

Natural Organic Matter as Reductant for Chlorinated Aliphatic Pollutants

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Humic acids (HA) are ubiquitous redox-active compounds of natural aquatic and soil systems. Here we studied the potential of HA as reductants for chlorinated aliphatic pollutants. To avoid artifacts potentially involved when studying chemically reduced HA, we prepared electrochemically reduced soil, aquatic and synthetic HA, and anthrahydroquinone-2,6-disulfonic acid (AHQDS), a model compound for hydroquinone moieties in HA. Both reduced HA and AHQDS reduced hexachloroethane (HCE) at appreciable rates. Some reduction of HCE by HA, however, occurred even before electrochemical reduction of the humic acids. This indicates that a small fraction of reduced moieties in HA persists at oxic conditions for some time. The initial reaction followed pseudo-first-order reaction kinetics, and tetrachloroethylene was the only halogenated product. The relatively small variations in carbon-normalized rate constants, k_{DOC} , found indicate that despite inherent variations in concentration, accessibility, and reactivity of redox-active groups in HA of various origins their overall dechlorination activity is fairly constant. However, HCE transformation rate constants and reducing capacities of different HA did not correlate. Rate constants normalized to both carbon content and reducing capacity of HA clearly indicate that reduced functional groups in different HA exhibit different reactivities. Our results together with the fact that reduced HA can be formed by a variety of microbiological and chemical processes suggest that HA could play a significant role as reductants in the reductive transformation of subsurface contaminants and that such a process could potentially be enhanced at contaminated sites by addition of reducible natural organic matter.

Introduction

Chlorinated organic compounds are produced in large quantities and are widely used as solvents and chemicals in various industries. As a result of their widespread use, improper handling, and disposal practices and due to accidental spills, toxic chloroorganic compounds can be found ubiquitously in the environment (for a review, see ref 1). In addition to these anthropogenic sources, certain chloroorganic compounds are produced naturally (2, 3).

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Microorganisms are known to dechlorinate both aliphatic and aromatic compounds (1) but also a number of naturally occurring chemical reductants including Fe(II) sulfides (4) and various forms of Fe(II) present at (5, 6) or within iron minerals, e.g., green rusts (7), clay minerals (8), and cements (9) are capable of transforming chlorinated compounds. Laboratory and field studies showed that in anoxic sediments, soils and aquifers both biotic and abiotic transformation of aliphatic chloroorganic compounds may occur simultaneously (10–12).

In the presence of such solid-phase bulk reductants soluble electron carriers could facilitate pollutant reduction by accepting electrons from the solid bulk reductant and transferring the electrons to the pollutant. Components of biological electron-transfer systems such as corrinoid systems (13, 14) and porphyrins and quinones (15–17) as well as humic substances (18–20) have been shown to stimulate pollutant transformation. Humic substances are redox-active organic compounds, which are ubiquitously present in soils and sediments, as well as in surface- and groundwater. Their major functional groups are carboxylic acids, phenolic and alcoholic hydroxyls, and ketone and quinone groups, the latter considered as the main redox active moieties of humic substances (18, 21).

In recent years evidence has been accumulated that humic acids (HA) and particularly their quinone moieties can play an important role as an electron shuttle to metal oxides (e.g., iron(III) oxides) in microbial redox processes and in the biodegradation of priority pollutants (for a review, see ref 22). However, it is not clear from the previous studies which factors were actually responsible for the stimulation of the reduction rates in the presence of HA. When using hydrogen sulfide as reducing agent for HA, reactive mercaptoquinones may be formed which can reduce the pollutants and supplement the redox activity of reduced quinone moieties (23, 24). In experiments where Fe(II), Ti(III), or Pd/H₂ is used for the reduction of HA, either complexed metal ions or traces of metals can remain in the HA and may be involved in the subsequent transformation of the pollutants.

The objectives of our study were thus to determine (i) whether soil, aquatic, and synthetic HA can be reduced electrochemically, (ii) whether reduced HA in *native* form can transform pollutants, and (iii) the redox capacity of humic acids before and after reduction.

To test reduced native humic acids for their potential of reductively transforming chlorinated pollutants and to avoid artifacts caused by reactive secondary species such as mercaptoquinones formed during the reduction process, we reduced different humic acids electrochemically. Aquatic, soil, and synthetic HA as well as anthraquinone-2,6-disulfonic acid (AQDS), a model compound for quinone moieties in natural organic matter, were reduced in aqueous solution in a specially designed electrochemical cell. The reactivity of the reduced native HA was investigated using hexachloroethane (HCE) as probe compound. The highly reactive HCE enabled us to investigate the transformation reactions within a reasonable period of time and to compare our results to previous studies where HA were reduced by other reductants.

Experimental Section

Chemicals. Potassium hexacyanoferrate(III), sodium dihydrogenphosphate monohydrate (>99%), methanol (HPLC grade), and anthraquinone-2,6-disulfonic acid sodium salt (95%) were purchased from Fluka AG (Buchs, Switzerland). Hexachloroethane (>99%), tetrachloroethylene (>99%), and

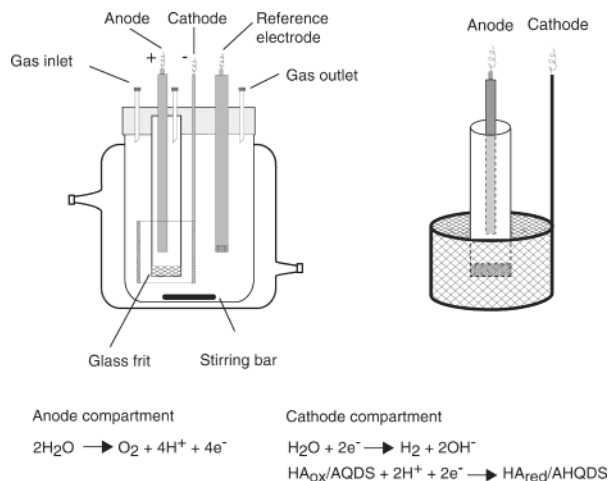


FIGURE 1. Scheme of the electrochemical cell used for the reduction of AQDS and humic acids.

bromoform (>99%) were purchased from Aldrich Chemie (Switzerland).

Three different types of humic acids (soil, aquatic, and synthetic HA) were used for the experiments. Soil humic acids were extracted from a Ferralsol soil using the classical, solubility-based fractionation (25), purified according to the standard isolation protocol from the International Humic Substance Society (IHSS) (26), except for the last dialysis step, and freeze-dried. Characterization of the soil and the humic acids and the detailed isolation procedure was described previously (27). Aquatic humic acids (Suwannee River) were purchased from the IHSS. Synthetic humic acids were synthesized by iron-catalyzed polymerization of a mixture of different phenols. To a mixture of pyrogallol, phloroglucinol, and hydroquinone (333 mg each) in 10 mL of NaOH (0.01 M) was added 50 mg of poorly crystallized Fe(III) hydroxide, prepared as described by Lovley and Phillips (28). The suspension was stirred thoroughly under air, and the pH was controlled regularly and kept at pH 12 with NaOH (10 M). After 150 h, the suspension was centrifuged (13 000 g, 30 min) to remove Fe(III) hydroxide and alkali-insoluble organic precipitates (humins). The supernatant was acidified to pH 1 with 6 N HCl and kept at 4 °C for 24 h to allow precipitation of the humic acids. After centrifugation (13 000 g, 30 min), the precipitated humic acids were resuspended in 15 mL of water, acidified to pH 1 with 6 N HCl, and centrifuged again. The humic acids were freeze-dried and stored at 4 °C.

Solutions. A stock solution of 200 μM anthraquinone-2,6-disulfonic acid (AQDS) was prepared in phosphate buffer (100 mM, pH 7), degassed, and flushed with nitrogen. Solutions of humic acids were prepared by adding 10 mg of humic acids to 100 mL of phosphate buffer (100 mM, pH 7). After stirring for 24 h, the solutions were filtered (cellulose) to minimize the possibility of microbial degradation of the chlorinated compounds in the kinetic experiments, degassed, and flushed with nitrogen. The carbon content in the humic acid solutions was determined by catalytic combustion (TOC Analyzer model High TOC II, Elementar, Hanau, Germany).

A methanolic spiking solution of hexachloroethane ($\sim 90 \mu\text{M}$) was prepared in nitrogen-sparged methanol.

Electrochemical Cell. The electrochemical cell consisted of a glass vessel closed with a Teflon cover including the cathode (platinated titanium grid), the anode (platinated titanium bar), the reference electrode (Ag/AgCl), and a gas inlet and outlet for flushing the headspace with nitrogen (Figure 1). The cathode compartment (about 100 mL volume), which could be stirred with a magnetic stirrer bar, was separated from the anode compartment by a glass frit. At the

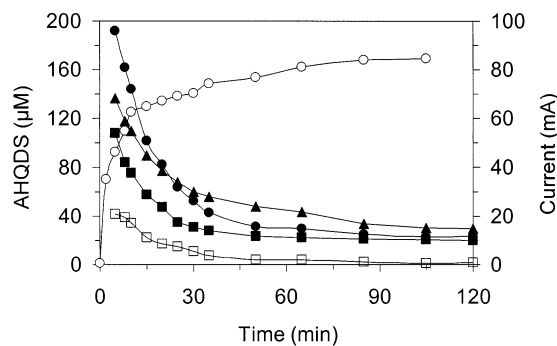


FIGURE 2. Electrochemical reduction of anthraquinone-2,6-disulfonic acid (AQDS, 200 μM) and aquatic humic acids (HA) (0.1 mg/mL). Plotted are the concentration of the reduced form of AQDS, i.e., anthrahydroquinone (AHQDS, O), the current during the reduction of AQDS (\bullet) and during the reduction of aquatic HA (\blacktriangle), the background current in a phosphate buffer without AQDS or HA (\blacksquare), and the residual current for the AQDS sample (\square , current of AQDS sample minus current of phosphate buffer control). The current curves obtained for the reduction of soil HA and synthetic HA looked similar to the curves obtained with aquatic HA (graphs not shown).

anode water was oxidized releasing molecular oxygen, electrons, and protons. The anode compartment was bubbled with N_2 , thus preventing the produced oxygen to diffuse through the glass frit into the cathode compartment. The released electrons traversed the external circuit to the cathode where they engaged in the reduction of AQDS and humic acids, respectively, and, to a smaller extent, of protons producing hydrogen which probably contributes also to the reduction of the AQDS and the HA.

Although we used a 100 mM phosphate buffer system, the pH of the solutions increased by almost one pH unit after the dissolution of the humic acids and the AQDS and the subsequent reduction (Table 2). This pH increase is probably due to the production of hydroxyl ions at the cathode, but obviously other unknown processes must be involved because a pH increase is also observed in the nonreduced controls after dissolution of the HA.

Prior to the use for the kinetic experiments, the solutions of the electrochemically reduced HA and of AHQDS were nitrogen-purged to remove traces of hydrogen, which are formed during the reduction process at the cathode, thus avoiding artifacts in the kinetic experiments caused by the hydrogen.

The reduction potential at the electrodes during the reduction was maintained constant using a potentiostat (model LB81 M, Bank IC, Clausthal-Zellerfeld, Germany). The voltage between the reference electrode and the cathode was maintained at a set point reference voltage of -0.75 V , and the voltage between the anode and the cathode was adjusted to maintain the set point reference potential. The reference electrode tip was positioned by a Haber-Luggin-capillary at a few mm from the cathode to prevent voltage drops that might result from solution resistance.

Experimental Procedures. The kinetic experiments with AHQDS and reduced humic acids, respectively, were conducted in 14-mL glass flasks sealed with a Viton-stopper (Maagtechnik, Duebendorf, Switzerland) and an aluminum crimp cap. Ten milliliters of the humic acid solution (for concentrations of dissolved organic carbon see Table 2) or AHQDS solution (about 170 μM ; see Figure 2) was injected into a nitrogen-purged serum flask followed by the release of the overpressure from the flask into the syringe. The experiment was started by adding 100 μL of a methanolic stock solution of hexachloroethane ($\sim 100 \mu\text{M}$) with a gastight Hamilton syringe through the Viton-stopper resulting in a hexachloroethane concentration of about 1.0 μM in all

TABLE 1. Reducing Capacity^a of Different Humic Acids (HA) before and after Electrochemical Reduction (Means \pm Standard Deviation)

sample	reducing capacity (mequiv e ⁻ /g C in HA)			relative diff (%) ^b
	before reduction	after reduction	difference	
soil HA	1.55 \pm 0.04	2.46 \pm 0.24	0.91	37
aquatic HA ^c	3.48 \pm 0.21	9.11 \pm 0.62	5.63	62
synthetic HA	21.78 \pm 1.01	31.44 \pm 1.51	9.65	31
Aldrich HA ^d	1.62 \pm 0.38	2.13 \pm 0.23	0.51	24

^a Amount of electrons transferred to Fe(III) in potassium ferricyanide K₃[Fe(CN)₆] ($E_h = +430$ mV). ^b Amount of electrons taken up by the humic acids during the reduction relative to the reducing capacity of the humic acids after the reduction. ^c Suwannee River HA from the International Humic Substance Society (IHSS). ^d Values calculated from refs 30 and 34.

experiments. The flasks were stored on a horizontal shaker (80 rpm) at 25 °C in the dark.

At appropriate time intervals, 250 μ L samples were withdrawn with a gastight syringe after injection of an equal volume of nitrogen gas. The sample was added to a vial for GC-analysis already containing 250 μ L of hexane. For extraction, the vial was closed immediately and shaken on a vortex mixer for 30 s.

The volume of the headspace in the serum flasks (4 mL at the beginning of the experiment) increased with each sampling step allowing more of the volatile chlorinated compounds to partition into the headspace. Aqueous concentrations were corrected for the amount of compound that partitioned into the headspace according to ref 23.

Besides the transformation by reductants, adsorption to and diffusion through the Viton-stoppers were also responsible for reduction of the concentrations of the halogenated compounds in the flask. Control flasks set up with buffer and halogenated compounds (1 μ M) without reductant revealed losses of about 20% of the initial PCE and HCE within 200 h. The measured concentrations in the kinetic experiments with reductant were corrected by this factor assuming a constant loss over the time. Due to the low hydrolysis rate of HCE in H₂O (Table 2) this process should not contribute significantly to the disappearance of HCE in the flasks.

At the beginning of the kinetic experiments with HCE, in the reaction vials a molar ratio of electron donor (HA or AHQDS) to acceptor (HCE) of about 340 or greater was present. After 50–100 h, when several samples had been withdrawn of the reaction vial, only 10–30% of the initial redox equivalents were present indicating that more redox equivalents were consumed than were transferred to HCE. Also, the k_{DOC} values decreased and deviated from “first-order” rate constants during the course of the reaction

(Figure 4). Therefore k_{DOC} values were determined for the first 50–100 h. We assume that traces of oxygen were introduced into the reaction vial by diffusion through the Viton stoppers with time and by the sampling procedure and therefore part of the reduced HA did not react with HCE rather than with the oxygen. This assumption was supported by control experiments with reduced humic acids but without hexachloroethane which were stored and sampled with the normal time frequency in an O₂-free glovebox. These controls showed almost complete recovery of the introduced reducing equivalents.

Analytical Procedures. The hexane extracts were analyzed by GC on a Carlo Erba HRGC 5160 equipped with an autosampler (Carlo Erba A200S) and an electron capture detector (Carlo Erba ECD 400 with a Ni-63 source). The injector and detector temperatures were 250 °C and 300 °C, respectively. A 30 m DB-624 column (J&W Scientific, Folsom, U.S.A.) was used (i.d. 0.32 μ m, 1.8 μ m film thickness) with the following temperature program: 60 °C (1 min), 10 °C/min to 120 °C (0 min), 10 °C/min to 175 °C (0 min), 35 °C/min to 250 °C (5 min). Hydrogen gas and a mixture of argon (90%) and methane (10%) were used as carrier and makeup gas, respectively. The hexane extracts contained bromoform (1.5 μ M) as internal standard. The hexane extracts (1.5 μ L) were injected splitless. Concentrations were calculated by comparison with a linear calibration curve and normalized to the internal standard.

AHQDS was quantified by UV absorption (450 nm). The redox state of the humic acids prior and after electrochemical reduction was determined by measuring their reducing capacity, i.e., the amount of electrons which were transferred to K₃[Fe(CN)₆] by the humic acids (29) as described by Benz et al. (30).

Results and Discussion

Electrochemical Reduction and Reducing Capacities. Humic acids (HA) and anthraquinone-2,6-disulfonic acid (AQDS) were reduced in aqueous solution at a platinated titanium cathode. The reduction of AQDS to the corresponding hydroquinone (AHQDS) was observable visually by the color change from the oxidized to the reduced form. Quantification with UV absorption spectroscopy showed that within 120 min more than 170 μ M of AQDS was reduced to AHQDS (Figure 2). Parallel to the formation of AHQDS the current decreased from initial values > 100 mA to steady-state values of about 10 mA at the end of the reduction.

Controls containing phosphate buffer but no AQDS gave plots of similar shape but started at lower currents and showed lower currents over the complete time of the reduction. This current was probably caused by reduction of traces of oxygen present in the solutions but also by background electrolysis of water. The plot for the residual

TABLE 2. Carbon-Normalized Second-Order Rate Constants (k_{DOC}) for the Transformation of Hexachloroethane (HCE) to Perchloroethylene (PER) by Different Electrochemically Reduced HA (at 25 °C) and by AHQDS,^a the Reduced Form of AQDS^{a,g}

system	pH	DOC (mg/L)	k_{DOC} (d ⁻¹ (mg C/L) ⁻¹)	k_{DOC}^c (d ⁻¹ (μ equiv e ⁻ /L) ⁻¹) ^b	recovery ^c (%)
soil HA	7.8	47.0	0.00296 \pm 0.00100	0.00120	86.3 \pm 4.2
aquatic HA ^d	8.0	49.2	0.00147 \pm 0.00063	0.00016	80.8 \pm 5.0
aquatic HA (control) ^e	7.8	49.2	0.00028 \pm 0.00010	0.00008	81.7 \pm 8.4
synthetic HA	7.9	13.3	0.01140 \pm 0.00280	0.00036	83.7 \pm 4.1
synthetic HA (control) ^e	7.9	13.3	0.00997 \pm 0.00209	0.00046	84.5 \pm 2.3
AHQDS	8.2	33.6	0.01458 \pm 0.00155		90.0 \pm 4.0
H ₂ O ^f	7.0		1.0 \times 10 ⁻¹⁰		

^a Anthra(hydro)quinone-2,6-disulfonic acid. ^b Rate constants normalized to the DOC and to the measured reducing capacities of the individual humic acid solutions (shown in Table 1). ^c Average and standard deviation (HCE + PER) relative to the amount of HCE added in %. ^d Suwannee River HA from the International Humic Substance Society (IHSS). ^e HA dissolved in phosphate buffer (pH 7) under oxygen exclusion (glovebox) without electrochemical reduction. ^f k_{obs} (HCE in H₂O) (d⁻¹) at 25 °C (35). ^g Values are the pseudo-first-order rate constants k_{obs} (not shown) divided by the dissolved organic carbon (DOC) concentration.

current (current obtained for the AQDS reduction minus current of phosphate buffer control, Figure 2) clearly showed the expected decrease of the current with time due to the cumulative reduction of the AQDS.

For the reduction of the three different HA (soil, aquatic, and synthetic) the decrease of the current with time was similar with slightly smaller values compared to the reduction of AQDS (Figure 2). As the redox state of HA cannot be quantified using UV absorption, the reducing capacity of HA was determined by a redox titration using potassium ferricyanide ($E_h = +430$ mV). The reducing capacities of all investigated HA were substantially higher after electrochemical reduction compared to the nonreduced HA (Table 1). However, HA that were not electrochemically reduced also contained a significant amount of reduced moieties, which reacted with ferricyanide (and with HCE; see later). The reducing capacities obtained for the nonreduced HA in this study were in the same range as values obtained by iodine titration for different nonreduced HA from the International Humic Substance Society (IHSS) (31).

Significant reducing capacities of nontreated HA were also reported by Benz et al. (30) for sediment and commercial HA (values for commercial HA are included for comparison in Table 1). Apparently, a certain fraction of reduced functional groups persists in reduced form even after extraction and cleanup of natural HA under oxic conditions. The persistence of reductive capability in dissolved natural organic matter (NOM) in the presence of oxygen and a suggestion of slow reactions NOM moieties with molecular oxygen was already mentioned by Macalady and Ranville (32). Micelle-like aggregates of HA (33) that are unfolded only under certain conditions in solution rendering accessible some functional groups may be responsible for the protection of the reduced functional groups from oxidation by atmospheric oxygen.

By the electrochemical reduction method applied in this study 0.91–9.65 mequiv electrons were taken up per g C in the HA corresponding to 31–62% of the total reducing capacity of the reduced HA (Table 1). The synthetic HA took up more electrons than the natural HA. The aquatic HA showed the highest relative electron uptake among all humic acids samples investigated in this study. These results reflect differences in the content of redox active groups or differences in the reactivity of reduced redox-active groups in the titration with ferricyanide. The synthetic HA, which were polymerized out of a mixture of phenols, contain more redox active groups per g carbon than natural aquatic and soil HA, which typically also contain constituents of proteins and carbohydrates (24).

The reducing capacities of electrochemically reduced HA measured in our study (Table 1) were in the same range (2.46 mequiv e^- per g C for soil HA) or higher (9.11 and 31.44 mequiv e^- per g C for aquatic and synthetic HA, respectively) than values reported for "Aldrich" HA reduced chemically by Pd/H₂ (30). The authors measured a reducing capacity of 2.13 mequiv electrons per g C for Aldrich HA (calculated with a carbon content of 39%; see ref 34). This lower value may indicate either that Aldrich HA contains less redox-active moieties than aquatic and synthetic HA or the redox moieties in Aldrich HA have a lower reactivity with ferricyanide. Another explanation could be that the electrochemical reduction used in our study is more effective or less specific to redox-active functional groups than the Pd/H₂ reduction used for the Aldrich HA. In particular the values measured for the reducing capacity of the reduced synthetic HA (0.37 mmol electrons per mmol of carbons) were remarkably high. They were in the range of values calculated for a system of one quinone group per six carbon atoms (0.33 mmol electrons per mmol of carbons). This result indicates both the high content of hydroquinone groups in the reduced synthetic

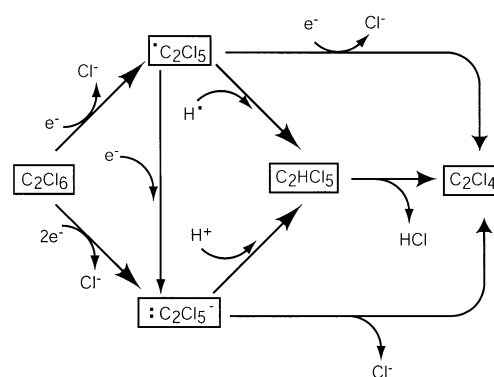


FIGURE 3. Potential pathways for the reductive transformation of hexachloroethane C_2Cl_6 to tetrachloroethylene C_2Cl_4 (adapted from ref 19).

HA and the effectivity of the electrochemical reduction method.

Our results show that both the quinone model compound AQDS and natural HA can be reduced electrochemically. This reduction method prevents potential side reactions with reducing agents. Thus the formation of reactive adducts such as mercaptoquinones and the presence of remaining metal ions or metals in the reduced humic acids can be avoided and the reduction leads to reduced HA in native form. Therefore, electrochemically reduced HA can be used for the investigation of the reactivity of reduced functional groups in HA. However, one must be aware that in nature not only pure HA but also combinations of HA with various reductants (e.g., Fe(II), ...) are involved in redox reactions.

Transformation of HCE. Both reduced humic acids and AHQDS reduced HCE to tetrachloroethylene (PER, C_2Cl_4) as a single product. C_2HCl_5 , which can be formed from HCE (C_2Cl_6) after a one- or two-electron transfer and subsequent H-radical abstraction or protonation (Figure 3), respectively, followed by a fast elimination reaction in aqueous solution leading to PER, was not detected. Figure 4 shows typical plots of the concentration of HCE and PER in the presence of soil HA and AHQDS, respectively. Similar results as for the soil HA were obtained with the aquatic and synthetic HA (data not shown).

It is known that redox reactions of nitroaromatic and chlorinated pollutants with phenolic compounds show strong pH dependence. This was shown by other authors for the reaction of NOM with nitroaromatic compounds in the presence of hydrogen sulfide (18) and for the reaction of AHQDS with HCE (19). Dunnivant et al. (18) showed that the carbon normalized rate constants for the transformation of nitrobenzene by NOM and 5 mM H₂S increased from $5.9 \times 10^{-6} h^{-1}$ (mg of C/L)⁻¹ at pH 5.5 to 2.4×10^{-5} (pH 6.5), 6.4×10^{-5} (pH 7.5), and finally to $1.3 \times 10^{-4} h^{-1}$ (mg of C/L)⁻¹ at pH 8.6, corresponding to an about 20-fold increase within 2 pH units. With increasing pH values phenolic hydroxyl groups dissociate and phenolate ions rather than hydroxyl groups are the reactive groups thus increasing the reaction rates. Here we carried out all kinetic experiments in the same pH range.

In all kinetic experiments with HCE, the reaction was pseudo-first-order with respect to HCE, at least during the first 50–100 h of the experiment (Figure 4c). Pseudo-first-order rate constants, k_{obs} , were determined according to

$$[HCE]_t = [HCE]_0 e^{-k_{obs}t} \quad (1)$$

and

$$\ln[HCE]_t = \ln[HCE]_0 - k_{obs}t \quad (2)$$

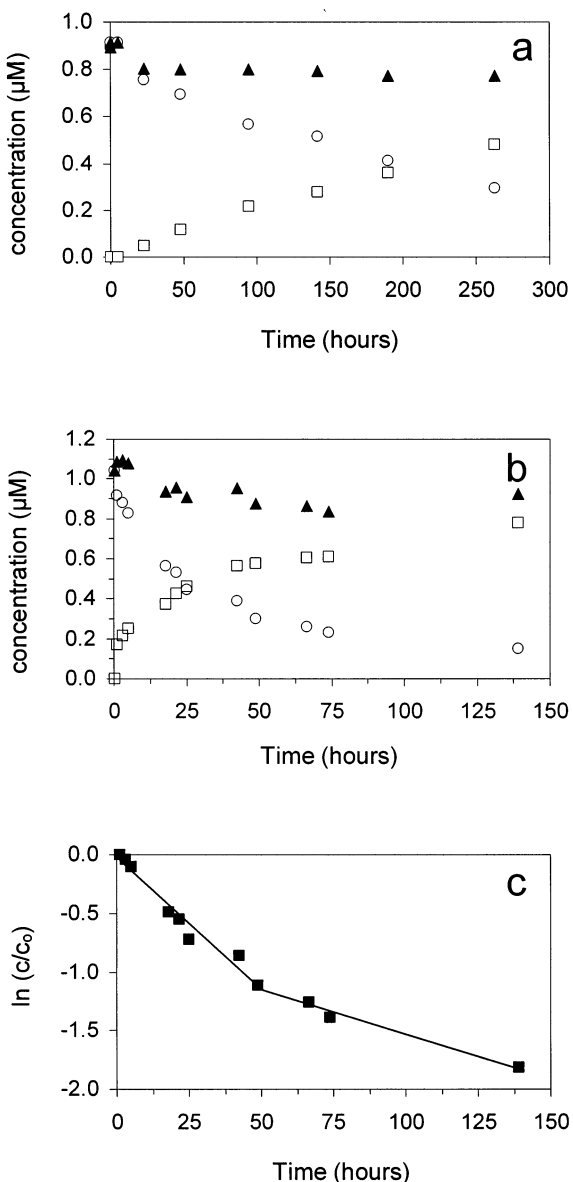


FIGURE 4. Examples for the transformation of HCE in aqueous solution in the presence of (a) 0.1 g/L electrochemically reduced soil humic acids at pH 7.8 and 25 °C and (b) about 170 μM AHQDS at pH 8.2 and 25 °C. Plotted are the concentration of hexachloroethane (○) and tetrachloroethylene (□) and the mass balance (▲). (c) The semilogarithmic plot of relative concentration (c_0 HCE = 0.92 μM) versus time in the presence of reduced soil humic acids shows how the pseudo-first-order kinetics deviates after around 50 h.

where $[\text{HCE}]_t$ and $[\text{HCE}]_0$ are the concentrations of HCE at time t and zero, respectively. Due to the different solubilities of different HA and due to their different content of impurities (i.e., inorganic components) the dissolved organic carbon (DOC) concentration varied in the experiments with different HA. Dunnivant et al. (18) showed that the rate constants for the transformations of nitroaromatic compounds by natural organic matter (with hydrogen sulfide as bulk reductant) were a linear function of the total organic carbon content in the solutions. Therefore in our study carbon-normalized rate constants

$$k_{\text{DOC}} = k_{\text{obs}}/[\text{DOC}] \quad (3)$$

were determined for the HCE transformation (Table 2).

AHQDS showed the highest HCE transformation rate (Table 2). Among the investigated HA, the highest k_{DOC} values

were obtained for the synthetic HA ($0.01140 \pm 0.00280 \text{ d}^{-1}$ (mg C/L^{-1})). This is consistent with the observation that the synthetic HA have the highest overall reducing capacity (Table 1) (assuming a similar reactivity of the redox active groups with ferricyanide). The soil HA showed a higher k_{DOC} ($0.00296 \pm 0.00100 \text{ d}^{-1}$ (mg C/L^{-1})) than the aquatic HA ($0.00147 \pm 0.00063 \text{ d}^{-1}$ (mg C/L^{-1})). The fact that the aquatic HA had a higher reducing capacity (Table 1) but a lower HCE transformation rate than the soil HA shows that reduced functional groups in different classes of HA exhibit different reactivities. This important result is demonstrated by calculating rate constants normalized to the reducing capacities of the humic acid solutions (Table 2).

The different reactivities for soil and aquatic HA observed in our experiments could indicate that the hydroquinone groups in the soil HA have a higher reactivity toward HCE than the hydroquinone groups in the aquatic HA. Furthermore, different $\text{p}K_a$ values of the hydroxyl groups depending on their chemical environment in the different investigated humic acids could lead partly to deprotonated and thus more reactive hydroxyl groups, i.e., phenolate ions. Other explanations for the different reactivity could be that there are differences in the accessibility of the redox-active groups or that in the soil HA other redox-active constituents such as humic-metal-complexes are more reactive than the redox-active groups in the aquatic HA. Natural humic acids contain complexed metal ions which are strongly bound to the humic acids and which are not completely removable even with strong acids (see ref 30 and discussion in ref 31). Although it was shown that these metal ions can participate in chemical and microbiological redox reactions of humic acids, the exact mechanisms and extent of their contribution is rather unclear.

HCE transformation rates were also determined with nonreduced HA. To this end, the aquatic and synthetic HA were tested for reactivity with HCE without previous electrochemical reduction. The measured rate for the nonreduced aquatic HA was significantly lower than the rate obtained with electrochemically reduced aquatic HA (Table 2), whereas the rate for the nonreduced synthetic HA was only slightly lower than the rate for the reduced counterparts. In both cases the rates were significantly higher than the rates measured for HCE transformation in water without addition of a reductant ($1.0 \times 10^{-10} \text{ d}^{-1}$ at pH 7 and 25 °C; see ref 35). This is consistent with the fact that the reducing capacities of the soil, aquatic, and synthetic HA showed the presence of significant amounts of reduced functional groups already without previous electrochemical reduction (Table 1). The HCE transformation rates obtained with these nonreduced HA indicated that the redox equivalents present in the nonreduced HA reacted not only with ferricyanide during the determination of the reducing capacity but also with HCE in the kinetic experiments.

Environmental Significance

In this study, we successfully demonstrated the electrochemical cathodic reduction of humic acids. The presented results may have important implications for the understanding of processes occurring during the application of electroremediation techniques where metals and organic contaminants are to be removed from polluted sites. When currents are applied in soils, charged and noncharged particles (e.g., metal ions or aromatic hydrocarbons) are transported by electromigration and electroosmosis (for overviews see refs 36–38). However, redox reactions of humic substances at the electrodes and their influence on the pollutant behavior have not yet been investigated. As a consequence of our study we suggest that during electroremediation, reduced humic acids are formed at the cathode. These reduced humic acids can contribute to the transformation of pollutants (e.g., dechlorination of chlorinated

pollutants or reduction and mobilization of metal ions). This means that besides a direct reductive transformation of pollutants in electromediation (39, 40) an indirect electron transfer via humic acids is possible.

Furthermore, the results of our kinetic experiments demonstrate that reduced humic acids can transform HCE, a probe compound for chloroorganic pollutants. In natural environments, reduced humic acids are a product of a variety of microbiological processes, e.g. iron(III)-reduction, sulfate-reduction, halorespiration, methanogenesis, and fermentations (30, 41, 42). Chemical reaction with reductants such as hydrogen sulfide or metal sulfides can also produce reduced humic acids (24). The multiplicity and significance of these reduction processes further suggest that humic acids have a high potential to contribute to pollutant reduction at contaminated sites. The findings that in reduced humic acids even after exposure to oxic environment a significant reducing capacity for HCE persists suggests that reduction by HA may also occur in natural environments where oxic and anoxic microzones change within short distances. Our results may thus be useful for developing a remediation technology based on pumping quinone-rich natural organic matter fractions (e.g., tree bark extracts) into contaminated sites to stimulate pollutant transformation.

Acknowledgments

We gratefully acknowledge M. Elsner, Dr. T. Hofstetter, Prof. R. Schwarzenbach, and L. Zwank for critically reviewing the manuscript. This work was part of the European Union project "Protection of Groundwater Resources at Industrially Contaminated Sites (PURE)" and was supported by the Swiss Federal Office for Education and Science (grant no. BBW 99.0396).

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Received for review August 30, 2002. Revised manuscript received April 4, 2003. Accepted April 4, 2003.

ES0201808