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Effect of arsanilic acid on anaerobic methanogenic process: Kinetics, inhibition and biotransformation analysis

Hai-Long Wang[a], Zhen-Hu Hu[a,b,*], Zi-Lin Tong[a,b], Qiao Xu[a,b], Wei Wang[a,b], Shoujun Yuan[a,b]

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

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ABSTRACT

Arsanilic acid (4-aminophenylarsonic acid) is widely used in the poultry and animal industries as a feed additive in the diets. Nearly all the added arsanilic acid is excreted unchanged in manure resulting in the risk of arsenic contamination. In this study, the effects of arsanilic acid on the kinetics, inhibition of methanogenic process and its biotransformation were investigated. The methane yield was not affected by arsanilic acid loading at concentration <0.46 mM, while the methane production was completely inhibited at concentration of 0.92 mM. The IC_{50} of arsanilic acid in this study was 0.47 mM. After 115 days of incubation, 37–59% of the added arsanilic acid was degraded. The species analysis indicated that at lower initial arsanilic acid concentration, the soluble inorganic arsenic mainly existed in the species of arsenate (As(V)), while at higher initial arsanilic acid concentration (>0.460 mM), the soluble inorganic arsenic mainly existed in the species of arsenite (As(III)), which explains why higher arsanilic acid concentration has severe inhibition to methanogens.

1. Introduction

Arsanilic acid (4-aminophenylarsonic acid, $C_6H_8NO_3As$), an aromatic organic-arsenic, has been extensively used as feed additive in the poultry and pig farms for improving feed efficiency and growth rate, and controlling coccidial intestinal parasites [1–3]. The approved dosages as additives in animal feed are usually at 20–50 mg/kg, and most of the supplemented arsanilic acid is excreted without chemical change in manure [4]. There were 800–1000 tons of arsanilic acid being consumed every year in the United States, and the leaching and runoff resulted in the release of arsanilic acid and its metabolites from manure into water body and soil, causing the risk of arsenic contamination [5]. Adsorption removal of organoarsenic from aqueous solution has been reported in the previous references [6,7].

Since arsanilic acid is high water soluble, irrigation and runoff would mobilize it in the environment after land application of arsanilic acid contaminated manure and litters and discharging of wastewater from farms [8], which results in the risk of arsenic contamination in the environment [9]. Guo et al. observed the inhibition of organoarsenic on the biological wastewater treatment processes at concentration exceeding 40 mg/L [10]. Yao et al. has reported that organic arsenic compounds accumulated in crops, and their degradation products arsenite (As(III)) and arsenate (As(V)) accumulated in water spinach and turnip [11–13]. Therefore, there are increased concerns on the arsenic pollution from organoarsenic additives.

Anaerobic digestion is a very common treatment way for the high concentration of organic wastewater generated during pig and poultry production. It has been reported that anaerobic methanogenesis was inhibited by arsanilic acid at a concentration of 1 mM to acetate-utilizing methanogens [14]. However, to hydrogen-utilizing methanogens, there was little or no inhibition during the initial 4 days exposure to arsanilic acid, and the inhibition significantly increased with the prolonged exposure time [14]. The reason why the inhibition markedly increases with exposure time is not clear. Several studies have reported that biological transformation of organoarsenic under anaerobic environments, which resulted in the formation of As(V) and As(III) [8,15]. As(III) and As(V) can be readily leached into surface water and groundwater [16,17], and are more toxic to organisms than organoarsenic. The actual concentration of arsanilic acid in animal wastewater is lower

* Corresponding author at: Department of Civil Engineering, Hefei University of Technology, Hefei 230009, Anhui, China. Tel.:+86 551 2904144; fax: +86 551 2902066.
E-mail address: zhhu@hfut.edu.cn (Z.-H. Hu).

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than 1 mM. However, the impact of low concentration of arsanilic acid on acetate-utilizing methanogens, and the fate and speciation of arsanilic acid during anaerobic digestion process is very limited.

The aim of this study was to investigate the inhibition of arsanilic acid on anaerobic acetate-utilizing methanogens, to analyze the inhibitory parameters through modified Gompertz equation, and to investigate the biodegradation and speciation of arsanilic acid under anaerobic conditions. The investigation of fate and speciation of arsanilic acid during anaerobic digestion might be able to explain the increased toxicity of arsanilic acid to acetate-utilizing methanogens with prolonged exposure time, and to provide suggestion for the removal of arsenic from the effluent after anaerobic digestion.

2. Materials and methods

2.1. Chemicals

Arsanilic acid was purchased from Alfa Aesar (98% purity) without further purification before use. Stock solution (1000 mg/L) was prepared by dissolving the determined amount of arsanilic acid into deionized water. All other chemicals used in this study were of analytical grade.

2.2. Anaerobic methanogenic sludge

Anaerobic methanogenic sludge was collected from Zhu Zhuanjing wastewater treatment plant, Hefei, China with volatile suspended solids (VSS) concentration of 27 g/L. After collection, the anaerobic sludge was acclimated in the laboratory at 37 °C for two months as the inoculums.

2.3. Anaerobic inhibitory assays

The inhibitory effect of arsanilic acid on anaerobic methanogenesis was carried out in 250 mL serum bottles with work volume of 150 mL using batch cultures at 37 °C. The inhibition assays composed of two parts. In the first part, various sludge concentrations were investigated at an arsanilic acid concentration of 0.092 mM, and the corresponding inoculated sludge concentration (as mixed liquor volatile suspended solids, MLVSS) was 482 mg/L, 1446 mg/L, 2450 mg/L, and 4820 mg/L, respectively. The acclimated sludge was diluted to the desired concentration with mineral medium under N_2 gas conditions. In the second part, various arsanilic acid loadings (0 mM, 0.092 mM, 0.230 mM, 0.460 mM, 0.61 mM, 0.76 mM, and 0.920 mM) were applied at a MLVSS concentration of 1446 mg/L. Anaerobic assay without arsanilic acid addition was used as the control. The nutrient medium used in the assays had the following composition (g/L): KH_2PO_4, 0.066; CH_3COONa, 3.81; $NaHCO_3$, 2.00; NH_4Cl, 0.287. Acetate was used as the sole carbon source and was added at the beginning of the experiment. Arsanilic acid was supplemented into the bottles by injecting a calculated volume of stock solution. Serum bottles were incubated statically in an incubator and were mixed once per day. Each test was carried out in triplicate and the incubation was lasted over 115 days.

The mixed medium and headspace were flushed with N_2 for 2–3 min to maintain anaerobic conditions, and then the bottles were sealed with butyl rubber. Biogas volume was measured by a glass syringe once per day during the initial 20 days and the corresponding composition of the biogas was analyzed by a gas chromatography. The cumulative methane production was calculated. Liquid samples were collected at predetermined time intervals and centrifuged for 10 min at 10,000 rpm. The supernatant was filtered by 0.45 μm cellulose membrane under N_2 conditions for the analysis of arsanilic acid and arsenic species.

2.4. Kinetics analysis

Modified Gompertz equation has been used to describe the accumulative hydrogen production process and anaerobic digestion process of cattail by rumen microorganisms [18,19]. In this study, the modified Gompertz equation was applied to describe the methane production for analyzing the inhibitory effect. The modified Gompertz equation was given as:

$$P_{CH_4} = CH_{4\,max} \times \exp\left\{\exp\left[\frac{R_{max,CH_4} \times e}{CH_{4,max}}(\lambda - t) + 1\right]\right\} \quad (1)$$

By differentiating Eq. (1), the methane production rate was expressed as

$$r_{CH_4} = R_{max,CH_4} \times \exp\left\{\exp\left[\frac{R_{max,CH_4} \times e}{CH_{4,max}}(\lambda - t) + 1\right] + \left[\frac{R_{max,CH_4} \times e}{CH_{4,max}}(\lambda - t) + 1\right] + 1\right\} \quad (2)$$

where P_{CH_4} is the cumulative methane production (mL CH_4/flask); $CH_{4,max}$ is the potential maximum cumulative methane production (mL CH_4/flask); R_{max,CH_4} is maximum methane production rate (mL CH_4/flask/day); r_{CH_4} is the methane production rate (mL CH_4/flask/day); t is the incubation time (day); λ is the lag phase time (day); and R^2 is the correlation coefficient.

The experiment data were non-linearly simulated with software First Optimization (7D-Soft High Technology Ltd., China) and Origin 7.5 (OriginLab Ltd., USA). The corresponding parameters in both Eqs. (1) and (2) were estimated. Correlation coefficient (R^2) values were used to evaluate the fitness between the experimental data and estimated values.

The inhibition of arsanilic acid to methanogenesis was expressed by the specific methanogenic activities, which were calculated from the slope of the cumulative methane production versus time based on the results obtained from the second part of the inhibitory assays. Since the biomass concentrations added in the second part of the inhibitory assays were same, the specific methanogenic activities can be simplified as maximum methane production rate, which was calculated from Eq. (1). Thus, the inhibition of arsanilic acid to methanogenesis was described by inhibition index as Eq. (3).

$$\text{Inhibition index}\ (\%) = 100 - \left[100 \times \frac{R_{max,CH_4}}{\bar{R}_{max,CH_4}}\right] \quad (3)$$

where $\bar{R}_{max,CH_4}$ is the R_{max,CH_4} of the control (mL CH_4/flask/day).

2.5. Analysis

Arsanilic acid was analyzed by a high performance liquid chromatography (LC-20AD, Shimadzu Ltd., Japan) with an ultraviolet detector (UV) at 260 nm, a C18 Inertsil ODS-3 column (4.6 mm × 250 mm), and a HPLC guard column (4.6 mm × 10 mm). The mobile phase (pH 5.9) consisted of 46 mM potassium phosphate monobasic, 2 mM formic acid and 10% (v/v) methanol at a flow rate of 1.0 mL/min.

The methane content in the biogas was analyzed by a gas chromatography (Model SP-6890, Lunan Co., China) equipped with a thermal conductivity detector. The temperature of injector, detector and column was maintained at 100, 100 and 90 °C, respectively. Argon was used as carrier gas at a flow rate of 30 mL/min.

The concentration of inorganic arsenic in the solution was determined by an atomic fluorescence spectrometer (AFS-8220, Beijing