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

JIE JIANG,[†] IRIS BAUER,[†] ANDREA PAUL,[‡,§] AND ANDREAS KAPPLER*,[†]

Geomicrobiology, Center for Applied Geosciences, University of Tuebingen, Germany, and BAM, Fed. Inst. for Materials Research and Testing, Richard-Willstaetter-Str. 11, 12489 Berlin, Germany

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Arsenic is a redox-active metalloid whose toxicity and mobility strongly depends 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 E_h-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 phosphorylation. 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 pK_a values of 2.2 and 6.9 for the first deprotonation steps of arsenate and a first pK_a value of 9.3 for arsenite, the protonated, uncharged form of arsenite (H_3AsO_3) and the anionic forms of arsenate ($H_2AsO_4^-$ and $HAsO_4^{2-}$) are the predominant species at neutral pH. Therefore, arsenate sorbs strongly to (positively charged) surfaces of several common metal oxides such as iron oxy(hydr)oxides 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 TiO_2 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 microbal 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

* Corresponding author phone: +49-7071-2974992; fax: +49-7071-5059; e-mail: andreas.kappler@uni-tuebingen.de.
[†] University of Tuebingen.
[‡] Fed. Inst. for Materials Research and Testing.
[§] Former address: IGB, Institute of Freshwater Ecology and Inland Fisheries, Mueggelseedamm 301, 12587 Berlin, Germany.

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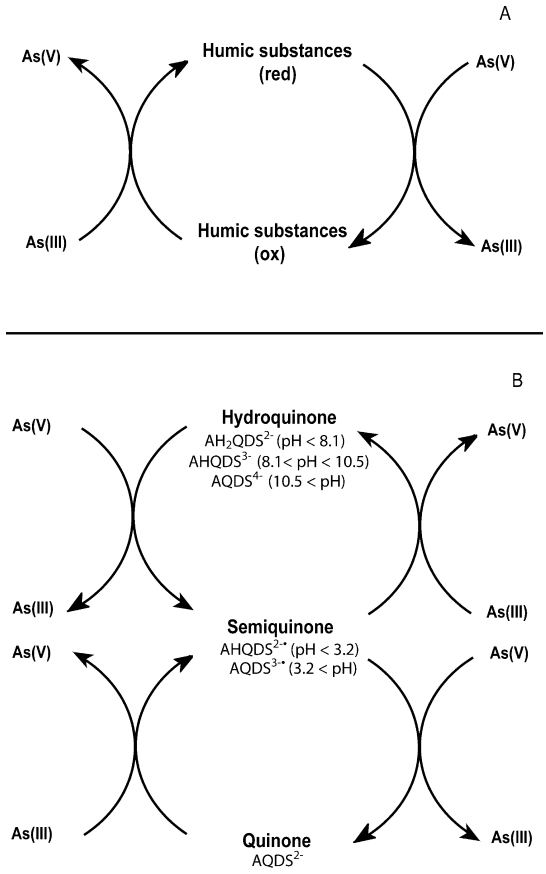

FIGURE 1. Scheme illustrating reduction of As(V) and oxidation of As(III) (A) by reduced and oxidized humic substances (HS) or (B) by the fully reduced (hydroquinone), partially reduced (semiquinone) and oxidized form (quinone) of anthraquinone -2,6-disulfonate ($AQDS^{2-}$), a model compound for quinone moieties in humic substances.

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 $AQDS^{2-}/AH_2QDS^{2-}$ 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 thermodynamic calculations.

Materials and Methods

Preparation of Solutions. Aqueous solutions of AQDS (4 mM) were chemically reduced at pH 7 by H_2 gas in the presence of a palladium catalyst (*18*). The reduction could be followed visibly by a distinct color change from light yellow to an orange color which stayed stable over days and even weeks 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 μm, 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 ($AsHNa_2O_4 \cdot 7H_2O$) 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_3PO_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 μM was used.

Cw X-band ESR-Spectra were recorded at 25 °C using an ESR spectrometer (MiniScope MS 300, Magnettech 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 (α,α-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, Brand GmbH, Germany). They were then placed into quartz glass tubes with an inner diameter of 4 mm and closed with plastic caps (Magnettech 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 μM As(III) and 450 μM $AQDS^{2-}$ (molar ratio of reduced to oxidized compound of 1:5, oxidant in excess) or 330 μM As(III) and 67 μM $AQDS^{2-}$ (molar ratio of reduced to oxidized compound of 5:1, reductant 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

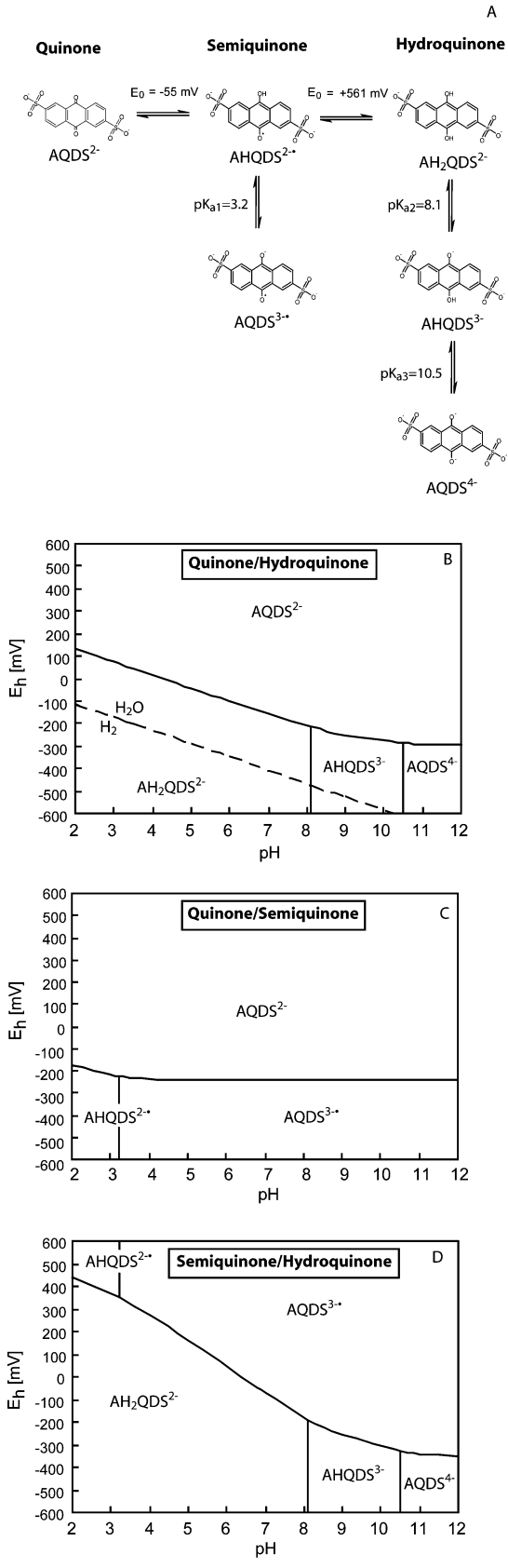

FIGURE 2. (A) Redox potentials and pK_a values for the anthraquinone (quinone), anthrasemiquinone (semiquinone), and anthrahydroquinone-2,6-disulfonate (hydroquinone) system. Standard reduction potential (E_0) values of half-reactions are given in (*38*). E_h-pH diagrams of the redox couples quinone/hydroquinone (B), quinone/semiquinone (C) and semiquinone/hydroquinone (D). The equations for calculation of E_h in (B), (C), and (D) are given in the Supporting Information (Thermodynamic calculations S1).

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 μM As(V) incubated at pH 3 and 7 with a solution containing 67 μM chemically reduced $AQDS^{2-}$ (molar ratio of 5:1), 4.9 and 39.3 μM 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 μM) was incubated at pH 3 and 7 with 67 μM microbially reduced $AQDS^{2-}$ (molar ratio of 5:1), 2.2 and 1.9 μM 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 μM As(V) and 450 μM 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 μM As(III) and 450 μM reduced $AQDS^{2-}$ (molar ratio of reduced arsenic species to reduced $AQDS^{2-}$ of 1:5) or 330 μM As(III) and 67 μM 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]

	spins/L[b]	concentration of semiquinone radicals [μM]	90 μM As(III) + 450 μM reduced $AQDS^{2-}$ (As(V) [μM])	330 μM As(V) + 67 μM reduced $AQDS^{2-}$ (As(III) [μM])
		chemically reduced $AQDS^{2-}$		
pH 11	2.5×10^{20}	408.3[c]	57.4 ± 10.9	—
pH 7	5.6×10^{18}	9.2[d]	12.7 ± 8.0	39.3
pH 3	—	—	0.4 ± 0.7	4.9
		microbially reduced $AQDS^{2-}$		
pH 11	2.9×10^{20}	480.4[c]	49.4 ± 20.0	—
pH 7	6.0×10^{18}	9.9[d]	20.7 ± 3.1	1.9 ± 2.6
pH 3	—	—	11.5 ± 10.2	2.2 ± 0.4

[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 —. [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 (100:1) at pH 11 (Figure 4B and C). [d] This value is used to estimate the ratio of semiquinone to hydroquinone (1:50) at pH 7 (Figure 4B and C).

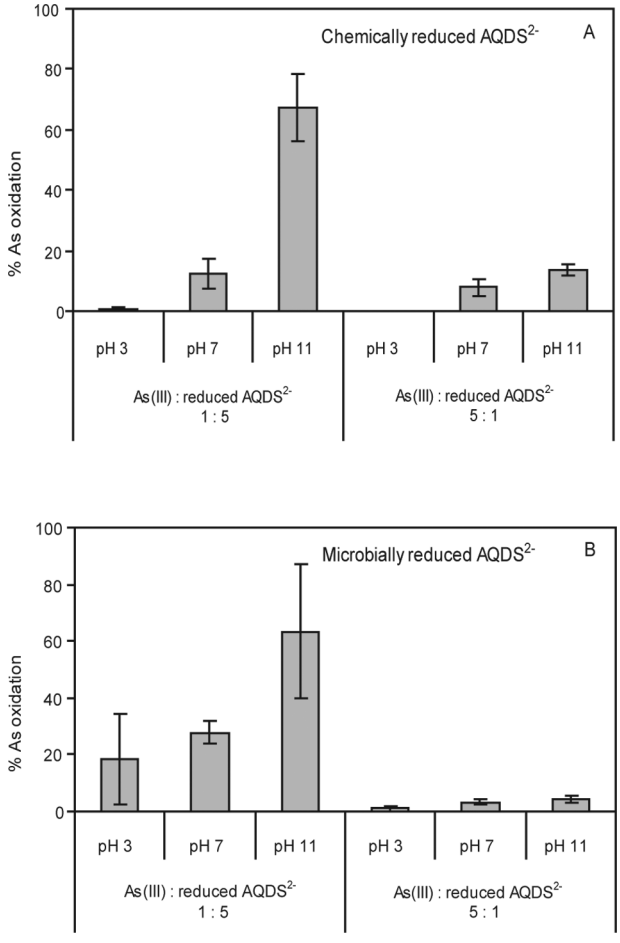

FIGURE 3. Oxidation of As(III) by chemically (A) and microbially (B) reduced $AQDS^{2-}$. The initial concentrations were 90 μM As(III) and 450 μM reduced $AQDS^{2-}$ (molar ratio 1:5) or 330 μM As(III) and 67 μM reduced $AQDS^{2-}$ (molar ratio 5:1).

(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 μequ/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 μequ/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 support the values of As(III) oxidation of up to 67% observed in our study. From our experiments and the results obtained with 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 7. 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-disulfonic 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

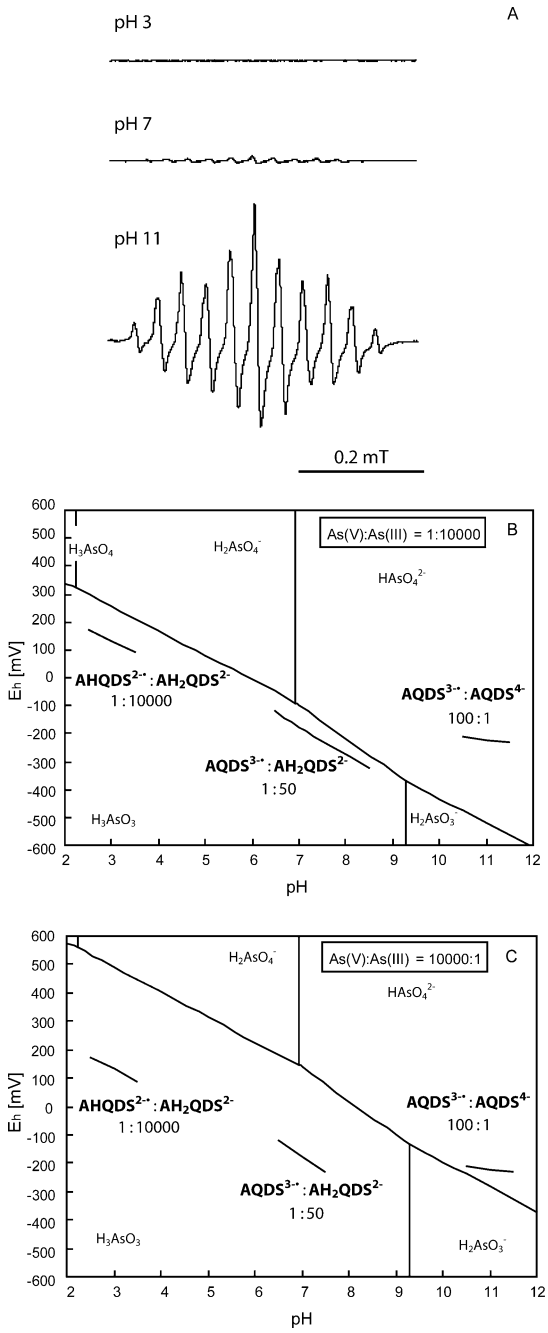

FIGURE 4. ESR spectra of microbially reduced $AQDS^{2-}$ measured at pH 3, 7, and 11 (A) at a microwave power of 0.1 mW and a modulation amplitude of 100 mG. E_h-pH diagrams (B and C) for the As-AQDS system at 25 °C based on thermodynamic constants and pK_a values of As oxyanions (*6*), in combination with standard redox potentials (E_0) (*38*) and pK_a values of the AQDS system (discontinuous lines) (*41*). The ratios of quinone redox couples (10000:1, 1:50 and 100:1), are derived from radical measurements. (B) represents the scenario in which mainly As(V) is present in the beginning of the experiment and (C) represents the scenario in which mainly As(III) is present at the beginning of the experiments.

to pH 11, significantly lower signal intensity at pH 7 and spectra indicating no radicals at pH 3. The latter result is in agreement with the general observation of enhancement of subsequent reactions of free radicals by protonation at low pH, yielding a smaller net effect. On the other hand, alkaline pH is well-known to stabilize semiquinone radical anions, an effect also observed for humic matter, where highest spin 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 6.0×10^{18} 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 7.4×10^{18} 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_h-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_h 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 ($AQDS^{3-\bullet}$/$AQDS^{4-}$) (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 (ΔG, Gibbs free energy) for As(III) oxidation. As can be seen in Supporting Information Table S1, ΔG values for the oxidation of As(III) by semiquinones become more positive with decreasing pH values. At pH 11, ΔG 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 ΔG 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_h-pH diagram (Figure 4B) where the redox potentials of $AHQDS^{2-\bullet}/AH_2QDS^{2-}$ (pH 3) and $AQDS^{3-\bullet}/AH_2QDS^{2-}$ (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 $AQDS^{2-}$ reduction experiments may actually have been slightly higher than in the setups used for ESR measurements, potentially explaining the deviation from stoichiometric As(III) oxidation and semiquinone content.

While we observed a high redox activity of reduced $AQDS^{2-}$ in our As(III) oxidation experiments, in setups with As(V), we saw only a small amount of As(V) reduction by reduced $AQDS^{2-}$ solutions. From the E_h-pH diagram (Figure 4C), it can be seen that the E_h of the As(V)/As(III) redox couple at pH 3 and 7 is higher than the E_h of the redox couples $AHQDS^{2-\bullet}/AH_2QDS^{2-}$ and $AQDS^{3-\bullet}/AH_2QDS^{2-}$ but lower than the E_h of the redox couple $AQDS^{3-\bullet}/AQDS^{4-}$ at pH 11. This explains our observation that there was some reduction of As(V) at pH 3 and 7 and no reduction at pH 11. Calculations of ΔG values (Supporting Information Tables S2 and S3) also showed negative values for the reduction of As(V) at pH 3 and 7 by the hydroquinone with either the semiquinone or the quinone as end product but values close to zero or even positive at pH 11. This indicates that As(V) reduction is expected to occur at pH 3 and 7 but not at pH 11. However, although the ΔG values for the pH 3 and pH 7 reactions are rather negative (−60 to −103 kJ/mol, depending on the end product of hydroquinone oxidation to either the semiquinone or hydroquinone level), we observed only little As(V) reduction. The reason for this behavior is probably the very slow kinetics for As(V) reduction in the range of months or even years (*39*).

The reduction of As(V) to As(III) is accompanied by the oxidation of reduced $AQDS^{2-}$ while it is unclear whether the reduced $AQDS^{2-}$ is oxidized back to the semiquinone or even to the quinone level. However, the products of this reaction can be estimated from the ΔG calculations in Supporting Information Tables S2 and S3: At pH 3 and pH 7, ΔG is more negative when the fully oxidized quinone is the end product of hydroquinone oxidation (Supporting Information Table S3) compared to hydroquinone oxidation to the semiquinone level (Supporting Information Table S2) suggesting that reduced $AQDS^{2-}$ is probably oxidized to the fully oxidized quinone during As(V) reduction.

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.

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.

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Supporting Information Available

Figure S1, Thermodynamic calculations S1, and Tables S1, S2, S3. This material is available free of charge via the Internet at http://pubs.acs.org.

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