Arsenic Redox Changes by Microbially and Chemically Formed Semiquinone Radicals and Hydroquinones in a Humic Substance Model Quinone

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Received November 4, 2008. Revised manuscript received March 16, 2009. Accepted April 1, 2009.

Arsenic is a redox-active metalloid whose toxicity and mobility strongly depend on its oxidation state, with arsenite (As(III)) being more toxic and mobile than arsenate (As(V)). Humic substances (HS) are also redox-active and can potentially react with arsenic and change its redox state. In this study, we show that semiquinone radicals produced during microbial or chemical reduction of a HS model quinone (AQDS, 9,10-anthraquinone-2,6-disulfonic acid) are strong oxidants. They oxidize arsenite to arsenate, thus decreasing As toxicity and mobility. This reaction depends strongly on pH with more arsenite (up to 67.3%) being oxidized at pH 11 compared to pH 7 (12.6% oxidation) and pH 3 (0.5% oxidation). In addition to As(III) oxidation by semiquinone radicals, hydroquinones that were also produced during quinone reduction reduced As(V) to As(III) at neutral and acidic pH values (less than 12%) but not at alkaline pH. In order to understand redox reactions between arsenite/arsenate and reduced/oxidized HS, we quantified the radical content in reduced quinone solutions and constructed Eₚ-pH diagrams that explain the observed redox reactions. The results from this study can be used to better predict the fate of arsenic in the environment and potentially explain the occurrence of oxidized As(V) in anoxic environments.

Introduction

Arsenic is a known carcinogen and mutagen. Its elevated concentration in ground and surface water in various countries throughout the world causes large problems for the local population (1). Microbial activity as well as hydrogeological conditions can be responsible for As release from As-rich aquifer sediments into groundwater (2–4).

Arsenic can exist in four oxidation states: As(−III), As(0), As(III) and As(V) with As(V) and As(III) being the dominating oxidation states in the environment (5). The toxicity of arsenic varies with its chemical speciation. Arsenate (As(V)) is a molecular analog of phosphate and inhibits oxidative phos-

phorylation. Arsenite (As(III)) is more broadly toxic because it binds to sulfhydryl groups, impairing the function of many proteins. Also the mobility of As in aquatic environments depends on arsenic redox state with As(III) being the more mobile species (1). With pKₐ values of 2.2 and 6.9 for the first deprotonation steps of arsenate and a first pKₐ value of 9.3 for arsenite, the protonated, uncharged form of arsenite (H₃AsO₃⁻) and the anionic forms of arsenate (H₄AsO₄⁻ and H₅AsO₅²⁻) are the predominant species at neutral pH. Therefore, arsenate sorbs strongly to (positively charged) surfaces of several common metal oxides such as iron oxy(hydrox)ides and aluminum oxides. In contrast, the uncharged arsenite binds less strongly to minerals leading to a higher overall mobility (5).

Cycling among different valence states of arsenic and chemical species in soils and natural waters is influenced by abiotic and biotic processes (6). Manganese dioxides can oxidize arsenite chemically (7). Photooxidation of As(III) can be catalyzed by TiO₂ and dissolved Fe(II) (8, 9). In addition to chemical reactions, also the activities of arsenic-metabolizing microbes affect the speciation of arsenic. Certain prokaryotes use arsenic oxyanions for energy generation by oxidizing arsenite, respiring arsenate or even in photosynthesis (5, 10, 11).

Some sediments from which As-release was observed contained high amounts of natural organic matter (NOM) (12). It was shown that NOM can not only complex arsenic but can also change its redox state by both oxidizing As(III) and reducing As(V) (Figure 1A) (13, 14). However, the reaction mechanisms of this process, in particular the responsible redox-active functional groups in humic substances (HS), were not identified.

HS were shown to be reduced by a variety of microorganisms (15–19) and to transfer the accepted electrons to chlorinated hydrocarbons (20, 21), to Fe(III) minerals (22) and to other metal ions (23, 24). Quinones were suggested to function as the main electron accepting moieties in HS (25, 26). Recently, Ratasuk and Nanny identified three groups of reactive sites in a variety of HS among which two were of quinoid nature (27). Scott et al. provided evidence that semiquinone radicals are produced during microbial HS reduction (25) but free radicals were also detected in nonreduced HS and soils (28, 29). The radical concentrations detected by Scott et al. correlated with the electron accepting capacity (i.e., amount of electrons that can be accepted by HS) of HS. This also points to the importance of quinones as redox active functional groups in HS (25) as well as the fact that polyphenolic-rich NOM fractions exhibited a higher electron accepting capacity than carbohydrate-rich fractions (30). It does not, however, rule out the possibility of other redox active functional units such as sulfuryl groups, aromatic constituents and complexed metal ions being also involved in HS redox reactions (30, 31).

Although evidence exists in the literature that NOM undergoes redox reactions with arsenic, these reactions were neither quantified nor investigated on a mechanistic basis. However, since NOM can not only lead to redox transformations but also to complexation of arsenic via metal-bridging mechanisms (13), quantification of arsenic speciation can be hindered. Therefore, we used the chemically defined compound AQDS (9,10-anthraquinone-2,6-disulfonic acid, Figures 1B and 2) as a model compound for quinoid moieties in NOM which does not interfere with arsenic analysis. As mentioned above, quinones are suggested to represent the most important redox-active functional group in HS, although it is known that other redox-active moieties besides quinones...
exist in HS. It has been shown that AQDS is also able to shuttle electrons from microorganisms to iron(III) hydroxides (15) and so far no evidence exists that it is toxic at low concentrations for iron-reducing microorganisms. Furthermore, the redox potential of the AQDS\(^{2-} \)/AH\(_{2}\)QDS\(^{2-}\) redox couple (~160 mV at pH 7) is in the range of redox potentials given for HS in the literature (32, 33). The objectives of this study therefore are the quantification of arsenic redox transformations by quinone, semiquinone, and hydroquinone moieties in AQDS, and the identification of the role of free radicals (semiquinones) in arsenic redox transformations. With the help of these results, we will explain our findings on a mechanistic basis by thermo-dynamic calculations.

**Materials and Methods**

**Preparation of Solutions.** Aqueous solutions of AQDS (4 mM) were chemically reduced at pH 7 by \(\text{H}_2\) gas under anoxic conditions. UV—vis absorption spectroscopy showed that a new peak for reduced AQDS\(^{2-}\) appeared after a few minutes (~406 nm) and simultaneously the AQDS\(^{2-}\) peak (~328 nm) decreased and was not recognizable anymore at the end of reduction (not shown). After reduction, solutions were filtered (0.22 \(\mu\)m, cellulose acetate) to remove the Pd catalyst.

Microbially reduced AQDS\(^{2-}\) was prepared by reduction of AQDS\(^{2-}\) (4 mM) by *Geobacter sulfurreducens* with acetate (10 mM) in minimal medium (19). When the reduction was complete, cells were removed by filtration (see chemical reduction). We recently showed that after filtration no dissolved organic carbon (DOC) was present in solution indicating that also no redox-active organic compounds were released from the cells to the supernatant (19).

As source of As(III), a 0.05 M sodium arsenite solution (NaAsO\(_2\)) was prepared, disodium hydrogen arsenate heptahydrate (As\(\text{HNa}_2\text{O}_4\cdot7\text{H}_2\text{O}\)) was used as a source of As(V).

**Experimental Setups for As-AQDS Redox Reactions.** Solutions of AQDS\(^{2-}\) and reduced AQDS\(^{2-}\) were adjusted from pH 7 to pH 3 and 11 with anoxic 0.1 M HCl or 0.1 M NaOH and mixed with solutions of As(III) and As(V) in an anoxic glovebox (100% N\(_2\)). No buffer was used. When reduced AQDS\(^{2-}\) solutions at pH 7 were mixed with either As(III) or As(V), a slight increase in the pH values was observed 2–3 min after mixing and an increase of 1–1.5 pH units was observed after one day. When reduced AQDS\(^{2-}\) solutions adjusted to pH 3 and 11 were mixed with As(III) or As(V), an increase to pH values of 3.2–3.7 and a decrease to pH values of 10.4–10.9, respectively, were observed within a few minutes. Thereafter, the pH did not change anymore in these setups. Vials were closed with O\(_2\)-tight Teflon-coated butyl rubber stoppers. Samples were incubated for 1 day in the dark on a rotary shaker outside of the glovebox at 200 rpm. Additional analysis after 7 days showed no further reaction (Supporting Information Figure S1). We therefore assumed the reaction to be complete (in steady-state) after 1 day and continued sampling after 1 day for all further experiments.

**Analytical Methods.** As(III) and As(V) were quantified using a high performance liquid chromatograph (Shimadzu, LC-10Avp) equipped with an anion exchanger column (PRP-x100, Hamilton) and a diode array UV detector. The mobile phase was 30 mM H\(_3\)PO\(_4\) (flow rate 0.8 mL/min, column temperature 50 °C). As(III) and As(V) were detected by UV absorption at 200 nm. To quantify As(III) and As(V) concentrations, a calibration curve in the range of 5–100 \(\mu\)M was used. CW X-band ESR-Spectra were recorded at 25 °C using an ESR spectrometer (MiniScope MS 300, Magnetotech GmbH, Berlin, Germany) at a microwave power of 0.1 mW. For the determination of g-values, an internal manganese standard (Mn\(^{2+}\) in ZnS) was measured simultaneously with the samples. Spin concentrations were calculated using DPPH (\(\alpha\-\alpha\)/diphenyl-picryl-hydrazyl) dissolved in toluene as a reference (29). Absolute radical concentrations were calculated after double integrating the AQDS spectrum and quantitative comparisons to the DPPH spectrum. Reduced AQDS solutions were filled into graduated quartz capillaries (Blaubrand Intramark Mikropipettes, Brand GmbH, Germany) in an anoxic glovebox and the capillaries were sealed with a vinyl sealing kit (Haematocrit Sealing Compound, Blaubrand GmbH, Germany). They were then placed into quartz glass tubes with an inner diameter of 4 mm and closed with plastic caps (Magnetotech GmbH, Berlin, Germany) and parafilm to maintain anoxic conditions throughout the measurement.

**Results and Discussion**

**Arsenic Redox Transformations by Oxidized and Reduced AQDS\(^{2-}\).** To investigate the oxidation of As(III) by the oxidized form of AQDS\(^{2-}\), mixtures of either 90 \(\mu\)M As(III) and 450 \(\mu\)M AQDS\(^{2-}\) (molar ratio of reduced to oxidized compound of 1.5, oxidant in excess) or 330 \(\mu\)M As(III) and 67 \(\mu\)M AQDS\(^{2-}\) (molar ratio of reduced to oxidized compound of 5:1, oxidant in excess) were incubated at pH 3 and 7. In all setups, no oxidation of As(III) was observed. Experiments with As(V) and AQDS\(^{2-}\) at the same concentrations and molar ratios of As to quinone also showed no reactions. Thus, both
As(III) and As(V) are stable in the presence of AQDS\(^{2-}\) under all pH conditions investigated.

In contrast, when As(V) was incubated with chemically reduced AQDS\(^{2-}\) (potentially including both semiquinones and hydroquinones) at different pH values, reduction of As(V) was observed under some conditions (Table 1). At initial concentrations of 330 \(\mu M\) As(V) incubated at pH 3 and 7 with a solution containing 67 \(\mu M\) chemically reduced AQDS\(^{2-}\) (molar ratio of 5:1), 4.9 and 39.3 \(\mu M\) As(V) were reduced to As(III), respectively (Table 1). At pH 11, no As(V) was reduced by chemically reduced AQDS\(^{2-}\). When As(V) (330 \(\mu M\)) was incubated at pH 3 and 7 with 67 \(\mu M\) microbially reduced AQDS\(^{2-}\) (molar ratio of 5:1), 2.2 and 1.9 \(\mu M\) As(V) were reduced, respectively. This difference in As(V) reduction by microbially reduced AQDS\(^{2-}\) compared to chemically reduced AQDS\(^{2-}\) might be due to differences in the reaction progress during microbial and chemical reduction of AQDS\(^{2-}\). Microbially reduced AQDS\(^{2-}\) was harvested after 3 days which was the experimentally determined time it takes for microbial reduction of AQDS\(^{2-}\) by Geobacter sulfurreducens. In contrast, chemical reduction of AQDS\(^{2-}\) by \(H_2\) was finished after one day. It is possible that the different incubation times during microbial and chemical reduction of AQDS\(^{2-}\) lead to slightly varying ratios of oxidized vs reduced AQDS\(^{2-}\) species in our samples yielding different extents of As redox transformations.

As it already has been observed for chemically reduced AQDS\(^{2-}\), As(V) could also not be reduced by microbially reduced AQDS\(^{2-}\) at pH 11. As a next step, we intended to favor the reduction of As(V) by providing an excess of reductant. The initial concentrations were changed to 90 \(\mu M\) As(V) and 450 \(\mu M\) chemically or microbially reduced AQDS\(^{2-}\), yielding a molar ratio of oxidized to reduced compound of 1:5. However, although we observed As(V) reduction when less reduced AQDS\(^{2-}\) was present, under the same conditions but with higher concentrations of reduced AQDS\(^{2-}\), no reduction of As(V) was observed at all pH values tested (pH 3, 7, 11) (not shown). This was confirmed in several independent experiments but the reason for this rather surprising behavior remained unknown. Our observations confirm the results obtained by Redman et al. (13) who observed a slight reduction of As(V) only by one out of six different NOM fractions showing that NOM in general is not a very good reductant for As(V).

Experiments with As(III) (the reduced arsenic species) incubated with reduced AQDS\(^{2-}\) solutions were initially set up as mere control experiments. Identical to the experiments described above for As(V) and reduced AQDS\(^{2-}\), experiments were set up with mixtures of either 90 \(\mu M\) As(III) and 450 \(\mu M\) reduced AQDS\(^{2-}\) (molar ratio of reduced arsenic species to reduced AQDS\(^{2-}\) of 1:5) or 330 \(\mu M\) As(III) and 67 \(\mu M\) reduced AQDS\(^{2-}\) (molar ratio of 5:1) at pH 3, 7, and 11. We found that both chemically and microbially reduced AQDS\(^{2-}\) oxidized As(III) to a significant extent especially at high pH values, independent of whether As(III) or reduced AQDS\(^{2-}\) was present in excess (Figure 3 and Table 1). At a molar ratio of As(III):reduced AQDS\(^{2-}\) of 5:1, 13.7 \(\pm\) 1.7% and 4.5 \(\pm\) 1.3% of As(III) was oxidized at pH 11 by chemically and microbially reduced AQDS\(^{2-}\), respectively. At a ratio of 1:5 (excess reduced AQDS\(^{2-}\)) even up to 67.3 \(\pm\) 11.3% (chemically reduced AQDS\(^{2-}\)) and 63.3 \(\pm\) 22.6% (microbially reduced AQDS\(^{2-}\)) of As(III) was oxidized at pH 11 (Figure 3). At the same ratios at pH 7, 12.6 \(\pm\) 4.9% and 27.9 \(\pm\) 4.0% As(III) and at pH 3, 0.6 \(\pm\) 1.0% and 18.3 \(\pm\) 16.0% As(III) were oxidized by chemically and microbially reduced AQDS\(^{2-}\), respectively. Again, as mentioned above, these differences between setups containing chemically and biologically reduced AQDS\(^{2-}\) could be due to slight differences in ratios of oxidized vs reduced AQDS\(^{2-}\) species after reduction.

Similar to our experiments with the HS quinone model compound AQDS\(^{2-}\), previous experiments with aquatic NOM.
TABLE 1. Radical Concentrations of Chemically and Microbially Reduced Solutions of AQDS$^{2-}$ and Amounts of As(III) Oxidized and As(V) Reduced$^{a}$

<table>
<thead>
<tr>
<th>pH</th>
<th>Spins/L$^2$</th>
<th>Chemically Reduced AQDS$^{2-}$</th>
<th>Microbiologically Reduced AQDS$^{2-}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>$2.5 \times 10^{20}$</td>
<td>408.3$^c$</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>$5.6 \times 10^{18}$</td>
<td>9.2$^c$</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>$-\text{m}^{-1}$</td>
<td>0.4$^c$</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>$2.9 \times 10^{20}$</td>
<td>480.4$^c$</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>$6.0 \times 10^{18}$</td>
<td>9.9$^d$</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>$-\text{m}^{-1}$</td>
<td>11.5$\pm$10.2</td>
<td></td>
</tr>
</tbody>
</table>

$^{a}$ The radical content was determined by ESR spectroscopy and is given for 450 µM reduced AQDS$^{2-}$ solutions at pH 3, 7, and 11. The amounts of As(III) oxidized and As(V) reduced are given for experiments with 90 µM As(III) and 330 µM As(V) incubated with 450 and 67 µM reduced AQDS$^{2-}$ solutions, respectively. Concentration below detection limit is indicated by $-\text{m}^{-1}$. $^b$ The accuracy of spin concentrations within this method was ~5% for AQDS$^{2-}$ at pH 11 and ~10% for AQDS$^{2-}$ at pH 7. $^c$ This value is used to estimate the ratio of semiquinone to hydroquinone (1:50) at pH 7 (Figure 7B and C). $^d$ This value is used to estimate the ratio of semiquinone to hydroquinone (1:50 at pH 7 (Figure 7B and C).

![Figure 3. Oxidation of As(III) by chemically (A) and microbially (B) reduced AQDS$^{2-}$. The initial concentrations were 90 µM As(III) and 450 µM reduced AQDS$^{2-}$ (molar ratio 1:5) or 330 µM As(III) and 67 µM reduced AQDS$^{2-}$ (molar ratio 5:1).](image)

(Suwannee river NOM, SRNOM) by Redman et al. (13) also showed significant oxidation of As(III) and only slight reduction of As(V) under some conditions. When they incubated nonreduced SRNOM (10 mg C/L) with 1 µM As(III) at pH 6, 43% As(III) was oxidized after 90 h. Similar percentages of As(III) oxidation were observed in the presence of both aquatic and terrestrial NOM.

In order to compare the results for As(III) oxidation and As(V) reduction by SRNOM obtained by these authors to the values that we obtained in our experiments using AQDS$^{2-}$ on a quantitative basis, it is necessary to estimate their (hydro)quinone to As ratios in contrast to our own. This can be done via an estimation of the reducing capacities (i.e., amount of electrons transferred from NOM to an electron acceptor) of the NOM fractions used in their study. Recently, we determined the reducing capacity of Suwannee River humic acid (SRHA) (19) to be 1.6 µequiv/mg C. This value can be applied as an estimate for the reducing capacity of SRNOM utilized by Redman et al. (13), since SRHA and SRNOM have a similar carbonyl content (http://www.ihss.gatech.edu/). Employing this reducing capacity, 10 mg C/L of SRHA (also SRNOM) yield a reducing capacity of 16 µequiv/L, referring to 8 µM reduced AQDS$^{2-}$ (2-electron transfer process). As they used 1 µM As(III), the ratio of reduced quinones to As(III) can approximately be calculated as 8:1 which indicates that in their experiments an excess of reduced quinone was present similar to our 450 µM reduced AQDS$^{2-}$ vs 90 µM As(V)/As(III) experiments. Consequently, the values of As(III) oxidation by nonreduced HS of 40–70% observed in their study and the values of As(III) oxidation determined in our experiments using HS in the literature we conclude that, although HS are able to both oxidize As(III) and reduce As(V), they are generally much better oxidants than reductants for arsenic.

Radical Content of Microbially and Chemically Reduced AQDS$^{2-}$: The As(III) oxidation by reduced AQDS$^{2-}$ observed in our experiments suggests that a good oxidant, most probably semiquinone radicals, was formed during microbial and chemical reduction of AQDS$^{2-}$. We therefore investigated chemically and microbially reduced AQDS$^{2-}$ solutions at pH 3, 7, and 11 by ESR spectroscopy which yielded typical ESR spectra of 11 lines (Figure 4A), indicating that semiquinone radicals were formed. The appearance of 11 lines is in agreement with the results of Geimer and Beckert (34), who also reported 11 line spectra for the AQDS radical anion at pH 6. The observed radical has a $g$-value of 2.0040 determined in comparison to DPPH with $g = 2.0035$. The spectrum corresponds to three groups of two equivalent protons with hyperfine splitting constants of $a_{H1} = 0.36$ G, $a_{H2} = 0.42$ G, and $a_{H3} = 1.2$ G, respectively. In addition, sodium with a hyperfine splitting constant $a_{Na}$ of 0.055 G needs to be included in the model to describe the features of the spectrum at pH 11 in Figure 4A since sodium 9,10-anthraquinone-2,6-disulfonyl was used as a source of AQDS$^{2-}$.

With regard to signal intensity, the same pattern of ESR spectra was observed both for chemically and microbially reduced AQDS$^{2-}$: high signal intensity for samples adjusted
concentrations were found at alkaline pH for humic acids (35), fulvic acids (36) and NOM (29).

Based on the ESR spectra, radical concentrations of 408.3 and 480.4 µM were calculated at pH 11 for the semiquinone anion radical of chemically and microbially reduced AQDS$^{2-}$, respectively (Table 1). This suggests that under the given conditions approximately 100% of the initially present AQDS$^{2-}$ (450 µM) were present as semiquinone and not as hydroquinone. At pH 7, a radical concentration of 9.9 µM in microbially reduced AQDS$^{2-}$ solutions corresponding to 6.0 \times 10^{18} \text{ spins/g} was determined. This value is close to the value that has been published previously by Scott et al. (25) for reduced AQDS$^{2-}$ at pH 6.7 to be 7.4 \times 10^{18} \text{ spins/g}.

In our experiments, the radical content remained constant over several days (data not shown), which is in agreement with the results of Hocking and Mattar (37) who report stable anthraquinone radicals under anoxic conditions over one year. The authors attributed the high stability of anthraquinone radicals to their symmetrical structure and the possibility to delocalize electrons over the ring system. Additionally, it was previously shown that free radicals even exist in nonreduced HS (25, 28). The fact that such radicals are even present after exposure to oxic conditions shows that they are very persistent. This could be related either to steric protection of reduced functional groups from oxygen inside the aromatic framework of HS, or to the fact that these radicals have a redox potential that is not favorable for oxidation by O$_2$.

Thermodynamic Basis of As(V)/As(III) Redox Reactions with Quinone, Semiquinone and Hydroquinone Moieties. Interactions of As(III) and As(V) with oxidized and reduced quinone species are complex to understand on a quantitative basis due to different $pK_a$ values of semiquinones and hydroquinones (Figure 2A) as well as of As(III) and As(V) species (Figure 4B and C). Depending on the species, different redox potentials are relevant. It has to be considered that redox transformations of As(V) and As(III) are two-electron transfer steps, whereas the reduction of the quinone to the semiquinone or the further transformation of the semiquinone radical to the hydroquinone are one-electron transfer steps. To construct $E_0$-pH diagrams for different As-AQDS$^{2-}$ systems (Figure 4B and C) we used semiquinone radical concentrations determined by ESR spectroscopy (Table 1) and thermodynamic data available in the literature (38). For $E_0$ calculations of the As(V)/As(III) redox couple we used ratios of 1:10000 and 10000:1, respectively, representing starting conditions when mainly As(III) (oxidation experiments) or As(V) (reduction experiments) were present.

In our As(III) oxidation experiments, the redox potential of the As(V)/As(III) couple at pH 11 is far more negative than the redox potential for the semiquinone/reduced AQDS$^{2-}$ couple (AQDS$^{2-}$/AQDS$^{-}$) (Figure 4B). This suggests that based on thermodynamics, the semiquinone is a good oxidant for As(III) at this pH value. The larger the difference between redox potentials of the semiquinone/reduced AQDS$^{2-}$ pair and the As(V)/As(III) pair, the higher the thermodynamic driving force ($\Delta G$, Gibbs free energy) for As(III) oxidation. As can be seen in Supporting Information Table S1, $\Delta G$ values for the oxidation of As(III) by semiquinones become more positive with decreasing pH values. At pH 11, $\Delta G$ is negative (~57 kJ/mol). Therefore, As(III) is expected to be oxidized at high pH values. Indeed, this is exactly what we observed in our experiments in which we measured the highest percentage of As(III) oxidation at pH 11 (Figure 3) where we also quantified the highest amount of radicals (Table 1 and Figure 4A).

We also saw some As(III) oxidation by semiquinones at pH 3 and 7 (Figure 3) where $\Delta G$ values are positive (15 and 23 kJ/mol, respectively) and therefore, no oxidation is expected to occur. The unfavorable thermodynamic condi-
tions for As(III) oxidation at pH 3 and 7 by semiquinones can also be seen in the $E_0$-pH diagram (Figure 4B) where the redox potentials of $\text{AHQDS}^2+/\text{AHQDS}^-$(pH 3) and $\text{AQDS}^2+/\text{AH}_2\text{QDS}^2$ (pH 7) are lower than or quite close to the values for As(V)/As(III). A possible explanation for these results is that radicals at lower pH are not as stable as at pH 11 and their concentrations probably varied significantly under slightly different conditions. Support for this explanation comes from the fact that we observed increasing accuracy for measurements of radical concentrations at lower pH (Table 1). As a consequence, the actual radical concentrations in the $\text{AQDS}^2^-$ reduction experiments may actually have been slightly higher than in the setups used for ESR measurements, potentially explaining the deviation from stoichiometric oxidations between Humic Model Quinones and Arsenic.

Our study points towards an indirect influence of oxidized quinones in HS as electron acceptors occurs in the environmental systems. This is supported by our results, which showed that semiquinone radicals and hydroquinones, as well as quinone/reduced quinones (including semiquinones and hydroquinones), have no direct effect on the redox speciation of As. In contrast, semiquinones and hydroquinones lead to abiotic transformations by reduced HS, as electron shuttles in the metabolic network of environmental systems. This is consistent with previous findings that reduced quinones (i.e., semiquinones and hydroquinones) have no direct effect on the redox speciation of As.

Aquifers in Bangladesh containing high concentrations of dissolved As were shown to contain Fe(III)-reducing microbes (2) and also significant amounts of NOM (12). Based on the fact that Fe(III)-reducing microorganisms can also reduce NOM/HS (15, 18, 19, 40), it is expected that reduced quinones (i.e., semiquinones and hydroquinones) are formed. These processes could explain the presence of As(V) that was observed in some anoxic aquifers (6). From our results, the As(III) oxidation by NOM reported previously (13) can now be explained on a mechanistic basis. However, care still has to be taken regarding the transfer of our results to HS redox reactions. First, HS can vary to large extents in their composition and can contain very high concentrations of or almost no quinoid moieties. Our results are especially applicable to HS with high quinone contents. Second, in complex systems containing reduced quinones and a mixture of As(III) and As(V) (as probably present in environmental systems), the extent of As(III) oxidation and As(V) reduction will depend on the geochemical parameters, e.g. pH and the ratios of As(V)/As(III) as well as of quinone/reduced quinones (including semiquinones and hydroquinones). The present study in combination with previous research (19, 32) improved our understanding of the role of HS as electron shuttles in the metabolic network of environmental redox processes. However, our findings should be used in future studies to quantitatively confirm As redox processes mediated by semiquinone radicals and hydroquinones in the environment by following As redox transformations simultaneously with radical measurements in HS.

Environmental Implications of Redox Reactions between Humic Model Quinones and Arsenic. Our study showed that semiquinone radicals and hydroquinones, as they are present in HS and potentially being produced during microbial or chemical reduction of HS, can change the redox state of arsenic and thus its mobility and toxicity. Therefore, an in-depth knowledge about the radical formation in HS by chemically and microbiologically induced mechanisms as well as about the environmental conditions under which As transformation by reduced HS occurs brings forth a better understanding of how As is mobilized into drinking water and of ways it can be removed.

Our study clearly demonstrated that the oxidized form of quinones has no direct effect on the redox speciation of As. In contrast, semiquinones and hydroquinones lead to abiotic As redox changes. However, it is possible that microbially catalyzed oxidation of As(III) using the oxidized form of quinones in HS as electron acceptors occurs in the environment which would suggest an indirect influence of oxidized quinones on As redox speciation.


ES803112A