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Biodegradation and speciation of roxarsone in an anaerobic granular sludge system and its impacts

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HIGHLIGHTS

- Arsenic species in the anaerobic granular sludge system with HPLC–HG-AFS analysis.
- Arsenic distribution in the liquid and anaerobic granular sludge.
- Structure of anaerobic granular sludge affected by roxarsone and its metabolites.
- SEM analysis of anaerobic granular sludge in the absence and presence of roxarsone.

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ABSTRACT

Roxarsone (3-nitro-4-hydroxy benzene arsenic acid) is an organoarsenic feed additive and has been widely used in the poultry industry to prevent coccidiosis and improve feed efficiency. The presence of roxarsone and its degradation products results in the instability of the anaerobic methanogenic process. This study investigated the degradation and speciation of roxarsone in an anaerobic granular sludge (AGS) system and the impacts of roxarsone and its degradation products on the structure of AGS. Roxarsone inhibited methane production, and the added roxarsone was rapidly degraded into 3-amino-4-hydroxyphenylarsonic acid (HAPA). After 240 days of incubation, the distribution of arsenic differed between the aqueous solution and the AGS in the assays of 20 and 350 mg/L roxarsone. Species analysis indicated that HAPA was completely degraded in all of the assays with roxarsone addition after 240 days of incubation. Species distribution was affected by the phases and the initial concentration of roxarsone added. The concentration of As(III) was higher than that of As(V) in both the aqueous solution and the AGS in all assays with roxarsone addition. The toxicity of roxarsone and its degradation products resulted in changes in the structure and the microorganism species in the AGS.

1. Introduction

Roxarsone (3-nitro-4-hydroxy benzene arsenic acid), as an organoarsenic feed additive, has been widely used in the poultry industry to prevent coccidiosis and improve feed efficiency [1,2]. Most of the supplemented roxarsone was excreted into manure without chemical change [3]. Land application of roxarsone contaminated manure resulted in the arsenic pollution in soil and surface water through runoff, while the discharging of wastewater from poultry farms also caused the pollution in surface water [4]. Roxarsone in the environment was slowly converted into inorganic arsenic species through biotic and abiotic pathways [5]. Inorganic arsenic, such as arsenate and arsenite, is more toxic than roxarsone [6,7]. Therefore, roxarsone in manure is a hazardous chemical compound [8].

Anaerobic digestion has been widely used in the treatment of municipal and industrial wastewaters [9]. Anaerobic treatment processes are more cost-effective than aerobic treatment processes for high concentrations of organic wastewater in terms of methane recovery and less sludge production [10,11]. Stolz et al. have reported that roxarsone was transformed to 3-amino-4-hydroxyphenylarsonic acid (HAPA) and inorganic arsenic under anaerobic conditions, which inhibited the methanogenic process and resulted in the instability of the anaerobic digestion process [12].

Anaerobic granular sludge (AGS) in upflow anaerobic sludge blanket (UASB) reactors has been used to treat several types of

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Table 1
Characteristics of AGS.

Component	Content (%)
Moisture	85.20
VS	6.50
Ca	13.48
P	7.75
Zn	0.10
Cu	0.01
Ni	0.05
Cr	0.04
Mg	0.33

Note. The content of metals is based on the dry matter of AGS; VS: volatile solid.

high concentrations of organic wastewater [13]. AGS can be considered as a spherical biofilm consisting of billions of anaerobic microorganisms [14], and it has advantages over common anaerobic sludge flocs, such as good cohesion and quick settling ability [13]. AGS is composed of three-layered structures: the outer layer consists of cocci, rods and filamentous bacteria, primarily colonized by acidogens, H_2-consuming organisms; the middle layer is predominately made up of bacterial rods in an ordered arrangement, such as H_2-producing acetogens and H_2-consuming organisms; and the interior layer is formed of large numbers of methanothrix-like cells, and large cavities are notable in the internal cores of the granules [9]. Costa et al. has reported that detergent shock loads resulted in structural changes to the AGS [15].

Although investigations of the degradation of roxarsone under anaerobic sludge flocs have been reported [3,16], the degradation and speciation of roxarsone in the AGS system, and the influence of roxarsone and its metabolites on the structure of AGS remain unknown. The aim of this study was to investigate the degradation and speciation of roxarsone in the AGS system and the impacts of roxarsone and its degradation products on the structure of AGS, to explain why roxarsone causes instability of the anaerobic methanogenic process.

2. Materials and methods

2.1. Chemicals and AGS

Roxarsone (purity >99%, CAS NO.: 121-19-7, molecular formula: $C_6H_6AsNO_6$, molecular weight: 263.04) and HAPA (purity >97%, CAS NO.: 2163-77-1, molecular formula: $C_6H_8AsNO_4$, molecular weight: 233.05) were purchased from Sigma. The stock solution was prepared by accurately dissolving 0.35 g of roxarsone in 1.0 L deionized water. Among the chemicals used in this study, methanol was of HPLC grade, and others were all of analytical reagent grade.

AGS (black, average diameter: 2–4 mm) was collected from a 40 L UASB reactor in the laboratory. The characteristics of the AGS are listed in Table 1. AGS contained 14.8% dry matter, of which volatile solids were 43.9%.

2.2. Degradation of roxarsone by AGS

Three concentrations (0, 20, 350 mg/L) of roxarsone were examined in the batch experiments, representing blank, low concentration and high concentration of roxarsone. The blank (0 mg/L) was set as the control to investigate the anaerobic digestion without roxarsone contamination. The assay of 20 mg/L was set to simulate the concentration that possibly appeared in the digestion of poultry litter or wastewater. For investigating the impacts of extreme roxarsone concentration on the anaerobic digestion, a high concentration of 350 mg/L roxarsone was applied in this study. Each test was carried out in triplicate. The degradation assays were conducted in 250-mL serum bottles. In each experiment, 3.0 g of AGS and 0.5 g of sucrose were added into serum bottles with 100 mL of working volume. For methanogens, the suitable pH for methane production ranged from 6.5 to 7.5 [17]. Thus, the initial pH values were adjusted to approximately 7.5 with $NaHCO_3$. Before cultivation, loaded bottles were flushed with nitrogen gas for 3 min to create anaerobic conditions and then sealed with butyl rubber plugs and shaded with black plastic bags. Since anaerobic digestion in UASB reactors was often operated at mesophilic conditions [18], 37 °C was selected as the cultivation temperature in this study. The bottles were statically cultured and mixed once per day [12].

The degradation experiments were composed of two parts, a short-term experiment (40-day incubation) and a long-term experiment (240-day incubation). Cortinas et al. have reported that 99% of HAPA, which come from the reduction of roxarsone, was degraded over 229 days of incubation [16]. For identifying the speciation of arsenic after complete degradation, a 240-day incubation was carried out in this study. In the short-term assays, biogas production and methane content were measured every 24 h. The concentrations of roxarsone and HAPA were measured every 24 h in the first four days and every 48 h in the following six days, followed by every 72 h until the end of the experiment. In the long-term experiments, the distribution and speciation of arsenic, including arsenite [As(III)], arsenate [As(V)], dimethylarsenate [DMA(V)], monomethylarsenate [MMA(V)], and total dissolved arsenic in aqueous solution and in the AGS were measured after 240 days of incubation. The morphology in the surface and interior of the AGS was also analyzed to investigate the impacts of roxarsone on the AGS.

2.3. Sample preparation

After collection, liquid samples were immediately centrifuged at 10 000 rpm for 10 min, and the supernatant was filtered through 0.22-μm cellulosic membrane filters for the analysis of organic and inorganic arsenic.

For determination of total arsenic. For measuring the total arsenic concentration in aqueous solution and in the AGS, liquid samples and AGS were digested using aqua regia (HNO_3/HCl, at a ratio of 1:3) in a water bath at 100 °C for 2 h. Filtered aqueous solution (1 mL) was digested with 3 mL of aqua regia, or 0.25 g AGS withdrawn from each bottle was digested with 3 mL of aqua regia under the same conditions. After digestion, the digested solution was reduced by 1% (w/V) thiourea and diluted to 25 mL for analysis.

For determination of arsenic species. To guarantee that homogeneous samples were tested, a unified sampling method was applied. After 240 days of incubation, liquid samples were collected, centrifuged and filtered for the analysis of arsenic species. For measuring arsenic species in the AGS, an aliquot of 0.25 g AGS was withdrawn from the bottle. The AGS was first comminuted into small particles and then mixed with 5 mL deionized water for extraction of the soluble arsenic, and this extraction process was performed five times. Next, the extracted liquid was combined, centrifuged and filtered with a 0.22-μm cellulosic membrane filter for analysis. For investigating the arsenic species in the microorganisms of AGS, the solid residue after water extraction was mixed again with 25 mL distilled water and then put in an ultrasonic cell disruptor (Noise Isolating Tamber, Ningbo Scientz Biotechnology CO., China) to break down microorganisms. The conditions of disruption were as follows: power, 495 W; treatment time, 6 min. After ultrasonic treatment, the samples were centrifuged and filtered again as mentioned above. The supernatant was collected for analysis.

For scanning electron microscopy analysis. The AGS samples (10–15 granules), withdrawn randomly from each bottle, were cross-sectioned using a blade prior to the fixation protocol [19]. Samples were fixed with 2.5% glutaraldehyde for 10 h, sheltered