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Inhibition of the partial nitritation by roxithromycin and Cu(II)

Qiong Guo [a,b], Zhi-Jian Shi [a,b], Jia-Li Xu [a,b], Chen-Chen Yang [a,b], Mei Huang [a,b], Man-Ling Shi [a], Ren-Cun Jin [a,b,*]

[a] College of Life and Environmental Sciences, Hangzhou Normal University, Hangzhou 310036, China
[b] Key Laboratory of Hangzhou City for Ecosystem Protection and Restoration, Hangzhou Normal University, Hangzhou 310036, China

HIGHLIGHTS

- The IC_{50} of ROX and Cu(II) on PN sludge were 346 and 74.3 mg L^{-1}, respectively.
- The response–concentration effects were simulated by various inhibition models.
- NOB was more sensitive than AOB to ROX.
- The combined toxicity of ROX and Cu(II) was shown to be synergistic.
- FTIR was used to investigate changes in the functional groups of the PN sludge.

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ABSTRACT

To facilitate the application of partial nitritation (PN) – anaerobic ammonium oxidation process in nitrogen removal from livestock wastewater, the inhibition of roxithromycin (ROX) and Cu(II) on the PN sludge was examined using a respirometric method. The results showed that the IC_{50} of ROX and Cu (II) on PN sludge were 346 and 74.3 mg L^{-1}, respectively. The relative specific respiration rate (SRR) of ammonia-oxidizing bacteria (AOB) decreased from 87.4% to 17.7% with the ROX concentration increased from 0 to 500 mg L^{-1}. When the concentration of Cu(II) increased from 0 to 160 mg L^{-1}, the SRRs of AOB and nitrite-oxidizing bacteria decreased by 85.5% and 11.2%, respectively. According to the isobole plots analysis, combined suppression by ROX and Cu(II) was synergistic. Fourier transform infrared spectroscopy analyses showed that ROX exposure altered the positions of C—O bonds, and the intensity of the absorption peak at 2100 cm^{-1} changed under Cu(II) exposure.

1. Introduction

A combination of partial nitritation (PN) and anaerobic ammonium oxidation (anammox) has been one of the most innovative developments in biological wastewater treatment for high nitrogen removal efficiency in recent years. Partial nitritation provides nitrite for anammox at a lower energy cost and with a lower environmental footprint than other methods (Jemaat et al., 2014; Jin et al., 2013a). In a PN reactor, ammonium is partially oxidized to nitrite by accumulated ammonia-oxidizing bacteria (AOB), whereas nitrite-oxidizing bacteria (NOB) are eliminated or inhibited; thus, nitrite can accumulate in the reactor without further oxidation (Rongsayamanont et al., 2014). The PN reactor must include an effluent that is suitable for the subsequent anammox stage, i.e., an effluent with a nitrite/ammonium ratio near 1.

However, PN is sensitive to various conditions, such as dissolved oxygen (DO), temperature, inflow variation, pH and reactor configuration (Durán et al., 2014; Gu et al., 2012; Hao et al., 2002; Rathnayake et al., 2015). Several studies have also documented the effects of xenobiotics or inhibitors present in wastewater, such as quinoline, *o*-cresol and NaCl (Fu and Zhao, 2015; Jemaat et al., 2014; Jin et al., 2007; Wan et al., 2014). Livestock wastewater, which contains high concentrations of ammonia nitrogen, requires pre-treatment with biotechnological processes such as PN. The treatment efficiency depends on the composition of the wastewater and the operation conditions. In recent decades, large quantities of drugs have been used in veterinary medicine (Andreu et al., 2009) or as growth promoters at sub-therapeutic doses in swine, cattle and poultry. As Gao et al. (2012) reported, more than

* Corresponding author at: College of Life and Environmental Sciences, Hangzhou Normal University, Hangzhou 310036, China.
E-mail address: jrczju@aliyun.com (R.-C. Jin).

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25,000 tons of antibiotics are used in China each year, and their global annual usage is estimated to range from 100,000 to 200,000 tons (Kümmerer, 2009). Residues of these veterinary drugs have been widely detected in environmental samples such as livestock wastewater. Large quantities of these pharmaceuticals and their primary metabolites are rapidly excreted after administration to animals (Yu et al., 2014), and they are particularly conspicuous in livestock wastewater, where they may influence or inhibit microbial activity during the biological treatment of nitrogen pollutants. Roxithromycin (ROX) is an emerging macrolide antibiotic that is widely used to eliminate Gram-positive, anaerobic bacteria, and its half-life in water is as long as 130 or 180 d (Choi et al., 2008; Massé et al., 2014). The ROX concentration ranges between 44.7 and 130.2 ng L^{-1} in mainstream in Korea, while, in swine, beef, and poultry manures the concentration of the most commonly used antibiotics have been reported to be as high as 216 mg L^{-1} (Álvarez et al., 2010; Choi et al., 2008).

In addition to antibiotics, heavy metals, which are common livestock farming feed additives, are frequently found in piggery wastewater. Heavy metals can accumulate rapidly via relatively nonspecific metal transport systems; they are non-biodegradable and can accumulate in living organisms, thus inhibiting microbial activity in wastewater treatment systems (Scullion et al., 2007). Copper, a common heavy metal, can be bound as a cation into biofilm matrices and can even enter cells, and sulfides produced in anaerobic pockets can lead to CuS precipitation (Zhang et al., 2015). And its concentrations have been found with the highest level of 419, 656 and 356 mg L^{-1} in swine, poultry or dairy cattle wastes (Bolan et al., 2004).

The respiratory rate of the bacteria in activated sludge is the rate at which they use energy for various biological processes. This rate can be directly measured as the rate of heat production but is easier to measure indirectly from the rate of oxygen consumption. Any decrease in energy expenditure—or in the respiration rate—has a direct correlation with toxic effects on biological processes. The state of health of microorganisms can be easily deduced from their rate of respiration. Therefore, respiration is an essential activity of aerobic bacteria. For this reason, respiration inhibition is a significant and straightforward factor that can be used to assess the ecotoxicological risk of foreign substances in wastewater. In contrast to bioluminescence, activated sludge respirometry is a more direct method for measuring sludge activity and thus inhibition or toxicity (Ren, 2004).

Moreover, inhibitors are meaningful tools for studying the metabolic mechanisms and physiology of specific groups of microorganisms. In addition, 2 necessary conditions must be met: (1) the inhibitors must be able to coexist with nitrogen without reacting, and (2) the inhibitors must display prominent inhibition selectivity. With the help of a selective inhibitor, the effects of additives (toxic substances) on specific microorganismal groups can be clearly determined.

A preliminary feasibility study regarding the effects of toxic substances in livestock wastewater on the partial nitritation process was required with respect to the pre-treatment of livestock wastewater using PN. Moreover, the combined effects of ROX and Cu(II) on a partial nitritation system, especially with respect to the specific oxygen uptake rate (SOUR) of indigenous microbes in a lab-scale experiment, had not been explored.

Therefore, this study aimed to (1) quantify, using the respiration rate of bacteria in a partial nitritation sludge, the short-term effects of ROX and Cu(II); (2) investigate the biological selectivity of ROX and Cu(II) towards AOB and NOB; (3) assess the acute combined toxicity of ROX and Cu(II); and (4) identify changes to the functional groups present in sludge samples exposed to ROX and Cu (II) under varying conditions.

2. Materials and methods

2.1. Reactor and operating conditions

A 6.8-L internal-loop airlift reactor was operated in the continuous-flow mode at 30 ± 1 °C with a hydraulic retention time (HRT) of 26 h. Air was supplied at the bottom of the reactor. The DO concentration was maintained at 0.8–1.3 mg L^{-1}, and the level of free ammonia (FA) in the system was between 48.4 and 95 mg L^{-1}, which maintained partial nitritation. The seed sludge was obtained from a lab-scale partial nitritation reactor that was operated under similar conditions. The feed medium was devoid of organic carbon and contained 950 mg NH_4^+-N L^{-1}, and other conditions were set as previously described (Xing et al., 2013). The system ran with an NH_4^+-N removal efficiency (NRE) of approximately 43–59%, and the molar ratio of NO_2^--N:NH_4^+-N in the effluent ranged from 0.8 to 1.2.

2.2. Respirometric method and individual inhibition tests

The basis for the respirometric tests is that the respiration rate of activated sludge can be reduced in the presence of toxic substances. Measuring the oxygen uptake rate is the most common method of measuring the bacterial respiration rate.

A Strathtox respirometer (Strathkelvin Strathtox, Scotland, UK) was used to measure the toxicity of trade effluents entering the wastewater treatment plant (WWTP). Respiration inhibition is one of the preferred tests for heterogeneous cultures of microorganisms in an aqueous medium. This test measures the respiration inhibition caused by different concentrations of ROX and Cu(II) relative to the respiration in a control sample.

The laboratory temperature was set to 30 ± 1 °C. Tests were performed in six 20-mL glass tubes, and the synthetic sewage and test mixtures were added to each tube at different dilution ratios. Each tube contained a 2-mL sample of activated sludge that was obtained from the internal-loop airlift reactor. All solutions were prepared using an inorganic solution (as in the reactor operating conditions, except that a fixed initial substrate level was set at 100 mg NH_4^+-N L^{-1}). The readings were recorded in triplicate for each concentration and for the control mixture. Concentrations were selected to reflect the real ones of ROX and Cu(II) found in livestock wastes, and the concentrations used in the individual-effect experiments for each inhibitor are listed in Table 1. The individual acute toxicities of ROX and Cu(II) were determined at a fixed initial substrate level. When the initial substrate level was set to 100 mg NH_4^+-N L^{-1}, an initial ROX concentration gradient of 0, 100, 200, 300, 400 and 500 mg L^{-1} was adopted.

The specific respiration (SR) rate was calculated according to Eq. (1). Respiration inhibition was expressed with respect to the relative specific respiration rate (SRR), which was calculated according to Eq. (2).

$$SR = \frac{OUR}{VSS} \times 10^{-3} \quad (1)$$

where OUR is the oxygen uptake rate (mg L^{-1} h^{-1}) and VSS is the volatile suspended solids (g L^{-1}).

Table 1
The concentrations of various inhibitors in the individual-effect experiments.

Inhibitor	Concentration setting					
ROX	*i*	*1*	*2*	*3*	*4*	*5*
	Concentration (mg L^{-1})	100	200	300	400	500
Cu(II)	*i*	*1*	*2*	*3*	*4*	*5*
	Concentration (mg L^{-1})	40	80	120	160	200

i represents the concentration gradient *1*, *2*, *3*, *4*, *5*.