JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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pubs.acs.org/JAFC

# Biostimulation of Indigenous Microbial Communities for Anaerobic Transformation of Pentachlorophenol in Paddy Soils of Southern China

Manjia Chen,[†,‡,#] Kaimin Shih,[§] Min Hu,[‡] Fangbai Li,[*,‡] Chengshuai Liu,[*,‡] Weijian Wu,[†,‡,#] and Hui Tong[†,‡,#]

[†]Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou 510640, People's Republic of China

[‡]Guangdong Key Laboratory of Agricultural Environment Pollution Integrated Control, Guangdong Institute of Eco-Environmental and Soil Sciences, Guangzhou 510650, People's Republic of China

[§]Department of Civil Engineering, The University of Hong Kong, Hong Kong, People's Republic of China

[#]Graduate University of The Chinese Academy of Sciences, Beijing 100039, People's Republic of China

Supporting Information

**ABSTRACT:** This study explored biostimulation mechanisms with an electron donor and a shuttle for accelerating pentachlorophenol (PCP) transformation in iron-rich soils. The results indicated that indigenous microbial communities are important for PCP transformation in soils. Biostimulation of indigenous microbial communities by the addition of lactate and anthraquinone-2,6-disulfonate (AQDS) led to the enhanced rates of PCP dechlorination by the dechlorinating- and iron-reducing bacteria in soils. The electrochemical studies using cyclic voltammograms and microbial current measurements confirmed the high reduction potential and the large amount of electrons generated under biostimulation conditions, which were responsible for the higher rates of PCP transformation. After biostimulation treatments by the additions of lactate and/or AQDS during PCP dechlorination processes, microbial community analysis by the terminal restriction fragment length polymorphism (T-RFLP) method showed the abundance terminal restricted fragments (T-RFs), an indicator of bacterial abundance, which represents the dechlorinating- and iron-reducing bacteria, suggesting their critical roles in PCP dechlorination in soils.

**KEYWORDS:** *biostimulation, pentachlorophenol, reductive dechlorination, lactate, anthraquinone-2,6-disulfonate*

## INTRODUCTION

Polychlorinated phenols (CPs) are among the most popular and commonly used pesticides and herbicides and are also principally wood preservatives.[1] After prolonged nonprotective usage, CPs enter nontarget uplands, wetlands, and aquatic environments, associating with colloidal and particulate matter and eventually settling in the soils.[2] The universal toxicity of CPs has long been recognized.[3] CPs accumulate in living bodies, resulting in damage to the liver, kidneys, blood, and nervous system.[4,5]

Pentachlorophenol (PCP) from the category of CPs has been widely used in China since the 1980s, which has generated harmful effects in aquaculture or soil systems.[6] PCP is relatively persistent in soil and undergoes slow natural attenuation with a long field half-life.[7] Therefore, exploring highly efficient bioremediation methods for accelerating the transformation rates of PCP in soils, which usually contain a complex microbial community structure, is important for soil remediation.

In situ bioremediation has been proposed as an optimal method for soil organic pollutant removal with reduced time and cost for restoration of contaminated soil sites.[8,9] However, the lack of understanding of the factors controlling the biodegradation of CPs in situ, especially the activities of indigenous microorganisms, represents a significant obstacle for the application of several bioremediation methods in various contaminated soils.[10] The principles of bioremediation are based on natural attenuation, bioaugmentation, and biostimulation.[11] The simplest method of bioremediation is natural attenuation, in which soils are only monitored for variations in pollution concentrations to ensure that the pollutant transformation is active.[12] Unfortunately, CPs usually require long attenuation periods, with half-lives reaching more than 45 days in soils.[7] Bioaugmentation is usually applied in cases where natural active microbial communities are present in low quantities or even absent, wherein the addition of contaminant-degrading organisms can accelerate the transformation rates.[13] In such cases, the adaptation of exogenous strains that exert highly efficient activities for pollutant transformation to new environments is a key challenge in implementation.[14] Biostimulation requires adjustment for the contaminated soils to provide indigenous microbial communities with favorable environments to enable them to degrade target pollutants.[11] Indigenous microorganisms are the key components in this case, and biostimulation is a promising in situ bioremediation method for soil cleanup.[15]

Biostimulation involving the addition of soil nutrients, trace minerals, electron acceptors, or electron donors enhances the

Received: October 11, 2011
Revised: February 29, 2012
Accepted: March 4, 2012
Published: March 4, 2012

Table 1. Treatment Methods and Kinetics Parameters of PCP Transformation under Different Experimental Conditions[a]

| | | PCP transformation kinetics parameter | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | DP soil | | | | TZ soil | | | |
| treatment | conditions | half-life (days) | $k$ ($day^{-1}$) | $R^2$ | $p$ | half-life (days) | $k$ ($day^{-1}$) | $R^2$ | $p$ |
| CK | sterile soil + PIPES + PCP | | | | | | | | |
| T0 | soil + PIPES | | | | | | | | |
| T1 | soil + PIPES + PCP | 3.84 | 0.82 | 0.995 | <0.01 | 7.63 | 0.37 | 0.982 | <0.01 |
| T2 | soil + PIPES + lactate + PCP | 3.11 | 1.27 | 0.992 | <0.01 | 3.44 | 1.59 | 0.994 | <0.01 |
| T3 | soil + PIPES + lactate + AQDS + PCP | 2.77 | 1.28 | 0.984 | <0.01 | 2.71 | 2.28 | 0.998 | <0.01 |

[a]The initial PCP, PIPE buffer, lactic acid, and AQDS concentrations were 19, 30, 10, and 0.2 mM, respectively.

anaerobic biotransformation of a wide range of soil contaminants.[16,17] Chlorinated compounds could also undergo reductive dechlorination when suitable electron-donating substrates are available.[18,19] Trichloroethene and perchloroethene are reported to be completely converted to ethane by microorganisms in a short span of time with the addition of lactate during biostimulation.[20] Electron shuttles, such as humic substances (HS), may play a significant stimulation role in the anaerobic biotransformation of organic pollutants through enhancing the electron transfer speed. Anthraquinone-2,6-disulfonate (AQDS) from the category of HS can serve as an electron shuttle to promote the reduction of iron oxides and transformation of chlorinated organic contaminants.[21]

Soil indigenous microorganisms could degrade and/or metabolize CPs, although a long span of time is required.[22,23] Biostimulation methods can be implemented in accelerating the transformation rates of CPs in soils with a number of stimulation procedures for activating indigenous bacteria.[19] In soils in southern China, primarily distributed as red soils, the reductive dechlorination of CPs possibly occurs because of the high iron mineral content and high microbial richness and abundance formed under the warm and rainy climate in this region.[24] However, the half-lives of CPs in these soils are still long under natural attenuation conditions.[24] Therefore, integrating the rich organic matter and high iron content in these soils and studying the biostimulation processes to enhance the transformation rates of CPs are important strategies for soil decontamination.

In the present study, the anaerobic dechlorination of PCP by indigenous microorganisms in soils collected from typical sites of southern China, as well as the mechanism clarifying the effects of biostimulation, are reported. The current research focuses on the activities of functional microbial communities, confirmed as electrochemical and microbial evidence of the biodegradation of PCP in soil microcosm settings under anaerobic conditions, to provide necessary information on the possible in situ bioremediation of CPs in contaminated soils.

## MATERIALS AND METHODS

**Soil Samples.** Samples of Anthrosol used in the study were collected from the towns of Dongpu (22° 41.640′ N, 113° 19.836′ E) and Tanzhou (22° 13.274′ N, 113° 25.128′ E) in Zhongshan City, Guangdong Province, China (abbreviated as DP and TZ soil, respectively). Sampling sites were selected where rice had been planted for at least 10 years and where chemicals (such as fertilizers and pesticides) have been regularly used. To avoid effects of the different content of CPs uptake by vegetation, sampling was carried out where plants with superficial roots are not present. At each sampling point, soils were collected from the surface soil (0−20 cm), in which four subsamples, with 25 × 25 cm surface, were taken and then mixed to obtain a bulk sample. After sieving using a 2 mm laboratory test sieve, the soils with field moisture content were immediately transported for storage at 4 °C prior to the batch experiments. The basic physicochemical properties of the soils were analyzed using the methods described previously,[25] and the results are presented in Table 1S in the Supporting Information (SI). The chlorinated phenol content of the two soils was determined using standard analytical methods, but was not detected. For the control treatments, sterile soil was obtained by $\gamma$-irradiation at 50 kGy.

**Chemicals.** PCP (98+% purity), AQDS (97+% purity), and 1,4-piperazinediethanesulfonic acid (PIPES, 98+% purity), were obtained from Sigma-Aldrich (St. Louis, MO, USA). The other analytical grade chemicals were obtained from Guangzhou Chemical Co., China. Deaerated deionized water was prepared by deoxygenating ultrapure water (18 MΩ-cm, Easy Pure'II RF/UV USA) with nitrogen for 2 h before storage in an anaerobic chamber for 12 h. The PCP stock solution (94 $\mu$M) was prepared by dissolving PCP in 1.0 M NaOH solution.

**PCP Transformation Experiments in Soils.** The experiments were conducted in triplicate at a constant pH of 7.0 ± 0.5, maintained using a 30 mM PIPES buffer solution. In the experiment, 20 mL serum bottles with silicone-lined septa and aluminum sealing caps were used. The batch experiment procedures are as follows: The soil samples (0.5 g, dry weight) were transferred into a vial, along with 10 mL of PIPES buffer solution. Lactate, AQDS, and PCP were added as necessary during the process to achieve concentrations of 10 mM, 200 $\mu$M, and 19 $\mu$M, respectively. Standard anaerobic reaction experiments were conducted. The bottles were purged with $O_2$-free $N_2$ for 15 min, and the gas volume in the bottles was approximately 9.9 mL. The details of the different treatments for the PCP transformation studies are provided in Table 1. After being uniformly mixed, the reaction suspensions were incubated at (30 ± 1) °C in the anaerobic chamber, and at given time intervals, the bottles were taken out for analysis.

**Iron Species, PCP, and Intermediate Analyses.** The HCl-extractable Fe(II) in the reaction suspension was extracted using 0.5 M HCl for 1.5 h.[26] The suspension was centrifuged, and the supernatant was then decanted for HCl-extractable Fe(II) analysis using the 1,10-phenanthroline colorimetric method at 510 nm on a UV−vis spectrophotometer (TU-1810PC, Beijing Purkinje General Instruments, China). The PCP in the soil suspension was extracted using water/ethanol mixtures (50:50 in volume) on a horizontal shaker (180 rpm) for 1 h.[27] The filtrate from the 0.45 $\mu$m syringe filter was collected for the high-performance liquid chromatography (HPLC) analysis of PCP concentration. Details of the HPLC analysis were described in our previous study.[28] The sample preparation and the extractant used for intermediate analysis were as described earlier by Fukushima et al.[29] The determination of intermediates was performed using a gas chromatograph (Thermo Fisher Trace-DSQ-2000) with electron ionization and Agilent silicon capillary column (0.25 mm × 30 m). The temperature ramp was as follows: 50 °C for 2 min, from 55 to 220 °C at 10 °C $min^{-1}$, from 220 to 280 °C at 120 °C $min^{-1}$, and, finally, a 280 °C hold for 10 min.

**Electrochemical Analyses for Characterizing the PCP Transformation Processes.** The potentiostat of the adsorbed Fe(II)

species was determined using cyclic voltammetry (CV) tests, which were conducted in a conventional three-electrode electrochemical cell using an Autolab potentiostat (PGSTAT 30, EcoChemie, The Netherlands). The three-electrode cell comprised a glassy carbon (GC) electrode as the working electrode, a counter electrode (platinum spiral), and a saturated calomel electron (SCE) as the reference electrode. The cyclic voltammograms were recorded using the Autolab potentiostat at a scan rate of 50 mV/s. The electrochemical measurements were conducted in the cell (25 mL) under presettled conditions similar to those of PCP degradation. Prior to each test, the electrolyte was deoxygenated by bubbling pure nitrogen gas for 30 min. The nitrogen gas was continuously bubbled over the surface of the electrolyte throughout the experiments. The curves started at −1.0 V, rose to 1.0 V, and then returned to −1.0 V.

The generated current test was conducted on microbial fuel cell (MFC) equipment composed of two identical rectangular chambers (5.0 cm × 5.0 cm × 3 cm) made of Perspex and separated by a cation exchange membrane (Electrolytica Inc., Amherst, NY, USA).[30] Each chamber contained an electrode consisting of a piece of carbon felt (3.5 cm × 2.5 cm × 0.5 cm). A Ti wire (0.5 mm in diameter) was inserted in the carbon felt to connect the circuit. Prior to use, the carbon felt was soaked in a hot $H_2O_2$ (10%, 90 °C) solution for 3 h, followed by rinsing with deionized water and drying at 60 °C. Lactic acid (10 mM) and/or AQDS (0.2 mM) in the PIPES buffer (100 mM, pH 7.0) were added into the anode chamber (99.99% $O_2$-free $N_2$, atmosphere) as needed. The catholyte containing 0.1 M potassium ferricyanide was added to the cathode chamber (atmospheric pressure). The cell voltages were recorded using a 16-channel voltage collection instrument (AD8223, China) by connecting the circuit with 1000 Ω external resistance.

**DNA Extraction, Polymerase Chain Reaction (PCR) Amplification, Terminal Restriction Fragment Length Polymorphism (T-RFLP) Analysis, and Clone Libraries.** Total soil DNA was extracted with the PowerSoil DNA isolation kit (Mo Bio Laboratories, USA). The bacterial 16S rDNA gene was amplified by PCR using the primer set 27F: '5-AGA GTT TGA TCM TGG CTC AG-3' and 1492R: '5-GGT TAC CTT GTT ACG ACT T-3'. The 27F primer was labeled at the 5' end with 6-carboxyfluorescein phosphoramidite (FAM). The PCR mixture was 1 μL of each primer (20 μM), 5 μL of the 10× PCR buffer, 4 μL of dNTP (2.5 mM), 0.25 μL of Taq polymerase (5 U $\mu L^{-1}$), 1 μL of template DNA, and 38.75 μL of ultrapure water. PCR was performed at 94 °C for 4 min, followed by 30 cycles at 94 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 1.5 min and then a final extension step at 72 °C for 10 min. Triplicate PCRs per sample were performed, and the labeled PCR products were checked on 1.2% agarose gels and purified with a commercial PCR purification kit (OMEGA Biotek, USA) according to the manufacturer's protocol.

Aliquots of purified PCR products were digested with restriction enzymes *Msp*I (TaKaRa Biotechnology, China) at 37 °C for 3 h according to the manufacturer's protocol. Digested PCR products were resolved by electrophoresis using an ABI 3730*xl* sequencer (Applied Biosystems, USA). GS-500 Rox was loaded as an internal size standard in each lane. $G_{ENESCAN}$ software was used to analyze fragment sizes and peak fluorescence intensities. The relative abundance of individual terminal restriction fragments (T-RFs) was calculated as the percentage of total peak area in a given T-RFLP profile. Only those T-RFs with relative abundance >1% were included in the analysis.

The 16S rRNA purified PCR products for clone library construction were ligated into vector PCR2.1-TOPO according to the manufacturer's instructions (Invitrogen, USA). Selected clones were grown in 1.5 mL of Luria−Bertani (LB) medium amended with 50 μg/mL ampicillin. Following harvesting, plasmid DNA was purified from cell pellets using a 96-well alkaline lysis procedure.[31] Clones were sequenced with an ABI 3730*xl* sequencer.

Sequence comparisons were performed using the stand-alone version of the BLAST program against the Silva SSU data sets using the threshold of e-value < $10^{-20}$.[32] Then, the taxonomy of 16S rRNA sequences was determined by pairing with the results of BLAST.

## RESULTS

**Anaerobic Transformation of PCP in the Soils.** The kinetics of PCP transformation in the DP and TZ soils under different conditions are shown in Figure 1. The trends of PCP

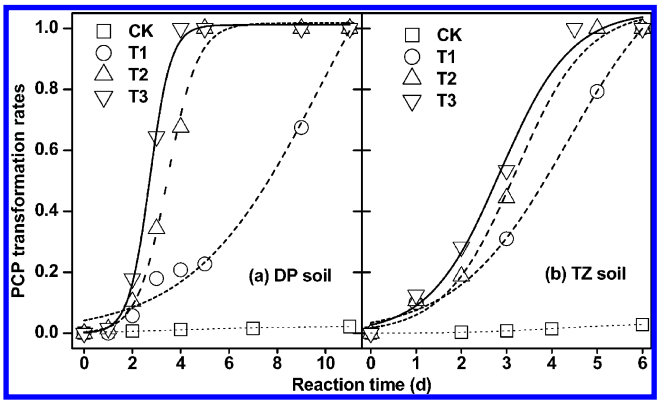


**Figure 1.** PCP transformation rates in DP (a) and TZ (b) soils, with theoretical value changes obtained from the modified microbial logistic growth equation presented as the solid lines. The reaction conditions of different treatments of CK-T3 are provided in Table 1. The initial PCP concentration was 19 μM. As used in the experiment, the PIPE buffer, lactic acid, and AQDS concentrations were 30, 10, and 0.2 mM, respectively.

removal rate curves were generally sigmoidal. A kinetics model modified from the microbial logistic growth eq 1 can well describe the PCP transformation process. The modified model is depicted as eq 2.

$$N(t) = A/(1 + B\,e^{-kt}) \quad (1)$$

$$(C_0 - C_t)/C_0 = A/(1 + B\,e^{-kt}) \quad (2)$$

$A$ is the maximum concentration of PCP, $B$ is the constant of a normal parameter, $k$ is a rate constant of PCP disappearance, and $C_0$ and $C_t$ are the concentrations of PCP at reaction times 0 and $t$, respectively. The kinetics values, that is, the rate constants ($k$), half-lives, relative coefficients ($R^2$), and significance level ($p$) values, for PCP transformation under different conditions were calculated and are listed in Table 1. In the sterile soils, only 2.2% of PCP in the DP soil and 2.8% in TZ soil were transformed after reaction for 6 days (control treatments in Figure 1). In the PIPES buffer, approximately 23% of PCP was transformed after incubation for 6 days in TZ soil that without sterile treatment (T1 treatment) showed a $k$ value of 0.37 $day^{-1}$. In DP soil, PCP transformation was more efficient than in TZ soil. Up to 97% PCP was transformed after incubation for 6 days, with a $k$ value of 0.87 $day^{-1}$. Compared with that in sterile soils, the accelerated PCP transformation rates in these two soils may be attributed to the activities of dechlorinating and iron-reducing bacteria in the soils.[33] As shown by the results of the T2 treatments (with PCP and lactate in the PIPES solution) in Figure 1, the PCP was transformed within 6 days in the soils, with $k$ values increased to 1.27 and 1.59 $day^{-1}$ in DP and TZ soils, respectively. With the further addition of AQDS (T3 treatment), the PCP transformation rates were further accelerated. In TZ soil, PCP was completely transformed in 3 days, with the $k$ value increased to 2.28 $day^{-1}$.

The intermediates of the PCP transformation were determined by gas chromatography−mass spectrometry anal-


dx.doi.org/10.1021/jf204134w | J. Agric. Food Chem. 2012, 60, 2967−2975

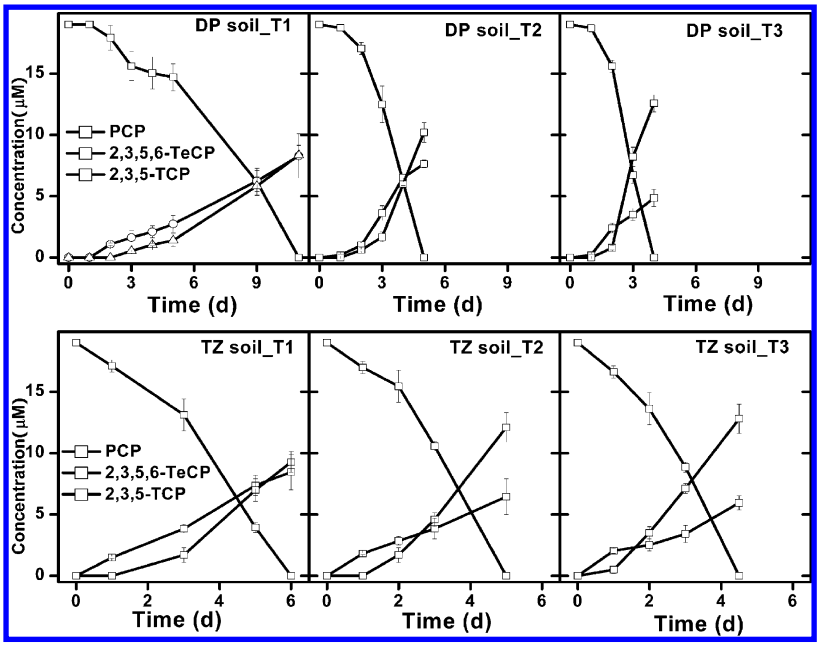


**Figure 2.** Anaerobic transformation of PCP and the intermediate concentration of 2,3,5-trichlorophenol (TCP) and 2,3,5,6-tetrachlorophenol (TeCP) in T1−T3 treatments.

ysis. (The chromatograms of PCP transformation after 2 days with TZ soil under T3 treatment are presented in Figure 1S in the SI.) The concentration changes of 2,3,5,6-tetrachlorophenol (TeCP) and 2,3,5-trichlorophenol (TCP) in all treatments in these two soils are also shown in Figure 2. 2,3,5,6-TeCP and 2,3,5-TCP, with one and two chlorines, respectively, dechlorinated in the PCP molecular structure, were determined in PCP transformation, and the concentrations were continuously increased, accompanied by PCP concentration decreasing, suggesting that the transformation processes underwent reductive dechlorination reaction under anaerobic conditions.[34]

The two soils used in the present study have high $Fe_2O_3$ contents and relatively high ratios of iron minerals (Table 1S of the SI). Iron cycle reactions may affect the transformation process, and the reduced Fe(II) in soils enhances the transformation of PCP in soil minerals.[24] Therefore, the iron reduction process accompanying the PCP transformation was also studied. The results of reduced 0.5 M HCl-extractable Fe(II) concentrations are presented in Figure 2S in the SI. The concentrations of 0.5 M HCl-extractable Fe(II) were in accordance with the PCP transformation rates. The highest concentrations of HCl-extractable Fe(II) were obtained when lactate and AQDS were present (T3 treatment) in both of the two soils. The highest $k$ values for PCP transformation were obtained as well (Figure 1). Along with the relatively lower $k$ values for PCP transformation in the T1 and T2 treatments, the concentrations of HCl-extractable Fe(II) also decreased. In the sterile soils, significantly lower extractable HCl-Fe(II) concentrations (approximately 1 mM) were detected, conforming to the negligible PCP transformation rates under these abiotic conditions.

**Electrochemical Behavior during PCP Transformation Processes.** Figure 3 illustrates the cyclic voltammetric (CV) analysis obtained using a saturated calomel electrode (SCE) to evaluate the Fe(II)-to-Fe(III) electron transfer behavior of the adsorbed Fe(II) in all treatments. Although various components in soils may pose the redox potential on CV analysis, the much higher content and active properties of iron species can give us the distinguishable results of Fe(II)-to-Fe(III) electron transfer in the studied soils, so as to provide direct evidence of redox potentials of the redox systems under different reaction conditions. No obvious redox peaks appeared in the cyclic voltammogram of the Fe species in the sterile soils, indicating the low reduction potentials, consistent with the low PCP transformation rates at these reaction conditions (Figure 1). When only PCP was present in the buffered nonsterile soil suspension (T1 treatment), the anodic peak of Fe(II) (0 to 0.1 V) and the cathodic peak of Fe(III) (−0.3 to 0 V) appeared because of the generated adsorbed Fe(II) species, suggesting the initiation of the PCP reductive transformation reactions in the soils. The anodic peak of Fe(II) in the DP soil exhibited a more negative shift compared with that in the TZ soil, suggesting the higher reduction potential in DP soil, in accordance with its higher PCP transformation rates under this condition. Under biostimulation conditions with the presence of lactate and/or AQDS (T2 and T3 treatments), the peaks of the Fe species exhibited higher intensities. The presence of AQDS yielded a pair of redox peaks at −0.28 and −0.41 V because of its electron transfer potential. The more obvious changes in the presence of AQDS were the negative shift of Fe(II)/Fe(III) pair peak values and the increased intensity of peaks, resulting in the increased reduction potentials of the reaction system and the accelerated PCP transformation rates.

An MFC device was used to quantify the generated electrons during PCP transformation processes in the reaction systems. The current variations are shown in Figure 4. A significantly higher cell current was generated in the DP soil system than in the TZ soil system. When only PCP was present in the buffered nonsterile soil suspension (T1 treatment), the current increased as the reaction progressed in both soils. However, overall, the reading was low (<0.05 mA) in these two soils with indigenous organic matter contents of about 4 and 4.9% (Table 1S of the SI) for the supply of electron donors. In the presence of both lactate and AQDS under biostimulation conditions (T3 treatment), the generated current increased to 0.175 and 0.075

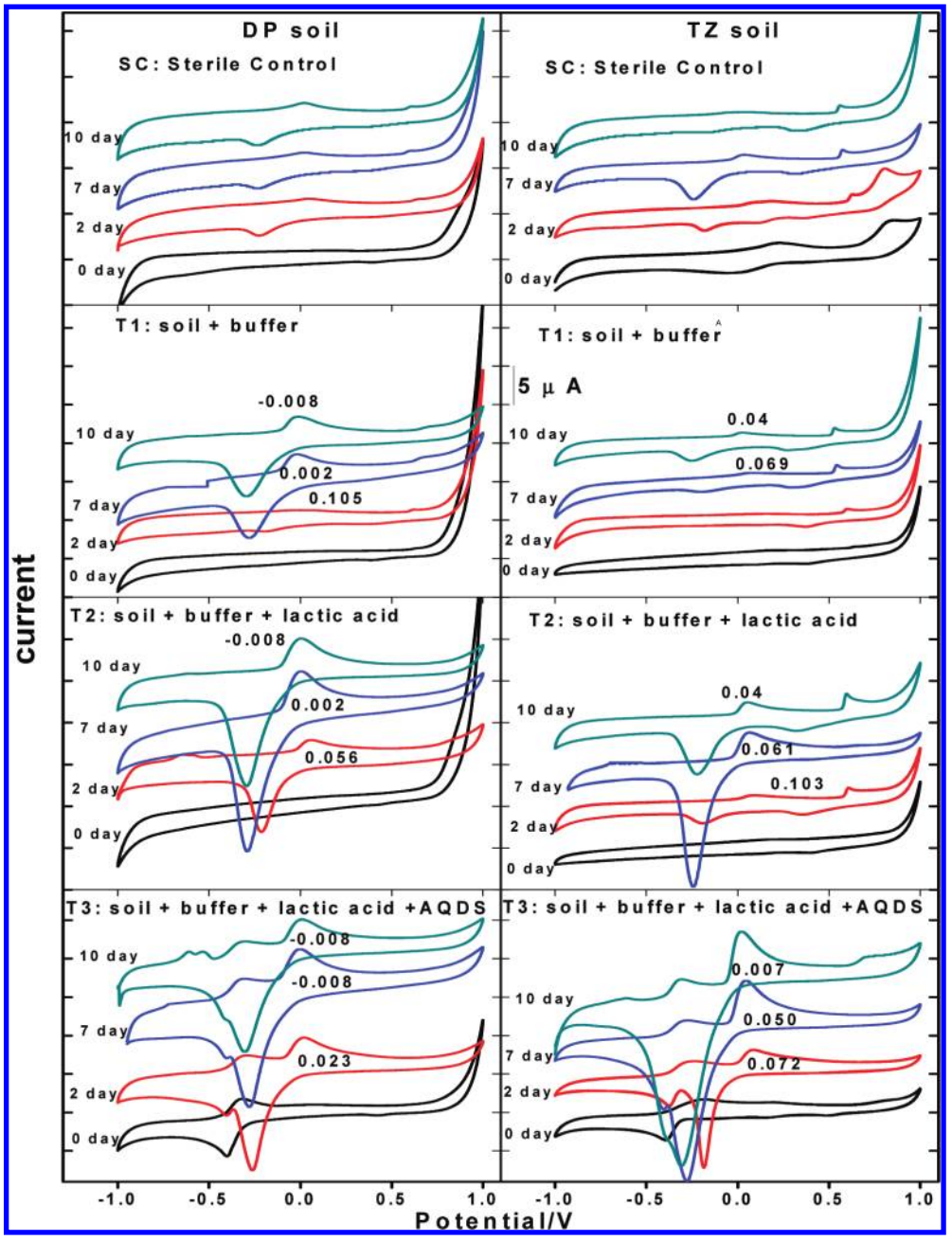


**Figure 3.** Details of the peak evolution in the cyclic voltammograms in DP and TZ soils in the first 10 days of reaction. The reaction conditions are shown in Table 1.

mA in 1.7 days in the DP and TZ soil systems, respectively. Following two additional supplies of lactate nutrient, another two corresponding peaks with even higher current readings were observed under the biostimulation conditions.

**Identification of Important Microbes Accounting for Dechlorination.** Following observations of some functional diversity and similarities among T0−T3 treatments, the structure of microbial communities was analyzed to determine if lactate and/or AQDS caused population shifts. Terminal restriction fragment length polymorphism (T-RFLP) was chosen as an initial assessment method to evaluate major changes in the diversity of T0−T3 treatments after incubation for 7 days. A total of 25/22 distinct T-RFs was identified in all T-RFLP profiles obtained in DP/TZ soils. The average number of T-RFs per sample was about 13, but varied from sample to sample (Figure 5). Details of the dominant T-RFs length presented in the soils are shown in Table 2. The soils with PIPES buffer (T0) showed the most diverse community structure. In DP soil, seven T-RFs were detected in all treatments (Figure 5a). Peaks 4 (154 bp, *Clostridium* sp.), 7 (268 bp, *Helicobacter* sp.), 9 (303 bp, uncultured bacterium), and 13 (531 bp, *Clostridium* sp.) showed significant increases in T1 treatment. Peaks 8 (288 bp, *Selenomonas* sp.) and 10 (521 bp, *Clostridium* sp.) were the dominant peaks in T2 and T3 treatments. The same major T-RFs were typically found in all PCP-treated treatments (T1−T3) in TZ soil, but with significant differences in relative abundance. Peaks 8 and 9 (509 bp, *Clostridium* sp.) in T2 and T3 treatments showed significant increases (Figure 5b). These results indicate that the corresponding species were enriched by biostimulation. The


dx.doi.org/10.1021/jf204134w | J. Agric. Food Chem. 2012, 60, 2967−2975

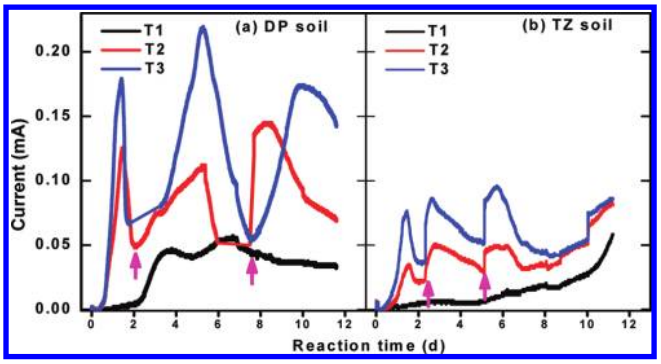


**Figure 4.** Currents generated in the MFC during the PCP transformation in the soils. The reaction conditions of different treatments of T1−T3 are provided in Table 1. Additional lactic acid was added to T2 and T3 treatments at the times indicated by the vertical arrows.

changes in these two soil communities were analyzed using principal component analysis (PCA) of T-RFLPs,[35] and the results are presented in Figure 6. In DP soil, all of the treatments could explain 52.562% of the T-RF variability by PC1 and 33.515% by PC2 (Figure 6a). In TZ soil, all of the treatments could explain 33.093% of the T-RF variability by PC1 and 28.712% by PC2 (Figure 6b). Figure 6 also shows that T2 and T3 grouped relatively closely together in these two soils. To determine the identity of these enriched fragments, approximately 20 plasmids were partially sequenced for each microcosm. In addition, dominant T-RF fragments in these two soils were also evaluated by clone library. The taxonomic identity of the enriched and dominant fragment was determined by comparison with the known sequences in the Silva database (Table 2). Dominant fragments (154, 288, 509, 521, and 531 bp), being increased after incubation with PCP treatments, corresponded to *Clostridium* sp., which have a high degree of similarity to iron-reducing, denitrificating,[36] and dechlorinating[37] bacteria.

## DISCUSSION

A number of CPs, for example, DCEs and dichloromethane, can act as carbon sources for the anaerobic respiration of some microorganisms in anaerobic soil conditions and, therefore,

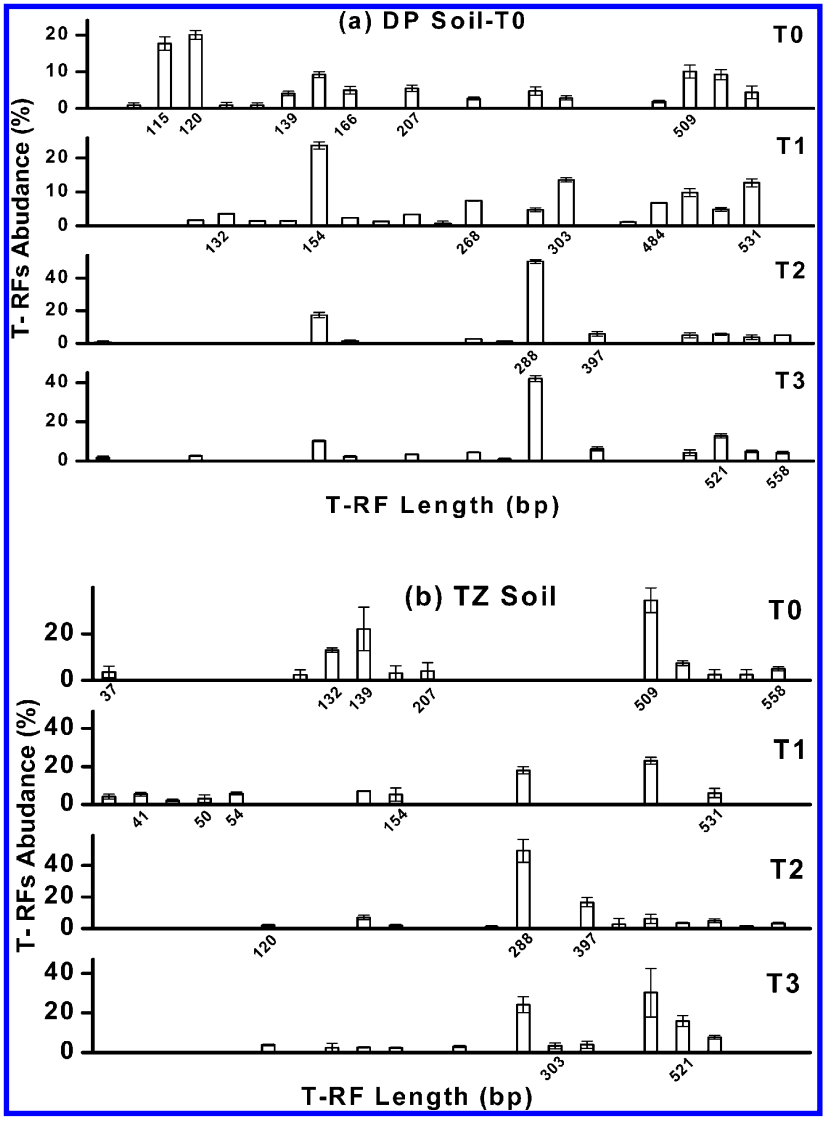


**Figure 5.** T-RFLP analysis of bacteria retrieved from nonsterile soil incubated with T0−T3 treatments for 7 days. The reaction conditions of different treatments of T1−T3 are provided in Table 1.

**Table 2. Summary of Dominant T-RF Lengths Present in All Treatments**

| | | T-RF abundance (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | DP soil | | | | TZ soil | | | | | |
| peak | T-RF length (bp) | T0 | T1 | T2 | T3 | T0 | T1 | T2 | T3 | predicted genus association | accession no. |
| 1 | 115 | 17.7 | | | | | | | | Planctomycetaceae | FJ543038 |
| 2 | 120 | 20 | 1.7 | | | | | | | Planctomycetaceae | FJ543038 |
| 3 | 139 | 4.1 | 1.5 | | | | | | 3.9 | uncultured *Clostridia* bacteria | AJ306803 |
| 4 | 154 | 9.2 | 23.7 | 17.5 | | 22.0 | 7.0 | 7.0 | | *Clostridium* sp. | AB486047 |
| 5 | 166 | 4.9 | 2.4 | | 10.3 | 3.1 | 5.2 | | | *Bacillus* sp. | HM099642 |
| 6 | 207 | 5.5 | 3.4 | | 2.2 | | | | | unknown | GU305789 |
| 7 | 268 | 2.7 | 7.4 | | 3.3 | 4.0 | | | | *Helicobacter* sp. | CP002184 |
| 8 | 288 | 4.8 | 4.8 | 50.3 | 4.4 | | | | | *Selenomonas* sp. | AY349410 |
| 9 | 303 | 2.8 | 13.6 | | 42 | | 18.0 | 49.2 | 24.1 | unknown | FJ189542 |
| 10 | 509 | 10.1 | 9.8 | | | | | | | *Clostridium* sp. | GQ356959 |
| 11 | 521 | 9.2 | 4.9 | 5.0 | 4.2 | 34.9 | 23.0 | 6.1 | 30.3 | *Clostridium* sp. | GQ461617 |
| 13 | 531 | 4.4 | 12.8 | 5.7 | 12.8 | 7.4 | | 3.5 | 6.0 | *Clostridium* sp. | AB486159 |
| 14 | 132 | | | | | 13.0 | | | | *Rhodobacter* | CP000143 |

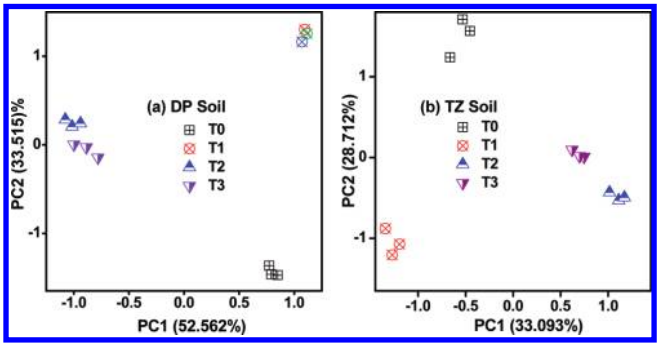


**Figure 6.** Principal component analysis of microbial communities retrieved from nonsterile soil incubated with T0−T3 treatments for 7 days. The reaction conditions of different treatments of T1−T3 are provided in Table 1.

several microorganisms have been isolated for dechlorination.[11] Iron species in soils are also important for the reductive transformation of chlorinated compounds, and the dechlorination capability of Fe(II) species adsorbed on the surface of minerals in soils was determined.[24] However, the natural dechlorination process of CPs in soils may be ultimately slow. Therefore, accelerating this process with biostimulation to enhance the activities of the related microorganisms is necessary. In soils with high iron content, for example, the soils used in the present study, the dechlorinating and iron-reducing bacteria are important for dechlorination. Biostimulation should be employed in both kinds of microorganisms to accelerate the dechlorination rates.

Under anaerobic conditions, soil iron usually undergoes a dissimilatory reduction process,[24] and the contaminant transformation also requires a long time. Without the microbial communities for PCP transformation and iron reduction, the generated amounts of active Fe(II) species were low (Figure 2), and the abiotic reaction for PCP dechlorination was negligible in both sterile DP and TZ soils. The reductive transformation processes of PCP in the soils occurred by electron transfer, with different rates and amounts affecting the PCP transformation rates. Under this condition, the peaks of Fe(III)-to-Fe(II) electron transfer were almost invisible by cyclic voltammetric analysis, with a small number of electrons being transferred under this condition.

The microbially mediated PCP transformation may depend on the species and the abundance of active microbial communities. The indigenous content of organic matter, although with limited content (Table 1S), can act as the electron donor for the respiration of microorganisms.[38] Therefore, with the controlled nonsterile soils under appropriate water content, the related dechlorinating and iron-reducing bacteria are activated by accepting electrons from the organic matter for anaerobic inspiration to increase the PCP transformation rates. The PCP transformation rate was higher in DP soil than in TZ soil, which was attributed to the higher abundance of the dechlorinating and iron-reducing bacteria in DP soil, as indicated by the T-RFLP results.

Biostimulation is an efficient and promising method for accelerating the transformation of contaminants, especially for in situ soil remediation.[11] In the present study, the biostimulation treatments can result in a complete transformation of PCP in a shorter time frame. The use of soils in optimal conditions with appropriate water content can also be considered an efficient stimulation method for the anaerobic respiration of microorganisms and has been reported as a biostimulation treatment for stimulating the activities of microorganisms[15] to increase the amount of transferred electrons and subsequently accelerate the PCP transformation rates. Electron donors, such as acetate, formate, pyruvate, and lactate, can support and stimulate the biotic dechlorination processes.[33] Lactate is a good electron donor for the respiration of a wide range of microorganisms in contaminant transformation.[34] The dechlorinating and iron-reducing bacteria identified in the present study can also utilize lactate as an electron donor to stimulate the reductive dechlorination of PCP. The continuous dechlorination in lactate-fed systems with higher electron concentration transferred and increased the amount of generated active Fe(II) species, as shown in the MFC results (Figure 4). When supplied with sufficient electron donors, PCP transformation rates in DP and TZ soils were almost identical because of the activities of the dechlorinating and iron-reducing bacteria.

Humic substances may play a significant role in the anaerobic respiration of microorganisms because it can accelerate the electron transfer rate.[39] AQDS from the category of HS could shuttle electrons to iron oxides and chlorinated compounds by acting as a redox mediator.[21] In the current study, the presence of both lactic acid and AQDS in soils generated high current

intensity and quickly resulted in complete PCP transformation. When AQDS was present, the generated current and transferred electron amount were largely increased because of the significant biostimulation effect of the dechlorinating and iron-reducing bacteria. The bacteria in soils were more active when lactate was added as an electron donor. These bacteria then transferred electrons to iron minerals for generation of active Fe(II) species, in which AQDS acted as an electron shuttle to accelerate the process. Higher concentration of Fe(II) ultimately increased the transformation rates of PCP.[24] The PCP transformation process was also accelerated with the biostimulation of dechlorinating bacteria, which were more active in accepting electrons through anaerobic respiration and then transferring electrons to the PCP. The electron transfer from electron donors to bacteria could be mediated and catalyzed by AQDS, resulting in more efficient transferring rates, subsequently achieving complete PCP dechlorination in a shorter period. Lactate was continuously required for the needed high concentration of electrons and high PCP transformation rates. As indicated by Figure 4, both T2 and T3 treatments subsequently had rapid drops in current, presumably attributable to the fast depletion of lactate that should be available for microbial activity. However, AQDS, as the catalyst for mediation, was not consumed in the reaction system. The initial added amount was sufficient for the electron transfer throughout the whole dechlorination processes.

The biostimulation methods conducted by providing electron donors and shuttles can obviously increase the electron transfer rates, ultimately accelerating PCP transformation rates in iron-rich soils. However, the fundamental critical factor in the soil was the microbial processes. On the basis of the results of microbial community analysis, the iron-reducing and dechlorinating bacteria (154, 288, 509, 521, and 531 bp) were the critical factors for PCP transformation. Under biostimulation conditions, that is, with the additional presence of electron donors of lactate and electron shuttles of AQDS, the PCP in soils would be rapidly transformed in a shorter period. In the future, functional microbial communities, including the specific bacterium functional group, the functional gene, and microbe activity, should be given greater attention to better apply the decontamination function of biostimulation in soil remediation in situ.

## ■ ASSOCIATED CONTENT

### Ⓢ Supporting Information

Table 1S, Figure 1S, and Figure 2S. This material is available free of charge via the Internet at http://pubs.acs.org.

## ■ AUTHOR INFORMATION

### Corresponding Author

*(F.L.) Phone: +86 20 87024721. Fax: +86 20 87024123. E-mail: cefbli@soil.gd.cn. (C.L.) E-mail: csliu@soil.gd.cn.

### Funding

The present work was supported by grants from the National Natural Science Foundation of China (No. 41025003), the "973" Program (2010CB134508), the International Foundation for Sciences (IFS, AC/19668), and the Guangzhou Technology Support Project (2009ZI-E541).

### Notes

The authors declare no competing financial interest.

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