Design of Quinonoid-Enriched Humic Materials with Enhanced Redox Properties

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The primary goal of this work was to develop quinonoid-
enriched humic materials with enhanced redox properties
that could be used as potentially effective redox mediators
and reducing agents for in situ remediation of soil and
aquatic environments. Two different strategies were
formulated and tested to derive these materials. The first
strategy called for the oxidation of phenolic fragments
associated with the humic aromatic core. In a second
strategy, polycondensation of these phenolic fragments was
carried out with hydroquinone and catechol. The oxidized
derivatives and copolymers obtained were characterized
using elemental and functional group analyses, and capillary
zone electrophoresis. The redox properties were evaluated
using ESR spectrometry and reducing capacity deter-
minations. The reducing capacities of copolymers ranged
between 1 and 4 mmol/g, which were much higher than
the parent material and the oxidized derivatives. Hence,
preference should be given to the copolycondensation
approach. The quinonoid-enriched humics are nontoxic,
water soluble, and resistant to biodegradation; thus, they
could be applied as soil amendments to reduce highly mobile
oxoanions of heavy metals and radionuclides, or as
redox mediators to enhance in situ bioremediation. Otherwise,
cross-linked copolymers could be created to serve as
inexpensive reductants in permeable reactive barriers
designed to remove highly oxidized contaminants from
polluted groundwaters.

Introduction

Humic substances (HS) are ubiquitous redox-active com-
pounds in the environment. In recent years, evidence has
been accumulated that HS and particularly their quinonoid
moieties can play an important role as electron shuttles in
microbial redox reactions involved in the biodegradation of
priority pollutants (for an overview, see refs *1–4*). In addition,

they are known to take part in abiotic reduction of metals
and organic contaminants (*5–8*). Operating as redox media-
tors and reductants, it is plausible for HS to function within
technologies designed to bring about in situ remediation of
soil and aquatic environments polluted with redox-sensitive
contaminants (*9–11*).

Humic-based redox agents possess such important ad-
vantages as low susceptibility to biodegradation, nontoxicity
to living organisms, and low production costs from inex-
pensive raw materials such as low-rank coals, peats, sapropels,
and composts. The primary factor limiting application of HS
in remediation technologies is the inherent variability in redox
properties observed among humic materials and their
fractions (*3, 12*).

In this work, we sought to control the redox properties
of humic materials, and we used chemical modification to
enrich the aromatic core with quinonoid moieties. Two
different strategies were formulated and tested for this
purpose. The first constituted an oxidation of the phenolic
fragments present in the aromatic core to quinones using an
appropriate oxidant (e.g., Fenton's reagent, Fremy's salt).
Copolycondensation was the second strategy in which natural
humic material and dihydroxybenzenes (hydroquinone and
catechol) served as counterpartners in a formaldehyde
condensation. Both reaction pathways are schematically
shown in Figure 1.

Coal-derived humics consisting mostly of aromatic core
(*13*) were considered the best candidates for this modification.
Among them, leonardite humic acids are the most widely
produced on industrial scale (*14*), and their use herein could
render results transferable to a range of existing laboratory
and pilot scale studies. Hence, leonardite humic acids were
selected as the parent material for these studies.

To the best of our knowledge, previous investigations
involving HS modifications focused on understanding the
structure of humics (*15*) or their bioavailability (*16*), and not
on enhancing or altering their reactive properties for the
purpose of augmenting their utility for environmental
remediation. The commercial production of modified HS is
limited to HUMASORB, a multipurpose contaminant ad-
sorbent obtained by the cross-linking of HS that reduces
their solubility (*17*), but does not enhance their redox activity.
It may be the case, however, that cross-linking quinonoid-
enriched humic materials will yield redox-active humic
adsorbents suitable for a use in redox reactive barriers. The
objective of this work was to develop and test chemical
modification strategies for preparing humic materials with
enhanced redox activity.

Materials and Methods

Humic acids of leonardite (CHP) used for further modifica-
tions were isolated from commercially available potassium
humate (Powhumus) produced by Humintech Ltd., Germany.
For this purpose, a known weight of potassium humate was
dissolved in distilled water and centrifuged to separate and
discard any insoluble mineral components. The supernatant
was then acidified to pH 2 with concentrated HCl and
centrifuged. The precipitate of humic acids (HA) was col-
lected, washed with distilled water, desalted using electrodi-
alysis, evaporated at 60 °C, and stored in a desiccator over
P_2O_5.

Reagents of analytical grade were used for the following
purposes: concentrated solutions of HCl and NaOH were
used for the isolation of CHP; KH_2PO_4 and $Na_2HPO_4 \cdot 12H_2O$
were used in the preparation of buffers; $NaNO_2$, Na_2SO_3, 25%
ammonia solution, $KMnO_4$, glacial acetic acid, KCl, methanol,

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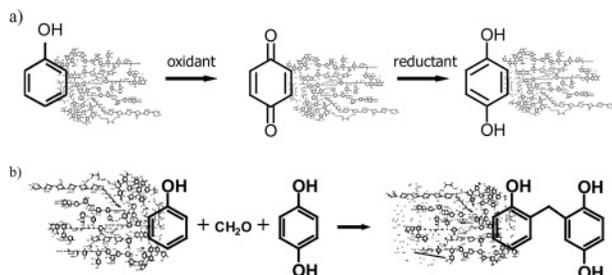
FIGURE 1. Schematic reaction pathways for synthesis of quinonoid-enriched humic materials: (a) subsequent oxidation and reduction of available phenolic fragments of humic backbone; (b) formaldehyde condensation between phenolic fragments of humic aromatic core and hydroquinone yielding humic copolymer with pendant hydroquinone units.

and acetone were used for the synthesis of Fremy's salt; $K_2S_2O_8$ was used for Elbs' oxidation; and $FeSO_4 \cdot 7H_2O$ and a 50.85% H_2O_2 solution were used in Fenton's oxidation. Hydroquinone, catechol, and a 35% solution of formaldehyde used for condensation were of reagent grade. Diethyl ether used for extraction was of analytical grade. All remaining reagents were of analytical grade and were used without further purification.

Oxidation protocols of humic materials with different oxidants and corresponding references are summarized in Table 1.

All oxidized products were precipitated by acidification of the reaction mixture with HCl until pH 1, washed with distilled water, desalted using dialysis, and rotor-evaporated at 60 °C.

Reduction of oxidized humic derivatives was conducted using sodium sulfite. Oxidized derivatives (1.5 g) were dissolved in 1 mL of a concentrated ammonia solution followed by dilution to 50 mL with distilled water. Sodium sulfite (2 g, 15 mmol) was added to the solution and stirred for 1 h at room temperature. The reduced products were isolated as described above for oxidized derivatives.

Formaldehyde polycondensation was conducted between CHP, hydroquinone, and catechol under conditions adopted from ref *23*. CHP (1 g) was dissolved in a small amount of concentrated sodium hydroxide; this solution was then diluted with distilled water, and its pH was adjusted to 7 to give a final volume of 50 mL. The amounts of hydroquinone and catechol were 100, 250, and 500 mg containing 2, 5, and 10 mmol of ArOH, respectively, per 1 g of CHP containing 5 mmol of acidic groups (ArOH+COOH) (see Table 2). The monomer was added in the presence of catalytic amounts of oxalic acid followed by 0.5, 0.8, and 1 g of a 35% solution of formaldehyde. The mixture was stirred for 1 h at 100 °C and rotor-evaporated at 60 °C. The product was washed with distilled water, dialyzed, evaporated at 60 °C, and stored in a desiccator over P_2O_5.

Copolymerization with *p*-benzoquinone was conducted following ref *24*. CHP (1 g) was dissolved in a small amount of concentrated KOH solution and diluted to 50 mL with 0.1 M potassium dihydrophosphate. The amounts of *p*-benzoquinone added per 1 g of CHP were 100, 250, and 500 mg. The reaction mixture was stirred for 1 h at 25 °C. The product was isolated as described above for the oxidized derivatives.

Extractive purification of humic copolymers was conducted using diethyl ether. For this purpose, a humic copolymer was dissolved either in water at slightly alkaline pH (8.5–9.0) for capillary-electrophoresis analyses or in phosphate buffer at pH 6.0 for redox-capacity determinations. The obtained solutions were extracted three times with diethyl ether at 1:3 (vol:vol) ratio. For electrophoretic measurements, the ether extract was evaporated under nitrogen, dissolved in slightly alkaline water solution at pH

8.5–9.0, and used for further measurements along with the extracted solution. The latter was used for redox capacity measurements as well.

Elemental analyses (C, H, N) were performed on a Carlo Erba Strumentazione elemental analyzer. Ash content was determined manually. Oxygen content was calculated as a difference.

Acidic group analyses were carried out as described elsewhere (*25*) using barium hydroxide and calcium acetate techniques for determination of total and carboxylic acidity, respectively. The content of phenolic groups was calculated as a difference between the total and carboxylic acidity.

Capillary zone electrophoresis (CE) separations were conducted as described in refs *26, 27* using a Beckman Coulter PA/CE 5510 system (Beckman Instruments, Fullerton, CA), equipped with an on-column diode array detection, an autosampler, and a power supply capable of delivering up to 30 kV. The separations were performed using a noncoated, open fused-silica capillary from Polymicro Technologies (Phoenix, AZ) (50 μm ID, 57 cm total length, 50 cm to the diode array detector). The separation buffer was 20 mM sodium carbonate (pH 11.4). Sample injections (20 nL) were achieved using pressure mode for 20 s at 0.5 psi. The separation was carried out by applying 25 kV to the anode. Concentration of humic materials in the samples was 1 g/L.

ESR spectra were measured using laboratory made spectrometer operating at 100 kHz field frequency. Spin quantity was determined by double spectra integration using IBM-compatible PC. Sugar coal with a spin concentration of 0.92×10^{16} spin/g was used as a standard.

Reducing capacities of humic materials were determined using ferricyanide as oxidizing agent as described in ref *28*. 0.07 M phosphate buffer at pH 6 was nitrogen-purged for 30 min and used for preparing all solutions. Concentrations of stock solutions of $K_3Fe(CN)_6$ and humics were 0.5 mM and 100 mg/L, respectively. The optical densities of control solutions containing HS and $K_3Fe(CN)_6$ and of reference solutions containing only HS and only $K_3Fe(CN)_6$ were measured in the maximum of ferricyanide absorbance, at 420 nm using 662 photometer (Metrohm). The obtained values were used to calculate the reducing capacity of HS as follows:

$$\text{reducing capacity (mmol/g)} = \frac{\Delta C(K_3Fe(CN)_6)}{C_{HS}}$$

where $\Delta C(K_3Fe(CN)_6)$ represents the decrease in concentration of $K_3Fe(CN)_6$, mmol/L; and C_{HS} is the mass concentration of HS in the reference solution, g/L.

Results and Discussion

Synthesis and Structural Studies of the Quinonoid-Enriched Humic Materials. Leonardite humic acids (CHP) were used for all modifications. Five oxidants differing in mechanisms of reaction with phenols were used to oxidize the aromatic core of CHP. The oxidized derivatives obtained were further reduced using a mild reductant sodium sulfite. A list of the obtained derivatives is shown in Table 2.

To synthesize quinonoid-enriched humic materials based on polycondensation reactions, the hydroquinoic and catecholic fragments were incorporated into the structure of humics using a formaldehyde polycondensation technique. In addition, radical copolymerization with *p*-benzoquinone was used to obtain hydroquinone-enriched derivatives. The reactions were run at three different monomer-to-humics ratios: 100, 250, and 500 mg of monomer per g of humic material. For each reaction, the corresponding homopolymer was obtained, without addition of humic material as described in refs *23, 24*. Nine humic copolymers and three homopolymers were obtained (Table 2).

TABLE 1. Protocols of Oxidation Procedures of Leonardite Humic Material — CHP

oxidation reaction	humic material	oxidant	reaction conditions
Fremy's salt $(KSO_3)_2NO$ (18)	CHP (3 g), 1 mL of concentrated NH_3, 50 mL of dist. water, pH adjusted to 7	Fremy's salt (10 g), 100 mL of 0.3 M KH_2PO_4	oxidant was added with continuous stirring and stirred for 1 h at $T = 20\ °C$
chromium trioxide (CrO_3) (19)	CHP (3 g) sonicated in 50 mL of glacial acetic acid, and cooled to 0 °C	CrO_3 (3.6 g), 10 mL of glacial acetic acid, and cooled to 0 °C	oxidant was added with continuous stirring at 0 °C in ice bath, and stirred for 1 h
potassium ferricyanide (20)	CHP (3 g), 1 g of solid KOH, and 150 mL of dist. water	solid potassium ferricyanide 7.4 g	solid oxidant was added with continuous stirring, and stirred for 2 h
Elbs' reagent (21)	CHP (3 g), 50 mL of 10% NaOH, and 50 mL of dist. water	a saturated aqueous solution of potassium persulfate (4 g)	oxidant was added with continuous stirring, and stirred for 1 h at $T < 20\ °C$
Fenton's reagent (22)	CHP (3 g), 1 mL of concentrated NH_3, and 100 mL of dist. water	$FeSO_4·7H_2O$ (0.028 g) dist. water, ascorbic acid	oxidant was added, 2 g of a 50.85% H_2O_2 solution, stirred for 1 h at $T = 25\ °C$

TABLE 2. Atomic Ratios and Content of Acidic Groups in the Modified Humic Materials

| sample	description	atomic ratios		acidic groups, mmol/g		
		H/C	O/C	TA[a]	COOH	ArOH[b]
CHP	leonardite humic acids	0.85 ± 0.02^c	0.38 ± 0.01	5.3 ± 1.0	4.2 ± 0.2	1.1 ± 0.2
Hydroxylated Derivatives of CHP						
OFr	Fremy's-oxidized CHP	0.81 ± 0.01	0.42 ± 0.01	5.8 ± 0.8	4.5 ± 0.1	1.3 ± 0.2
RFr	Na_2SO_3-reduced OFr	0.81 ± 0.01	0.42 ± 0.01	5.8 ± 0.8	4.8 ± 0.1	1.0 ± 0.24
OEl	Elbs's-oxidized CHP	0.85 ± 0.03	0.38 ± 0.01	6.0 ± 0.2	5.2 ± 0.1	0.8 ± 0.04
REl	Na_2SO_3-reduced OEl	0.89 ± 0.03	0.38 ± 0.01	6.3 ± 0.2	5.0 ± 0.1	1.3 ± 0.04
OFe	Fenton's-oxidized CHP	0.92 ± 0.02	0.45 ± 0.02	6.9 ± 2.7	4.2 ± 0.1	2.7 ± 1.1
RFe	Na_2SO_3-reduced OFe	0.85 ± 0.01	0.38 ± 0.02	6.3 ± 0.9	4.4 ± 0.1	1.9 ± 0.3
OHx	$K_3Fe(CN)_6$-oxidized CHP	0.84 ± 0.04	0.38 ± 0.02	7.2 ± 0.2	4.1 ± 0.1	3.0 ± 0.1
RHx	Na_2SO_3-reduced OHx	0.86 ± 0.03	0.36 ± 0.02	6.7 ± 2.3	4.6 ± 0.1	2.1 ± 0.8
OCr	CrO_3-oxidized CHP	1.14 ± 0.05	0.63 ± 0.04	8.5 ± 0.2	2.7 ± 0.5	5.8 ± 1.3
RCr	Na_2SO_3-reduced OCr	1.02 ± 0.05	0.51 ± 0.03	5.5 ± 0.8	1.7 ± 0.5	3.9 ± 1.6
Copolycondensated/Copolymerized Materials						
HQHQ	hydroquinone homopolymer	0.94 ± 0.01	0.38 ± 0.01	6.2 ± 2.1	0.2 ± 0.1	6.0 ± 3.3
HQ100	HQ:CHP ratio of 100:1000 mg	1.18 ± 0.03	0.26 ± 0.01	8.2 ± 2.9	4.3 ± 0.3	4.0 ± 1.7
HQ250	HQ:CHP ratio of 250:1000 mg	1.15 ± 0.02	0.34 ± 0.01	8.0 ± 1.5	3.6 ± 0.1	4.4 ± 1.0
HQ500	HQ:CHP ratio of 500:1000 mg	1.20 ± 0.02	0.38 ± 0.02	7.3 ± 0.5	3.1 ± 0.2	4.3 ± 0.6
CTCT	catechol homopolymer	0.84 ± 0.02	0.30 ± 0.02	9.8 ± 2.0	1.0 ± 0.2	8.8 ± 3.5
CT100	CT:CHP ratio of 100:1000 mg	0.76 ± 0.01	0.43 ± 0.01	7.5 ± 1.1	4.0 ± 0.1	3.5 ± 0.6
CT250	CT:CHP ratio of 250:1000 mg	0.80 ± 0.01	0.40 ± 0.02	7.8 ± 0.2	3.7 ± 0.1	4.1 ± 0.2
CT500	CT:CHP ratio of 500:1000 mg	0.81 ± 0.02	0.41 ± 0.03	8.0 ± 1.3	3.6 ± 0.8	4.6 ± 1.8
BQBQ	p-benzoquinone homopolymer	0.87 ± 0.02	0.46 ± 0.02	7.9 ± 0.4	2.8 ± 0.2	5.1 ± 0.6
BQ100	BQ:CHP ratio of 100:1000 mg	1.05 ± 0.02	0.37 ± 0.01	7.3 ± 2.1	5.3 ± 0.3	2.0 ± 0.7
BQ250	BQ:CHP ratio of 250:1000 mg	0.71 ± 0.2	0.37 ± 0.03	8.5 ± 0.3	5.4 ± 0.1	3.1 ± 0.15
BQ500	BQ:CHP ratio of 500:1000 mg	1.01 ± 0.02	0.34 ± 0.01	8.0 ± 1.1	3.9 ± 0.1	4.1 ± 0.7

[a] TA stands for total acidity. [b] Calculated as a difference between total acidity and COOH. [c] $\pm$ stands for one standard deviation from triplicate measurements.

Data on atomic ratios and content of acidic groups in the modified humic materials are given in Table 2. Data for CHP are typical for coal HA (13). The low H/C atomic ratio (0.85) is indicative of the high aromaticity of this material. All hydroxylated derivatives, except for those oxidized wtih CrO_3, are characterized with an H/C ratio very close to CHP. The same is true for the catecholic copolymers. The considerably higher H/C ratios for OCr and RCr derivatives are the result of underestimating the carbon content in these samples (49.5% and 55.1%, respectively), a potential consequence of the high ash content determined for both samples (20.95% and 19.5%, respectively). For CrO_3-treated samples, an elevated ash content may result when Cr is firmly bound to humic material and not removed by dialysis and cation exchange. At the same time, higher values of H/C (1.15−1.2) are evident in all hydroquinonic copolymers due to the cross-linking of hydroquinone novolac polymers. This is in line with the elevated H/C ratio found for the hydroquinonic homopolymer (1.33) as compared to the theoretical value of 0.86 for the linear homopolymer containing one methylene group per monomeric unit.

Elemental composition of the p-benzoquinone homopolymer (BQBQ) is close to the theoretical values for polyhydroxybenzoquinone (C, 58.06%; H, 3.23%; O, 38.71%) and to reported experimental values (21, 26, 27). H/C value of 0.7 observed for BQ250 copolymer is a clear outlier as confirmed by the large experimental error in the determination (28%).

As with H/C ratios, all hydroxylated derivatives, except for those treated with CrO_3, were characterized with O/C values close to the parent humic material. CrO_3-oxidized derivatives possessed much higher O/C values of 0.63 and 0.51, which could result from underestimating the carbon content and, in turn, overestimating the oxygen content in these high-ash samples. For catecholic copolymers, slightly higher O/C values were observed, whereas HQ100 and HQ250 copolymers were characterized with slightly lower O/C values as compared to CHP. Again, as with H/C ratios, no direct
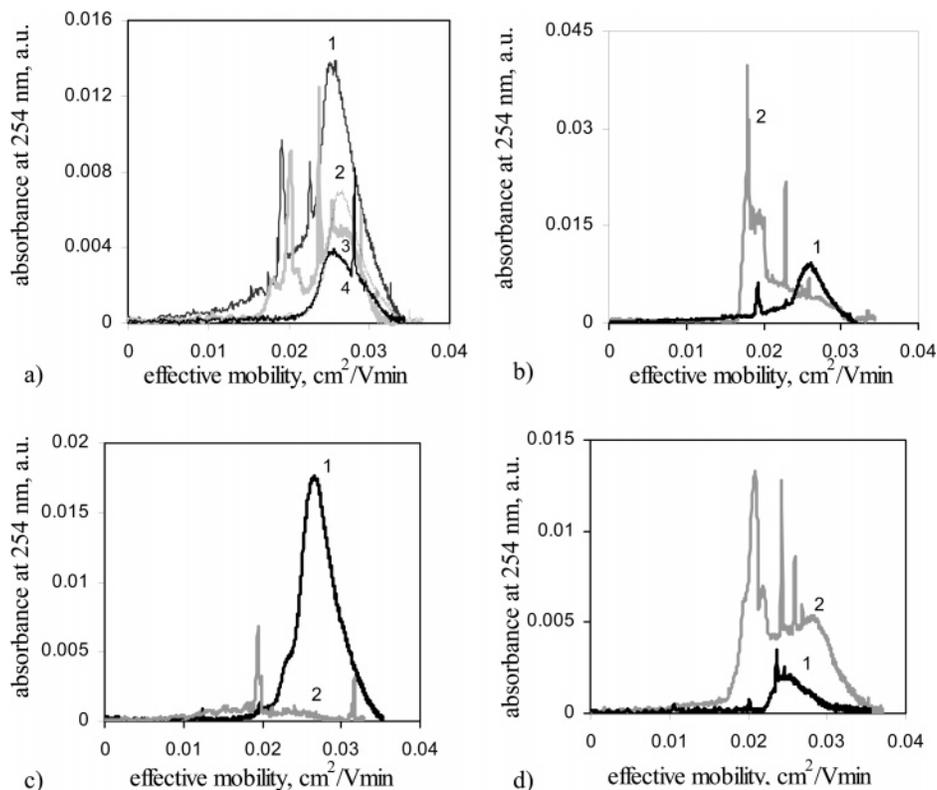
FIGURE 2. Electropherograms of copolymers and parent humic material dissolved at pH 8.85 (a): (1) HQ250; (2) BQ250; (3) CT250; (4) CHP; and of nonextractable residues (1) and ether-extracts (2) of copolymers obtained using liquid−liquid extraction from water solutions at pH 8.85. The ether extract was evaporated and then dissolved in water of pH 8.85: (b) HQ250; (c) BQ250, (d) CT250. All separations were conducted using carbonate buffer at pH 11.4.

relationship was observed between monomer-to-humic ratio and O/C values.

The content of phenolic hydroxyls (ArOH) is of particular importance for modified humic materials as it provides a direct estimate of the amount of phenolic hydroxyls incorporated into the parent material and, hence, indicates the degree of modification. From Table 2, it can be seen that any observed increase in the content of ArOH groups is slight among the hydroxylated derivatives (except for OEl, Ocr, and HCr). The higher ArOH values observed among CrO_3-treated derivatives are a consequence of underestimating the carboxylic acidity resulting from the high ash content in these derivatives.

The lower ArOH value for OEl can be explained by preferred oxidation of side chains of the aromatic rings that results in an increase in carboxyl group content. At the same time, a remarkable increase in the ArOH content can be seen for all copolymers. The content of phenolic hydroxyls reaches maximum values of 4.4−4.6 mmol/g for hydroquinonic and catecholic formaldehyde copolymers, which is a factor of 4 greater than that of parent material. A somewhat lower content is seen for *p*-benzoquinone copolymers (4.1 mmol/g).

Results indicate polycondensation was more effective at transforming parent humic material than direct oxidation. This conclusion was also confirmed by FTIR and ^{13}C NMR spectroscopic studies as well as by size exclusion chromatography (results are not shown). Polycondensation would have produced structural changes in parent material via the formation of humic-catechol and humic-hydroquinone graft-polymers. To prove this and discount the likelihood that homopolymeric admixtures of hydroquinone and catechol were produced, the humic copolymers were analyzed using capillary zone electrophoresis (CZE).

CZE was selected as the method of choice due to the polyelectrolytic properties of humic copolymers and phenolic oligomers. CZE is known as a powerful tool for the analysis of complex mixtures of polyelectrolytes that enables their separation as a function of charge density (*26*). CZE separations of humic copolymers were conducted under conditions adapted from previous studies on characterization of natural humics and synthetic humic like polymers (*27*). The separation buffer with pH 11.4 was chosen to achieve maximal ionization of all carboxylic and phenolic groups present in the polymers. Under these conditions, we found it feasible to separate oligomers in the high mobility range (*27*). Humic copolymers were dissolved in water at the lowest pH value of 8.85 that still provided for high solubility. Typical electropherograms for different copolymers are shown in Figure 2a. Oligomeric admixtures appear to be present with the hydoquinone- and catechol-copolymers obtained by formaldehyde condensation. At the same time, no oligomers can be seen with *p*-benzoquinone copolymers. Results suggest the dialysis technique is less effective in cases where phenolic oligomers are relatively hydrophobic and of high molecular weight.

To determine whether extraction with organic solvent could serve as a viable alternative to the dialysis, humic copolymers were extracted with diethyl ether using liquid−liquid extraction from the slightly alkaline copolymer solution. The ether extracts and nonextractable residues were further dissolved at pH 8.85 and separated under conditions described previously (Figure 2b−d).

From Figure 2, it is clearly evident the oligomers present in hydroquinone- and catechol-copolymers are almost completely extracted into the ether phase. The ether-extractable fraction of the copolymers consisting of polyphenolic oligomers has lower mobility as compared to the nonextractable fraction. The latter is represented by quinonoid-enriched humic copolymers, which possess a mobility that is both higher and similar to the more acidic leonardite HA.

TABLE 3. Redox Properties of the Modified Humic Materials[a]

sample	reducing capacity, mmol/g	free-radical content, spin/g $\times 10^{-16}$
CHP and Hydroxylated Derivatives		
CHP	0.6 ± 0.1	64.8
OFr	0.7 ± 0.1	41.4
RFr	0.7 ± 0.1	70.8
OEl	0.6 ± 0.1	41.0
REl	0.6 ± 0.1	56.1
OFe	1.3 ± 0.1	35.3
RFe	0.8 ± 0.1	58.7
Homopolymers and Humic Copolymers		
HQHQ	2.2 ± 0.2	0.2
HQ100	1.2 ± 0.2; 1.6 ± 0.2[b]	50.7
HQ250	2.7 ± 0.1; 3.6 ± 0.1[b]	53.2
HQ500	4.0 ± 0.1; 3.5 ± 0.2[b]	71.7
CTCT	2.1 ± 0.1	2.1
CT100	2.0 ± 0.1	43.6
CT250	3.0 ± 0.1; 3.3 ± 0.2[b]	63.7
CT500	2.9 ± 0.4; 3.4 ± 0.2[b]	59.3
BQBQ	3.9 ± 0.1	251.1
BQ100	1.2 ± 0.1	213.3
BQ250	1.1 ± 0.1	213.3
BQ500	2.0 ± 0.1; 2.6 ± 0.2[b]	340.1

[a] $\pm$ stands for one standard deviation from triplicate measurements.
[b] Denotes redox capacity of the copolymer measured after diethyl ether-extraction.

Results show that both hydroquinone- and catechol-copolymers obtained using formaldehyde condensation can be efficiently purified using liquid−liquid ether extraction from slightly alkaline aqueous solutions. At the same time, p-benzoquinone-copolymers have almost none of the oligomeric admixtures and can be purified using dialysis. Results confirm polycondensation of leonardite humic material and selected monomeric dihydroxybenzenes leads to the formation of corresponding copolymers and not simply mixtures of humic materials and hydroquinone- and catechol-homopolymers.

Redox Properties of the Obtained Derivatives. To characterize redox properties of the hydroxylated derivatives and copolymers, the reducing capacity and stable free radical content were determined as described in refs 28 and 12, respectively. The reducing capacity reflected the amount of electrons transferred by humic materials to Fe(III) in potassium ferricyanide $K_3[Fe(CN)_6]$ ($E_h = +0.43$ V). Hence, this parameter characterized the amount of contaminant that the quinonoid-enriched material could treat. The measurement of free radicals was to determine the role of semiquinone-type free radicals in the redox reactions of humic materials widely discussed in the literature (2, 12, 29, 30). To quantify the amount of semiquinoic radicals in all humic samples, ESR spectroscopy was used. The results on redox capacity and the free radical content in samples of modified humics are given in Table 3.

The measured reducing capacity of CHP is 0.6 mmol/g, which is corroborated by other data published for coal-derived humics (8, 31). Data on the reducing capacity of modified humics show the oxidation approach to be significantly less efficient than polycondensation. Oxidation leads to an increase in redox capacity only among derivatives from Fenton's oxidation. The corresponding sample (OFe) is characterized with a 2-fold increase in reducing capacity (1.3 mmol/g) as compared to CHP. For the same sample, the content of phenolic hydroxyls is 2 times higher than the parent material.

The reducing capacities of homopolymers are much higher as compared to CHP. Corresponding values for formaldehyde homopolymers (HQHQ and CTCT) are, re-

spectively, 2.2 and 2.1 mmol/g, whereas for p-benzoquinone (BQBQ) it approaches 3.9 mmol/g. Such substantial differences could stem from the low solubilities of HQHQ and CTCT as compared to BQBQ samples under the conditions created. This could lead to a general underestimation of reducing capacity for homopolymers. For all humic copolymers obtained, the redox capacity values are much higher than those of parent HA and vary from 1.1 to 4.0 mmol/g. These results are in the range of values reported under similar conditions for natural humics from soil (1.09 mmol/g), peat (2.29 mmol/g), and freshwater (6.5 mmol/g) (12). A direct relationship between the monomer-to-humic ratio and redox capacity of the copolymers is observed only for hydroquinone. Among those copolymers generated under the minimum monomer-to-humics ratio (100 mg per g CHP), a maximum increase in redox capacity (270%) is found for the catechol copolymer. The highest redox capacity of 4.0 mmol/g is observed for HQ500. All p-benzoquinone humic copolymers have lower redox capacities than those of hydroquinone and catechol. It could be that the conjugated aromatic rings present in the structure of BQ-copolymers stabilize the semiquinone moieties and prevent an oxidation to quinones. Despite their relatively high reducing capacities, applications of phenolic homopolymers could result in adverse environmental impacts not seen with the nontoxic humics. Hence, for purposes of expediting environmental remediation, the use of homopolymers would be less plausible than modified humic materials.

Based on the CZE studies revealing the presence of oligomeric admixtures in the humic copolymers obtained by formaldehyde polycondensation, the redox capacities of six copolymers (HQ100, HQ250, HQ500, CT250, CT500, and BQ500) were measured after liquid−liquid extraction with ether. The corresponding results are given in Table 3 and denoted with a "b". Data show ether extraction yielded a 10−30% increase in the reducing capacity for all humic copolymers, except for CT500. For this copolymer, the performed t-test ($P > 95\%$) shows that the reducing capacity of the purified copolymer does not differ statistically from that of the crude copolymer. An increase in the reducing capacity observed for the extracted copolymers is associated with the breakdown of quinhydrone-type structures produced by oligomeric quinones with humic copolymers, a consequence of removing the latter with ether extraction. Hence, purification by liquid−liquid ether extraction would be recommended for the synthesis of humic copolymers both to ensure greater reactivity and to avoid secondary contamination upon subsequent application in environmental remediation.

The measurements of free radical content (Table 3) were used to examine trends in the reducing capacity of the modified humic materials. The positive correlation between the "electron-accepting capacity" and free radical content was reported by Scott et al. (2). Our titration results, however, did not reveal correlation with the free radical contents in the modified humic materials. Scott et al. (2) determined free radical contents using conventional ESR methods on HA solutions, whereas our correlations were made with stable free radical concentrations measured on solid HA samples (Table 3). Differences between solid HA and aqueous solutions of HA substantially affect the formation and stability of free radicals (12, 30) that may explain a lack of correlation. For all oxidized derivatives, a decrease in free-radical content is observed as compared to parent material. For the reduced derivatives, however, the free radical content is almost equal to that of the parent material. This can be explained assuming the semiquinoic radicals present in the parent material are converted to quinones during oxidation and regenerated during the reduction of oxidized derivatives. For all humic copolymers obtained by formaldehyde polycondensation,

the free radical content was very close to the parent HS. The highest free-radical contents were observed for *p*-benzoquinone homopolymer and for BQ-copolymers with CHP. This can be explained by a radical mechanism of polymerization of *p*-benzoquinone resulting in the formation of conjugated structures that facilitate delocalization of unpaired electrons (*32*). In turn, this can be indicative of maximum antioxidant capacities of *p*-benzoquinone-modified humic materials.

Environmental Implications. In this study, we have successfully demonstrated the possibility of enhancing redox activity of humic materials by incorporating into their structure well-defined quinonoid moieties, hydroquinone and catechol units. These results may have important implications for ensuring control over redox properties of humic-based redox-mediators and reductants needed for their successful application in remediation strategies/technologies targeted for redox sensitive metals and organic contaminants. As a consequence of our study, quinonoid-enriched humic materials are developed, which possess considerably higher reducing capacities, and, hence, their use over natural humics provides for greater remediation efficiencies. Reported values of formal electrode potentials for natural HS vary from +0.328 to +0.78 V (*12, 33–35*). The synthesized quinonoid-enriched copolymers are expected to display redox potentials closer to the upper range due to the presence of well-defined redox centers such as catechol and hydroquinone (E_h = +0.7 and +0.78 V, respectively). This is consistent with the similarity of redox potentials observed within water-soluble hydroquinones and polymeric hydroquinones (*36, 37*), and it holds promise of higher redox potential for modified materials. Direct electrochemical measurements of electrode potentials and reversibility of redox transformations of the developed materials are currently underway. The first experimental confirmation of higher reduction potential of the quinonoid-enriched derivatives has been obtained for the case of Np(V) (*38*). Hence, evidence exists that these modified humics can be also used to reduce U(VI), Tc(VII), and V(V), which cannot be reduced with natural humics (*3, 5, 6*). The reduced forms of these metals pose much lower risks to environment due to the lower solubility of corresponding hydroxides.

An available supply of humics possessing a variety of quinonoid reactive centers will give unique opportunities to probe the selection of electron shuttling interactions mediated by humics between ecotoxicants and diverse microorganisms (for a review, see ref *1*). Thus, there now exist potential opportunities for using quinonoid-enriched humics as biostimulators to accelerate microbial redox transformations of contaminants.

Finally, there are additional benefits provided by the low cost of producing humic copolymers and the ease of using them in remediation. The cost of producing modified humics is expected to be quite low, first because the chemical modification procedure is based on well understood processes (e.g., phenolformaldehyde condensation), and, second, because raw humic materials (leonardite and other lignites) are inexpensive and their available supplies are plentiful. Being nontoxic and water soluble, quinonoid-enriched humics are easily introduced into the subsurface for purposes of effecting remediation, or they can be safely applied as soil amendments over large vegetated areas through cost-effective irrigation or aerial broad-casting systems (crop duster).

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