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Characterization of arsenic species in the anaerobic granular sludge treating roxarsone-contaminated wastewater

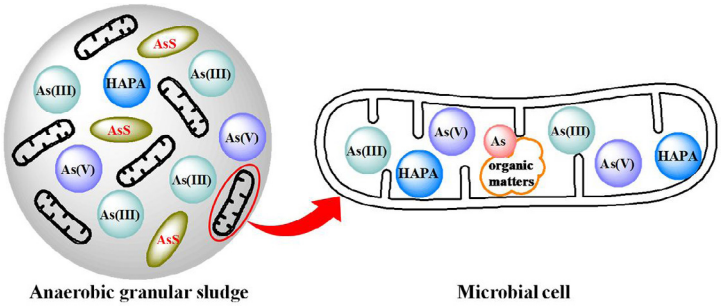
Peilun Ji [a], Rui Tang [a], Ping He [a], Shoujun Yuan [a,b], Wei Wang [a,b,*], Zhen-Hu Hu [a,b,*]

[a] School of Civil Engineering, Hefei University of Technology, Hefei 230009, China
[b] Institute of Water Treatment and Wastes Reutilization, Hefei University of Technology, Hefei 230009, China

HIGHLIGHTS

- Total arsenic concentration was accumulated to 8246.69 ± 378.93 μg·g^{-1} in the AGS.
- About 74.6% of arsenic could be leached out and 25.4% was precipitated in the AGS.
- As(III) was predominant arsenic, followed by As(V) and HAPA in the AGS.
- Precipitated arsenic in the AGS was in the form of AsS.

GRAPHICAL ABSTRACT



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ABSTRACT

Roxarsone (4-hydroxy-3-nitrophenylarsonic acid), an organoarsenic feed additive, has been widely used in animal feed. Significant amount of arsenic enters into anaerobic granular sludge (AGS) in roxarsone-contaminated wastewater treatment process. However, the characterization of bonded and precipitated organoarsenic and inorganic arsenic in the AGS is still not clear. The characterization of arsenic species in the AGS treating roxarsone-contaminated wastewater was investigated in this study. Total arsenic concentration in the AGS was 8246.69 μg·g^{-1}. A series of extraction processes were performed to analyze arsenics in the AGS. As(III) (1384.71 μg·g^{-1}) and 4-hydroxy-3-aminophenylarsonic acid (HAPA, 439.05 μg·g^{-1}) were the dominant inorganic and organic soluble arsenic species, respectively. Similar arsenic species were detected in the cellular bonded arsenics. Field emission scanning electron microscope coupled with energy dispersive spectroscopy (SEM-EDS), X-ray photoelectron spectroscope (XPS) and X-ray diffraction (XRD) analysis confirmed that the precipitated arsenic was in form of realgar (AsS). Cellular bonded arsenic had the highest percentage (42.2%), followed by soluble arsenic (32.4%) and precipitated arsenic (25.4%). The result from this study suggests that most of arsenics in the AGS could be leached out and has risk to the environment, and arsenic precipitation could be used as a remediation technique in bioreactors treating organoarsenic-contaminated wastewater.

1. Introduction

Roxarsone (4-hydroxy-3-nitrophenylarsonic acid), an organoarsenic feed additive, has been widely used to prevent coccidial intestinal parasites, promote animal growth, and improve feed efficiency [1–3]. Most of the added roxarsone is excreted into the manure [2]. It has been estimated that approximately 1.7 × 10^5–3.1 × 10^7 kilograms of arsenic was discharged into environment through pig manure and chicken litter every year in China [4]. Arsenic is one of the most prevalent environmental carcinogens with wide distribution, strong carcinogenicity and high toxicity [5–7]. The use of organoarsenicals in animal production is one

* Corresponding authors at: School of Civil Engineering, Hefei University of Technology, Hefei 230009, China.
E-mail addresses: dwhit@126.com (W. Wang), zhhu@hfut.edu.cn (Z.-H. Hu).

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source of arsenic being introduced to the environment [8]. The discharging of organoarsenic contaminated wastewater from animal farms also caused arsenic contamination [9]. Although roxarsone has relative low toxicity, the released toxic inorganic arsenic species, such as arsenate (As(V)) and arsenite (As(III)) from roxarsone degradation are severe risk to the environment [10,11].

Under anaerobic conditions, roxarsone was rapidly transformed to 4-hydroxy-3-aminophenylarsonic acid (HAPA) and then slowly degraded to inorganic arsenic [12]. Organic and inorganic arsenics can be adsorbed on the microorganism surface by electrostatic interaction [13]. Due to the similar chemical structure to phosphate, As(V) can be taken into bacterial cell through phosphate transporters. Through aqua-glyceroporins, As(III) can also enter bacterial cell [14], which is bonded in the cell by forming As(III) complexes with chelating peptides or proteins containing thiol groups [15]. Inorganic arsenic can form insoluble sulfide minerals, such as arsenopyrite (FeAsS), realgar (AsS) and orpiment (As_2S_3) [16,17], which can be used to immobilize arsenic for removing arsenic [18,19].

Toxic organic pollutants and metals in wastewater have seriously threatened the health and environment security, and biological treatment has been considered as an efficient way [20–22]. In biological treatment, the upflow anaerobic sludge blanket (UASB) reactor has been extensively used for the treatment of wastewater because of low cost, methane production and high organic loading rate [23,24]. In UASB rector, anaerobic granular sludge (AGS) plays the decisive role in wastewater treatment. In the anaerobic degradation of roxarsone, significant amount of organic and inorganic arsenics entered into AGS from aqueous solution, which affected the USAB performances and AGS structure [25]. The disposal of AGS containing high concentration of arsenics will result in arsenic contamination in the environment.

Inorganic arsenic speciation in the environment has been investigated a lot, and the soluble species of organoarsenic and its degradation products in the AGS have also been reported before [25–27]. To the best of our knowledge, the speciation of bonded and precipitated organoarsenic and its degradation products in the AGS have not been reported before. The objective of this study was to investigate arsenic species in the AGS treating roxarsone-contaminated wastewater, including soluble, bonded and precipitated arsenics. A better understanding of the arsenic species in the AGS can regulate and control the degradation and transformation of roxarsone in anaerobic reactor, and also provide useful suggestion for the disposal of the contaminated AGS.

2. Materials and methods

2.1. Materials

The AGS used in this study was collected from a UASB reactor, which has been operated for more than 300 days with synthetic wastewater containing roxarsone. The average diameter of AGS was 2–4 mm and the total solid (TS) content of AGS was 8.2%. The methanogenesis was strongly inhibited in the UASB reactor [26]. The working volume and internal diameter of the UASB were 3.5 L and 100 mm, respectively. The UASB was operated at 35 ± 1 °C, and influent flux was 2.0 L d^{-1} and hydraulic retention time was 1.75 d. The synthetic wastewater contained the following ingredients (mg·L^{-1}): sucrose (1000.0), NH_4Cl (107.5), KH_2PO_4 (22.5), $NaHCO_3$ (1500.0), $MgSO_4{\cdot}7H_2O$ (42.5), $CaCl_2$ (13.0), $FeCl_3{\cdot}6H_2O$ (4.1), $NiSO_4{\cdot}7H_2O$ (5.3), $MnCl_2{\cdot}4H_2O$ (1.1), $CoCl_2$ (0.6), $ZnCl_2$ (0.6) and $CuCl_2{\cdot}2H_2O$ (0.2). In synthetic wastewater, 13.2 mg L^{-1} of roxarsone was added to simulate livestock wastewater [27].

Alkaline protease (activity ≥ 200 units mg^{-1}) was purchased from Amresco. Roxarsone (purity > 99%, CAS NO.: 121-19-7, molecular formula: $C_6H_6AsNO_6$, molecular weight: 263.04) and sodium dodecyl sulfate (SDS, chemically pure, CAS NO.: 151-21-3, molecular formula: $C_{12}H_{25}NaO_4S$, molecular weight: 288.38) were purchased from Sinopharm Chemical Reagent Co., China. Other chemicals used were all of analytical grade except methanol, which was of HPLC grade.

2.2. Sample preparation

After collection, the AGS was washed with deionized water to remove organic and inorganic arsenics in the surface for three times prior to analysis.

To determine the total arsenic concentration in the AGS, 0.5 g washed AGS was digested with 15 mL double-diluted aqua regia (HNO_3/HCl = 1/3, v/v) for 2 h at 100 °C in a water bath. After that, the digested solution was diluted to 50 mL with ultrapure water for analysis. And the experiments were performed in triplicate.

To determine extracellular soluble arsenic species, the AGS were extracted using 2% (w/v) K_2HPO_4 with 1.5% (v/v) H_3PO_4 mixed solution. 0.5 g washed AGS was grinded and mixed with 10 mL extraction solution in 50 mL centrifuge tube, and then agitated in an oscillation bath at 60 °C. The extraction process was conducted for three times, and lasted 16 h, 4 h and 2 h, respectively. After extraction, the extracted solution was mixed together and centrifuged at 10,000 rpm for 15 min, and the supernatant was filtered with 0.22-μm cellulosic membrane filter for analysis. The experiments were performed in triplicate and the solid residue was collected for the next step analysis.

To determine intracellular soluble arsenic species, the solid residue collected from above extraction step was mixed with 10 mL extraction solution again and put in an ultrasonic cell disruptor (Noise Isolating Tamber, Ningbo Scientz Biotechnology Co., China) for breaking down microbial cells. The disruption lasted for 3 min at a power of 495 W. After that, the same extraction processes (in oscillation bath for 3 times at 60 °C) were conducted and the collected extraction solution was centrifuged and filtered as mentioned above for analysis. The experiments were performed in triplicate and all the solid residue was collected for next step analysis.

Cellular arsenic, which was partly bonded by cellular materials (such as protein) tightly and as a result, was difficult to extract in above two steps. Hence, further pretreatment and extractions were carried out to dissolve bonded cellular arsenics. The solid residue was firstly hydrolyzed with alkaline protease to release bonded arsenics. The solid residue was mixed with alkaline protease at enzyme/substrate ratio of 1:50 (w/v) and then agitated in an oscillation bath for 10 h to dissolve organic matters (mainly protein). For getting high enzyme activity, the pH of the mixture was adjusted to 10–11 and maintained at 45 °C. After the centrifugation and filtration processes, the liquid was used for analysis. The solid residue was further mixed with 2% (w/v) SDS, which is extremely efficient surfactant and can dissolve protein well, in an ultrasonic cleaner (KQ5200DE, Kunshan Ultrasonic Instruments Co., China) for 4 h at room temperature to further release the bonded cellular arsenic. Then the same centrifugation and filtration processes as mentioned above were carried out to separate the supernatant and solid residue. The residual arsenic in the solid was considered as precipitated arsenic. The solid residue was washed by ultrapure water and freeze dried for further spectroscopic analyses.

2.3. Analytical methods

The concentrations of roxarsone and its metabolite HAPA were determined by a high performance liquid chromatography (HPLC, LC-20AD, Shimadzu Co., LTD Japan) with a diode array detector (SPD-20A) at 264 nm and a C18 column (4.6 mm × 150 mm,