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Enhancing Roxarsone Degradation and *In Situ* Arsenic Immobilization Using a Sulfate-Mediated Bioelectrochemical System

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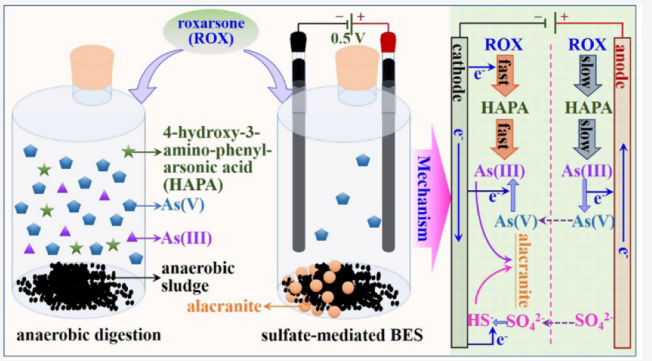
ABSTRACT: Roxarsone (ROX) is widely used in animal farms, thereby producing organoarsenic-bearing manure/wastewater. ROX cannot be completely degraded and nor can its arsenical metabolites be effectively immobilized during anaerobic digestion, potentially causing arsenic contamination upon discharge to the environment. Herein, we designed and tested a sulfate-mediated bioelectrochemical system (BES) to enhance ROX degradation and *in situ* immobilization of the released inorganic arsenic. Using our BES (0.5 V voltage and 350 μM sulfate), ROX and its metabolite, 4-hydroxy-3-amino-phenylarsonic acid (HAPA), were completely degraded within 13−22 days. In contrast, the degradation efficiency of ROX and HAPA was <85% during 32-day anaerobic digestion. In a sulfate-mediated BES, 75.0−83.2% of the total arsenic was immobilized in the sludge, significantly more compared to the anaerobic digestion (34.1−57.3%). Our results demonstrate that the combination of sulfate amendment and voltage application exerted a synergetic effect on enhancing HAPA degradation and sulfide-driven arsenic precipitation. This finding was further verified using real swine wastewater. A double-cell BES experiment indicated that As(V) and sulfate were transported from the anode to the cathode chamber and coprecipitated as crystalline alacranite in the cathode chamber. These findings suggest that the sulfate-mediated BES is a promising technique for enhanced arsenic decontamination of organoarsenic-bearing manure/wastewater.



INTRODUCTION

For several decades, roxarsone (ROX), 4-hydroxy-3-nitro-phenylarsonic acid, has been extensively used as a supplement in animal feeds to stimulate animal growth and to control coccidial parasites.[1] Due to the potential health hazards associated with residual trace arsenic in animal meats,[1,2] several countries, e.g., Europe, the USA, Australia, and China, have forbidden the use of organoarsenic feed additives.[3,4] However, ROX is still being used in many countries worldwide, e.g., India and Brazil (Table S1).[2,4,5] The dosage of ROX in feed is 20−50 mg kg^{-1}, of which >90% is ultimately excreted, thus typically ending up in the animal manure or in wastewater.[6,7] A survey of eight animal feeding operations in Beijing, China, indicated that animal manure contained up to ∼120 mg kg^{-1} arsenic.[8] Through naturally occurring microbial processes, ROX is converted into various arsenical metabolites such as 4-hydroxy-3-amino-phenylarsonic acid (HAPA), arsenite (As(III)), arsenate (As(V)), and methylated arsenic.[1,3] Fisher et al.[3] found that 70−75% of the total arsenic contained in poultry manure was easily mobilized due to its high water solubility. This mobile fraction of arsenic can enter into soils, surface and/or groundwater, or even accumulate in crops and aquatic products.[9,10] Considering the substantial risks of human exposure, more attention should be paid to arsenic contamination of livestock manure and wastewater.

Currently, livestock manure/wastewater treatment occurs mostly through anaerobic bioprocesses.[6] Under anaerobic conditions, while ROX is easily transformed to HAPA through the reduction of its nitro group to an amino group, HAPA is resistant to further degradation.[11−13] As HAPA is water-soluble, it is easily discharged into the natural environment with effluents from wastewater treatment plants. Once discharged, HAPA can eventually degrade into more toxic As(III) through photocatalytic or microbial degradation in the natural environment, i.e., surface waters,[14] threatening water quality and security. To avoid this, arsenic should be immobilized prior to wastewater discharge. Inorganic arsenic rather than organoarsenicals can be effectively immobilized through coprecipitation with ferric iron (Fe^{3+}) or sulfide (S^{2-}).[15,16] However, the low biodegradation efficiency of HAPA under anaerobic conditions limits the opportunities for effectively bioconverting ROX into inorganic arsenic. Im-

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portantly, even if only a small fraction of HAPA is transformed into inorganic arsenic, this fraction often fails to be efficiently immobilized in anaerobic reactors without or only low concentrations of sulfate.[13,17,18] For instance, Ji et al.[17] quantified the arsenic contained in the sludge in a 300-day operated upflow anaerobic sludge blanket (UASB) reactor, of which ∼75% remained soluble. Furthermore, elevated concentrations of dissolved arsenic, especially inorganic arsenic, have shown to inhibit microbial activities, thereby adversely affecting the performance of bioreactors.[12,18] Based on these previous findings, it is evident that the key for controlling the exposure and health risks associated with ROX is to enhance HAPA degradation and *in situ* immobilization of the inorganic arsenic that is produced.

Bioelectrochemical systems (BESs) have been proposed as an alternative technique for organic contaminant removal, hydrogen recovery, and heavy metal(loid) (e.g., arsenic) remediation.[19,20] BESs can facilitate biochemical processes by accelerating extracellular electron transfer[19] and therefore potentially assist to overcome the limitations exerted by the low degradation efficiency of HAPA. Shi et al.[20] reported that HAPA degradation was significantly accelerated when a voltage of 0.5 V was applied in anaerobic bioreactors. Nevertheless, its terminal product, As(V), still persisted in the dissolved form and therefore required subsequent disposal. *In situ* immobilization that relies on the stimulation of sulfate-reducing conditions is a commonly used technique for inorganic arsenic remediation.[15,21,22] Through sulfate reduction, dissolved arsenic is often transformed to As-bearing sulfides minerals, e.g., AsS and As_2S_3, and thus is immobilized.[17,22] Sulfate exists ubiquitously in livestock wastewater. Therefore, a sulfate-mediated BES can potentially be an effective technique for organoarsenic-contaminated manure/wastewater treatment.

However, such a treatment process, i.e., one that combines the BES with the stimulation of sulfate-reducing conditions, has not previously been considered for arsenic removal from organoarsenic-contaminated manure/wastewater. The objectives of this study were therefore (a) to investigate the feasibility of enhancing ROX degradation and arsenic immobilization simultaneously using a sulfate-mediated BES and (b) to elucidate the mechanisms that control this process. The feasibility and efficiency of such a treatment process were evaluated by experimentally determining the ROX degradation rates and the corresponding arsenic immobilization efficiency. The mechanisms were explored using a double-cell BES, where the transformation of arsenic and sulfate was compared between the anode and cathode chambers.

MATERIALS AND METHODS

Inoculums and Synthetic Wastewater. Sludge obtained from a municipal wastewater treatment plant (Hefei, China) was acclimatized with the supplement of glucose and sulfate (shown in the Supporting Information, SI) and used as inoculums. Synthetic wastewater was used in this study. According to the survey reported by Makris et al.,[23] arsenic concentration in the swine wastewater could reach ∼20 μM. A slightly higher, but still broadly representative, concentration of 50 μM ROX was added to the synthetic wastewater in this study to ensure more reliable measurements of arsenic precipitation in sludge. To guarantee the capability of sulfate to completely immobilize arsenic in any form of sulfides, the sulfate concentration was set to 350 μM. Since swine wastewater typically contains 1000−30 000 mg L^{-1} COD and ∼50% is recalcitrant to biodegradation,[24] 500 mg L^{-1} glucose was used to simulate biodegradable organic pollutants. The synthetic wastewater also contained essential mineral elements for which details are provided in the SI.

Bioelectrochemical Reactors. Two types of bioelectrochemical reactors, i.e., single- and double-cell reactors, were used in this study. The single-cell reactors (Figure S1a) incorporated an undivided cylindrical electric cell with a working volume of 300 mL. The double-cell reactor (Figure S1b) consisted of two separated cylindrical electric cells, which were linked via an intersecting column. The intersecting column contained an anion-exchange membrane for controlling the diffusion of anions. The working volume for each cell was 120 mL. For either single- or double-cell reactors, a graphite electrode pair was employed and a saturated calomel electrode (241 mV vs normal hydrogen electrode (NHE)) was used as a reference electrode. The voltage of 0.5 V was supplied by an external constant-voltage power supply (APS3005S, ATTEN), since this voltage was demonstrated to effectively stimulate organoarsenic degradation.[20]

Experimental Design. The experimental work was performed in two phases (Table 1). Phase I involved the setup and operation of a series of single-cell reactor experiments (assays 1−5) to investigate the efficiency of simultaneously degrading ROX and immobilizing inorganic arsenic *in situ* and several key factors. Assay 1 was set up as the control group and was therefore operated without sulfate amendment or voltage application. Assay 2 was supplemented with 350 μM sulfate to exclusively investigate the role of sulfate reduction on microbial ROX degradation and arsenic immobilization. Again, no voltage was applied. Assay 3 was operated without inoculums to clarify the significance of microbes on the transformation reactions. Two sulfate-mediated BESs were applied with 0.5 V voltage (assays 4 and 5) and assay 5 was spiked with 500 mg L^{-1} glucose, to evaluate the effect of labile organic substrates on ROX biodegradation and arsenic immobilization in the sulfate-mediated BES.

Table 1. Experimental Conditions in Assays 1−6

assay	sludge (g-VS L^{-1})[a]	glucose (mg L^{-1})	ROX (μM)	SO_4^{2-} (μM)	voltage (V)	reactor type
			Phase I			
1	0.5	500	50			single[b]
2	0.5	500	50	350		single
3		500	50	350	0.5	single
4	0.5		50	350	0.5	single
5	0.5	500	50	350	0.5	single
			Phase II			
6	0.5	500	50	350	0.5	double[c]

[a]VS, volatile solid. [b]Single-cell reactor. [c]Double-cell reactor.

Phase II was carried out in a double-cell bioelectrochemical reactor (assay 6) to elucidate the mechanisms that control the enhanced ROX degradation and arsenic precipitation using the sulfate-mediated BES. Both the cathode and anode chambers of a double-cell reactor were supplemented with the same wastewater proxy ingredients, i.e., with 50 μM ROX, 350 μM sulfate, and 500 mg L^{-1} glucose. While applying a voltage of 0.5 V, dissolved arsenic and sulfur species, immobilized arsenic within the sludge, and the electrode potentials were routinely

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monitored in both chambers over the whole experimental period.

Swine wastewater was normally neutral or weakly alkaline.[25] Given that sulfate reduction occurs within the pH range of 6−8[26] and sulfide-driven arsenic precipitation preferentially occurs under weakly acidic conditions,[27,28] an initial pH of 6.0 was chosen for all assays to satisfy both conditions. Mesophilic anaerobic digestion (at 30−35 °C) is one of the typical techniques for waste/wastewater treatment and has higher efficiency and stability compared to anaerobic digestion at room temperature.[29] Therefore, all experiments were carried out under anaerobic conditions at 35 °C for 32 days. During incubation, liquid samples were routinely collected from each reactor, immediately centrifuged at 8000 rpm for 10 min, and filtered through a 0.22 μm cellulosic membrane for chemical analyses. Sludge samples were withdrawn from each assay at the end of each experiment for the determination of arsenic concentrations and speciation.

Analytical Methods. Concentrations of total arsenic and individual arsenic species were determined by liquid chromatography-hydride generation-atomic fluorescence spectrometry (HPLC-HG-AFS, AFS-8200, Beijing Titan) following previously reported methods.[11,17] The detection limits for total arsenic, inorganic arsenic, and organoarsenicals were 0.01, 0.05, and 1.0 μM, respectively. Arsenic contained in the sludge was extracted using sequential extraction to determine the amount of adsorbed arsenic (As_{ads}).[17] The sequential extraction method included four steps, each with a specific reagent: (i) a phosphate solution (ii) a phosphate solution + ultrasonic treatment (iii) an alkaline protease, and (iv) sodium dodecyl sulfate. After sequential extraction, the recalcitrant arsenic that remained in the sludge was characterized using an X-ray photoelectron spectroscope (XPS, ESCALAB 250Xi, Thermo Scientific) and X-ray diffraction (XRD, X'Pert PRO MPD) with Cu Kα radiation (λ = 1.5406 Å) to determine the occurrence of precipitated arsenic (As_{pre}). Arsenic adsorbed on the electrodes was extracted three times using the phosphate solution + ultrasonic treatment mentioned above, and the extracts were combined for the determination of arsenic concentrations. The electrode potential was measured using a data acquisition system (2700 multimeter, Keithley) connected with the reference electrode.

Sulfate concentrations were determined using a modified turbidimetric method.[30] Dissolved sulfide concentrations were determined using a methylene blue spectrophotometric method with a detection limit of 0.2 μM.[31] Significant differences were assessed by the analysis of variance at a significance level of 0.05 using Origin 9.0.

RESULTS AND DISCUSSION

Enhanced ROX Degradation in the Sulfate-Mediated BES. *Effect of Sulfate Amendment.* The role of microbes on ROX degradation was investigated by comparing assays 1−3. In assays 1 and 2 with inoculums, ROX rapidly degraded into HAPA, whereas in assay 3 without inoculums, ROX persisted in its original form over the whole experimental period (Figure 1a). Therefore, it is evident that ROX degradation was microbially mediated.[32] The effect of sulfate on ROX degradation in the absence of any voltage was assessed by comparing assay 1 without sulfate and assay 2 with sulfate. In both assays, ~99% of ROX degraded into HAPA within 9 days, indicating that sulfate amendment had no obvious influence on reductive transformation of the ROX's nitro group. With the

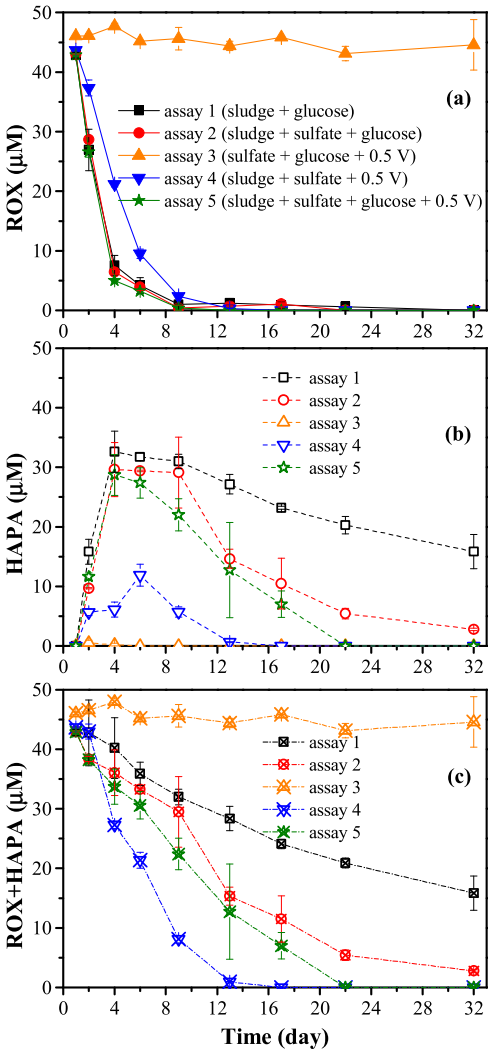

Figure 1. ROX degradation in assay 1 (with 500 mg L^{-1} glucose and without sulfate and voltage application), assay 2 (with 350 μM sulfate and 500 mg L^{-1} glucose and without voltage application), assay 3 (without sludge), assay 4 (with 0.5 V voltage and 350 μM sulfate and without glucose), and assay 5 (with 350 μM sulfate, 500 mg L^{-1} glucose, and 0.5 V voltage).

degradation of ROX, HAPA reached a maximum concentration of 32.6 and 29.6 μM on day 4 and then decreased to 15.8 and 2.8 μM, respectively, in assays 1 and 2. The organoarsenic (including ROX and HAPA) removal in assay 2 with sulfate amendment eventually reached 95%, >25% higher than that in assay 1 without sulfate amendment (68%, Figure 1c). HAPA degradation followed first-order reaction kinetics, and the HAPA degradation rate in assay 2 was 0.10 d^{-1}, 2.3 times higher compared to assay 1 (0.03 d^{-1}, Table S3). This indicates that sulfate amendment stimulated HAPA degradation, which was the rate-limiting step for anaerobic biotransformation of ROX to inorganic arsenic, especially in the presence of labile organic substances.[12,20] Heterotrophic sulfate-reducing bacteria (SRB) can utilize various organics including aromatic compounds as electron donors and carbon sources.[33] HAPA, as aromatic arsenic, could also be utilized under sulfate-reducing conditions, which would explain the enhanced HAPA degradation.

To confirm this hypothesis, a series of sulfate reduction experiments with different electron donors were performed

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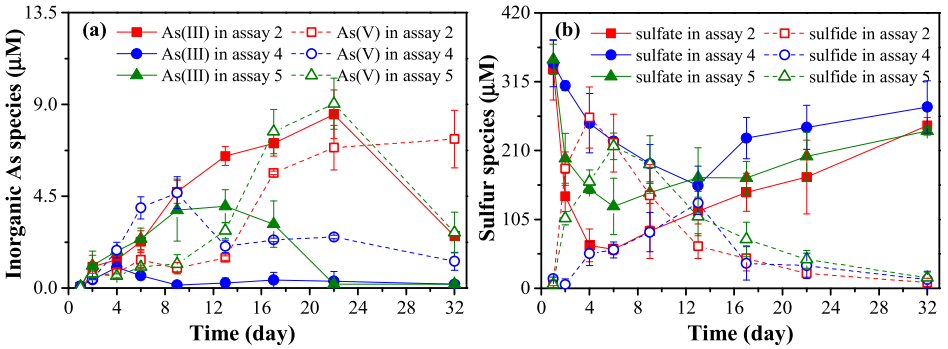

Figure 2. (a) Inorganic arsenic transformation and (b) sulfate reduction in assay 2 (without voltage), assay 4 (with 0.5 V voltage and without glucose), and assay 5 (with 0.5 V voltage and 500 mg L^{-1} glucose).

(Figure S2). When HAPA was used as the sole electron donor, the sulfate reduction rate was 38.0 μmol g^{-1} VS d^{-1}, which was >3 times of that when no electron donor was supplied (11.6 μmol g^{-1} VS d^{-1}). Meanwhile, the HAPA degradation rate was enhanced by 133.3% when sulfate was amended (Figure S2a). This demonstrated that the enhanced HAPA degradation can be ascribed to the utilization of HAPA as the electron donor for sulfate reduction.

Combined Effect of Sulfate Amendment and Voltage Application. The combined effect of voltage application and sulfate amendment on ROX degradation was investigated by comparing assay 5 applied with a voltage of 0.5 V and voltage-free assays 1 and 2. All ROX was converted to HAPA within 9 days in the three assays with comparable reduction rates of ~0.5 d^{-1} (Table S2), indicating that neither sulfate amendment nor voltage application exerted any obvious impacts on the reduction of ROX's nitro group. However, HAPA was completely exhausted on day 22 in assay 5, at least 10 days faster compared to the voltage-free assays (assays 1 and 2) (Figure 1). The HAPA degradation rate in assay 5 (0.14 d^{-1}) was accelerated by 367% compared to assay 1 (Table S3), demonstrating that the C−As bond cleavage of HAPA was further enhanced under combined functions of sulfate amendment and voltage application. The application of higher voltages exhibited an increasing capacity for decomposing HAPA (Figure S3a). These results indicate that the voltage application accelerated extracellular electron transfer for microbial metabolism.[19] HAPA functioned as an alternative electron donor for sulfate reduction; therefore, its degradation was enhanced. Note that HAPA degraded completely within 22 days in the sulfate-mediated BESs regardless of the presence of glucose (assay 5) or not (assay 4). Our previous work[20] found that HAPA was resistant to degradation in the presence of 3810 mg L^{-1} acetate in the sulfate-free BES applied with the same voltage. This suggests that sulfate-reducing conditions effectively mitigate the inhibitory effects of organic substances on HAPA degradation.

When the sulfate-mediated BES, supplied with a voltage of 0.5 V, was applied to an environmental sample of swine wastewater spiked with 50 μM ROX, 34.8% higher arsenic removal efficiency and 107% higher As_{pre} fraction in the sludge were achieved compared to only anaerobic digestion (Figure S4a and b). Simultaneously, COD removal was also enhanced using the sulfate-mediated BES (Figure S4c). These results confirmed that the sulfate-mediated BES is a promising approach for treating organoarsenic-contaminated livestock wastewater. Notably, the sulfate-mediated BES applied with a voltage of 0.5 V was less efficient for the treatment of the environmental wastewater compared to the synthetic wastewater. This suggests that some aqueous species present only in the swine wastewater exerted inhibitory impacts on both organoarsenic degradation and arsenic precipitation, which need to be identified and comprehensively evaluated in our future work.

***In Situ* Arsenic Immobilization in the Sulfate-Mediated BES.** *Transformation of Inorganic Arsenic and Sulfate in Solution.* The dissolved inorganic arsenic and sulfur species were monitored during the experiment. In the two sulfate-mediated BESs (assays 4 and 5), inorganic arsenic was gradually released due to HAPA degradation, reaching a maximum concentration of 4.8 μM on day 9 in assay 4 and of 10.8 μM on day 17 in assay 5 (Figure 2a). The inorganic arsenic concentrations in both assays were much lower than the observed maximum in the corresponding voltage-free assay (assay 2, 15.4 μM). On day 32, inorganic arsenic concentrations eventually declined to 1.5 μM in assay 4 and to 2.9 μM in assay 5, both concentrations being much lower compared to assay 2 (9.9 μM, Figure 2a). This demonstrates the superior performance of the sulfate-mediated BES in removing inorganic arsenic from solution. Additionally, As(III) was dominating in assay 2, while it remained at a significantly lower concentration and was easily oxidized to As(V) in the sulfate-mediated BES (assay 4 and 5). The facile oxidation of As(III) to As(V) in assays 4 and 5 could be caused by the high potentials of the anode (+(0.39−0.97) V vs NHE, Figure S5a). de Matos et al.[34] reported that ~50 μM As(III) exerted an adverse influence on the growth and metabolism of SRB. As(V) was shown to be up to 60 times less toxic than As(III).[16] Therefore, the dominance of As(V) in the sulfate-mediated BES could have created more favorable conditions for sulfate reduction and the associated sulfide-driven arsenic immobilization.

Aqueous sulfur speciation further clarified the interactions between sulfate and arsenic. In the voltage-free assay (assay 2), sulfate was rapidly reduced to sulfide, which reached a maximum concentration of 260.4 μM within 4 days (Figure 2b). At the time, >75% of the arsenic persisted as ROX or HAPA (Figure 1b), which could not react with sulfide to form any precipitates.[28] In the sulfate-mediated BESs (assays 4 and 5), microbial sulfate reduction was decelerated. Under the electrochemical stimulation, the anode potentials remained + (0.39−0.97) V versus NHE (Figure S5a), exceeding the optimal ORP range for microbial sulfate reduction.[33] The high potential caused an increase of *Thiobacillus* and *Sulfurisoma*

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abundance from 0.07 to 0.91−2.81% and from 0.03 to 0.40−0.64%, respectively (Figure S6b). The two gena were responsible for the reoxidization of sulfide.[26] These explained the deceleration of sulfate reduction in the sulfate-mediated BES. The deceleration of sulfate reduction sustained the availability of sulfide for the formation of arsenic-bearing sulfides. After the 32-day experiment, total observed sulfur losses ranged from 67.5 to 98.9 μM. Given the corresponding decrease in inorganic arsenic concentrations (Figure 2a), we hypothesize that a fraction of the sulfide coprecipitated with arsenic.

Previous studies have shown that As(V) reduction is expected to proceed preferentially according to the comparison of the redox pair of As(V)/As(III) and SO_4^{2-}/H_2S.[28] This phenomenon was also observed in assay 2 without voltage application, whereas sulfate reduction appeared to proceed first in the single-cell BES (assays 4 and 5). This was mainly attributed to the difference in the microbial community between these assays. A higher abundance of *Desulfobulbus* was observed in the sulfate-mediated BES compared to assay 2 (Figure S6). This As(V)-respiring SRB preferred to reduce sulfate first and then As(V).[28]

Arsenic Mass Balance. The arsenic mass balance was evaluated to determine the fate of arsenic in each reactor (Figure 3a). The initial amount of ROX in each assay was 15.0 μmol. In the voltage-free assay without sulfate (assay 1), 55.7% of arsenic remained in solution and only 34.1% was immobilized in the sludge. Compared to assay 1, arsenic immobilization was enhanced in the voltage-free assay with sulfate (assay 2), in which arsenic in solution and sludge accounted for 27.4 and 57.3% of the total arsenic, respectively. This demonstrates the stimulatory role of sulfate amendment on arsenic immobilization. In the sulfate-mediated BESs (assays 4 and 5), only 3.1−9.8% of the initially amended arsenic remained in solution, while 75.0−83.2% was immobilized in the sludge. This indicates that arsenic immobilization was further enhanced when sulfate amendment and voltage application were combined.

Several previous studies indicated that arsenic could be immobilized through adsorption by sludge and coprecipitation with other ions.[17,35] To better understand the mechanism underlying the enhanced arsenic immobilization in the sulfate-mediated BES, As_{ads} and As_{pre} in the sludge were determined using a sequential extraction protocol[17] (Figure 3a). There was little difference in As_{ads} (accounting for 23.5−27.7% of the total arsenic) among all assays whether the sulfate or voltage was applied or not. Interactions between arsenic and surface functional groups of sludge are considered as the primary mechanisms for arsenic adsorption.[36] Our previous study found that the adsorption of arsenic was negatively impacted by increasing pH, ionic strength, and phosphate ions.[35] Given that the same mass of inoculum and the almost identical synthetic wastewater were employed in each experiment, its capacity for adsorption should be similar. In contrast, the influence of both sulfate amendment and voltage application on arsenic precipitation was much more pronounced: In assay 2 (with sulfate amendment), As_{pre} was up to ∼30% of the total arsenic, i.e., four times of that in assay 1 (without sulfate, 7.1%). This indicates the stimulatory role of sulfate amendment on arsenic precipitation, most likely caused by the formation of arsenic-bearing sulfides.[15,28] In the sulfate-mediated BESs (assays 4 and 5), As_{pre} accounted for 51.5−58.9% of the total arsenic, i.e., ∼8 times of that in assay 1.

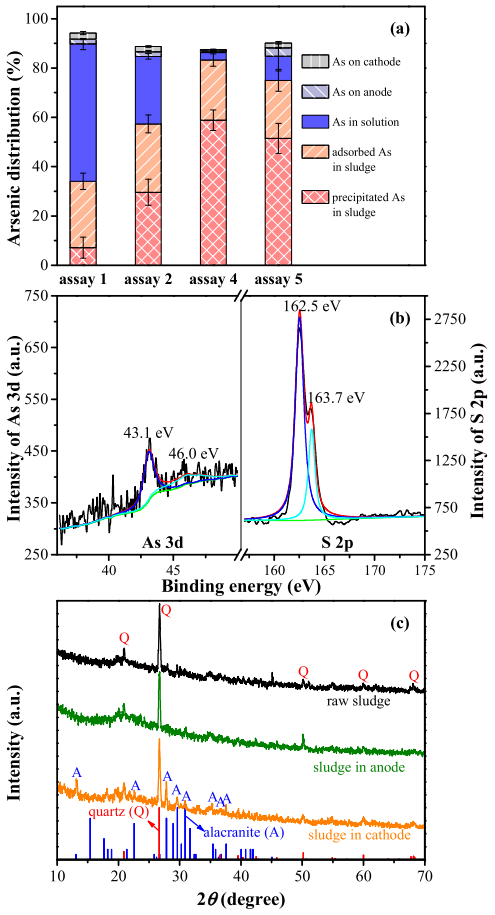

Figure 3. (a) Arsenic distribution in assay 1 (without sulfate and voltage application), assay 2 (with 350 μM sulfate and without voltage application), assay 4 (with 0.5 V voltage and without glucose), and assay 5 (with 500 mg L^{-1} glucose and 0.5 V voltage) after 32 days, (b) XPS spectrum, and (c) XRD pattern for precipitated arsenic species in the sludge.

Arsenic precipitation was therefore further enhanced by the combination of sulfate amendment and voltage application presumably because the applied voltage accelerated the extracellular electron transfer for sulfide-driven arsenic precipitation.[19]

Characterization of Precipitated Arsenic. To identify the specific mineral phase(s) that sequestered arsenic in the sulfate-mediated BES, the residual sludge samples after the sequential extraction were characterized using XPS and XRD. However, both methods failed to identify any As-bearing mineral in the raw sludge or the sludge from single-cell BES, most likely due to the low content of As_{pre} in the samples. For the sludge in the cathode chamber of the double-cell BES, the XPS spectrum displays an As 3d peak at a binding energy of 43.1 eV, corresponding to As−S binding energy,[37] and two S 2p peaks at 162.5 and 163.7 eV, corresponding to S−As and S−S binding energy,[38] respectively (Figure 3b). This indicated the occurrence of realgar/realgar-like minerals in the cathode chamber. The XRD pattern of the sample in the cathode chamber shows peaks at 2θ values of 13.2, 22.7, and 27.9°, which is in good agreement with the standard XRD pattern of alacranite (PDF#25−0057), a realgar-like mineral, while the XRD pattern of the sample in the anode chamber remained almost identical with that of the raw sludge (Figure 3c). This was probably associated with a lower potential in the cathode

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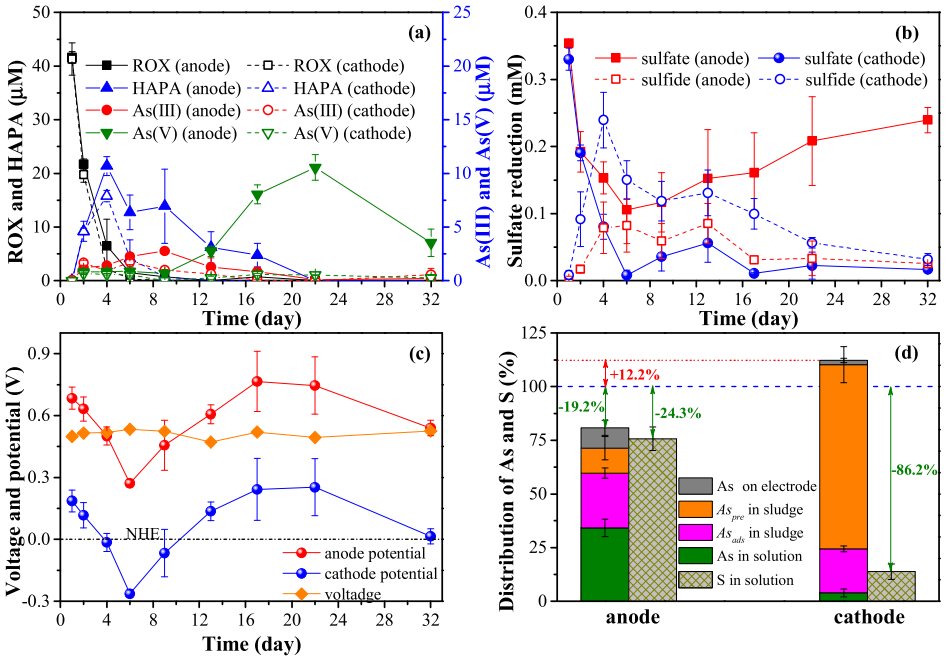

Figure 4. (a) ROX degradation, (b) sulfate reduction, (c) potential change over the course of the whole experiment, and (d) distribution of arsenic and sulfur at the end of the experiment in the double-cell sulfate-mediated BES with 0.5 V voltage application.

chamber, which favored microbial sulfate reduction.[33] Thus, sulfide-driven arsenic precipitation prominently occurred in the cathode chamber, allowing it to be identified via XRD. The full width at the half-maximum (FWHM) value of alacranite (0.29°) at a 2θ of 29.7° was close to that of quartz, a highly crystalline mineral.[21] The Scherrer domain size of alacranite was calculated at 26 nm using the Scherrer equation (eq S1).[39] Therefore, the alacranite identified was crystalline. Alacranite was previously reported to be produced by an As(V)-respiring microbe, strain MPA-C3, under mesophilic anaerobic conditions.[40] In this process, the ArrA-catalyzed reduction of As(V) to As(III) plays an important role. Given that the *arrA* gene of strain MPA-C3 is highly homologous (99% BLAST identity) with that of a *Desulfitobacterium* species,[40] a *Desulfobacteraceae* genus identified mainly in the cathode (the relative abundance of 2.1 and 0.4% in the cathode and anode chambers, respectively, Figure S6a) might be responsible for the formation of alacranite in this study.

Mechanism for Enhanced ROX Degradation and Arsenic Immobilization. To better understand the mechanisms underlying ROX degradation and arsenic immobilization in the sulfate-mediated BES, the fate of ROX in the anode and cathode chambers of the double-cell BES was investigated separately (Figure 4a). The results showed that the rates for ROX reduction (0.87 d^{-1}) and HAPA degradation (0.63 d^{-1}) in the cathode chamber were 1.6 and 4.8 times of those in the anode chamber (Tables S2 and S3). According to previous literature,[32,41] As(III) is the direct product of the C−As bond cleavage of organoarsenicals. Therefore, both ROX reduction and HAPA degradation are electron-consuming processes. The electrons required partially originated from the anaerobic digestion of the amended or endogenous organic substrates.[20] The lower cathode potentials (between −0.26 and +0.25 V vs NHE, Figure 4c) created more favorable conditions for anaerobic digestion compared to the anode potential (+(0.27−0.77) V vs NHE),[42] thereby enhancing ROX's reduction and C−As bond cleavage within the cathode chamber. On the other hand, the low cathode potential allowed the enrichment of SRB, e.g., *Desulfomicrobium*, *Desulfobulbus*, and Desulfobacteraceae (Figure S6a). These heterotrophic SRB can utilize a wide variety of organic substrates, including glucose, volatile fatty acids (VFAs), and aromatic compounds.[43,44] HAPA, as an aromatic arsenical compound, could also be utilized by SRB, further enhancing the HAPA degradation. This explains why HAPA degradation was accompanied by sulfate reduction over the first 6 days (Figure 4a,b).

Previous studies[20,32] indicated that inorganic arsenic and o-aminophenol were generated simultaneously accompanied by the degradation of HAPA through a microbial process under anaerobic conditions. Similar results were obtained in our study, where As(III) was produced with the degradation of HAPA. The generated As(III) concentrations peaked on day 9 in the anode chamber and on day 6 in the cathode chamber. It then decreased to below the detection limit in both chambers (Figure 4a). In the anode chamber, As(III) depletion was accompanied by an increase in As(V) concentrations after day 9, indicating the oxidation of As(III) to As(V). Using the Nernst equation, the ORP required for the oxidation of As(III) to As(V) was between +0.54 V and +0.62 V versus NHE. The anode potential of up to +0.77 V versus NHE therefore enabled the As(III) oxidation. In the anode chamber, the ORP decreased rapidly and remained low during days 0−9, since the amended glucose and the generated VFAs provided a stable electron source.[45] After 9 days, the labile carbon substrates were exhausted causing the potential to gradually increase and remain at >0.50 V versus NHE for the remaining duration of the experiment. This allowed the anode to function as an electron acceptor for As(III) oxidation.[46] In the cathode chamber, As(III) was depleted without any other dissolved arsenic species generated, and the dissolved sulfide kept being consumed simultaneously (Figure 4b). Further, the precipitation of alacranite was detected in the cathode chamber

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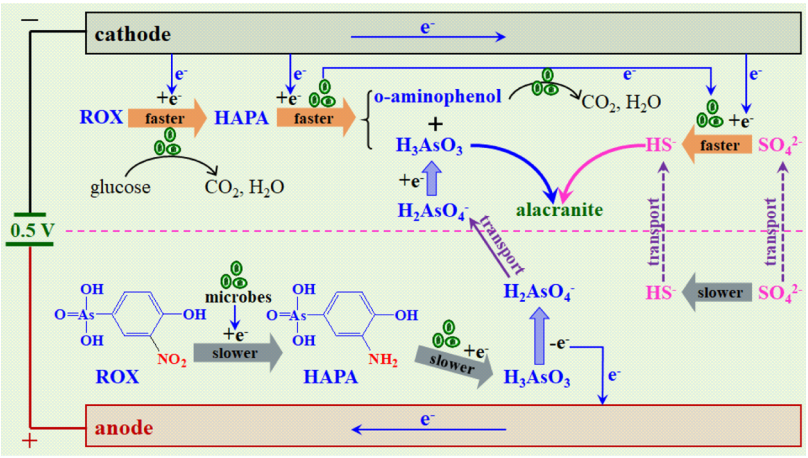

Figure 5. Mechanism for ROX degradation and arsenic immobilization in the sulfate-mediated BES.

(Figure 3c). These results confirmed the occurrence of sulfide-driven arsenic precipitation in the cathode chamber.

To reveal the transport of arsenic and sulfur between both chambers, their mass balance was evaluated (Figure 4d). The total amount of arsenic was initially the same, i.e., 6.0 μmol, in each chamber. After the 32-day experiment, total arsenic decreased by 19.2% in the anode chamber and increased by 12.2% in the cathode chamber, indicating the migration of arsenic from the anode to the cathode chamber. Given the existence of an anion-exchange membrane, only strongly polar anions (e.g., SO_4^{2-}, HS^-, and negatively charged inorganic arsenic) could diffuse freely. In both chambers, pH was between 5.0 and 6.5 (Figure S7), and therefore, the dominant forms for As(III) and As(V) were H_3AsO_3 and $H_2AsO_4^-$, respectively.[47] Only the negatively charged, As(V) could pass through the membrane. As_{pre} in the cathode chamber accounted for 85.8% of the initial arsenic, which was >7 times of that in the anode chamber (11.7%). Total sulfur in the solution decreased by 86.2% in the cathode chamber and by 24.3% in the anode chamber. This confirms that sulfide-driven arsenic precipitation principally occurred in the cathode chamber. Based on these results, we propose a conceptual model that describes the mechanism for enhanced ROX degradation and arsenic immobilization in the sulfate-mediated BES (Figure 5).

Environmental Implications. This study demonstrated that the sulfate amendment and voltage application had synergistic effects, which simultaneously enhanced organo-arsenic transformation to inorganic arsenic and *in situ* arsenic precipitation. The sulfate-mediated BES also effectively mitigated the negative effects of labile organic matter on organoarsenic degradation,[20] improving its adaptability in treating practical manure/wastewater. Therefore, the sulfate-mediated BES can potentially be an effective technique to treat organoarsenic-contaminated manure/wastewater for many countries worldwide,[2,5] where organoarsenic feed additives are currently in use.

Our proposed conceptual model comprises multiple processes responsible for ROX degradation and arsenic immobilization. A refined quantification of the contribution of each process will be helpful to better understand the relative importance of each mechanism and how to optimize the design and operation of the sulfate-mediated BES. For example, the utilization of HAPA as the electron donor for sulfate reduction plays an important role, as it determines not only the HAPA degradation rate but also the extent of sulfide-driven arsenic precipitation in the sulfate-mediated BES. However, HAPA is not an exclusive electron donor in these systems as numerous other organic substrates, e.g., glucose and VFAs, can also be utilized by SRB,[43,44] which will affect the efficiency of inorganic arsenic removal. Experimentally quantifying the contribution of each process, i.e., the utilization rate of HAPA or other organic substrates by SRB, is challenging. Therefore, geochemical modeling[48−51] will be an important complementary tool for a more rigorous interpretation of the collected biochemical data.

Additionally, sulfate-driven arsenic precipitation reduced the mobility and bioaccessibility of inorganic arsenic and consequently preserved microbial communities responsible for wastewater decontamination. The stability of arsenic-bearing sulfides is largely dependent on environmental conditions.[52] Under low-redox conditions, e.g., the cathodic condition in our BES, arsenic-bearing sulfides remain very stable.[22,28] However, fluctuating environmental conditions occur during sludge disposal, e.g., Fe^{3+} concentration, redox, and pH, which may mobilize As_{pre}.[16,52] Therefore, further studies to evaluate the risk of arsenic release when the sludge-containing As_{pre} is disposed of are needed.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.0c06781.

Details of other characteristics of the wastewater and inoculums, supplementary analytical, and calculation methods; four tables showing the regulatory status of ROX as feed additives worldwide and kinetics for ROX and HAPA degradation; seven figures illustrating BES reactor construction, role of HAPA on the sulfate-reducing process, effects of organic matter and voltage on ROX degradation, performance of the sulfate-mediated BES treating real swine wastewater, variation of SRB and sulfur-oxidizing bacteria abundance, and variation of pH and potentials (PDF)

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Notes

The authors declare no competing financial interest.

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