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ORIGINAL PAPER

Simultaneous biodegradation of phenol and carbon tetrachloride mediated by humic acids

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Abstract The capacity of an anaerobic sediment to achieve the simultaneous biodegradation of phenol and carbon tetrachloride (CT) was evaluated, using humic acids (HA) as redox mediator. The presence of HA in sediment incubations increased the rate of biodegradation of phenol and the rate of dehalogenation (2.5-fold) of CT compared to controls lacking HA. Further experiments revealed that the electron-accepting capacity of HA derived from different organic-rich environments was not associated with their reducing capacity to achieve CT dechlorination. The collected kinetic data suggest that the reduction of CT by reduced HA was the rate-limiting step during the simultaneous biodegradation of phenol and CT. To our knowledge, the present study constitutes the first demonstration of the simultaneous biodegradation of two priority pollutants mediated by HA.

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Keywords Phenol · Carbon tetrachloride · Humic acids · Redox mediator

Abbreviations

HA	Humic acids
ETC	Electron-transferring capacity
TEA	Terminal electron acceptor
RM	Redox mediators
AQDS	Anthraquinone-2,6-disulfonate
CT	Carbon tetrachloride
CS	Anaerobic sludge from a factory of candies
PS	Anaerobic sludge from a paper-mill factory
S	Sediment
VS	Volatile solids
VFA	Volatile fatty acids
AH_2QDS	Anthrahydroquinone-2,6-disulfonate
R^2	Coefficient of determination
SCP	Soil of cocoa plantation
SDF	Soil of deciduous forest in San Luis Potosí, Mexico
GWC	Compost produced with gardening wastes
IHSS	International Humic Substance Society
EAC	Electron accepting capacity
μEq	Micro-electron equivalents
CF	Chloroform
DCM	Dichloromethane
k_{d2}	Second-order rate constant
k_{d1}	First-order rate constant

Introduction

Humic acids (HA) represent the most abundant organic matter fraction in terrestrial and aquatic environments. HA are formed from the decomposition of plant, animal and microbial cells and tend to be more recalcitrant than precursor materials. They consist of a skeleton of alkyl/aromatic units cross-linked mainly by oxygen and nitrogen groups with the major functional groups being carboxylic acid, phenolic and alcoholic hydroxyls, ketone and quinone groups, among them quinone groups are considered the main electron-transferring functional groups in HA (Stevenson 1994; Watanabe et al. 2009).

Due to their electron-transferring capacity (ETC), HA and quinones analogues have been shown to have three distinct roles supporting the abiotic and microbial redox transformation of different priority pollutants: as terminal electron acceptor (TEA) for microbial respiration (Lovley et al. 1996; Benz et al. 1998; Bradley et al. 1998; Cervantes et al. 2000, 2001a, 2008), as electron donors to achieve the microbial reduction of TEA with a more positive standard redox potential (Lovley et al. 1999; Field et al. 2000; León-García et al. 2007; Van der Zee and Cervantes 2009) and as redox mediators (RM) channeling reducing equivalents from an electron donor to electron-accepting pollutants (Fig. 1).

Previous reports have shown the microbial reduction of HA or quinones coupled to the anaerobic oxidation of ecologically relevant substrates such as acetate, hydrogen, propionate, lactate, phenol, *p*-cresol, toluene, vinyl chloride and dichloroethene (Bradley et al. 1998; Cervantes et al. 2001a, b, 2008). Moreover, the anaerobic oxidation of these substrates occurs in anaerobic environments despite the presence of alternative electron acceptors, such as sulfate and nitrate (Cervantes et al. 2008; Minderlein and Blodau 2010). Reduced HA or hydroquinones can also serve as electron donors for the microbial reduction of different relevant electron acceptors, such as nitrate, nitrite, nitrous oxide, perchlorate, arsenate and selenate (Bruce et al. 1999; Coates et al. 2002; Aranda-Tamaura et al. 2007). HA and quinone model compounds [e.g. anthraquinone-2,6-disulfonate (AQDS), 1,4-benzoquinone, 1,4-naphthoquinone, juglone, menaquinone, lawsone] have also been demonstrated to accelerate the microbial and abiotic reductive transformation of azo dyes, nitroaromatics and polychlorinated pollutants by serving as RM increasing the redox reactions by several orders of magnitude (Curtis and Reinhard 1994; Rau et al. 2002; Cervantes et al. 2004; Doong and Chiang 2005; Li et al. 2009; Liu et al. 2009).

Although evidence collected during the last two decades clearly indicates a great potential for applying HA for remediation purposes, further information is needed in order to elucidate the main mechanisms involved in the redox reactions mediated by HA and to enhance their applicability. For instance, Perminova et al. (2005) suggested that the principal factor limiting the application of HA in remediation technologies is the intrinsic variability in redox properties observed among humic materials and their fractions. Indeed, the ETC greatly varies among HA extracted from different organic rich environments (Ratasuk and

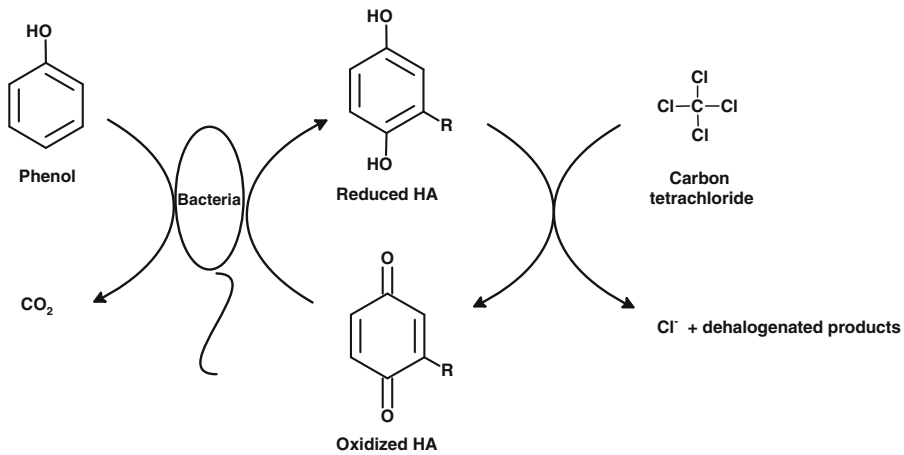

Fig. 1 Mechanism proposed for the simultaneous biodegradation of phenol and CT mediated by humic acids

Nanny 2007) and scarce information is available in the literature clarifying the appropriate HA source demanded in redox reactions for remediation purposes. Moreover, very limited information has been reported to elucidate the rate-limiting step during the redox biotransformation of contaminants. In some cases, the reduction of RM by different microorganisms has been pointed out as the rate-limiting step during redox reactions (Rau et al. 2002). In contrast, other studies evidenced that the reduction of electron-accepting contaminants by reduced HA or hydroquinones is the rate-limiting step (Rau et al. 2002; Jiang and Kappler 2008). Therefore, further research is demanded in order to elucidate the mechanisms limiting the catalytic effects of HA during the anaerobic (bio)transformation of priority pollutants.

The aim of the present work was to study the kinetic aspects involved during the simultaneous biodegradation of phenol and carbon tetrachloride (CT) mediated by HA (Fig. 1). Phenol and CT were selected as model pollutants because they frequently contaminate soils and water bodies due to their widespread industrial use and high incidence due to improper disposal, leaking storage tanks, and spills (Cervantes et al. 2000, 2004). The study assesses the capacity of three anaerobic consortia to oxidize phenol with the humic model compound, AQDS, as TEA; evaluates the capacity of three sources of HA as reducing agent of CT; and selects the proper inoculum and source of HA to achieve the simultaneous removal of phenol and CT.

Materials and methods

Inocula and basal medium

Anaerobic granular sludge samples were collected from two full-scale upflow anaerobic sludge bed (UASB) reactors treating effluents from a factory of candies (CS) (San Luis Potosi, Mexico) and from a paper-mill factory (PS) (Eerbeek, The Netherlands). Additionally, a sediment (S) was collected from a lagoon (Marland) contaminated with hydrocarbons located in Ebano (San Luis Potosí, Mexico). The content of volatile solids (VS) in these anaerobic consortia were (in % wt/wt): 4.98, 9.94 and 3.75 respectively, for CS, PS and S. The basal medium used in all batch experiments was prepared as previously described (Cervantes et al. 2000). The basal medium was flushed with N_2/CO_2 (80%/20%) by passing this gas mixture through the liquid bulk for 10 min and was used without sterilization in all the experiments.

Biodegradation of phenol with AQDS as TEA

Incubations were conducted in batch mode by duplicate in glass serum bottles with a liquid volume of 50 mL. Anaerobic basal medium supplemented with AQDS (5 mM) was transferred directly to the glass serum bottles, which were inoculated by adding 2 g VS/L of each consortium and sealed with Teflon stoppers and aluminum caps. Vials were then flushed with N_2/CO_2 (80%/20%) for 10 min. Finally, phenol was added as substrate at the initial concentration of 1.35 mM. Controls without phenol were also incubated to correct for endogenous AQDS reduction. All bioassays were incubated at 28°C. The pH of the medium was controlled at 7.2 by a bicarbonate/CO_2 buffer (60 mM).

To determine if the evaluated consortia were also able to degrade phenol under methanogenic conditions, incubations lacking AQDS were included. Controls without phenol were also included to correct for the endogenous methanogenic activity. Samples from every experimental unit were periodically taken, centrifuged and filtered (0.2 μm), for determining the concentration of volatile fatty acids (VFA), such as acetate, propionate and butyrate, as well as to measure the reduction of AQDS [as anthrahydroquinone-2,6-disulfonate (AH_2QDS)]. Production of methane was determined by monitoring the composition of the produced biogas. All bioassays were incubated at 28°C in the dark with mild shaking.

AQDS reduction and CH_4 production rates were determined using the maximum slope observed on linear regressions considering at least three sampling points. The coefficient of determination (R^2) was higher than 0.9 for all microbial activities calculated.

Reduction of CT by HA

Three different sources of HA were tested, which originated from a soil of cocoa plantation (SCP), soil of deciduous forest in San Luis Potosí, Mexico (SDF) and compost produced with gardening wastes (GWC). HA were extracted with NaOH 0.1 M (Sigma-Aldrich, solution titrated to 0.0987 M) under a nitrogen atmosphere, according to the standardized procedure of the International Humic Substance Society. Table 1

Table 1 General characteristics and electron accepting capacity of each HA evaluated

Code	Description	Localization (state) and geographic coordinate	Typical vegetation		Soil pH [a]	EAC (μEq/gHA)
			Species	Genus		
SDF	Soil of deciduous forest of San Luis Potosí	San Luis Potosí: 22°5′0″N, 100°38′0″W	*Quercus rugosa Neé*	*Quercus*	5.65	174.9 ± 66.9
SCP	Soil from cocoa plantation	Pichucalco, Chiapas: 17°30′N, 93°07′W	*Theobroma cacao* L.	*Theobroma*	5.23	163.2 ± 21.3
GWC	Compost of gardening wastes	Pilot station at University of Guadalajara			6.69	347.1 ± 10.2

[a] Determined directly on the sample with a soil pH electrode

EAC was chemically determined in a H_2/Pd reaction system including 4 g HA/L

shows the general characteristics of the HA evaluated. Chemical reduction experiments were performed in batch mode by triplicate in glass serum bottles with a liquid volume of 50 mL. Serum flasks were filled with basal medium containing HA (4 g/L), Pd as catalyst (8 pellets) and sealed with Teflon stoppers and aluminum caps. The vials were flushed with H_2 for 1 h to saturate the vials with this reducing agent and were incubated for one week to achieve complete reduction of HA. Table 1 shows the electron accepting capacity (EAC) of the tested HA determined by the ferrozine technique (Lovley et al. 1996). After complete reduction of HA, Pd pellets were removed and solutions were diluted with anaerobic basal medium to establish an initial reducing capacity of HA at 150 micro-electron equivalents (μEq)/gHA. Reduced HA solutions were then transferred (in an anaerobic chamber with a N_2/H_2 atmosphere) to serum flasks sealed with Teflon stoppers and aluminum crimps and previously flushed with N_2/CO_2 (80%/20%) for 20 min. Finally, CT (100 μM referred to the liquid volume) was added to the serum flasks (from a concentrated anaerobic stock solution). The assays were incubated at 28°C in the dark with a mild shaking. The concentration of CT and products derived from its reductive dechlorination, such as chloroform (CF) and dichloromethane (DCM), was determined over time to assess the rate of dechlorination of CT.

Simultaneous biodegradation of phenol and CT mediated by HA

The assays were conducted in batch mode by triplicate in glass serum bottles with a liquid volume of 30 mL. Anaerobic basal medium was directly transferred to the vials, which were then inoculated with 0.01 g VS/L of sediment from Marland lagoon (inoculum S). SA derived from SCP (4 g/L) were used as RM during these experiments. All vials were sealed with Teflon stoppers and aluminum caps and flushed with N_2/CO_2 (80%/20%) for 10 min to establish anaerobic conditions. After that, phenol was added as external electron donor at the initial concentration of 1.35 mM**.** Before addition of CT, phenol oxidation and reduction of HA were monitored. Once phenol oxidation linked to the reduction of HA was observed, CT was added at the initial concentration of 30 μM. Endogenous controls lacking phenol were included in order to document the coupling between phenol oxidation and CT dechlorination. Moreover, controls lacking HA were also included in order to assess their catalytic effects. Finally, sterile controls without inoculum were also included in the experimental protocol in order to identify potential physicochemical processes (e.g. adsorption) involved during phenol and CT removal. All experimental treatments were incubated at 28°C in the dark with a mild shaking.

Analytical methods

The concentration of AH_2QDS was determined on anaerobically collected samples in an anaerobic chamber by UV/visible spectrophotometry (Thermo-Scientific Genesys 10 UV) at 450 nm using a calibrating curve of AQDS chemically reduced by dithionite as previously described (Cervantes et al. 2000).

The production of methane was quantified in 100 μL headspace samples in a gas chromatograph (GC, Agilent Technologies 6890 N series) equipped with a thermal

conductivity detector and a column Hayesep D (Alltech, Deerfield, Illinois, USA) with the following dimensions: 3.048 m × 3.18 mm × 2.16 mm. Nitrogen was used as carrier gas with a flow-rate of 12 mL/min. Temperatures of the injection port, oven and the detector were 250, 60 and 250°C, respectively. Nitrogen was used as carrier gas with a flow-rate of 12 mL/min.

The removal of CT and the production of volatile chlorinated hydrocarbons such as, CF and DCM were determined in 50 μL headspace samples by gas chromatography (GC, Agilent technologies 7890 series) coupled to a micro cell electron capture detector. Separation was achieved with a 5% phenyl-95% dimethyl-polysiloxane (30 m × 32 mm ID, 0.25 μm (d_f)) HP-5 fused-silica capillary column from Agilent Technologies (Little Falls, DE). Helium was used as carrier gas at 1 mL/min column flow-rate and nitrogen was the makeup gas at 59 mL/min. The temperatures of injector and detector were maintained at 200 and 260°C, respectively. The oven temperature was programmed from 45°C (1 min hold) to 63°C (5°C/min).

Phenol was analyzed by gas chromatography (GC, Agilent technologies 7890 series) coupled to a flame ionization detector (FID). Separation was achieved with a 5% phenyl-95% arilen-siloxane (30 m × 0.250 mm ID, 0.25 μm (d_f)) DB-5MS fused-silica capillary column from Agilent Technologies. Helium was used as carrier gas at 33 cm/s column flow-rate and nitrogen was the makeup gas at 30 mL/min. The temperatures of injector and detector were maintained at 250 and 300°C, respectively. The oven temperature was programmed from 35°C (1 min hold) to 280°C (8°C/min).

The concentration of VFA acetate, propionate and butyrate were measured using a capillary electrophoresis ion analyzer (Agilent G1600A, Waldbronn, Germany) equipped with a capillary column (50 lm ID_ 72 cm).

Results

Anaerobic biodegradation of phenol

Among the different consortia studied, sediment S was the only consortium capable of anaerobically oxidizing phenol with the AQDS as TEA. The maximum respiratory rate achieved by this inoculum during the anaerobic oxidation of phenol was 7.04 ± 0.37 μEq/gVS h; in contrast, no coupling between phenol degradation and AQDS reduction was detected with consortia CS and PS after 40 days of incubation.

On the other hand, complete conversion of phenol to methane was accomplished by both anaerobic granular sludges tested (inocula CS and PS). The maximum methane production rates calculated were 5.9 ± 7.1 and 3.46 ± 2.9 μEq/gVS h, respectively, for CS and PS consortia. In both cases, complete phenol conversion was based on stoichiometric recovery of methane (>95%) and no detection of phenol after 50 days of incubation. Meanwhile, sediment S did not show any methanogenic activity with phenol as electron donor.

Reduction of CT by HA derived from different environments

Three different sources of HA were chemically reduced in a H_2/Pd reaction system and evaluated for their capacity to dechlorinate CT under abiotic conditions with reduced HA as a sole electron donor. Figure 2 shows the reduction of CT by the reduced HA evaluated. In all cases, the reduction of CT occurred without any lag phase. HA extracted from SCP showed the highest reducing capacity for the reductive dehalogenation of CT. After 16 days of incubation, 47.3% of the CT initially added (100 μM) was reduced by SCP, whereas only 25% reduction occurred with HA derived from SDF and GWC. These results contrast with the EAC quantified (Table 1), in which, GWC showed the highest value, indicating that EAC was not related to the capacity to reduce CT.

CF was the only dehalogenation product detected from CT dechlorination by reduced HA in all cases. The high level of recovery obtained at the end of the incubation period suggests that unidentified dechlorination products accounted for a minor fraction of reduced CT (Table 2). Dehalogenation of CT by reduced HA was most accurately described by second-order kinetics (Fig. 3). The second-order rate constants (k_{d2}) were 1.8×10^{-5}, 8.2×10^{-6} and 8×10^{-6} μM/h when reduced HA derived from SCP, SDF and GWC were used as reducing agents, respectively. Therefore, the k_{d2} value observed with HS from SCP corresponded to a twofold increase respect to the dechlorination rate observed with the other HA sources. There was no reduction of CT in chemical controls including non-reduced HA.

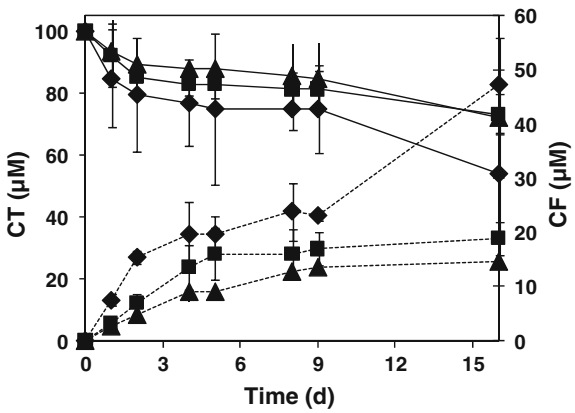

Fig. 2 Time course of dechlorination of CT linked to CF formation by three HA (4 g/L) samples previously reduced with H_2/Pd. *Filled diamond* SCP, *filled square* SDF, *filled triangle* GWC, *solid line* CT reduced, *dotted line* CF produced. Results represent average from triplicate incubations

Simultaneous biodegradation of phenol and CT mediated by HA

HA derived from SCP showed the highest capacity to transfer electrons to CT. Likewise, sediment S was the only consortium able to oxide phenol using AQDS as TEA. Thus, these sources of HA and inoculum were used to study the simultaneous biodegradation of phenol and CT mediated by HA. Figure 4 shows the reduction of CT and oxidation of phenol under the different treatment conditions evaluated. In the presence of HA (Fig. 4a), complete reduction of CT was observed after 3 days of incubation and significant reduction of CT occurred even in endogenous controls lacking phenol. In the absence of HA (Fig. 4b), the reduction of CT was completed after 10 days of incubation. However, in this case, endogenous activity did not promote CT dechlorination. In both cases, phenol was not completely consumed. In the presence of HA, only 46 ± 1.58 mg/L (0.20 μM) was oxidized, whereas in controls lacking HA 88 ± 3.2 mg/L (0.40 μM) of phenol was removed. Therefore, it is evident in both cases that the coupling between phenol degradation to CT reduction occurred in sediment incubations.

Table 3 shows dehalogenation rates and mass balances obtained in the different treatments evaluated after 3 days of incubation. Dehalogenation of CT followed first-order kinetics and the first-order rate constants (k_{d1}) calculated in the presence of HA derived from SCP, with and without of phenol, were 0.025 and 0.015/h, respectively. For biological controls lacking HA, the k_{d1} value was 0.010/h. Thus, the presence of HA increased 2.5-fold the k_{d1} value compared to the control incubated in the absence of HA. HA also promoted a significant dechlorination of CT even in endogenous controls lacking phenol.

The impact of HA was also reflected in an increase in the efficiency of dechlorination by this consortium. Certainly, 77% of CT reduction occurred in sediment incubations including phenol and HA, 50% in endogenous control supplied with HA and 44% in incubations provided with phenol but lacking HA. CF and DCM were the dehalogenation products detected in all cases. Meanwhile, not significant reduction of CT and oxidation of phenol occurred in sterile controls (without consortium).

Further experiments to elucidate the role of HA on CT dechlorination pathway revealed that chemically reduced HA derived from SCP did not have the capacity to reduce CF further under abiotic conditions. Furthermore, CF could not be dechlorinated in biologically active sediment incubations when

Table 2 Second order rates constants (k_{d2}) and mass balance for the dechlorination of CT by the three sources of HA evaluated after 16 days of incubation

HA	k_{d2} μM/h	CT_i μM	CT_f μM	CF_p μM	Reduction (%)	Recovery (%)
SCP	1.8×10^{-5}	102.3 ± 3	53.9 ± 20	47.3 ± 8.7	45.8	98.9
SDF	8.2×10^{-6}	98.5 ± 1.1	73.2 ± 6.4	19 ± 2.9	19.28	93.6
GWC	8×10^{-6}	98.9 ± 2.2	72.3 ± 9.3	14.8 ± 0.8	14.96	88

Initial concentration: CT 100 μM, HA 4 g/L. Results represent average from triplicate incubations

SCP soil of cocoa plantation, *SDF* soil of deciduous forest in San Luis Potosí, Mexico, *GWC* compost produced with gardening wastes, CT_i initial concentration of CT, CT_f final concentration of CT, CF_p produced concentration of CF

Reduction = $(CFp/CT_i) \times 100$; % Recovery = $(CT_f + CF_p)/CT_i \times 100$

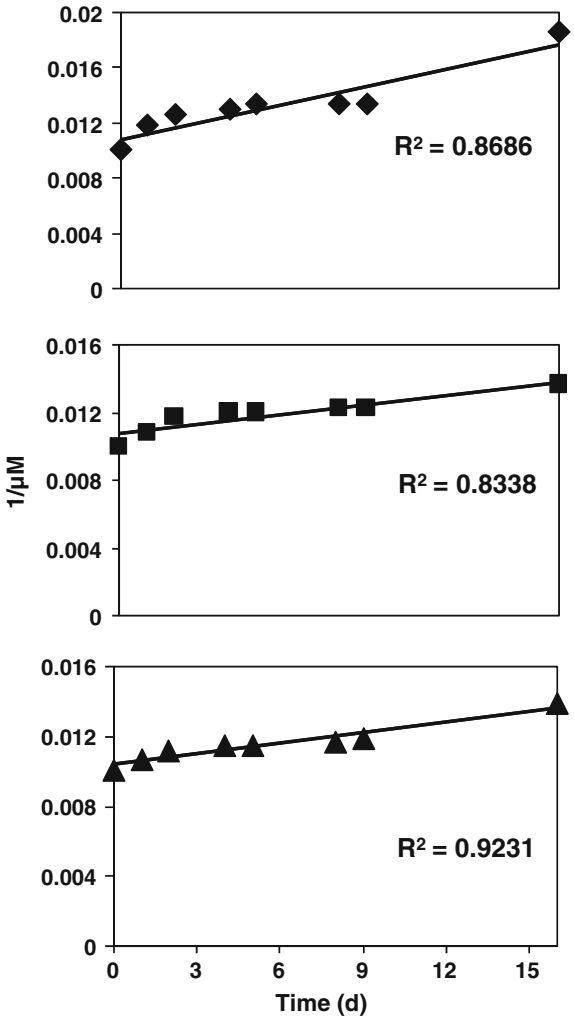

Fig. 3 Second-order kinetics during the dechlorination of CT by different HA samples previously reduced with H_2/Pd. *Filled diamond* SCP, *filled square* SDF, *filled triangle* GWC. Results represent average from triplicate incubations

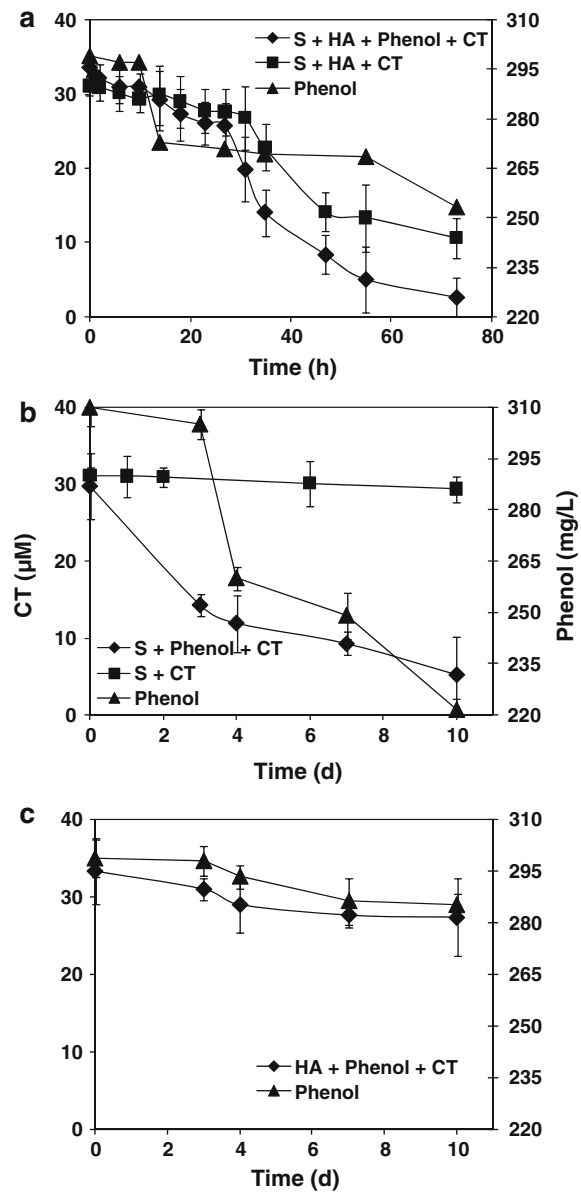

Fig. 4 Impact of HA during the simultaneous biodegradation of CT and phenol **a** in presence of HA from SCP as RM **b** biological control lacking HA **c** chemical control without sediment. Results represent average from triplicate incubations

reduced HA were supplied as a unique electron donor. Therefore, these results suggest that the redox mediating capacity of HA were only involved in the first step of CT dechlorination (e.g. CT to CF). Nevertheless, the presence of HA in sediment incubations supplied with phenol as electron donor promoted a larger extent of CT reduction, including higher production of DCM probably due to kinetic enhancement. Namely, by promoting a faster reduction of CT to CF by the presence of HA, a higher concentration of CF would have then been available for further dechlorination in sediment incubations, thus achieving an overall more efficient dechlorination process.

Discussion

The present study indicates that HA were able to mediate the simultaneous biodegradation of phenol and CT, by increasing the rate and extent of oxidation of phenol and dechlorination of CT by the studied consortium. Certainly, addition of HA in sediment incubations increased the rate of oxidation of phenol

Table 3 First order rate constants (k_{d1}) and mass balance for the dechlorination of CT by the different culture conditions evaluated after 3 days of incubation

Culture conditions	k_{d1} (h^{-1})	CT_i (μM)	CT_f (μM)	CF_p (μM)	DCM_p (μM)	Reduction (%)	Recovery (%)
A	0.025	33.5 ± 1.18	5 ± 4.5	6.24 ± 0.7	19.8 ± 1.6	77.7	92.6
B	0.015	31.0 ± 1.16	13.3 ± 1.9	7.5 ± 5.1	8 ± 4.1	50	92.9
C	0.010	29.7 ± 4.2	14.9 ± 1.4	2.8 ± 1.5	10.5 ± 2.2	44	94.9
D	0.000	31.1 ± 1.18	30.9 ± 1.25	0	0	0	99
E	0.000	33.3 ± 0.18	31 ± 1.6	0	0	0	93

Initial concentration: CT 30 μM, HA 4 g/L. Results represent average from triplicate incubations

(A) S + HA + Phenol + CT, (B) S + HA + CT, (C) S + Phenol + CT, (D) S + CT, (E) HA + Phenol + CT

S Sediment, *HA* Humic acids, *CT* Carbon tetrachloride, *CF* Chloroform, *DCM* Dichloromethane, CT_i initial concentration of CT, CT_f final concentration of CT, CF_p produced concentration of CF, DCM_p produced concentration of DCM

Reduction = $(CFp + DCMp/CT_i) \times 100$; % Recovery = $(CT_f + CF_p + DCMp)/CT_i \times 100$

as compared to controls lacking HA in which methanogenesis was the prevailing process. Moreover, the enhanced phenol oxidation was coupled to CT dechlorination, which was also increased both in terms of rate and extent of dechlorination (Table 3). The role of HA during the coupling between phenol oxidation and CT dechlorination was further emphasized by the higher rate and extent of CT reduction observed in HA-phenol-amended incubations compared to the results obtained in HS-amended endogenous controls lacking phenol. Although several studies have previously been documented the impact of HA by increasing the rate and extent of biodegradation of a wide variety of priority pollutants (Van der Zee and Cervantes 2009), the present work reports for the first time the simultaneous biodegradation of two contaminants mediated by HA acting as effective RM (Fig. 1).

The results from the present study also indicate that the EAC depended on the source and characteristics of HA and that the EAC was not related to its ETC to achieve CT dechlorination. Indeed, HA derived from SCP showed the lowest EAC among the different HS studied, but accomplished the reduction of CT to CF at a rate 2.2-fold faster as compared to the dechlorination rate observed with HA derived from SDF and GWC. Thus, the results suggest that HA derived from SCP have electron-transferring functional groups with a greater reactivity towards CT dechlorination than that expected from redox functional groups present in the other two HA sources. Several studies have reported that the properties of HA such as size, molecular weight, elemental composition, structure and the number and position of functional groups vary depending on the origin and age of the precursor material, and affect directly the ETC of these materials (Ratasuk and Nanny 2007).

The anaerobic biodegradation of CT in the presence of quinones or HA has been reported in previous studies. For instance, Cervantes et al. (2004) who evaluated the dechlorination of CT by anaerobic sludge in the presence of AQDS and HA, reported that the addition of these redox mediators significantly increased the k_{d1} value during CT dechlorination. However, the type of the substrate provided during this study significantly affected the rate of CT dechlorination. In the presence of AQDS (20 μM) and with glucose, acetate and methanol as electron donors, the k_{d1} values after 27 days of incubation were, 0.174, 0.141 and 0.105/day respectively. In the present study, the k_{d1} value obtained after 73 h of incubation was 0.6/day, which is higher than previously reported with redox mediators including AQDS, riboflavin and HA (Cervantes et al. 2004; Guerrero-Barajas and Field 2005). Thus, the use of phenol as an electron donor did not limit the CT dechlorination process neither in the presence nor in the absence of HA.

Considering the kinetic data collected during the biological reduction of AQDS linked to phenol oxidation and from the chemical reduction of CT by reduced HA, the results suggest that the first step in Fig. 1 (biological reduction of quinones with phenol as electron donor) was 17.22-fold faster than the second step (transfer of reducing equivalents from reduced HA towards CT dechlorination) and therefore, this last step is considered the rate-limiting step in the whole process.

Indeed, the reduction rate of AQDS was 14.09 μEq/L h during the oxidation of phenol in sediment incubations, whereas the transfer of electrons from reduced HA reduced to CT occurred at 0.818 μEq/L h. Although these calculations were made with the humic model compound, AQDS, previous studies have documented that the reduction rate of AQDS and HA proceed at similar rates by different humus-reducing microorganisms (Lovley et al. 1996). Thus, the kinetic data obtained with AQDS could be extrapolated with those expected with HA.

The fact that the reduction of CT by previously reduced HA under abiotic conditions was most accurately described by second-order kinetics (Table 2) implies that the reaction rate depended on the concentration of both reduced redox functional groups in HA and CT. Previous studies have also reported second-order kinetics describing the reduction of other polyhalogenated pollutants, such as hexachloroethane, by reduced HA (Kappler and Haderlein 2003). However, dechlorination of CT coupled to phenol oxidation in sediment incubations followed first-order kinetics, probably because redox functional groups in HA could be regenerated through the microbial oxidation of phenol after transferring the electrons for CT dechlorination.

Conclusions

The results presented in this study indicate that HA can serve as an effective RM during the simultaneous biodegradation of phenol and CT. Further experiments revealed that the electron-accepting capacity of HA derived from different organic-rich environments was not associated with their reducing capacity to achieve CT dechlorination. The collected kinetic data suggest that the reduction of CT by reduced HA was the rate-limiting step during the simultaneous biodegradation of phenol and CT. To our knowledge, the present study constitutes the first demonstration of the simultaneous biodegradation of two priority pollutants mediated by HA.

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