + Fe(II), (b) HS01, (c) HS01 + AQDS, (d) HS01 + α-FeOOH and (e) HS01 + α-FeOOH + AQDS

	DDT			DDD			DDE		
matrix	k_{obs} (d^{-1})	R^a	$t_{1/2}$ (d)	k_{obs} (d^{-1})	R^a	$t_{1/2}$ (d)	k_{obs} (d^{-1})	R^a	$t_{1/2}$ (d)
a	0.044	0.982	15.9	0.005	0.924	147.4	0.018	0.679	39.6
b	0.033	0.995	20.7	0.003	0.992	266.5	0.018	0.993	37.9
c	0.043	0.981	16.0	0.004	0.966	187.3	0.020	0.977	34.1
d	0.066	0.900	10.6	0.014	0.847	48.8	0.040	0.887	17.2
e	0.076	0.841	9.1	0.017	0.791	40.8	0.045	0.903	15.3

[a] R: Pearson's correlation coefficient.

incubation. Results in Figure 3a showed no DDT was detected, while a large amount of DDD appeared, which was quantified as 90% of the initial DDT concentration, suggesting that DDD was the major product during this period. Besides DDD, tiny amounts of four other intermediates were identified as DDE, DDMU (1-chloro-2,2-bis(*p*-chlorophenyl)-ethylene), DDNU (1,1-bis(*p*-chlorophenyl)ethylene) and DBP (dichlorobenzophenone), indicating DDD was dechlorinated further to DDMU and DDNU, among others. The possible pathway of DDT degradation in this study was proposed in the previous studies:[2,6] DDT was first dechlorinated to DDD and then formed dechlorination product DDMU following a dehydrochlorination process, DDMU and DDNU undergo reductive dechlorination, dehydrochlorination, and oxidation reaction to the end product DBP, which is not transformed further under such anoxic conditions.[28]

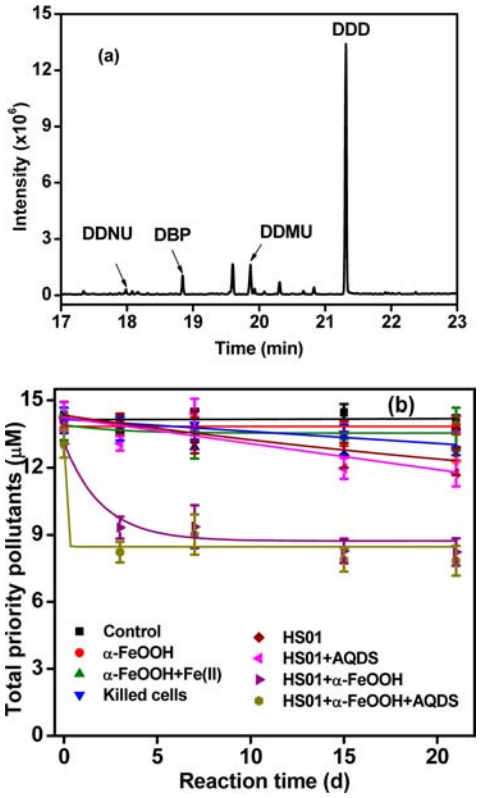

Figure 3. (a) Total ion current chromatograph of DDT transformation products by HS01 in the presence of α-FeOOH and AQDS after one-year incubation; (b) removal of total priority pollutants in eight treatments: control, α-FeOOH, α-FeOOH + Fe(II), killed cells, HS01, HS01 + AQDS, HS01 + α-FeOOH and HS01 + α-FeOOH + AQDS under anoxic conditions at 30 °C and pH 7.0 (PIPES). Error bars show standard deviation from triplicates.

The increase of DDD formation (Figure S3) coincident with DDT reduction in all the systems indicated that DDD is the major product under anoxic conditions. Figure 3b presented the sum of measured concentrations of the total priority pollutants (DDT, DDD, and DDE) to evaluate the overall success of the bioremediation process. The concentrations of total priority pollutants after 21 day's incubation are ranked in the order HS01 + α-FeOOH + AQDS < HS01 + α-FeOOH < HS01 + AQDS < HS01. Although DDT reductions were found in most of the reaction systems, the total priority pollutants concentrations decreased less than 20% after 21 day's incubation for HS01 and HS01 + AQDS. The presence of HS01 and α-FeOOH increased the removal efficiency of total priority pollutants to 40%, and the addition of AQDS further increased this value to 44%, but then the total priority pollutants kept at a stable concentration without any further decrease. Hence, the initial quick reduction of total priority pollutants may be attributed to the reaction of DDT to DDD. The DDT can be reduced in a short time with a half-life time just 7−16 days in different treatments, while the total priority pollutants decreased slowly, so it was necessary to further investigate the degradation of the other metabolites like DDD and DDE.

Transformation of DDD and DDE. Figure 4, panels a and b showed the decrease in DDD and DDE concentrations over reaction time for all systems, respectively. Even after 56 days of incubation, there were still 13.4, 12.8, 7.5, and 6.9 μM of DDD observed for HS01, HS01 + AQDS, HS01 + α-FeOOH and HS01 + α-FeOOH + AQDS, respectively. A comparison of pseudo first-order rate constants of DDT and DDD (Table 1) indicated that DDD reduction proceeds at a slower rate as compared to DDT reduction. This observation was consistent with the previous reports.[2,6] For DDE reduction, the pseudo-first-order rate constants of DDE followed the order: HS01 + α-FeOOH + AQDS > HS01 + α-FeOOH > HS01 + AQDS > HS01, which was the same as DDT and DDD.

According to the kinetic data of DDTs transformation, the half-life of DDTs in the different systems could be calculated (Table 1). The half-life times of DDT, DDE and DDD by HS01 were 15.9, 39.6, and 147.4 days, respectively, suggesting that DDD was more difficult to be reduced through biotransformation. Although the three aliphatic chlorines in DDT may be reductively removed, DDD, the first dechlorination product, is the major end-product of DDT degradation in most studies.[7,10] The presence of α-FeOOH and/or AQDS can all obviously shorten the lifetime of the DDTs biotransformation. It was also noted that the half-life time was even reduced

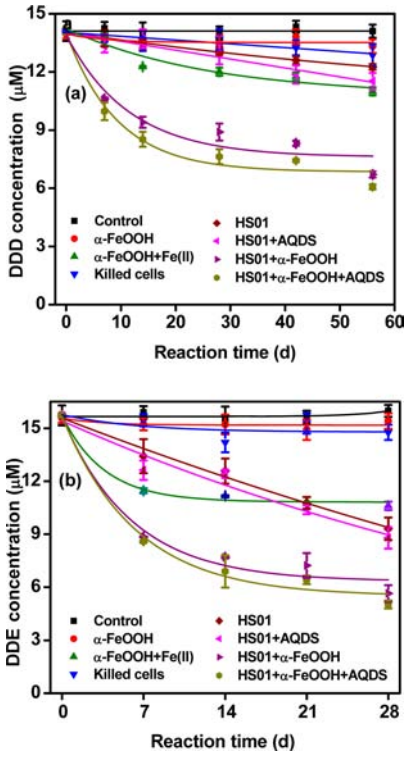

Figure 4. Kinetics of transformation of DDD (a) and DDE (b) in eight treatments under anoxic conditions at 30 °C and pH 7.0 (PIPES): control, α-FeOOH, α-FeOOH + Fe(II), killed cells, HS01, HS01 + AQDS, HS01 + α-FeOOH and HS01 + α-FeOOH + AQDS. Error bars show standard deviation from triplicates.

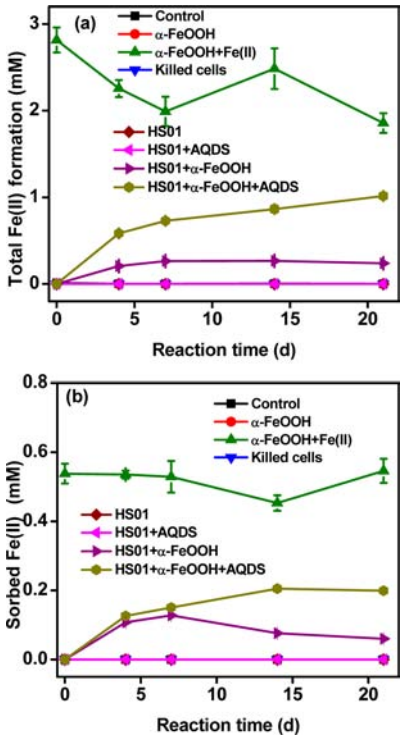

Figure 5. Total Fe(II) (a) and sorbed Fe(II) (b) formation vs time in eight treatments under anoxic conditions at 30 °C and pH 7.0 (PIPES): control, α-FeOOH, α-FeOOH + Fe(II), killed cells, HS01, HS01 + AQDS, HS01 + α-FeOOH and HS01 + α-FeOOH + AQDS. Error bars show standard deviation from triplicates.

to the minimum levels in the presence of α-FeOOH and AQDS together ($t_{1/2}$ of "HS01 + α-FeOOH + AQDS"), which were just 57%, 28%, and 38% of that for HS01 only ($t_{1/2}$ of "HS01"). Results presented in long-term incubation experiments (Figure 3a) showed that DDT was completely removed in the system of HS01 + α-FeOOH + AQDS after one year's incubation, and DDD was the major composition in the residual products.

DISCUSSION

The Role of HS01. On the basis of the above results, HS01 had the ability of reducing AQDS, DDTs, and α-FeOOH which has also been reported in our previous study.[2] Results from the experiment with killed cells (Figures 1−3) suggest that DDTs may be mainly reduced by HS01 through microbial metabolism, not due to the chemical reaction of cell surface. Hence, the strain HS01 should be the driving force of the DDTs transformation in the interaction of HS01, AQDS and α-FeOOH.

The Role of Iron Oxides. The experimental results indicated that the transformations of DDTs by HS01 were improved by the presence of α-FeOOH, which was consistent with our previous observation.[6] It was reported that the Fe(II) species formed by iron-reducing bacteria in subsurface environments may directly reduce chlorinated contaminants.[29] Figure 5a showed formation of total Fe(II) measured in the vials of different treatments. As time elapsed, the production of Fe(II) in the inoculated vials of HS01 increased gradually, and the amounts of total Fe(II) was higher with AQDS addition. According to Amonette et al.,[11] the reducing capacity of Fe(II) bound to iron mineral surface was more effective than dissolved Fe(II), because the surface hydroxyl groups may act as ligands to form inner-sphere bonds that increase the density of electrons around the adsorbed Fe(II) centers, and the sorption of multiple Fe(II) atoms in close proximity to each other may promote multiple-electron-transfer reactions of dechlorination. The sorbed Fe(II) concentrations in Figure 5b also showed a similar trend as those for total Fe(II). In addition, the reactivity of different iron species (Fe(II), α-FeOOH and α-FeOOH + Fe(II)) for DDTs reduction was also tested by using the kinetics experiments (Figures 1−3). Results showed that just α-FeOOH + Fe(II) had the capacity of reducing DDTs, indicating that the sorbed Fe(II) should be contributed to the DDTs reduction in the interaction system with HS01 and/or AQDS.

Upon the basis of the above results of Fe(II) and relevant studies,[10,30,31] the reactivity of different Fe(II) species should be dependent on their redox potential, and usually the mineral surface associated Fe(II) had a low value of redox potential and high reducing capacity. To explore the redox potential of different systems, the electrochemical tests were conducted using cyclic voltammetry (CV). Figure 6a showed CVs of different treatments after three days of incubation. The CVs of control, killed cells, or α-FeOOH systems exhibit no redox behavior, and the CV of HS01 + α-FeOOH system showed two distinct redox peaks, including an oxidation peak at 0.12 V and a reduction peak at −0.22 V. This pair of peaks is considered associated with the redox electrochemistry of Fe(III)/Fe(II) couple because their positions are in good agreement with previous observations for redox reactions of adsorbed Fe(II) on a mineral surface.[32] The CV of HS01 + AQDS displayed another pair of redox peaks that are expected to be the contributions of AQDS/AH_2QS.[33] Moreover, the peak current

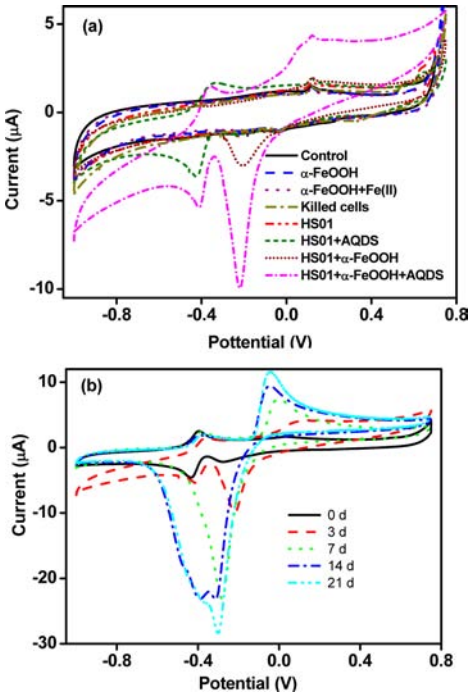

Figure 6. Cyclic voltammetries of a 3-day reaction in eight treatments under anoxic conditions at 30 °C and pH 7.0 (PIPES): control, α-FeOOH, α-FeOOH + Fe(II), killed cell, HS01, HS01 + AQDS, HS01 + α-FeOOH and HS01 + α-FeOOH + AQDS (a); cyclic voltammetries of HS01 + α-FeOOH + AQDS at different reaction times: 0 day, 3 days, 7 days, 14 days and 22 days (b).

signals increased to higher values with the addition of AQDS to the reaction media of HS01 + α-FeOOH, suggesting an increase in the amounts of Fe(II) in this system. All the treatments were tested as a function of time (Figure S4 and Figure 6b), and the peak of Fe(II) in α-FeOOH + Fe(II) disappeared quickly in 7 days. There were no significant changes for the systems of HS01 and HS01 + AQDS during the 22-day incubation. As time elapsed, the redox peak intensities of Fe(II)/Fe(III) in HS01 + α-FeOOH (Figure S4e) and HS01 + α-FeOOH + AQDS (Figure 6b) increased gradually, and their positions shifted toward a negative direction. These observations suggested that the reductive ability of Fe(II) species in the HS01 + α-FeOOH and HS01 + α-FeOOH + AQDS systems were enhanced as the increase of the biogenic Fe(II) formation.

The Role of AQDS. Numerous studies have suggested that AQDS could serve as electron shuttles to accelerate the extracellular electron transfer from the bacteria to the reducible substrates (e.g., iron oxides, chlorinate compounds, electrodes and so on).[34] The results in this study implied that HS01 was capable of reducing AQDS to AH_2DS, which could chemically transfer electrons to Fe(III) or DDTs with the regeneration of AQDS, as a result of enhancing the reduction rates of iron and DDTs. Upon the basis of the CVs (Figure S4), there was no significant change in the redox peaks of AQDS/AH_2DS under the different incubation times, while the peaks of Fe(III)/Fe(II) increased sharply after 22 days of incubation. The stable level of AQDS/AH_2DS peaks may be attributed to the equilibrium of AQDS to AH_2DS, while the increasing Fe(III)/Fe(II) peaks may result from the cycles of AQDS/AH_2DS; in other words, the role of AQDS in this system was also the electron shuttle mediating the extracellular electron transfer to the terminal electron acceptors including α-FeOOH and DDTs.

On the basis of the above discussion, HS01 was the original driving force to reduce AQDS, iron oxides and DDTs, while the reductive products (e.g., AH_2DS, sorbed Fe(II)) also facilitated the DDTs reduction. Therefore, the DDTs transformation in the HS01 + α-FeOOH + AQDS system may include three routes, including (I) directly reduced by HS01 via the bacterial metabolism; (II) reduced by biogenic Fe(II) from microbial and AQDS-mediated iron reduction; (III) reduced by the AH_2DS from microbial AQDS reduction. The positive effects of biogenic Fe(II) and AH_2DS would be the critical factors to enhance the biotransformation of DDTs by HS01.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The online version of this article contains Table S1, Figure S1, Figure S2, Figure S3 and Figure S4. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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