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The performance of activated sludge exposed to arsanilic acid and amprolium hydrochloride in sequencing batch reactors

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ABSTRACT

Arsanilic acid (ASA) and amprolium hydrochloride (AMP) are widely used as feed additives to control coccidial intestinal parasites and improve feed efficiency, but due to low metabolism most of the drugs are excreted by the animals unchanged and eventually end up in wastewaters. Little is known about the impacts of AMP and ASA on the performance of activate sludge in sequencing batch reactors (SBRs) as well as about their fate in wastewater treatment. In this study, the long-term performance of activated sludge in SBRs exposed to ASA and AMP was investigated. The COD removal and nitrification were not affected when the concentration of ASA or AMP was lower than 20 mg L^{-1}, but were markedly inhibited at 100 mg L^{-1} of ASA or AMP. The inhibition to of COD removal was reversible whereas the inhibition to nitrification was irreversible. Phosphate removal was not affected by the continuous exposure to ASA or AMP. ASA and AMP were very resistant to be degraded by the activated sludge in SBRs, and only a small quantity of ASA was degraded to inorganic arsenic (no more than 300 μg L^{-1}) in the form of As (III) and As (V).

1. Introduction

Large amounts of antibiotics and veterinary drugs, about 100,000–200,000 tons, are used around the world every year (Du and Liu, 2011). The usage of these compounds in China was about 25,000 tons per year (Hou et al., 2015), including more than 8000 tons as feed additives to treat infections and promote growth of animals (Ben et al., 2009). However, the majority of veterinary drugs is excreted via manure and urine without metabolism by animals (Sarmah et al., 2006). Residual veterinary drugs in wastewater, surface water and soil environments (Du et al., 2015; Gao et al., 2016; Kümmerer, 2009; Xu et al., 2007), may foster the spread of antibiotics-resistant bacteria and ultimately compromise the clinical or veterinary effectiveness of antibiotics (Bergeron et al., 2015; Boxall et al., 2003; Máthé et al., 2012; Wang et al., 2006). Amprolium hydrochloride (1- ([4-Amino-2-propyl-5-pyrimidinyl] methyl)-2-methylpyridinium chloride, AMP) and arsanilic acid (4-aminophenylarsonic acid, ASA) were widely used as feed additives to control coccidial intestinal parasites and improve feed efficiency (El-Kosasy et al., 2015; Jones, 2007). The approved dosage of AMP in animal feed is around 30 mg kg^{-1} (El-Kosasy et al., 2015), and 50–100 mg kg^{-1} for ASA (Wang et al., 2010). The yearly consumption of organoarsenic feed additives is approximate 1000 tons in China (Wang et al., 2010).

Sequencing batch reactors (SBR) are widely used in wastewater treatment plants (WWTPs) because of the unique advantages of this process configuration. Compared to conventional activated sludge, SBR is known to be simpler and offers more operational flexibility, saving more than 60% of operating cost (Singh and Srivastava, 2011). Furthermore, the SBR performs well for high biomass retention and organic carbon removal (Fernandes et al., 2013). Previous studies have reported that the performance of SBR was affected by the exposure to roxarsone (ROX), which is similar to ASA, and the removal of chemical oxygen demand (COD), nitrogen and phosphorus was inhibited (Guo et al., 2013; Liu et al., 2014). The SBR process with aerobic granular sludge can remove fluoroquinolones, but the efficiency was lower than expected (Amorim et al., 2014).

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Physical-chemical processes have been studied for the treatment of ASA, e.g. photo-oxidation (Czaplicka et al., 2014) and common chemical oxidation processes (Wang and Cheng, 2015). 37–59% of the added arsanilic acid was degraded after 115 days of incubation under anaerobic conditions (Wang et al., 2014). Little is known about the effect of AMP and ASA on the performance of SBR as well as about the fate of the compounds in the treatment process. Therefore, the aim of this study was to evaluate the performance of activated sludge in SBR with long-term exposure to ASA and AMP and their removal during the process.

2. Materials and methods

2.1. Chemicals

AMP and ASA were purchased from Sigma Aldrich (Steinheim, Germany) and Alfa Aesar (Heysham, UK), respectively. Methanol and acetonitrile were purchased from CNW Technologies (Düsseldorf, Germany) with HPLC grade. Other chemicals were purchased from Sinopharm Chemical Reagent Co. Ltd with analytical grade.

2.2. Synthetic wastewater

The synthetic wastewater contained (per liter of water): 445.2 mg $C_{12}H_{22}O_{11}$ (sucrose), 96 mg NH_4Cl, 28.22 mg $K_2HPO_4 \cdot 3H_2O$, 5.14 mg KH_2PO_4 (COD: N: P = 100: 5: 1), 100 mg $MgSO_4 \cdot 7H_2O$, 0.83 mg $FeCl_3 \cdot 6H_2O$, 7.5 mg $CaCl_2$, 0.25 mg $Al_2(SO_4)_3 \cdot 18H_2O$, 0.11 mg $ZnSO_4 \cdot 7H_2O$, 0.05 mg $MnSO_4 \cdot H_2O$, 0.05 mg $CoCl_2 \cdot 6H_2O$, 0.05 mg $NiCl_2 \cdot 6H_2O$, 0.05 mg $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.05 mg H_3BO_4, and 0.05 mg $CuCl_2$.

2.3. Set up and operation of the SBRs

Three parallel SBR reactors (R0, R1 and R2) each with working volume of 3 L were used in this experiment. The height and inner diameter of the reactor were 48.5 cm and 10 cm, respectively. Activated sludge from a municipal wastewater treatment plant (Hefei, China) was used as the inoculum. After washing with tap water, the activated sludge was equally divided into three parallel reactors. The system was operated in cycles by an automatic timer to control pumps for influent, aeration, and effluent discharge. The volume exchange ratios of three SBRs were all set at 70%. The SBRs were operated for two cycles per day at room temperature. Each cycle lasted for 12 h, with 10 min for feeding, 480 min for aeration, 180 min for settling, 5min for effluent discharge, and 45 min for idling. Hydraulic retention time (HRT) and sludge retention time (SRT) were 17 h and 16 d, respectively. The mixed liquor suspended solids (MLSS) was 3447 ± 351 mg L^{-1}, and dissolved oxygen (DO) in aerobic reaction period was 3.4 ± 0.3 mg L^{-1}. The pH of inflow was maintained at 7.5 ± 0.3 by dosing 0.5 M NaOH or 0.5 M HCl.

2.4. Experimental procedure

Three identical reactors run simultaneously and seven phases were segmented for each reactor, as listed in Table 1. One reactor was used as the blank control (R0) which was free of ASA and AMP in the influent. The other two reactors (R1 and R2) were continuously spiked with ASA and AMP in the influent, which were used to evaluate the response of the activated sludge.

2.5. Activity batch experiments

Biomass activity was determined through ex-situ experiments. Activated sludge was taken from the reactor at phase VI and IV. A blank control was always conducted throughout the experiments.

Table 1
The spiked concentrations of ASA and AMP in three SBRs.

Phase	Days	Influent (mg L^{-1})				Cycle time (h)
		R0		R1	R2	
		ASA	AMP	ASA	AMP	
I	0–29	0	0	0	0	12
II	30–49	0	0	5	5	12
III	50–69	0	0	10	10	12
IV	70–89	0	0	20	20	12
V	90–109	0	0	50	50	12
VI	110–139	0	0	100	100	12
VII	140–159	0	0	0	0	12

Nitrification and denitrification rates were evaluated by specific ammonium uptake rate (sAUR) and specific nitrate uptake rate (sNUR), respectively. The phosphorus uptake and release rate (sPUR, sPRR) were also determined for assessing the activity of phosphate accumulating organisms in aerobic and anaerobic conditions. In every test, 400 mL of activated sludge was washed with tap water and then placed in flasks. The temperature and pH were maintained at 25 ± 2 °C and 7.5 ± 0.3, respectively. DO was kept at 4.0 ± 0.5 mg L^{-1} in aeration phase. Ammonium (20 mg L^{-1}), nitrate (10 mg L^{-1}) and phosphorus (10 mg L^{-1}) were added to flasks for activity tests. Samples were collected from flasks every 10–20 min and the ammonium, nitrite, nitrate and phosphorus were measured. The sAUR, sNUR, sPUR and sPRR were determined by linear fitting of their concentrations over time by the constant concentration of volatile suspended solids (VSS).

2.6. Analytical methods

Liquid sample was immediately filtered through a cellulose acetate filter (0.22 μm pore-size) for the analysis of nitrate, nitrite and residual drugs. MLSS, VSS, COD, NH_4^+–N, PO_4^{3-}-P were determined according to the standard methods (APHA, 2005). Nitrate and nitrite were analyzed using ion chromatograph with conductivity detector (DIONEX, ICS-90, USA).

A high performance liquid chromatograph (HPLC, 1260 infinity, Agilent Technologies) with UV detector was used to measure the concentrations of ASA and AMP at 270 nm. The Wondasil C18 column (4.6 mm × 250 mm, 5 μm, GL Sciences Inc. Japan) was applied for the separation of ASA and AMP, and the mobile phase composed of acetonitrile, water and formic acid (25:75:1, V/V/V) at a flow rate of 1.0 mL min^{-1}.

Concentrations of total arsenic and total inorganic arsenic were measured with an atomic fluorescence spectrometer (AFS-8220, Titan Beijing LTD, China), (Zhang et al., 2014). The arsenic species were determined by a high performance liquid chromatography–hydride generation-atomic fluorescence spectrometer (HPLC–HG-AFS, SAP-10, Titan Beijing LTD, China), (Shi et al., 2014).

3. Results and discussion

3.1. Impacts on SBRs performance

3.1.1. COD removal

Fig. 1 depicts the COD removal efficiency in three SBRs exposed to ASA and AMP under different phases. The phase I was used to acclimate activated sludge and the COD removal efficiency in each reactor eventually reached around 95%. During the phases II to IV, the COD removal efficiency was not markedly influenced by the spiked ASA and AMP, indicating that the mineralization of organic matter was not affected when ASA and AMP concentrations were below 20 mg L^{-1}. However, from the beginning of phase V, the COD