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Inhibitory effects of sulfamethoxazole on denitrifying granule properties: Short- and long-term tests

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HIGHLIGHTS

- The effects of sulfamethoxazole on denitrifying granules were evaluated for the first time.
- The mathematical models used to quantify the performance fit the trial data well.
- Sulfamethoxazole changed the granule properties after long-term operation.
- The 100 mg L^{-1} sulfamethoxazole concentration had no adverse effects on the nitrogen removal performance.

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ABSTRACT

The broad-spectrum antibiotic sulfamethoxazole (SMX) was chosen to assess its short- and long-term effects on denitrifying granules. The SMX concentration and pre-exposure time in batch testing influenced the denitrifying granule activity. In the continuous-flow experiments, no inhibitory effects on the upflow anaerobic sludge blanket performance were observed at SMX concentrations up to 100 mg L^{-1}, probably because of functional redundancy and long-term acclimation. The specific denitrifying activity first decreased to a minimum of 49.3% and then recovered to a level comparable to the initial level as the SMX concentration increased. The changing trend of the extracellular polymer content was consistent with the specific denitrifying activity throughout the process, and relatively high EPS loss ratios (maximum loss of 61.8%) were observed. Additionally, the diameter of the denitrifying granules monophonically increased to a final value of 35.0%. This research provided the application of denitrifying granules to treat wastewater that contained antibiotic.

1. Introduction

The demand for medical antibiotics has significantly increased over the past three decades. Large amounts of veterinary antibiotics have been utilized to disease control and sterilization in livestock farms, and different types of antibiotics have been used as sub-therapeutics to promote animal growth. The misuse of antibiotics in China is common (Yan et al., 2013a,b; Zhang et al., 2010). Antibiotics originating from man-made input from wastewater disposal, fertilizer treatments, and aquaculture are frequently found in pharmaceutical wastewater, piggery wastewater, black water and municipal sludge (Kümmerer, 2009; Michael et al., 2013), which are featured by a high organic matter and high ammonium species contents.

Nitrogen and carbon-containing compounds are the main contaminants in wastewater that cause serious eutrophication (Guo, 2007; Smith et al., 1999). Anaerobic digestion united with methane gas production, as administered in reactors, is extensively applied and effective organic matter removal method (Hashimoto, 1983; Lo et al., 1994). Biological nitrogen removal is the crucial way of wastewater treatment technology at the moment, and the composite process of nitrification and denitrification is the most economical and reasonable way to nitrogen removal from wastewater (Bernet and Béline, 2009). However, a small amount of antibiotics are not thoroughly degraded during the anaerobic digestion process and have been present in the effluent (Michael et al., 2013).

The antibacterial properties of antibiotics may influence denitrifying bacteria and their denitrification rates (Conkle and White, 2012; Kandeler et al., 2006). Sulfamethoxazole (SMX) is a kind of sulfonamides that suppress folic acid production in bacteria, which is an essential prerequisite to the production of nucleic acids (Underwood et al., 2011). Antimicrobials such as SMX may

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coexist with NO_3^--N pollutant from wastewater or animals operations (Barber et al., 2009; Batt et al., 2006); thus, understanding the impacts of these antibiotics on naturally occurring biological nitrogen removal processes, for instance, denitrifying, is important. However, no studies have studied the response of denitrifying granules in the existence of SMX. Denitrifying bacteria might also go through starvation conditions in under-loaded bioreactors as a result of large flow fluctuations and component of wastewater. The impact of SMX on denitrifying bacteria may vary relying on whether the substrates are actively metabolized. Therefore, the lack of NO_3^--N and COD, which may decrease the resistance of denitrifying bacteria to SMX suppression, should be considered.

The effect of sulfonamide antibiotics on denitrification has been evaluated in a number of studies (additional details are shown in Table 1) (Ahmad et al., 2014; Conkle and White, 2012; Hou et al., 2014; Kotzerke et al., 2008; Underwood et al., 2011; Yan et al., 2013a,b); however, to date, there has been little agreement on how sulfonamide antibiotics influence this process in denitrifying biogranules. In this research, the acute toxicities impact of sulfamethazine (SMZ) on denitrifying granules was explored using batch tests. The impact of the original matrix level was analyzed, and the influence of starvation and pre-exposure were studied. Besides that, the long-term effects of SMZ on the performance of reactor and the attributes of the denitrifying granules were tracked.

2. Materials and methods

2.1. Experimental setup and inocula

An up-flow anaerobic sludge blanket (UASB) reactor (Fig. 1) was used to cultivate denitrifying granules. This reactor, which was composed of polymethyl methacrylate, had a working volume of 0.5 L (24-m height and 0.06-m diameter). The seeding denitrifying granules without previous SMX exposure were obtained from a laboratory-scale parent reactor operated steadily over a year at 35 ± 1 °C (Chen et al., 2015). The UASB reactor was initially inoculated with 0.5 L of denitrifying granular sludge with a volatile suspended solid/suspended solid (VSS/SS) ratio of 80%. These inoculation denitrifying granules were cultured under anaerobic conditions, with an average settlement rate of 6.04 ± 1.01 m h^{-1} and a particle diameter of 2.77 ± 0.94 mm. The extracellular polymeric substance (EPS) content was 338.3 ± 30.2 mg g VSS^{-1},

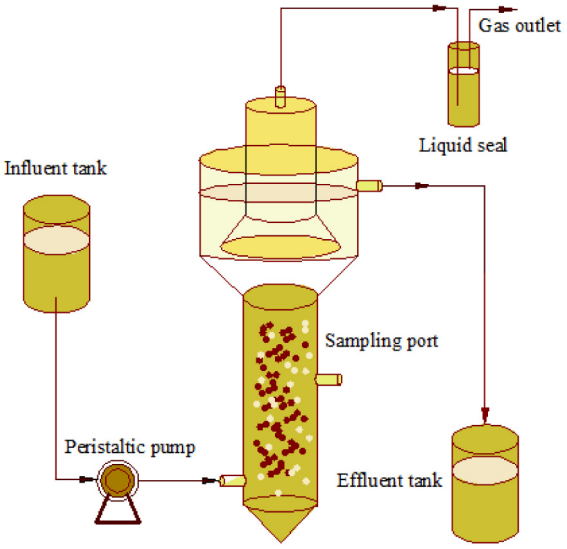

Fig. 1. The schematic representation of upflow anaerobic sludge blanket (UASB) reactor.

and the specific denitrifying activity (SDA) ranged between 422.0 and 674.6 mg NO_3^--N g volatile-suspended solid (VSS) d^{-1}. The UASB reactor was put in a temperature-controlled room at 35 ± 1 °C and run with a fixed hydraulic retention time (HRT) of 2.4 h. The reactor was set at a nitrogen loading rate (NLR) of 7 kg N m^{-3} d^{-1}. The sludge retention time of reactor was about 35 d. Considerable a certain number of antibiotics might flow into denitrifying-based reactors. Therefore, the influent SMX concentration setting in this study intended to assess the potential suppress of the denitrifying bacteria by antibiotics. The SMX concentrations are listed in Table 2.

2.2. Synthetic wastewater

The sodium nitrate and glucose concentrations were 700 mg NO_3^-N L^{-1} and 4200 mg COD L^{-1}, respectively. The chemical oxygen demand (COD) to nitrate nitrogen (COD/NO_3^--N) ratio of 6.0 was higher than the theoretical stoichiometric ratio (4.9) for complete denitrification (including bacterial growth)

Table 1
Summary of sulfonamide antibiotic effects on denitrification in the literature.

Microbial process	Chronic/ acute	Antibiotic				Effects		Environment	References
		Molecule	Nominal concentration	Duration	Quantification (loss)	(Approximate % changement)	Effective concentration		
Denitrification	C	Sulfamethoxazole	1.2 μg L^{-1}–0.5 g L^{-1}	Weeks	No	Inhibition (47%)	1.2 μg L^{-1}	Groundwater	Underwood et al. (2011)
Denitrification	C	Sulfamethoxazole	1–1000 μg kg^{-1}	Days	No	Inhibition	500 μg kg^{-1}	Soil	Conkle and White (2012)
Denitrification	C	Sulfamethoxazole	0.2 μg L^{-1}–50 mg L^{-1}	Weeks	Yes (93%)	Inhibition (39%)	50 mg L^{-1}	Sediment	Yan et al. (2013a, 2013b)
Denitrification	C	Sulfamethazine	0.01–1 mg L^{-1}	Days	Yes	Inhibition of nitrate removal (17%) and nitrite production (82%)[a]	0.01 and 1 mg L^{-1}, respectively	Groundwater	Ahmad et al. (2014)
Denitrification	C	Sulfamethazine	0.05–100 μg L^{-1}	Hours	Yes	Inhibition (20–30%)	50 ng L^{-1}	Sediment	Hou et al. (2014)
Denitrification	C	Sulfamethazine	10 and 100 mg kg^{-1}	Weeks	Yes (66–100%)	Inhibition (≈70%)	10 mg kg^{-1}	Soil	Kotzerke et al. (2008)

A and C, mean acute and chronic effect, respectively; an exposure was designed as chronic if the duration of the exposure reached a value higher than the life-span of the targeted organisms and eventually if the level of exposure was low (ng to μg L^{-1} or kg).

[a] Calculated by the authors.