Effect of *Aeromonas hydrophila* on Reductive Dechlorination of DDTs by Zero-Valent Iron

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This study presents a reductive transformation method that combines zerovalent iron (ZVI) and *Aeromonas hydrophila* HS01 with iron oxide reduction property to degrade DDT (1,1-trichloro-2,2-bis(4-chlorophenyl)ethane) under anoxic conditions. The results suggest that HS01 has weak capability in terms of reducing DDT to DDD (1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane) and nearly failed to reduce DDD or its transformed intermediates. The coexistence of ZVI and HS01 results in a slight enhancement of DDT degradation compared with the ZVI system alone. The reduction of intermediates by ZVI, however, can be obviously accelerated in the presence of HS01, and the addition of anthraquinone-2,6-disulfonic disodium salt (AQDS) can accelerate the transformation rates further, especially for intermediate reduction. The analysis of the amount and electrochemical properties of Fe(III)/Fe(II) indicates that the presence of HS01 with or without AQDS is beneficial to the reduction of Fe(III) to Fe(II), resulting in the removal of passivating ferric precipitates on the ZVI surface. A mechanism and pathway that clarify the roles of ZVI, HS01, and AQDS in the ZVI + HS01 + AQDS system for DDT transformation are proposed. The quick removal of surface ferric precipitates is thought to be the reason for the enhancement of the transformation of DDT and its intermediates.

KEYWORDS: DDT; zero-valent iron; *Aeromonas hydrophila*; dechlorination

INTRODUCTION

DDT residues in the environment are highly toxic to human beings and other organisms. Although it has been banned as an insecticide in many developed countries and China for over 30 years, DDT contamination of soil and groundwater remains a widespread environmental concern because of the persistence and stability of this pesticide. As products of natural DDT transformation, DDD and DDE (1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene) are also persistent organic pollutants (*1*, *2*). The US Environmental Protection Agency has classified DDT, DDD, and DDE as priority pollutants. Sequential reduction dechlorination of DDT and its transformation products into terminal non-halogenated compounds has received considerable attention from laboratory-scale (*3*, *4*) and field (*5*) studies. Among all the technologies concerned with the reductive transformation of DDT, permeable reactive barriers packed with zerovalent iron (ZVI) (*6*, *7*) represent an inexpensive and effective method, in which ZVI serves as a reducing agent for the abiotic reduction of DDT.

Previous studies that used ZVI to degrade chlorinated pollutants, however, have shown that this technology has an important drawback: the loss of ZVI reactivity in long-term treatment. This loss of reactivity can be attributed to the accumulation of passivating oxides and hydrogen gas (*8*, *9*). Therefore, there is a pressing need to maintain ZVI reactivity through the reductive dissolution of iron oxides and consumption of hydrogen gas. Fortunately, in nature, many iron-reducing microorganisms, such as *Goebacter*, *Aeromonas hydrophila*, *Shewanella putrefaciens*, and *Pyrobaculum* (*10*–*13*), are able to use iron oxides as electron acceptors, leading to the reduction of iron oxides into Fe(II) followed by the dissolution process. This reduction may cause the detachment of passivating oxides and leave fresh ZVI accessible to pollutant targets. In the meantime, *in situ* produced hydrogen can be removed because it is used as an electron donor for anaerobic respiration. In addition to these beneficial effects, some iron-reducing bacteria (*12*, *13*) can directly contribute to the reduction of organic chlorinated pollutants. Therefore, the combined use of ZVI and iron-reducing bacterium is expected to improve the reduction of chlorinated pollutants.

In this study, we combine ZVI and an anaerobic culture of *A. hydrophila* HS01 for DDT transformation. The influence of an electron shuttle, anthraquinone-2,6-disulfonic disodium salt (AQDS), on the reduction of DDT is studied. The intermediate products of DDT are more persistent than the parent chlorinated species; thus, the effect of HS01 on the further dechlorination of DDT by ZVI is also investigated. A mechanism and pathway are proposed on the basis of the kinetics and electrochemical results.

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MATERIALS AND METHODS

Experimental Materials. DDT (98%) and DDD (99.3%) were purchased from Supelco (USA). 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). DDMS (1-chloro-2,2-bis(4′-chlorophenyl)ethane) and DDNS (2,2-bis(4′-chlorophenyl)ethane) standard samples cannot be purchased; thus, quantitative and calibration data of DDMS were kept from DDD (*14*). High-performance liquid chromatography (HPLC)-grade acetone and *n*-hexane (Acros Organics, USA) were used without further purification. Zerovalent iron (Fe^0) powder with a particle size of 100 mesh and 99.9% purity was purchased from Tianjin Kermel Chemical Reagent Development Center, Tianjin, China, and used without any further purification. Its apparent density was 2.92 g cm^{-3}. Its specific surface area measured by BET analysis was 7.5 m^2 g^{-1}. Other chemicals of analytical grade were obtained from Guangzhou Chemical Co (China). All solutions were prepared in deionized water. Anhydrous sodium sulfate was purified by drying at 450 °C for 4.5 h.

Culture and Analysis of HS01. *A. hydrophila* HS01 (Deposition No. CCTCC AB 209165) was isolated from subterranean forest sediment from Sihui City, China. 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$_2$O): NaHCO$_3$ 2.5, KCl 0.1, NH$_4$Cl 0.25, and NaH$_2$PO$_4$ 0.68. Trace mineral and vitamin solutions were added at 1% (v/v) (*15*). The resting cell suspension of HS01 was resuspended in sterile buffer solution to an optical density of 2.1 to 2.3 (λ = 610 nm). A density of 2.166 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.

Batch Experimental Procedures. We used 100 mL serum bottles with Teflon-coated butyl rubber stoppers and crimp seals as test reactors. Iron powder (0.2 g) was preweighed in the test reactors. The reactors were then filled with 20 mL of buffer solution to keep the pH value at 7.0. Five batch experiments, including controls, were conducted in this study: (1) HS01 (1 mL of resting cells); (2) HS01 (1 mL of resting cells) + AQDS (36.833 mg L^{-1}); (3) ZVI (10 mg L^{-1}); (4) ZVI (10 mg L^{-1}) + HS01 (1 mL resting cells); (5) ZVI (10 mg L^{-1}) + HS01 (1 mL of resting cells) + AQDS (36.833 mg L^{-1}). Subsequently, 19 mL of the experimental medium and 100 μL of acetone-based stock solution of DDT or DDD (2 g L^{-1}) were added to each vial, while 18 g L^{-1} glucose was added into the HS01-containing systems as the electron donor. The mixture was then purged with a mixture gas (CO$_2$:N$_2$ = 2:8) for 30 min and sealed with Teflon-coated butyl rubber stoppers and crimp seals. The closed reactors were mixed using a rotary shaker at 130 rpm at 25 ± 0.5 °C. 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 carried out several times, and the sampling intervals depended on the degradation rates and were not always stable in the experiment. At the beginning, the sampling intervals were arranged in the 7th, 14th, 21st, 28th day. Some supplementary batch experiments were also carried out to improve the kinetic curves.

Analytical Methods. For the analysis of DDT and its transformed intermediates, the DDT and its intermediates were extracted from the samples by the ultrasonic extraction method according to ref *16*. The analysis of DDT and its intermediates 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, 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, DDMS, and DDNS was *m/z* 235. External standards of DDT and DDD 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 1000*g*, and the supernate filtered through a 0.2 mm pore size filter (Pall). Dissolved Fe(II) and Fe(III) were measured using the ferrozine method (*17*).

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. 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}.

Quality Assurance/Quality Control. A strict quality assurance and quality control program was implemented for the extraction method and analysis (*13*). Each sample was spiked with 4,4′-dichlorobiphenyl (PCB15) as a surrogate before extraction. Recoveries of the PCB15 surrogate were 75.5%−112.7%. The concentrations of DDT and DDD were determined quantitatively by the external standard method using peak area mode with PCB209 added as internal standard before GC−MS determination. The relative standard deviation values of DDT and DDD concentrations in eight replicated external standards ranged from 1.5% to 14.8%. The instrument detection limits of DDT and DDD were both 0.1 mg L^{-1}. For each batch of 15 samples, the method blank and external standards were measured.

Data Analysis. The degradation of DDT during the first seven-day reaction followed pseudo-first-order kinetics, and the apparent degradation rate constant (K_{obs}) was calculated according to the equation

$$-K_{obs}t = \mathrm{Ln}([\mathrm{DDT}]_t/[\mathrm{DDT}]_0)$$

where [DDT]$_t$ and [DDT]$_0$ are the concentrations of DDT at time *t* and time zero, respectively.

RESULTS AND DISCUSSION

DDT Transformation. Figure 1 shows the kinetics of DDT degradation and its intermediate formation in different reaction conditions over 42 days. From **Figure 1a**, we can observe that over 90% of the initial DDT is removed within seven days in the presence of ZVI, indicating that ZVI is capable of reducing DDT. This result accords with those of other reports (*6, 7*). To further enhance DDT transformation by ZVI, iron-reducing bacterium HS01 was introduced into the system, with the expectation that it will reduce iron oxide accumulation on the ZVI surface. Furthermore, AQDS was introduced as an electron shuttle to accelerate the electron transfer between the cell and ZVI, as well as enhance the DDT reduction rate. The results show that the addition of HS01 slightly improves DDT reduction by ZVI, causing a 94% removal of DDT within seven days. The utilization of AQDS as an electron shuttle can offer a potential means of further enhancing reduction; its use leads to the removal of about 96% of the DDT from the system. Control experiments with HS01 alone and HS01 + AQDS were also conducted; results from these experiments show that less than 20% of DDT is removed after a 42-day experimental period in both systems. This indicates that strain HS01 has limited ability to reduce DDT. The pseudo-first-order rate constants (K_{obs}) were calculated and shown in **Table 1**. The values of K_{obs} for each treatment are ranked in the order ZVI + HS01 + AQDS > ZVI + HS01 > ZVI > HS01 + AQDS > HS01. The system in which only HS01 is used is related to the lowest K_{obs} because of its weak ability to degrade DDT. The highest rate is obtained with the ZVI + HS01 + AQDS media, which reaches 0.395 day^{-1} with respect to DDT reduction. This result suggests that ZVI, HS01, and ZVI + HS01 all exhibit DDT transformation ability. The enhancement in HS01 with or without AQDS is minor compared with that in ZVI alone; hence, it is difficult to conclude whether HS01 with or without AQDS and ZVI exhibits coupling effects on DDT transformation. Therefore, the following work aims to investigate the effect of HS01 on product formation during DDT reduction by ZVI.
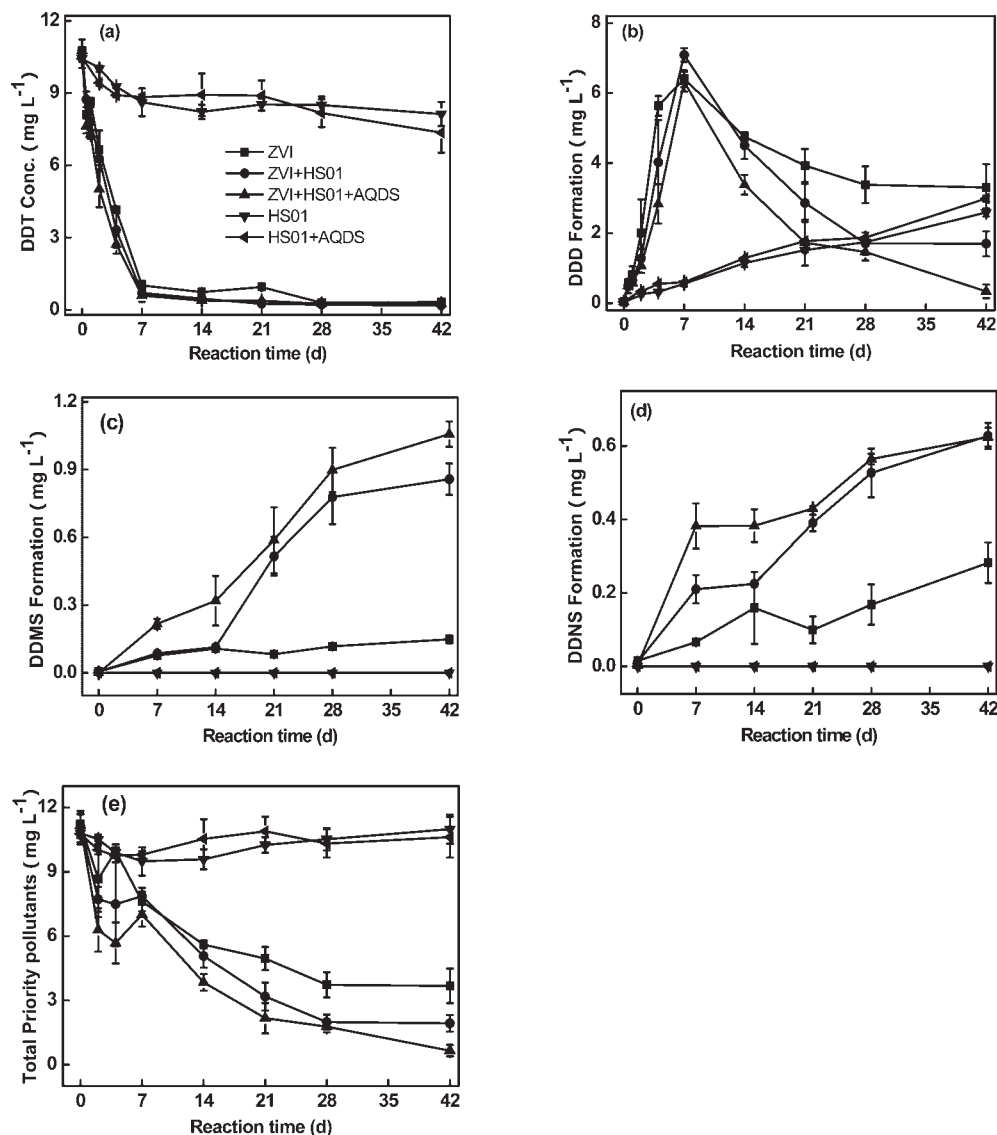
Figure 1. Transformation of DDT (**a**), related intermediates [DDD (**b**), DDMS (**c**), and DDNS (**d**)], and total priority pollutants (DDT + DDD + DDE) (**e**) in five systems: ZVI, ZVI + HS01, ZVI + HS01 + AQDS, HS01, and HS01 + AQDS. Error bars show standard deviation from triplicates.

Table 1. Kinetics Rate Constants (K_{obs}) of DDT, Total Priority Pollutants (DDT + DDD + DDE), and DDD in Five Systems: HS01, HS01 + AQDS, ZVI, ZVI + HS01, and ZVI + HS01 + AQDS

	DDT		Total priority pollutants		DDD	
	K_{obs} (day^{-1})	R^a	K_{obs} (day^{-1})	R^a	K_{obs} (day^{-1})	R^a
HS01	0.005 ± 0.002	0.519	0.001 ± 0.001	0.866	0.003 ± 0.001	0.749
HS01 + AQDS	0.006 ± 0.001	0.775	0.002 ± 0.001	0.999	0.004 ± 0.001	0.821
ZVI	0.320 ± 0.033	0.949	0.028 ± 0.004	0.869	0.043 ± 0.009	0.818
ZVI + HS01	0.378 ± 0.030	0.969	0.044 ± 0.005	0.904	0.063 ± 0.016	0.763
ZVI + HS01 + AQDS	0.395 ± 0.024	0.982	0.061 ± 0.005	0.959	0.090 ± 0.015	0.853

[a] *R*: Pearson's correlation coefficient.

Products. In biotic controls containing HS01 and HS01 + AQDS after a 42-day reaction, there is a slow increase in DDD concentration, which reaches a final value of 2.6 and 3.0 mg L^{-1}, or approximately 260 and 210 times higher than the DDE concentration. This result indicates that HS01 is capable of reducing DDT under anaerobic conditions, with DDD as the major product. Other intermediates (DDMS, DDNS, minor DDOH (2,2-bis-(4′-chlorophenyl)ethanol), and DBP (4,4′-dichlorobenzophenone)) are also simultaneously found, as shown in Figure S1 in the Supporting Information. In abiotic and biotic controls containing

ZVI, a pronounced increase in DDD concentration is observed in the first seven days of incubation (**Figure 1b**). Over the next 35 days, a continued decrease is observed, and the order of the decrease rate is ranked as ZVI + HS01 + AQDS > ZVI + HS01 > ZVI > HS01, indicating that there might be potential for DDD to be transformed into other products. Sayles (*6*) assumed that DDD is transformed to DDMS or other products. Our result shows an obvious increase in DDMS and DDNS during the 7 to 42-day period (**Figures 1c** and **1d**), suggesting that DDD is reductively dechlorinated further to DDMS and DDNS, among others.

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Figure 1e presents the sum of measured concentrations of the total priority pollutants (DDT, DDD, and DDE), which shows an obvious decline over time, indicating the overall success of the dechlorination process. The decrease rate in total priority pollutants also follows the order ZVI + HS01 + AQDS > ZVI + HS01 > ZVI. HS01 is capable of reducing DDT to DDD, but the total pollutant concentrations are not decreased, indicating the limited capability of HS01 in terms of DDD reduction. In the system that uses ZVI alone, the removal efficiency of total priority pollutants is 67%, whereas the removal efficiency of total priority pollutants in the ZVI + HS01 system increases to 83%. AQDS addition causes this value to rise to 94%. To further confirm the differences in DDT reductive dechlorination in the five systems, a long-term incubation experiment was conducted for one year using the results in Figure S2 in the Supporting Information. The results of the year-long experiment show that about 10% of DDTs is reduced by HS01 alone, whereas more than 90% of DDTs is reduced in the system with ZVI, particularly in the system with ZVI and HS01 (+AQDS). This indicates that the coupling of ZVI and HS01 (+AQDS) can accelerate the reduction rate of DDT and its intermediates.

DDD Transformation. Batch experiments using DDD at 10 mg L^{-1} as the substrate were also conducted with the five treatments. **Figure 2a** shows the decrease in DDD concentration over reaction time for all cases. DDD degradation rate, as a function of reaction media, follows a distinctly similar trend. After 21 days of incubation, the DDD concentration remains at 4.7, 3.4, and 2.3 mg L^{-1}, corresponding to ZVI alone, ZVI + HS01, and ZVI + HS01 + AQDS systems, respectively. A comparison of **Figures 1** and **2** indicates that DDD reduction proceeds at a slower rate compared with DDT reduction. This observation is consistent with that reported in previous studies (*4*, *6*). **Figures 2b** and **2c** show DDMS and DDNS formations, and suggest that the formation rates of the ZVI + HS01 and ZVI + HS01 + AQDS systems are obviously higher than that of the system using ZVI alone.

The kinetic studies (**Table 1**) show the enhanced degradation of DDT and DDD in the ZVI + HS01 system compared with that using ZVI alone. In addition, the subsequent reduction rate of DDD in ZVI-contained treatments (ZVI, ZVI + HS01, and ZVI + HS01 + AQDS) during the 7 to 42-day period also follows the order ZVI + HS01 + AQDS > ZVI + HS01 > ZVI. However, the gap between various treatments is larger than that for DDT transformation. In addition, the reduction rates of DDD are much slower than those for DDT; these findings are consistent with previous reports that revealed slower rates for successive dehalogenation by ZVI (*8*, *9*). It has been reported that the corrosion of ZVI and increase in pH favor the formation of iron hydroxide precipitate as a passivating layer that is responsible for the inhibition of further ZVI dissolution (*18*).

Dissolved Fe(II) and Fe(III) Generation. Numerous studies have investigated the factors that influence the efficiency of ZVI reduction. Matheson et al. (*19*) and Satapanajaru et al. (*9*) indicated that mass transport of substrate to the iron surface appears to be a key factor affecting dechlorination rate. Other operating parameters, including pH, Fe(II) concentration, surfactant, and iron source, have also been shown to influence the effectiveness on dechlorination by ZVI. In this study, the dissolved concentrations of Fe(II)/Fe(III) were examined. A high concentration of Fe(II) is found in the treatments where HS01 was added. For example, after a 4-day reaction, the Fe(II) concentrations detected are 142, 333, and 304 mg L^{-1} in relation to ZVI alone, ZVI + HS01, and ZVI + HS01 + AQDS treatments, respectively (**Figures 3a** and **3b**). The presence of this iron-reducing bacterium causes the bioreduction of Fe(III) to
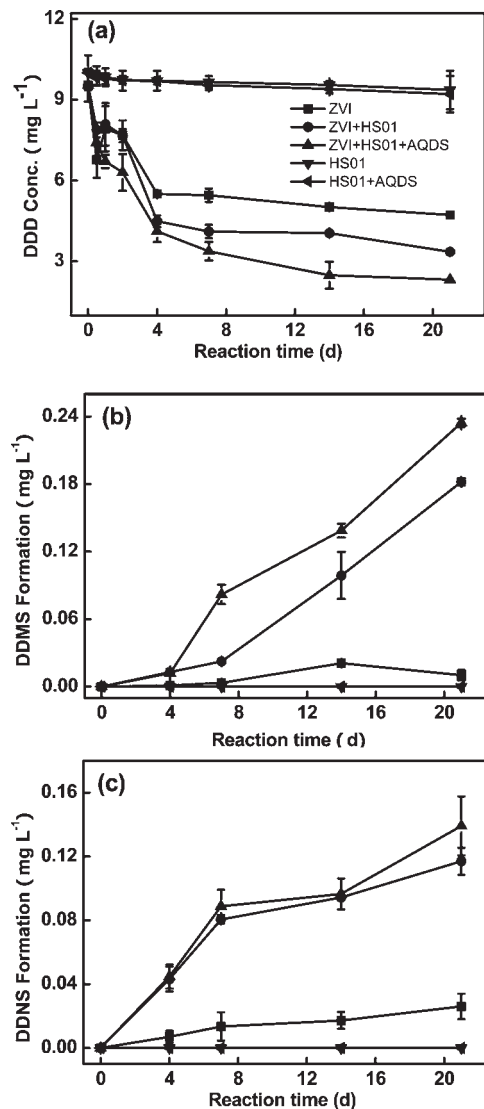

Figure 2. Transformation of DDD (**a**) and related intermediates [DDMS (**b**) and DDNS (**c**)] in five systems: ZVI, ZVI + HS01, ZVI + HS01 + AQDS, HS01, and HS01 + AQDS. Error bars show standard deviation from triplicates.

Fe(II), as suggested by the fact that Fe(III) does not accumulate over time. The ZVI + HS01 system reflects a dissolved Fe(III) concentration of 15 mg L^{-1}, which is a decrease of 15 times compared with that in the absence of HS01. The redox potential of Fe(II)/Fe(III) is also apparent in the electrochemical CV tests. **Figure 3c** shows voltammograms of different reaction solutions after seven days of operation. The voltammetric curve of bacteria-free supernatants does not exhibit any redox behaviors. By contrast, the CV of supernatants in the presence of HS01 shows two distinct redox peaks, including an oxidation peak at −0.22 V and a reduction peak at −0.48 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 (*20*). The CV obtained in supernatants containing HS01 and AQDS displays another pair of redox peaks that are expected to be the contributions of AQDS (*21*). Moreover, the peak current signals increase with the addition of AQDS to the reaction media, suggesting an increase in the amounts of dissolved Fe(II) available in this system. This effect is more pronounced with the addition of the electron shuttle. These
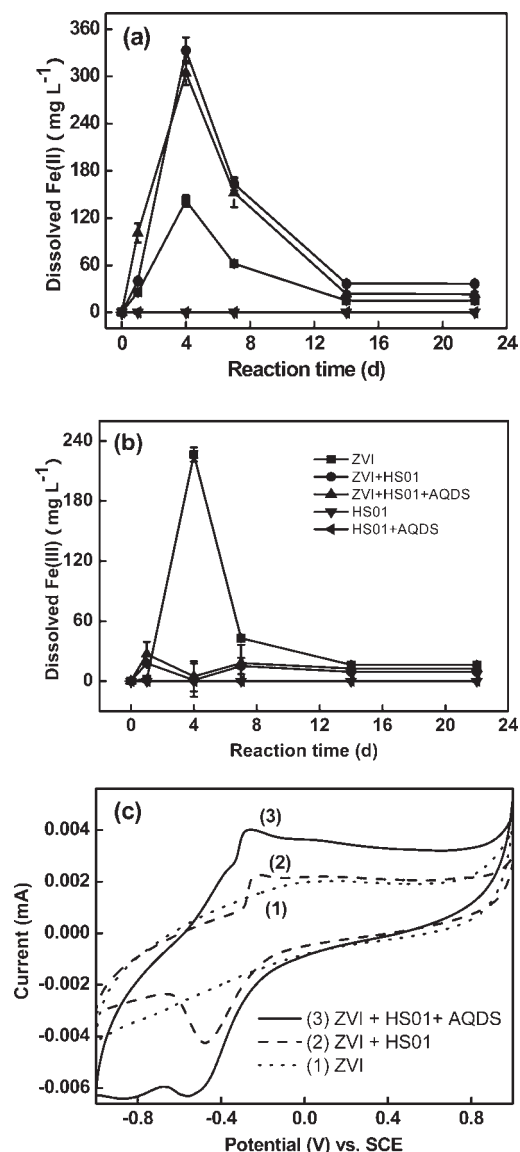
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Figure 3. Formation of dissolved Fe(II) (**a**) and Fe(III) (**b**) production and consumption in five systems: ZVI, ZVI + HS01, ZVI + HS01 + AQDS, HS01, and HS01 + AQDS. Error bars show standard deviation from triplicates. Cyclic voltammograms (**c**) obtained on a glass carbon electrode of different reaction conditions after 7 days of operation. The scan rate is 20 mV s^{-1}.

observations suggest that the presence of strain HS01 is beneficial to the dissolution of ferric precipitates to Fe(II).

Moreover, the changes in pH value in the five systems were measured (Figure S3 in the Supporting Information). The results show that no obvious change in the two systems without ZVI (HS01 and HS01 + AQDS) is observed. The pH values in the three systems with ZVI (ZVI, ZVI + HS01, and ZVI + HS01 + AQDS) increase by about one unit after 21 days of anaerobic reaction, indicating that the dissolution of ZVI is the main reason for pH enhancement. Increasing pH has been demonstrated to be a key factor that accounts for slower reaction kinetics as time proceeds, when pollutants such as metolachor (*22*), nitrite (*23*), or nitrate (*24*) are reduced by ZVI. An important explanation is the formation of secondary reductants [Fe(II) or Fe(II)-containing oxides and hydroxides] on the surface of ZVI (*23*). Various iron (hydr)oxides, such as goethite, that form at high pH can passivate the iron surface (*22, 25, 26*) and hinder the access of contaminant molecules to the ZVI surface (*27*).
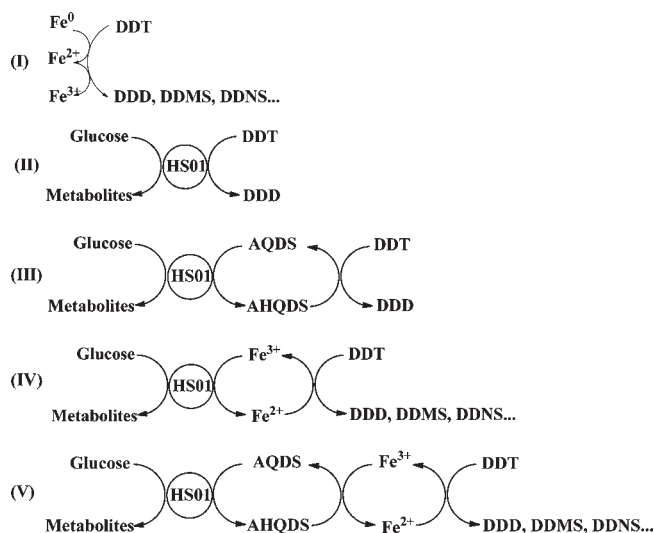

Figure 4. Proposed mechanism of DDT transformation by ZVI in five systems: ZVI, ZVI + HS01, ZVI + HS01 + AQDS, HS01, and HS01 + AQDS.

Proposed Mechanisms and Pathways of DDT Transformation. In the present study, a small amount of ZVI effectively removes 94% of DDT within seven days, exhibiting the strong DDT reduction capability of ZVI. However, the removal efficiency of total priority pollutants is merely 67%, indicating that although ZVI can remediate DDT-contaminated media, the dechlorination of total priority pollutants is incomplete. HS01 has been identified as an iron-reducing bacterium (*10, 11*), capable as well of reducing DDT to DDD, but does not contribute to the reduction of DDD to other transformed intermediates. For example, after 42 days of incubation, the total priority pollutant concentration is not decreased in the system using HS01 alone. The addition of HS01 improves the reactivity of ZVI in the anaerobic reactors, which suggests that combining biotic and abiotic reactions for DDT transformation can enhance the DDT reduction rate and, more important, lead to rapid and thorough dechlorination. In addition, AQDS plays an important role in accelerating the reduction of DDT, indicating that electrons are "shuttled" between microbes and terminal organic pollutants.

Based on the discussion above, the DDT transformation in the ZVI + HS01 + AQDS system may follow five routes (**Figure 4**), including (I) direct chemical reduction by ZVI; (II) direct reduction by HS01 via the metabolism of the bacterium; (III) AQDS-mediated DDT reduction by HS01; (IV) reduction by biogenic Fe(II) of microbial iron reduction; and (V) Fe(III)-AQDS-mediated reduction by HS01.

In the ZVI + HS01 + AQDS system, the above-mentioned methods play different roles in the enhancement of DDT transformation. To illustrate their roles in the entire process of DDT reduction, a pathway for the anaerobic transformation of DDT is proposed. By identifying these products, the DDT degradation pathway in the HS01 + ZVI system is proposed, as shown in **Figure 5**. HS01 and ZVI initially dechlorinate DDT to form DDD, and then form DDMS. DDMS and DDNS undergo oxidative reaction to DDOH and DBP, and are not transformed further under such anoxic conditions (*19*). The aforementioned results indicate that all the systems of ZVI, HS01, HS01 + AQDS, HS01 + ZVI, and HS01 + ZVI + AQDS might favor the reduction of DDT to DDD, and the reduction pathway of DDT to DDD can be driven by mechanisms (I)−(V). Because no obvious DDD reduction and product formation (DDD, DDMS and DDNS) in either the system of HS01 alone or HS01 + AQDS are observed, the reduction pathway of DDD to other products can be driven by
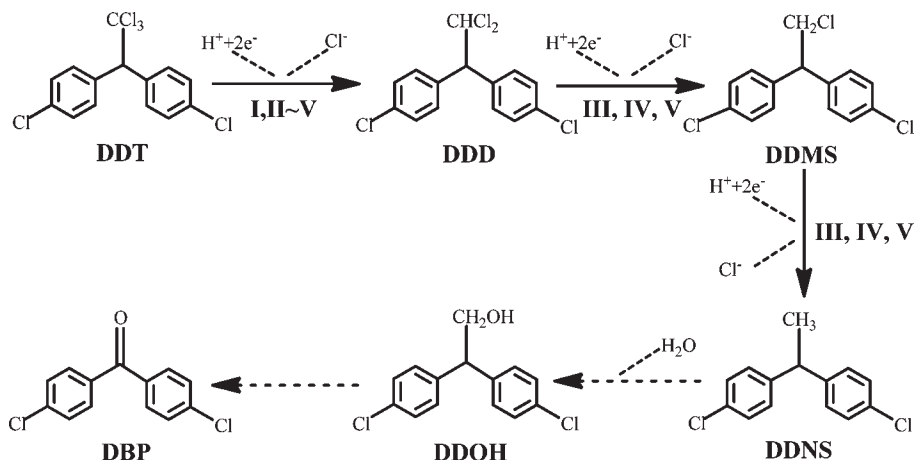
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Figure 5. Proposed pathway of anaerobic reductive transformation of DDT in this experiment.

mechanisms (I), (IV), and (V). In conclusion, HS01 with or without AQDS not only enhances DDT transformation but also accelerates the further reduction of other intermediate products.

As many researchers extensively researched on the pathway of DDTs, we have not found the new product and pathway of DDTs. The mechanisms of reduction by Fe^0 and iron reduction bacteria with/without AQDS were also studied before. However, the aim of this study is to clearly illustrate the mechanism in every step of DDT and its intermediate product transformation, and to make clear the role of the HS01, AQDS and ZVI in the whole process of the DDT transformation, especially for the intermediate product.

Reasons for the Positive Effect of HS01 on DDT Reduction by ZVI. On the basis of the above-mentioned discussion, we conclude that the addition of strain HS01 can positively affect the long-term performance of ZVI. This finding can be explained through the following aspects: (i) The iron-reducing bacterium causes the bioreduction of insoluble iron oxide to form biogenic Fe(II), further adsorbed on the oxide surface. A number of studies have suggested that mineral surface-bound Fe(II) species exhibit high reactivity for reductive transformation of carbon tetrachloride (27), DDT (13), and other chlorinated aliphatic hydrocarbons under anoxic conditions. This particular species is thus considered highly reactive for the reduction of additional contaminants, such as DDD, DDMS, and DDNS. (ii) The iron-reducing bacterium leads to the removal of passivating ferric precipitates on the ZVI surface, making fresh ZVI reactive sites susceptible to contaminants, and potentially facilitating complete DDD transformation. In the present study, we were not able to determine which mechanism plays the dominant role; it appears as though both are involved in the improvement of ZVI longevity. In addition, AQDS can further accelerate iron oxide reduction and lead to the enhancement of mechanisms (i) and (ii), as a result of the increase in DDT transformation.

Supporting Information Available: The GC–MS total ion current chromatogram, figure depicting concentration of DDT and its intermediates after a year of incubation, and plot of pH values as a function of time in the five systems. This material is available free of charge via the Internet at http://pubs.acs.org.

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