**Arsenic accumulation and volatilization in a 260-day cultured Upflow Anaerobic
Sludge Blanket (UASB) reactor**

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Abstract

Roxarsone has been extensively used as an organoarsenic feed additive. Part of supplemented roxarsone was discharged into wastewater. UASB reactor has been widely applied to treat high concentration of organics wastewater. However, there was little report about the arsenic accumulation from organoarsenic biotransformation in an UASB reactor. In this study, the accumulation and volatilization of arsenic in a 260-day cultured UASB reactor spiked with 13.2 mg/L roxarsone were investigated. In the anaerobic granular sludge (AGS), the total arsenic concentrations were $5,276.90 \pm 366.12$ mg/kg AGS at the bottom of anaerobic sludge bed and $10,661.92 \pm 539.63$ mg/kg AGS at the top of anaerobic sludge bed. Nearly 50% arsenic in the AGS was extracted under acidic conditions, and the main specie of the extracted arsenic was arsenite (As(III)). In the aqueous phases, roxarsone was rapidly transformed into 4-hydroxy-3-aminophenylarsonic acid (HAPA) and unknown organoarsenic, which was further converted to inorganic arsenic and monomethylarsenate (MMA(V)), in which As(III) was also the main species. Volatile arsenic from the reactor spiked with roxarsone was released at a rate of 134.93 ± 6.61 ng/d. Based on the mass balance analysis of arsenic, 23.40% of the influent arsenic was accumulated in the AGS and 0.002% was released in the form of volatile arsenic. The results from this study indicated that the operation of anaerobic digestion reactor treating organoarsenic-contaminated wastewater and the disposal of the residual AGS should be well managed.

Keywords: Accumulation; Anaerobic digestion; Organoarsenic; Roxarsone; UASB reactor; Volatile arsenic.

1. Introduction

Roxarsone (4-hydroxy-3-nitrophenylarsonic acid), as an organoarsenic additive, has been extensively used in the feeds of broiler chickens and swine to promote growth and feed efficiency, and to inhibit coccidial parasites [1, 2]. The common dosages of roxarsone are in the range of 20-50 mg/kg for poultry feed and of 50-100 mg/kg for swine feed [3]. Most of supplemented roxarsone is excreted into feces [4], which is further discharged into wastewater through washing or enter into soil through manure land application. Roxarsone is further converted to organic or inorganic arsenic compounds in the environment, resulting in anthropogenic arsenic contamination in the soil and surface water [2, 5], causing health risk to human.

Upflow anaerobic sludge blanket (UASB) reactor has been often used for the treatment of wastewater from animal farms [6, 7]. Under anaerobic conditions, roxarsone is firstly transformed to 4-hydroxy-3-aminophenylarsonic acid (HAPA) through the reduction of nitro group [8-10]. HAPA is further slowly converted into arsenate (As(V)) and arsenite (As(III)), and As(III) is the main inorganic arsenic species [11]. Although roxarsone has low toxicity, its degradation products As(III) and As(V) are more toxic than itself [12]. Our previous batch experiment showed that HAPA was the main product from roxarsone degradation after 16-day incubation, and was completely transformed to As(III) and As(V) after 240-day incubation [13]. The degradation of many pharmaceuticals such as amoxicillin and p-acetylsulfanilyl chloride and para-ester has been reported in the UASB reactors [14, 15]. However, the

1 information about the biotransformation of roxarsone in continuously operated UASB
2 reactors is still very limited.

3 Inorganic arsenic was easily accumulated in natural environments. It has been
4 reported that apparently 12% and 40% of influent arsenic was accumulated in
5 suspended solids and sediment, respectively [16]. In batch anaerobic digestion, our
6 previous study found that 56.7% of arsenic was accumulated in anaerobic granular
7 sludge (AGS) [13]. High arsenic concentration is toxic to microorganisms. For
8 example, methanogenesis and H₂S production was severely inhibited in salt marsh
9 sediments [17], and oxidative stress was induced in clams [18]. In addition, the
10 analysis of arsenic distribution and species can explain the inhibition from roxarsone
11 degradation. However, there were few reports about the arsenic accumulation in
12 continuously operated UASB reactor treating organoarsenic-contaminated wastewater.

13 Arsenic volatilization in natural environment is a vital part in the global arsenic
14 cycle, which could mitigate the toxicity of arsenic and achieve the bioremediation of
15 arsenic-contaminated water and soil [13, 19]. Methylation of arsenic, such as
16 monomethylarsine (MMA), dimethylarsine (DMA) and trimethylarsine (TMA), can
17 finally form volatile arsenic under anaerobic environments [20]. Soluble organic
18 matter in aqueous solution significantly affected the volatilization of arsenic [21].
19 UASB reactor is rich in soluble organic matter and operated under anaerobic
20 conditions. Therefore, arsenic volatilization might be occurred in the UASB reactor
21 treating organoarsenic-contaminated wastewater, which will be dangerous for the
22 operating workers. However, up to now, there is no information on the arsenic

1 volatilization in UASB reactors.

2 The aims of this study were to, (a) investigate the accumulation and distribution
3 of arsenic in the UASB reactor, (b) analyze the degradation of roxarsone and the
4 distribution of its metabolites in the anaerobic system, (c) and explore the arsenic
5 volatilization in the UASB reactor. The results from this research would improve the
6 management of anaerobic digestion system treating organoarsenic-contaminated
7 manure and wastewater.

9 **2. Materials and Methods**

10 *2.1 Synthetic wastewater*

11 Synthetic wastewater was used in this study, which was composed of the
12 following ingredients (mg/L in unit): Sucrose (1000.0), NH_4Cl (107.5), NaHCO_3
13 (1500.0), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (42.5), KH_2PO_4 (22.5), CaCl_2 (13.0), $\text{NiSO}_4 \cdot 7\text{H}_2\text{O}$ (5.3),
14 $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (4.1), $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (1.1), ZnCl_2 (0.6), $\text{CoCl}_2 \cdot 2\text{H}_2\text{O}$ (0.6) and $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$
15 (0.2). Additionally, 13.2 mg/L of roxarsone was added in the synthetic wastewater to
16 simulate livestock wastewater [13].

17 *2.2 Sampling and reactor operation*

18 Two identical UASB reactors, which were made of plexiglass cylinder with a
19 working volume of 3.5 L and an internal diameter of 100 mm, were used in this study,
20 as shown in Fig. 1. Seven sampling points (point a-g, as shown in Fig. 1) were set to
21 collect aqueous samples along the flow direction in both reactors for one week. AGS

1 samples were separately collected at the bottom (A) and top (B) of the anaerobic
2 sludge bed. The characteristics of suspended solids (SS) and volatile suspended solids
3 (VSS) of these sludge samples were listed in Table 1.

4 The reactor without roxarsone addition was set as the control reactor and the
5 other with continuous roxarsone addition of 13.2 mg/L was used to investigate the
6 accumulation and volatilization of arsenic. Both UASB reactors were operated at $35 \pm$
7 1°C for 260 days. The operation parameter and biogas production of both reactors
8 were listed in Table 2. The reactor with roxarsone addition was operated at lower pH
9 (6.82 ± 0.22) than the reactor without roxarsone addition. Methanogenesis was
10 seriously inhibited in the reactor with roxarsone addition, as listed in Table 2.

12 *2.3 Samples preparation*

13 **Liquid samples.** After collection, liquid samples were immediately centrifuged
14 at 8,000 rpm for 10 min, and the supernatant was filtered through 0.22 μm of
15 cellulosic membrane for the analysis of arsenic species. Then 1 mL of the filtrate was
16 further digested with diluted aqua regia consisting of HNO_3 , HCl , and deionized water
17 (1:3:4, v/v/v) at 100°C for 2 hours for the analysis of total arsenic.

18 **AGS samples.** 0.20 g of AGS was digested with 15 mL diluted aqua regia
19 consisting of HNO_3 , HCl , and deionized water (1:3:4, v/v/v) at 100°C for 2.5 hours.
20 The digested solution was reduced by the reductant composed of 1% (w/v) thiourea
21 and 5% (v/v) HCl for 30 min for the analysis of total arsenic in the AGS.

22 **Extraction of extracellular arsenic in the AGS.** Extracting solution was

1 composed of 20.00 g/L dipotassium hydrogen phosphate and phosphoric acid at a
2 ratio of 200:3 (v/v). Ten mL of extraction solution and 0.50 g of AGS were added to a
3 50-mL centrifuge tube, and then oscillated for 16 hours at 40°C. This extraction
4 process was performed three times. The extraction solution was combined together
5 and prepared as liquid samples for the analysis of arsenic species in extracellular AGS.
6 The residual AGS was further used for the extraction of intracellular arsenic.

7 **Extraction of intracellular arsenic in the AGS.** The residual AGS was further
8 disrupted by an ultrasonic cell disruptor (VCX150, SONICS Ltd, America) and the
9 same method as described in the extraction of extracellular arsenic was used to extract
10 intracellular arsenic. The extraction solution was prepared for the analysis of
11 intracellular arsenic species. The residue was collected for the further measurement of
12 insoluble arsenic in AGS.

13 **Insoluble arsenic in residue.** The residue was further digested in 10 mL of
14 diluted aqua regia at 100°C for 2.5 hours to measure the insoluble residual arsenic in
15 the AGS.

16 **Trapping of volatile arsenic.** For detecting the volatile arsenic, a trapping tube
17 was developed based on the method as described in previous literature [22]. In brief,
18 silica gel beads were impregnated with 10% AgNO₃ (w/v) for 2 h, and then placed in
19 an oven overnight at 160°C to remove H₂O. The tube containing AgNO₃-impregnated
20 silica gel beads was connected to the exhaust pipe of the reactor, and biogas was
21 pumped to the tube. Volatile arsenic was stabilized in silica gel by AgNO₃. A tube
22 without trapping volatile arsenic was set as the control to determine the background

values in the silica gel beads, AgNO_3 , and silicone rubber pipes. After trapping, volatile arsenic was eluted by 2% HNO_3 (v/v). The elution liquid was used to determine total volatile arsenic concentration.

2.4 Analytical methods

Morphology analysis of AgNO_3 -impregnated silica gel beads was conducted with SEM imaging (Sirion 200, FEI, USA). The concentration of total arsenic was measured using atomic fluorescence spectrometry (AFS-8220, Titan Beijing Ltd., China) with the mixed solution of 20.00 g/L KBH_4 and 5.00 g/L KOH as reductant, 5% HCl (v/v) as current-carrying solution, and argon as the carrier gas.

The concentrations of roxarsone and HAPA were detected by high performance liquid chromatography (HPLC, LC-20AD, Shimadzu Ltd., Japan) with detection wavelength at 260 nm. A C18 column (4.6 mm $\times$ 150 mm, 5 μm , Agilent Technologies, Japan) was applied for the separation of roxarsone, HAPA, and other organic arsenics. The mobile phase consisted of 50 mmol/L KH_2PO_4 , methanol and 10% (v/v) formic acid (90:10:0.1, v/v/v) with a flow rate of 1.0 mL/min.

Inorganic arsenic species were determined using a high performance liquid chromatography - hydride generation - atomic fluorescence spectrometer (HPLC-HG-AFS, Titan Beijing Ltd., China) with 15.00 mmol/L $(\text{NH}_4)_2\text{HPO}_4$ as the mobile phase (pH=6.0, adjusted with 10% (v/v) formic acid) at a flow rate of 1.0 mL/min. The arsenic species were separated with an anion-exchange column (250 $\times$ 4.1 mm, Hamilton PRP X-100) at 30°C. The reductant was composed of 20.00 g/L

1 KBH₄ and 5.00 g/L KOH, and the current-carrying solution was 7% (v/v) HCl. The
2 flow rates of the reductant and the current-carrying solution were 4.0 mL/min and 7.0
3 mL/min, respectively.
4

5 **3. Results and Discussion**

6 *3.1 Arsenic accumulation in AGS*

7 As shown in Fig. 2, the total arsenic concentrations in the AGS were $5,276.90 \pm$
8 366.12 mg/kg at the bottom of anaerobic sludge bed (A₁) and $10,661.92 \pm 539.63$
9 mg/kg at the top of anaerobic sludge bed (B₁) in the reactor with roxarsone addition,
10 which were three magnitudes higher than that in the reactor without roxarsone
11 addition ($p < 0.05$), which indicated that roxarsone in the influent caused obvious
12 arsenic accumulation in the AGS. Sever inhibition of methanogenesis occurred, as
13 shown in Table 2, because of high arsenic concentration in AGS. That indicated that
14 sludge treating arsenic-contaminated wastewater would be dangerous when it was
15 disposed of.

16 Insoluble arsenic concentrations, which were measured in the AGS at the
17 bottom (A₁) and top (B₁) of anaerobic sludge bed in the reactor with roxarsone
18 addition, were $2,486.99 \pm 59.30$ mg/kg and $6,378.40 \pm 123.78$ mg/kg, respectively.
19 Nearly half of total arsenic in the AGS in the reactor with roxarsone addition was
20 insoluble. The concentrations of arsenic in the AGS observed in the reactor without
21 roxarsone addition ranged from 8.38 ± 0.32 mg/kg to 13.64 ± 1.35 mg/kg, which

1 might come from tap-water and the background content of the AGS. Insoluble arsenic
2 concentrations in the reactor without roxarsone addition were 2.25 ± 0.12 mg/kg and
3 2.80 ± 0.15 mg/kg at the bottom (A_0) and top (B_0) of anaerobic sludge bed,
4 respectively, indicating that most of arsenic (73.13% - 79.45%) in the AGS in the
5 reactor without roxarsone addition was soluble under acidic conditions.

6 It is interesting that insoluble arsenic in the AGS at the top of anaerobic sludge
7 bed was more than that at the bottom in both reactors. That might be attributed to the
8 difference of arsenic species in aqueous phases and liquid retention time. The main
9 arsenic species at the bottom of anaerobic sludge bed (A_1) in the reactor with
10 roxarsone addition was organic arsenic, whereas that at the top of the anaerobic
11 sludge bed (B_1) was inorganic arsenic. Inorganic arsenic is easy to precipitate with
12 iron ions [23] and sulfur ions [24, 25]. In addition, the retention time of wastewater in
13 the inner was longer than that at the bottom because of the shape of the reactor, which
14 resulted in more reaction time for arsenic with other ions in the inner part of the
15 reactor.

16 Fig. 3 shows the distribution of acidic soluble arsenic species in the AGS in both
17 reactors. In the reactor without roxarsone addition, although 53.33% of acidic soluble
18 arsenic was As(III), total arsenic concentration was very low. Therefore, microbial
19 activity was not affected. Nevertheless, in the reactor with roxarsone addition, the
20 species in the AGS included As(III), As(V) and HAPA. Most of the soluble arsenic
21 was in the form of As(III) and As(V), especially high concentration of As(III) being
22 detected in the intracellular part in the AGS. It has been reported that methanogenesis

1 was completely inhibited as As(III) content approached 1 g/kg [17]. As shown in
2 Table 2, the methane yield of the reactor with roxarsone addition was significantly
3 lower than that of the reactor without roxarsone addition ($p < 0.05$), indicating that
4 severe inhibition occurred with roxarsone addition. Similar inhibition of arsenic on
5 methanogenic activity was reported in a recent study [26]. After 260-day incubation,
6 As(III) concentration reached 1937.01 ± 299.87 mg/kg in the AGS in the reactor with
7 roxarsone addition.

9 *3.2 Arsenic concentration in aqueous phases*

10 Fig. 4a shows the variation of total arsenic concentrations in aqueous phases
11 along the flow direction in both reactors. As expected, the total arsenic concentrations
12 in each part of the reactor with roxarsone addition were prominently higher than that
13 of the reactor without roxarsone addition ($p < 0.05$). The total arsenic concentrations in
14 the aqueous phases in points c and e were 2.08 ± 0.06 mg/L and 1.96 ± 0.03 mg/L in
15 the reactor with roxarsone addition, respectively, which were lower than that of other
16 five points. That might be due to the fact that the both parts (c, AGS bed; e, sludge
17 floating zone) gathered quantities of sludge, which has a good adsorption to organic
18 and inorganic arsenics [27]. The arsenic loss has also been reported from the
19 precipitation by anaerobic sludge [20]. Therefore, the arsenic accumulation in the
20 AGS can be the function of adsorption and precipitation together. The total arsenic
21 concentration in the reactor without roxarsone addition was very low and ranged from
22 1.98 ± 0.12 to 5.86 ± 1.01 μ g/L, which might be from the tap-water and sludge.

Fig. 4b shows arsenic species in aqueous phases in the reactor with roxarsone addition. Roxarsone concentration was quickly declined accompanied by the increasing HAPA concentration from sampling point a to point b in the reactor with roxarsone addition, meaning that roxarsone was rapidly reduced to HAPA through the reduction of nitro-group to amino group [28]. But the molar ratio of HAPA/roxarsone was only about 41%. Because inorganic arsenic was not detected in the sampling point b, it could be deduced that there might be other organic arsenic generated and HAPA was degraded slowly under anaerobic conditions [8]. As(III) reached maximum concentration of $511.20 \pm 13.33 \mu\text{g/L}$ at point e, and monomethylarsenate (MMA(V)) was detected at the concentration of $205.60 \pm 5.07 \mu\text{g/L}$ at the same position. It has been reported that the formation of MMA is the limiting step for subsequent methylation of arsenic [20]. Thus, MMA(V) being detected provided an essential condition for arsenic volatilization. The concentration of As(V) was very low in the reactor, but gradually increased in the effluent, which was related to the oxidation of As(III) to As(V) with exposing to the atmosphere in the effluent.

For confirming the unknown organic arsenic, three samples collected from the reactor with roxarsone addition were analyzed by HPLC-HG-AFS. As shown in Fig. 5, the retention time of inorganic arsenic and HAPA was at 2.16 min and 2.48 min, respectively. An unknown organoarsenic was detected in the chromatogram of samples from point d with a retention time of 3.25 min.

3.3 Arsenic volatilization

1 Volatile arsenic was trapped in both reactors. As shown in Fig. 6, AgNO_3 was
2 evenly conglomerated on silica gel beads according to the SEM image of a1 and a2.
3 After trapping volatile arsenic for 1 day, white granules (AgNO_3) were observed to
4 become dark. In addition, glossy surface of silica gel was covered by powdery
5 substances. That might be due to the immobilization of volatile arsenic on the surface
6 of AgNO_3 -impregnated silica gel beads.

7 As listed in Table 3, volatile arsenic trapped from the reactor with roxarsone
8 addition was significantly higher than that from the reactor without roxarsone addition
9 ($p=0.04$), which might be associated with the high arsenic content in the AGS in the
10 reactor with roxarsone addition, in which the total arsenic content reached $10,661.92$
11 ± 539.63 mg/kg (Fig. 2). According to previous report, higher concentration of arsenic
12 in the sludge resulted in more flux of volatile arsenic in sediment [21].

13 The generation of volatile arsenic could contribute to reduce the arsenic
14 contamination in wastewater, sludge, and soil [19]. However, volatile arsenic might
15 have adverse influence on air environment around digester, which was risk to human
16 and animals. Therefore, the wastewater with organoarsenic contamination from
17 poultry and swine farms should be well managed. Otherwise, the generated volatile
18 arsenic will emit into air and harm human and animal health.

19 20 *3.4 Mass balance analysis of arsenic*

21 In order to quantitatively analyze the mass balance of arsenic in both reactors,
22 the arsenic concentrations in the influent, effluent, and volatilization were measured

1 and calculated. Fig. 7 shows the result of mass balance of arsenic in both reactors.
2 Arsenic flow rate in the influent and effluent in the reactor with roxarsone addition
3 was 7.52 mg/d and 5.76 ± 0.24 mg/d (calculated according to roxarsone concentration
4 in synthetic wastewater and the influent flow), respectively, and volatile arsenic was
5 detected at a rate of 134.93 ± 6.61 ng/d. In other words, 23.40% of the influent arsenic
6 was accumulated in the AGS, and 0.002% was released in the form of volatile arsenic.
7 As comparison, arsenic flow rate in the influent and effluent in the reactor without
8 roxarsone addition was 11.72 ± 1.38 μ g/d and 5.76 ± 0.73 μ g/d, respectively, and
9 volatile arsenic was negligible. Hence, 50.85% of the influent arsenic was precipitated
10 in the AGS in the reactor without roxarsone addition.

11 The arsenic accumulation in the AGS indicated that the inhibition to
12 methanogens would be more serious with the long-term operation, and the disposal of
13 residual AGS should be well managed because of the high concentration of arsenic in
14 the sludge. Furthermore, high concentration of arsenic in the AGS provided favorable
15 conditions for the generation of volatile arsenic.

16

17 *3.5 Environmental significance*

18 Arsenic accumulation in UASB reactor was observed in this study, which would
19 cause serious inhibition on microbial activities, such as methanogenic activity [17, 26].
20 Serious inhibition on methanogenic activity suggested organoarsenic would
21 significantly impact the performance of UASB reactor. In addition, the disposal of
22 arsenic-contaminated sludge should be well managed for avoiding secondary

1 pollution. The occurrence of insoluble arsenic accumulation in AGS also indicated
2 part of arsenic was immobilized, indicating that arsenic immobilization in UASB
3 reactor would be a potential way to reduce arsenic toxicity and secondary pollution
4 [24]. For instance, inorganic arsenic was immobilized in the form of AsS and As₂S₃
5 by Fe-sulfide [25, 29]. Arsenic volatilization occurred in the UASB reactor treating
6 wastewater contaminated with roxarsone, emphasizing the importance to protect
7 human and animal from the volatilized arsenic, also indicating that
8 organoarsenic-contaminated poultry litter and pig manure should be well managed
9 because of the potential arsenic volatilization under anaerobic conditions [30].

11 **4. Conclusions**

12 In this study, the accumulation, distribution and volatilization of arsenic from
13 roxarsone degradation in a 260-day cultured UASB reactor were investigated. 13.2
14 mg/L of roxarsone was continually fed into the UASB reactor. In the AGS, 23.40% of
15 the influent arsenic was accumulated through adsorption and precipitation. And the
16 main speciation of acidic soluble arsenic was As(III). In aqueous phases, roxarsone
17 was rapidly transformed into HAPA and unknown organoarsenic along the flow
18 direction, which was further converted to inorganic arsenic and MMA(V) in the
19 reactor. In addition, 0.002% of the influent arsenic was released in the form of volatile
20 arsenic from the reactor with roxarsone addition. The results from this study indicated
21 that the operation of anaerobic reactors treating arsenic-contaminated wastewater

from poultry and swine farms and the disposal of residual AGS in the digester should be well managed.

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References

- [1] K.C. Makris, S. Quazi, P. Punamiya, D. Sarkar, R. Datta, Fate of arsenic in swine waste from concentrated animal feeding operations, *J. Environ. Qual.*, 37 (2008) 1626-1633.
- [2] D.J. Fisher, L.T. Yonkos, K.W. Staver, Environmental concerns of roxarsone in broiler poultry feed and litter in Maryland, USA, *Environ. Sci. Technol.*, 49 (2015) 1999-2012.
- [3] X. Liu, W. Zhang, Y. Hu, H. Cheng, Extraction and detection of organoarsenic feed additives and common arsenic species in environmental matrices by HPLC – ICP-MS, *Microchem. J.*, 108 (2013) 38-45.
- [4] J.R. Garbarino, A.J. Bednar, D.W. Rutherford, R.S. Beyer, R.L. Wershaw, Environmental fate of roxarsone in poultry litter. I. Degradation of roxarsone during composting, *Environ. Sci. Technol.*, 37 (2003) 1509-1514.
- [5] R. Liu, W. Xu, K. Wu, W. Gong, H. Liu, J. Qu, Species distribution of arsenic in sediments after an unexpected emergent discharge of high-arsenic wastewater

- 1 into a river, *Front. Env. Sci. Eng.*, 7 (2013) 568-578.
- 2 [6] W. Sun, R. Sierra-Alvarez, J.A. Field, Long term performance of an
3 arsenite-oxidizing-chlorate-reducing microbial consortium in an upflow
4 anaerobic sludge bed (UASB) bioreactor, *Bioresource Technol.*, 102 (2011)
5 5010-5016.
- 6 [7] E. Sahinkaya, A. Yurtsever, Y. Toker, H. Elcik, M. Cakmaci, A.H. Kaksonen,
7 Biotreatment of As-containing simulated acid mine drainage using laboratory
8 scale sulfate reducing upflow anaerobic sludge blanket reactor, *Miner. Eng.*, 75
9 (2014) 133-139.
- 10 [8] I. Cortinas, J.A. Field, M. Kopplin, J.R. Garbarino, A.J. Gandolfi, R.
11 Sierra-Alvarez, Anaerobic biotransformation of roxarsone and related
12 N-substituted phenylarsonic acids, *Environ. Sci. Technol.*, 40 (2006) 2951-2957.
- 13 [9] K.P. Mangalgiri, A. Adak, L. Blaney, Organoarsenicals in poultry litter: Detection,
14 fate, and toxicity, *Environ. Int.*, 75 (2014) 68-80.
- 15 [10] H. Liu, G. Wang, J. Ge, L. Li, G. Chen, Fate of roxarsone during biological
16 nitrogen removal process in wastewater treatment systems, *Chem. Eng. J.*, 255
17 (2014) 500-505.
- 18 [11] L. Shi, W. Wang, S.J. Yuan, Z.H. Hu, Electrochemical stimulation of microbial
19 roxarsone degradation under anaerobic conditions, *Environ. Sci. Technol.*, 48
20 (2014) 7951-7958.
- 21 [12] M.C. Kruger, P.N. Bertin, H.J. Heipieper, F. Arsène-Ploetze, Bacterial
22 metabolism of environmental arsenic--mechanisms and biotechnological
23 applications, *Appl. Microbiol. Biot.*, 97 (2013) 3827-3841.
- 24 [13] F.F. Zhang, W. Wang, S.J. Yuan, Z.H. Hu, Biodegradation and speciation of
25 roxarsone in an anaerobic granular sludge system and its impacts, *J. Hazard.
26 Mater.*, 279 (2014) 562-568.
- 27 [14] Z. Chen, H. Wang, Z. Chen, N. Ren, A. Wang, Y. Shi, X. Li, Performance and
28 model of a full-scale up-flow anaerobic sludge blanket (UASB) to treat the
29 pharmaceutical wastewater containing 6-APA and amoxicillin, *J. Hazard. Mater.*,
30 185 (2011) 905-913.

- 1 [15] W.C. Li, Q.G. Niu, H. Zhang, Z. Tian, Y. Zhang, Y.X. Gao, Y.Y. Li, O. Nishimura,
2 M. Yang, UASB treatment of chemical synthesis-based pharmaceutical
3 wastewater containing rich organic sulfur compounds and sulfate and associated
4 microbial characteristics, *Chem. Eng. J.*, 260 (2015) 55-63.
- 5 [16] H. Marcussen, I.T. Ha, C. Polprasert, P.E. Holm, Contents and mass balances of
6 cadmium and arsenic in a wastewater-fed fish pond of Hoang Mai, Hanoi,
7 Vietnam, *J. Environ. Sci. Heal. A*, 47 (2012) 2246-2253.
- 8 [17] D.G. Capone, D.D. Reese, R.P. Kiene, Effects of metals on methanogenesis,
9 sulfate reduction, carbon dioxide evolution, and microbial biomass in anoxic salt
10 marsh sediments, *Appl. Environ. Microb.*, 45 (1983) 1586-1591.
- 11 [18] R. Freitas, L. Salamanca, C. Velez, F.J. Wrona, A.M.V.M. Soares, E. Figueira,
12 Multiple stressors in estuarine waters: Effects of arsenic and salinity on
13 *Ruditapes philippinarum*, *Sci. Total Environ.*, 541 (2016) 1106-1114.
- 14 [19] J. Chen, G.X. Sun, X.X. Wang, L. Vd, B.P. Rosen, Y.G. Zhu, Volatilization of
15 arsenic from polluted soil by *Pseudomonas putida* engineered for expression of
16 the *arsM* Arsenic(III) S-adenosine methyltransferase gene, *Environ. Sci. Technol.*,
17 48 (2014) 10337-10344.
- 18 [20] A. Mestrot, W.Y. Xie, X. Xue, Y.G. Zhu, Arsenic volatilization in model
19 anaerobic biogas digesters, *Appl. Geochem.*, 33 (2013) 294-297.
- 20 [21] H. Huang, Y. Jia, G.X. Sun, Y.G. Zhu, Arsenic speciation and volatilization from
21 flooded paddy soils amended with different organic matters, *Environ. Sci.*
22 *Technol.*, 46 (2012) 2163-2168.
- 23 [22] A. Mestrot, M.K. Uroic, T. Plantevin, M.R. Islam, E.M. Krupp, J. Feldmann, A.A.
24 Meharg, Quantitative and Qualitative Trapping of Arsines Deployed to Assess
25 Loss of Volatile Arsenic from Paddy Soil, *Environ. Sci. Technol.*, 43 (2009)
26 8270-8275.
- 27 [23] C. Hohmann, E. Winkler, G. Morin, A. Kappler, Anaerobic Fe(II)-oxidizing
28 bacteria show as resistance and immobilize as during Fe(III) mineral
29 precipitation, *Environ. Sci. Technol.*, 44 (2010) 94-101.
- 30 [24] L. Rodriguez-Freire, R. Sierra-Alvarez, R. Root, J. Chorover, J.A. Field,

1 Biomineralization of arsenate to arsenic sulfides is greatly enhanced at mildly
2 acidic conditions, *Water Res.*, 66 (2014) 242-253.

3 [25] R. Liu, Z. Yang, Z. He, L. Wu, C. Hu, W. Wu, J. Qu, Treatment of strongly acidic
4 wastewater with high arsenic concentrations by ferrous sulfide (FeS): Inhibitive
5 effects of S(0)-enriched surfaces, *Chem. Eng. J.*, 304 (2016) 986–992.

6 [26] M. Shui, F. Ji, R. Tang, S. Yuan, X. Zhan, W. Wang, Z. Hu, Impact of roxarsone
7 on the UASB reactor performance and its degradation, *Front. Env. Sci. Eng.*, 10
8 (2016).

9 [27] J.C. Li, S.J. Yuan, W. Wang, F. Ji, Z.H. Hu, Adsorption characteristics of
10 4-hydroxy-3-aminophenylarsonic acid (HAPA) onto anaerobic granular sludge,
11 *Desalin. Water Treat.*, (2015) 1-12.

12 [28] J.F. Stolz, E. Perera, B. Kilonzo, B. Kail, B. Crable, E. Fisher, M. Ranganathan,
13 L. Wormer, P. Basu, Biotransformation of 3-nitro-4-hydroxybenzene arsonic acid
14 (roxarsone) and release of inorganic arsenic by *Clostridium* species, *Environ. Sci.*
15 *Technol.*, 41 (2007) 818-823.

16 [29] X. Xie, Y. Liu, K. Pi, C. Liu, J. Li, M. Duan, Y. Wang, In situ Fe-sulfide coating
17 for arsenic removal under reducing conditions, *J. Hydrol.*, 534 (2016) 42-49.

18 [30] T.M. Webster, R.R. Reddy, J.Y. Tan, J.D.V. Nostrand, J. Zhou, K.F. Hayes, L.
19 Raskin, Anaerobic disposal of arsenic-bearing wastes results in low
20 microbially-mediated arsenic volatilization, *Environ. Sci. Technol.*, (2016).

Figure captions

Fig. 1 Schematic diagram of the UASB reactor and sampling point distribution. (Point a-g were used to sample liquid, and A and B were used to sample AGS)

Fig. 2 The concentrations of total and insoluble arsenics in the AGS in both reactors. A_0 and B_0 were the bottom and top of the anaerobic sludge bed in the reactor without roxarsone addition, respectively; A_1 and B_1 were the bottom and top of anaerobic sludge bed in the reactor with roxarsone addition, respectively

Fig. 3 The acidic soluble arsenic species in AGS in both reactors. A_0 and B_0 were the bottom and top of the anaerobic sludge bed in the reactor without roxarsone addition, respectively; A_1 and B_1 were the bottom and top of the anaerobic sludge bed in the reactor with roxarsone addition, respectively

Fig. 4 Distribution and speciation of arsenic in aqueous phases in both UASB reactors. (a) total arsenic concentration in both reactors and (b) arsenic speciation in aqueous phases in the reactor with roxarsone addition. Point a-g were the sampling points in both reactors as shown in Fig.1

Fig. 5 The chromatogram of arsenic species detected in the reactor points of b and d with roxarsone addition by HPLC-HG-AFS with C18 column

Fig. 6 SEM analysis of $AgNO_3$ -impregnated silica gel beads without volatile arsenic (a1-a2) and with volatile arsenic (b1-b2). a1 and b1 showing the entire morphology (at 250 $\times$ and 35 $\times$ magnification, respectively), a2-b2 showing the surface (at 1000 $\times$ magnification)

Fig. 7 Mass balance of arsenic in both UASB reactors

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Fig. 2 The concentrations of total and insoluble arsenics in the AGS in both reactors. A₀ and B₀ were the bottom and top of the anaerobic sludge bed in the reactor without roxarsone addition, respectively; A₁ and B₁ were the bottom and top of anaerobic sludge bed in the reactor with roxarsone addition, respectively.

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3 **Fig. 3** The acidic soluble arsenic species in AGS in both reactors. A₀ and B₀ were the
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Fig. 7 Mass balance of arsenic in both UASB reactors

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Table 1 Characteristics of SS and VSS of the AGS in both reactors

Sampling points	SS (%)	VSS (%)
A ₀	10.34 ± 0.05	8.78 ± 0.06
B ₀	9.10 ± 0.01	6.79 ± 0.08
A ₁	9.51 ± 0.01	6.92 ± 0.02
B ₁	9.48 ± 0.27	5.95 ± 0.02

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Note: A₀, the bottom of anaerobic sludge bed in the reactor without roxarsone addition; B₀, the top of anaerobic sludge bed in the reactor without roxarsone addition; A₁, the bottom of anaerobic sludge bed in the reactor with roxarsone addition; B₁, the top of anaerobic sludge bed in the reactor with roxarsone addition; SS, suspended solid; VSS, volatile suspended solid.

1 **Table 2** Operation parameters and methane generation of both UASB reactors

	Roxarsone (mg/L)	Operation period (day)	Influent flux (L/d)	Hydraulic retention time (day)	pH	Production of methane (mL/d)
Without roxarsone addition	0	260	2.0	1.75	7.23 ± 0.26	473.50 ± 49.93
With roxarsone addition	13.2	260	2.0	1.75	6.82 ± 0.22	20.70 ± 6.45*

2 Note: *, is significant at the 0.05 level.

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Table 3 Volatile arsenic in both reactors

	Without roxarsone addition	With roxarsone addition
Yield of volatile arsenic (ng/d)	ND	134.93 ± 6.61 *

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Note: ND, not detected; *, is significant at the 0.05 level.

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