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Mechanistic model for interpreting the toxic effects of sulfonamides on nitrification

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HIGHLIGHTS

- Substituent groups have a substantial impact on the toxic effects of SAs.
- Mechanistic model was developed to interpret toxic effects of SAs.
- Toxic effects relate to quantum chemical descriptors and toxicodynamic features.

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ABSTRACT

Antibiotics are categorized as pseudopersistent compounds because of their widespread use and continuous emission into the environment. Biological systems such as active sludge and biofilms are still the principal tools used to remove antibiotics in wastewater treatment plants (WWTPs). Consequently, it is important to determine the relationship between toxic effects in biological WWTPs and the structural characteristics of antibiotics. In the present study, toxic effects of 10 sulfonamides (SAs) on nitrification in an active sludge system were studied. The toxicity results (half-effective concentrations, EC_{50}) indicated that the toxicity of sulfadimethoxine (SDM) is approximately 4 times as large as that of sulfadiazine (SD). Based on the toxicity mechanism and the partial least squares regression (PLS) method, an optimum quantitative structure-activity relationship (QSAR) model was developed for the test system. The mechanistic model showed that the pK_a, the binding energies between SAs with dihydropteroate synthetase ($E_{binding}^{SA-DHPS}$) and the binding energies between SAs with ammonia monooxygenase ($E_{binding}^{SA-AMO}$) are the key factors affecting the toxic effects of SAs on nitration process in active sludge system, following an order of importance of $E_{binding}^{SA-DHPS} > E_{binding}^{SA-AMO} > pK_a$.

1. Introduction

Antibiotics have been largely used in human and veterinary medicine, as well as in the feeding of domestic animals [1]. Due to a lack of proper guidance for use, many antibiotics and their metabolites, such as aminoglycosides, macrolides and sulfonamides (SAs), are mostly excreted as active compounds into the environment [2]. Thus, antibiotics in waste water treatment plants (WWTPs) were typically detected and the concentrations of them are generally in the lower μg-per-litre [3]. On the one hand, biological WWTPs using, for example active sludge and biofilm processes, are still the principal systems used to remove antibiotics [4]. Interestingly, on the other hand, bacteria are the target of antibiotic action and are also the key actors in biological wastewater treatment systems that employ active sludge and biofilm. Therefore, the potential effects of antibiotics on biological WWTPs have drawn great attention from researchers in recent years [5,6].

To date, effects of antibiotics on WWTPs were mostly focused on the microbial community structures and pollutant removal efficiency. Wang et al. [7] proved that the presence of antibiotics may affect microbial growth and microbial ecology especially under lower organic loading conditions; Deng et al. [8] investigated the microbial community at high level antibiotic wastewater, and the results showed that the microbial community structures in aerobic

Abbreviations: SAs, sulfonamides; WWWTP, wastewater treatment plants; QSAR, quantitative structure activity relationship; PLS, partial least squares regression; DHPS, dihydropteroate synthetase; AMO, ammonia monooxygenase; NOR, nitrite oxidoreductase.

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reactor were dramatically changed under streptomycin pressure. In the case of pollutant removal in WWTPs, nitrification is an important process of eliminating ammonium nitrogen ammonia and basically comprises of a two-step process: oxidation of ammonia to nitrite by ammonia monooxygenase (AMO) and oxidation of nitrite to nitrate by nitrite oxidoreductase (NOR) [9]. Recently, Prado et al. [10] studied the impact of tetracycline on a semi-industrial MBR process. Their results demonstrated that denitrification was slightly affected, but there was no effect on the elimination of organic matter or ammonium. Alighardashi et al. [11] revealed that the average rates of inhibition by erythromycin on nitrification at 10 mg L^{-1} and 20 mg L^{-1} were 46% and 72%, respectively; Campos et al. [12] demonstrated that oxytetracycline inhibited nitrification activity only above 100 mg L^{-1}. Previous studies have proven that the toxic effects of antibiotics on nitrification in WWTPs relate to their structural characteristics.

In the field of environmental toxicology and pharmacology, the effects of antibiotic structure on toxicity and pharmacological activity have been well established [13]. For instance, González-Pleiter et al. [14] determined the toxic effects of five antibiotics on a cyanobacterium, and the EC_{50} values ranged between 0.022 mg L^{-1} and 56.3 mg L^{-1}, with an order of toxicity of erythromycin> levofloxacin > norfloxacin > tetracycline > amoxicillin; Kim et al. [15] also revealed the effects of antibiotic structure on toxicity using three tested organisms (*Vibrio fischeri*, *Daphnia magna and Oryzias latipes*). To extensively investigate the relationship between structures and toxic effects, several mechanistic quantitative structure-activity relationship (QSAR) models have been developed on the basis of physical parameters, quantum chemical parameters and interaction parameters, in both single toxicity tests and mixture toxicity tests. Jiang et al. [16] reported that the toxicity of antibiotics on photobacteria was highly related to quantum chemical parameters, including the lowest occupied molecular energy and dipole moment. Based on QSAR and molecular docking approaches, our previous work [17] proved that the mechanisms for the different acute and chronic toxicities of a mixture (SA with trimethoprim) related to the receptors and actual binding concentrations. Recently, QSAR was also used to reveal the relationship between structures and their environmental behaviors (i.e., degradation characteristics) in WWTP systems [18,19]. Using a mechanistic QSAR model, Sudhakaran et al. [19] successfully explained how the structures of pollutants impact their ozonation degradation efficiencies. However, little is known about the relationship between the structures of antibiotics and their toxic effects on nitrification in WWTPs, and the possible mechanism for this relationship also remains unclear.

Sulfonamides (SAs) are antibiotics that act as competitive inhibitors of the enzyme dihydropteroate synthetase (DHPS) and have been effectively used in human and veterinary medicine. Consequently, several SAs, such as sulfamethoxazole (SMZ) and SDM, have been frequently detected in active sludge systems [20]. Therefore, the aims of the present study were (1) to evaluate the toxic effects of SAs on the nitrification process in an active sludge system and (2) to develop a mechanistic QSAR model to interpret the toxic effects of SAs on the nitrification process.

2. Materials and methods

2.1. Tested SAs

Ten SAs were selected as the tested chemicals in this study; including sulfapyridine (SPY), sulfadoxine (SFD), sulfadimidine (SM 2), sulfamerazine (SMI), sulfamethoxypridazine (SMP), sulfamethoxypyridazine (SCP), sulfafurazole (SIZ), SDM, SD and SMZ. All of the tested SAs were obtained from the Aladdin Chemistry Co. Ltd. (http://www.aladdin-e.com/ Shanghai, China) and were used without further purification (purity ≥ 99%); and the corresponding structures are listed in Fig. S1.

2.2. Nitrifying sludge cultures

Autotrophic nitrifying sludge was cultivated in sequencing batch reactor (SBR) (H 0.22 m × W 0.25 m × L 1 m with working volume 40 L) at about 21 °C (close to room temperature). Hydraulic retention time (HRT) and solids retention time (SRT) were set at 12 h and 30 days, respectively. The reactor was fed with inorganic synthetic wastewater consisting of ammonium chloride and sodium bicarbonate as nitrogen source and carbon source, respectively. The composition of the synthetic wastewater was shown in Table S1.

The SBR (mixed liquor volatile suspended solids (MLVSS), 3200 ± 100 mg L^{-1}) was operated at three cycles each day (cycle length 8 h). Each cycle consisted of 0.5 h feeding and 5 h aerating, followed by 1 h settling, 10 min decanting and 80 min for the remaining idle phase. In aerating periods, dissolved oxygen was maintained at about 3.5 mg L^{-1} with micro air compressor. Influent pH was adjusted to 7.5 by adding NaOH or HCl (4 mol L^{-1}). Initial concentrations of ammonia nitrogen (NH_4^+-N) were 30 mg L^{-1} at the starting stage and increased to 200 mg L^{-1}. After 60 days of cultivation, stable removal of ammonia nitrogen in the SBR was observed (over 98%, Fig. S2), suggesting good performance of the tested activated sludge system in terms of nitrogen removal.

2.3. Toxicity tests

Nitrification toxicity tests were carried out in 1000 mL Erlenmeyer flasks in a rotary shaker at 150 rpm and 21 °C, with dissolved oxygen of 3.5 mg L^{-1}. All the flasks were covered with aluminum foil to avoid possible photo-degradation effects of tested SAs. The toxicity test system (400 mL) consisted of 100 mL prepared autotrophic nitrifying sludge, and 100 mL synthetic wastewater (Table S1). Deionized water was last added to make the final volume in each flask 400 mL, resulting in the final concentration of ammonia nitrogen 200 mg L^{-1}.

The prepared autotrophic nitrifying sludge was obtained as follows: sludge was drawn after aerating stage from the parent SBR and then centrifuged at 150 rpm for five minutes, washed with NaCl solution (0.16 mol L^{-1}) for three times and resuspended in 400 mL deionized water. As for each tested SA, the toxicity test was uniformly conducted in triplicate at concentrations of 0 (control), 0.1, 0.32, 0.56, 0.8, 1.0, 1.32, 1.56, 1.8, 3.2 mmol L^{-1}.

The change in NH_4^+-N, nitrite–nitrogen (NO_2^--N), and nitrate–nitrogen (NO_3^--N) were measured to reveal the inhibition effects of the SAs on the nitrification process. Based on the pre-experiment results (Fig. S3), 8 h was chosen as the exposure duration of SAs upon the nitrifying active sludge system. The toxicity of each SA toward the nitrification process was expressed as an inhibition ratio (E or x) as follows:

$$E = x = \frac{N_{\text{control}} - N}{N_{\text{control}}} \times 100\% \quad (1)$$

where control was an average of the NH_4^+-N concentration exposed to the controls and N was an average of the NH_4^+-N concentration to the test SA.

2.4. Dose-response curve fitting

The derived concentration relationship data for the tested SAs were fitted to a dose-response model (Eq. (2)) [21]. The fitted function was evaluated by the determination coefficient (R^2) and the