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Degradation Sulfamethoxazole by Chlorination in Water Distribution

Systems: Kinetics, Toxicity, and Antibiotic Resistance Genes

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Declarations

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Consent to participate This research does not include human experiments.

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Abstract: Sulfamethoxazole (SMX) is a veterinary drugs and feed additives, which has been frequently detected in surface waters in recent years. This paper investigated the kinetics, evolution of toxicity and antibiotic resistance genes (ARGs) of SMX in reactions with free chlorine (FC) to evaluate the fate of SMX in batch reactors and water distribution systems (WDS). In the range of investigated pH (6.3 – 9.0), the SMX degradation had the fastest rate at close to neutral pH. The chlorination of SMX could be described by the first-order kinetics, with specific second-order rate constants in batch reactors of $(2.23 \pm 0.07) \times 10^2 \text{ M}^{-1} \text{ s}^{-1}$ and $(5.04 \pm 0.30) \times 10^1 \text{ M}^{-1} \text{ s}^{-1}$ for HClO and ClO^- , respectively. And in WDS of $(1.76 \pm 0.07) \times 10^2 \text{ M}^{-1} \text{ s}^{-1}$ and $(4.06 \pm 0.62) \times 10^1 \text{ M}^{-1} \text{ s}^{-1}$, respectively. The SMX degradation rate was also affected by pipe material, and the rate followed the order: stainless-steel pipe (SS) > ductile iron pipe (DI) > polyethylene pipe (PE). The flow rate from 0.7 to 1.5 m/s led to an increase of SMX degradation rate in DI, but the increase was limited. In addition, SMX could increase the toxicity of water initially, yet the toxicity reduced to the level of tap water after 2 hours chlorination. The relative abundance of ARGs (*sul1* and *sul2*) of tap water samples was significantly increased under different conditions including only use SMX, chlorination products of SMX, or pretreatment with SMX followed by chlorination.

Key words: Sulfamethoxazole (SMX); Water distribution system (WDS); Chlorination; Kinetics; Toxicity; Antibiotic resistance genes (ARGs)

1.Introduction

Since penicillin by Alexander Fleming was discovered in 1928, antibiotics have

been widely used to prevent human and animal infectious diseases (Huang et al. 2020, Wang et al. 2020, Zhang et al. 2015a). However, 60 – 80% of antibiotics are not absorbed after entering human and animal bodies but directly discharged from the body through feces or urine (Chen et al. 2017), which has become the main source of antibiotic residues in the environment. In addition, aquaculture industry (Tian et al. 2013), medical and pharmaceutical wastewater (Gobel et al. 2005, Lindberg et al. 2004) is also an important source of environmental antibiotic residue. Antibiotics are considered to be "pseudo persistent" pollutants because they continue to enter the ecosystem (Gulkowska et al. 2008), which has aroused great concern because of they may impose a potential risk to the non-target species (Chen et al. 2011).

Sulfonamides (SAs) are one of the most widely used antibiotics in the world (Gaffney et al. 2016), with more than 20,000 tons of SAs (excluding drugs used as herbicides) are introduced into the biosphere each year (Qiu et al. 2016). As a kind of fairly water-soluble polar compounds, SAs are weakly degraded in the nature, thus they can easily enter surface runoff or be leached into the groundwater. Luo et al. (Luo et al. 2011) reported that the concentration of trimethoprim, trimethoprim, sulfamethoxazole, sulfachloropyridazine in Haihe Rive was $(1.2 \pm 0.8) \times 10^2$ ng/L, $(1.2 \pm 0.8) \times 10^2$ ng/L, $(1.7 \pm 1.0) \times 10^2$ ng/L and $(1.8 \pm 1.4) \times 10^2$ ng/L, respectively. A survey (Jiang et al. 2011) of the Huangpu River found that the concentration of trimethoprim, sulfadiazine, sulfamethoxazole, sulfachloropyridazine was 62.39 ng/L, 40.55 ng/L, 623.27 ng/L, 58.29 ng/L, respectively. Li et al. (Li et al. 2017a) reported that the concentration of oxytetracycline reached 600 ng/L in tap water in Hong Kong. Therefore, the existence

of antibiotics is not only limited to the environmental water of nature, but also exists in the drinking water related to human beings. SAs concentrations in natural water are quite low, ranging from nanograms to micrograms per liter (ng/L – µg/L) (Zhang et al. 2018b), but it still has ecotoxicity at this level (Fent et al. 2006). For example, SAs can damage the human immune system, pose skin allergic reactions (Choquet-Kastylevsky et al. 2002) and even carcinogen risk (Shao et al. 2005), inhibit the germination of plants (Migliore et al. 2003), and cause ecological bioaccumulation in aquatic organisms (Peiris et al. 2017). Furthermore, they can promote the microbial genetic variation (Jeong et al. 2010), which may lead to the emergence of ARGs and antibiotic-resistant bacteria (Zheng et al. 2017). Guo et al. (Guo et al. 2014) investigated the ARGs levels in the Yangtze River Delta of China and detected the existence of 10 ARGs in the source waters of the 7 water treatment plants, among which the relative abundance of ARGs *sul1*, *sul2*, *tet (c)* and *tet (g)* is the highest, total concentrations of ARGs were above 10⁵ copies / mL. The *sul1* and *sul2* were also the most frequently detected sulfonamide ARGs in surface waters in Germany and Australia (Stoll et al. 2012). ARGs and antibiotic-resistant bacteria could enhance the potential for both new and old diseases to emerge (Mulvey & Simor 2009).

Various technologies were used to remove or degrade SAs in water, including biodegradation (Chen & Xie 2018, Yu et al. 2018), activated carbon and porous resin adsorption (Choi et al. 2008, Yang et al. 2011), membrane filtration (Rosman et al. 2018), free chlorination (Gaffney et al. 2016) and advanced oxidation (Beltran et al. 2008, Guo et al. 2015b). Among these methods, free chlorination is regarded as one of

the most effective technologies due to its low price, continuous disinfection, wide application scope and high oxidation efficiency for a variety of organic compounds (Dong et al. 2019, Dong et al. 2017). Nassar et al. (Nassar et al. 2018) have shown that FC can react with SAs in the first-order. Dodd et al. (Dodd &Huang 2004) established a degradation model of SMX by chlorine under different pH based on pseudo second-order kinetics, and detected the chlorination products by using GC-MS and LC-MS derivatization methods and proposed the degradation pathway (Dodd &Huang 2004, Gaffney et al. 2016). However, most SAs chlorination studies were generally conducted under lab-scale static conditions (e.g., beakers with buffered water, or deionized). The SAs chlorination and the potential influence of chlorination on ARGs in dynamic water distribution system (WDS) has been scarcely studied.

Sulfamethoxazole (SMX, CAS Number 723-46-6) is a typical SAs (Yao et al. 2018), which is mainly used as veterinary drugs and feed additives and its selected chemical properties were showed in Fig. S1. This study mainly includes the following aspects: (1) Investigate the relationship between the SMX chlorination and the concentrations of FC, pH, flow rate and pipe materials in batch reactor and WDS; (2) Determine the kinetics of SMX chlorination by FC, and analyze the difference between its degradation in batch reactor and WDS; (3) Evaluate the evolution of toxicity during the SMX chlorination in WDS; (4) Investigate the production of antibiotic resistance genes of pipeline bacteria under the exiting condition of only use SMX, chlorination products of SMX, or pretreatment with SMX followed by chlorination.

2. Materials and methods

2.1 Experimental Materials

SMX ($\geq 98\%$) was purchased from TCI (Shanghai, China), and FC stock solutions were prepared by dilution of commercial 5.5 – 6.5% sodium hypochlorite solution from Aladdin (Shanghai, China). N,N-diethyl-p-phenylenediamine (DPD) was purchased from Hach Company (Loveland, Colorado, USA) and it was used to measure chlorine concentration. HPLC-grade acetonitrile (ACN), methanol (MeOH) and phosphoric acid (H_3PO_4) were purchased from Sigma Aldrich (Shanghai, China). Other reagents, including dibasic sodium phosphate (Na_2HPO_4 , AR grade), sodium dihydrogen phosphate (NaH_2SO_4 , AR grade) and sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$, CP grade) was purchased from Sinopharm Chemical Reagent (Shanghai, China). The bacteria and reactivation reagent were from Hamamatsu Photonics (Beijing, China) were used to evaluate toxicity. The purified water obtained from a Millipore Milli-Q apparatus and tap water obtained from Hangzhou City.

2.2. Batch reactor and WDS

The degradation experiments of SMX were conducted in a batch reactor and WDS with using sodium hypochlorite (NaClO) as a disinfectant. Batch experiments were conducted in 500 mL brown glass beakers to exclude the influence of potential light (Fig. S2 (a)). The WDS system had three separate loops (each loop is 150 mm in diameter and 80 m in length), made of polyethylene pipe (PE), ductile iron pipe (DI), and stainless-steel pipe (SS). Each loop was equipped with an online monitoring system, circulation pump (which can adjust the flow rate), chemical injection ports and sampling tap, and each loop could be operated independently (Fig. S2 (b), (c) and

(d)).

2.3. Degradation experiments of SMX

Using fresh tap water washed each WDS loop at speed of 1 m/s for 20 min before WDS experiments. In the WDS experiments, experimental water was the tap water and its characteristics are shown in Table S1. The initial concentration of SMX was 200 µg/L, and experiments temperature was stabilized at 25 °C by an automatic control system. A 5% sodium hypochlorite solution was added in loops through chemical injection ports, until ultimately concentration of free chlorine reached the required values (0.4, 0.6, 0.8 and 1.0 mg/L). The pH levels (6.6, 7.0, 7.4, 7.8 and 8.2) and flow rates (0.7, 1.0, 1.3 and 1.5 m/s) were adjusted by phosphate buffer and circulation pump, respectively. In addition, the effects of pipe materials were examined by using three kinds of pipes (PE, DI, SS) due to they were commonly used in drinking water distribution system in China. In the batch reactors experiments, the pH levels were set as 6.3, 6.6, 6.9, 7.2, 7.5, 7.8, 8.1, 8.4, 8.7 and 9.0, and other conditions were consistent with WDS experiments. 10 mL samples were collected at 0, 5, 10, 15, 20, 25, 30, 35 and 40 min respectively, and then 1 mL of 100 mg/L Na₂S₂O₃ (as quenching agent) was immediately added to stop further reaction.

2.4. Determination of antibiotic concentration

The SMX in each sample was quantified by an Agilent 1200 Series HPLC, which equipped with a Zorbax Eclipse XDB-C18 column (4.6 mm × 150 mm, 5 µm) and a UV detector (G1314B, Agilent Technologies, Santa Clara, CA, U.S.). The column temperature was set at 30 °C and injection sample volume was 20 µL. The detection

wavelength was set at 270 nm. Pure acetonitrile and 1‰(v/v) phosphoric acid with the ratio of 80:20 was the mobile phase, and the flow was 0.8 mL/min.

2.5 Toxicity assessment of SMX degradation

Microtox method (the international procedure ISO 11348-3) (Buysse et al. 1989) was used to evaluate the toxic evolution of a SMX solution during chlorination in WDS (DI), which was based on the inhibition of the luminescence by *V. fischeri* (Barhoumi et al. 2016) marine bacteria with 15 min exposure. A multiscan spectrum M200 PRO (Tecan, Austria) was used. In order to prevent the inactivation of *V. fischeri* by FC, the FC in each water sample was quenched with 0.2 mol/L Na₂S₂O₃ and Na₂S₂O₃ itself did not inhibit *V. fischeri* (Wei et al. 2011). The calculation formula of the inhibition of light emissions was: inhibition = $(I_0 - I) / I_0$, where I and I₀ was the luminescent intensity of samples at different times and the luminescent intensity of the blank solution, respectively.

2.6. DNA extraction

Four group experiments were set up in this section, which were carried out in WDS (DI). A group represented the tap water samples that were placed for 6 days, as blank control; B group represented the tap water samples that were added SMX for 6 days; C group represented the tap water samples that were added the chlorinated products of SMX (obtained by solid phase extraction) for 6 days; D group represented the tap water samples that were pretreatment with SMX followed by chlorination for 6 days.

500 ml of above water samples were concentrated by 0.22 μm sterile filter

membranes until the membrane was clogged to capture bacteria. Then, the membranes were cut up with clean scissors and stored at -20°C in 50 mL centrifuge tubes. The total DNAs were extracted using an EZNA Water DNA Kit (Omega Bio-tek, Georgia, USA) and the extraction steps followed the manufacturer's instructions. The DNA concentration and purity were determined by 1.5% agarose gel electrophoresis and spectrophotometer analysis (NanoDrop ND-2000c, Thermo, USA), as previous reported by Chen (Chen & Zhang 2013).

2.7. DNA quantification by real-time quantitative polymerase chain reaction (qPCR)

Two sulfonamide resistance genes (*sul1* and *sul2*), and an integron gene (*int11*, used to reflect the transfer potential of horizontal gene) were selected for quantitative detection by StepOnePlus real-time PCR system (Applied Biosystems, CA, USA). In addition, 16S rRNA was also monitored to reflect relative abundance, and the calculation formula of RAs was: $\text{RAs} = \text{ARG copies} / \text{16S rRNA copies}$. The detailed primer information and the procedure of qPCR is shown in Table S2. Following previous research (Chen & Zhang 2013, Li et al. 2017b), the plasmids containing target genes were obtained by molecular cloning, and plasmids carrying target genes (serially 10-fold dilution) were used to generate standard curves (including 5 or 6 points).

The qPCRs were conducted in 96-well plates with a final volume of 20 μL mixture, containing 10 μL SYBR Premix Ex Taq (TaKaRa, Bio, Shiga, Japan), 7.2 μL DNA-free water, 2 μL template DNA and 0.2 μM forward and reverse primers. The temperature procedure was as the following: initial denaturation step for 180 s at 95°C and then 35 cycles of amplification containing denaturation for 30 s at 94°C , annealing for 30 s at

55 °C and extension for 60 s at 72 °C, with a final extension step for 600 s at 72 °C.

3. Results and discussion

3.1. Kinetics of SMX degradation

3.1.1. Effect of FC

Fig. 1 (a) and 1 (c) showed the SMX degradation at different free chlorine (0.4 – 1.0 mg/L), it can be seen that when $FC \geq 0.6$ mg/L and reaction time was more than 30 minutes, the SMX was almost completely degraded in both the batch and WDS tests. However, the degradation of SMX in the batch reactor was faster than that in WDS. At the concentration of 1.0 mg/L FC, it took about 15 min for the removal of SMX to reach 100% in WDS, while only 10 minutes in batch test. In addition, the degradation rate of the two reaction systems increased monotonously with the initial FC concentration increased (Fig. 1 (b) and Fig. 1 (d)). At the concentration of 0.4, 0.6, 0.8, and 1.0 mg/L of FC, the removal ratio of SMX in batch reactor increased from 52.9%, to 75.3%, 100% and 100%, and in WDS increased from 56.5%, to 66.5%, 85.7% and 92.6% respectively in 10 min.

[Fig. 1]

In order to better describe the correlation between reaction times and $\ln([SMX]_t/[SMX]_0)$ at different FC concentration, the first-order model was used (Eq. (1)):

$$-\frac{d[SMX]_t}{dt} = k_l[SMX]_t \quad (1)$$

Where k_l was the first-order rate constants, and $[SMX]_t$ was the SMX concentration at different times. The k_l and goodness of fit (R^2) of each initial FC

concentration in batch reactor was bigger than those in WDS (Table S3). The difference may have been caused by complex conditions of the WDS, where maybe occurred multiple reactions due to the presences of pipe contains scale (natural organic matter is its component (Lytle & Liggett 2016)) at the pipe wall (Fig S3), which consumed some FC (Dong et al. 2019). On the contrary, the conditions in the batch reactor were relatively stable, thus the linear correlation in backer tests was better than in WDS. However, the linear correlation in WDS was still satisfactory (all $R^2 \geq 0.966$), thus the SMX degradation by various initial FC concentrations in both the batch reactor and WDS were followed the first-order kinetics.

Previous studies have found that the degradation of Sulfadiazine the pipe network follows a second-order kinetics (Dodd & Huang 2004, Dong et al. 2019), so this possibility was also studied. The second-order rate kinetics constants (k_2) could be calculated by Eqs. (2) – (3):

$$-\frac{d[\text{SMX}]_t}{dt} = k_2[\text{FC}][\text{SMX}]_t \quad (2)$$

$$k_2 = \frac{k_1}{[\text{FC}]} \quad (3)$$

Where $[\text{FC}]$ was the concentration of FC. Fig. S4 (a) and Fig. S4 (b) showed that the relationship between k_2 and FC concentration was satisfactory linear correlation in both the batch reactor ($R^2 = 0.995$) and WDS ($R^2 = 0.960$). Therefore, the degradation of SMX in both the batch reactor and WDS also could be described by second-order model.

3.1.2. Effect of pH

[Fig. 2]

In the batch reactor (Fig. 2 (a)), after 40 min, the degradation amount of SMX by FC remained at 100% during pH 6.3 to pH 7.2 and decreased from 100% to 73.08% during pH 7.2 to pH 9.0. Fig. 2 (d) showed that the SMX degradation in the WDS and its results were similar to the batch tests, indicating that their highest efficiency of SMX degradation occurred at pH close to neutral ($\text{pH} \approx 7.0$). The first-order rate constants (k) of reactions between SMX and FC were obviously dependent on the pH value, as shown in Fig. 2(b) and Fig. 2(e). As shown in Table S4, the k increased from 0.117 to 0.139 min^{-1} in batch experiment and from 0.097 to 0.107 min^{-1} in WDS when the solution pH value increased from 6.3 to 7.0; Yet the k decreased from 0.139 to 0.027 min^{-1} in batch experiment and from 0.107 to 0.045 min^{-1} in WDS when the solution pH value increased from 7.0 to 9.0. Therefore, the fastest rate of SMX degradation also occurred at pH close to neutral. In addition, all R^2 of batch experiment were bigger than that of WDS when the set pH value was similar, and the reason had been discussed in section 3.1.1.

In the range of investigated pH, the acid-base equilibrium (Eqs. (4) – (5)) of HClO and the SMX may be used to explain the pH dependence behavior in the reaction of FC and SMX.

The two species of FC (i.e., HClO and ClO^-) and the three species of SMX (i.e.,

HSMX⁺, SMX, and SMX⁻) could be considered to react with each other, thus the dependence of k_{app} on solution pH can be modeled by Eqs. (7) – (8):

$$-\frac{d[SMX]_{all}}{dt} = k_{app}[SMX]_{all}[FC]_{all} = \sum_{i=1,2,j=1,2,3} k_{ij}\alpha_i\beta_j[SMX]_{all}[FC]_{all} \quad (7)$$

$$k_{app} = \sum_{i=1,2,j=1,2,3} k_{ij}\alpha_i\beta_j \quad (8)$$

Where $[FC]_{all} = [HClO] + [ClO^-]$; $[SMX]_{all} = [HSMX^+] + [SMX] + [SMX^-]$; k_{ij} is the species-specific second-order rate constants for reaction of $[FC]_{all}$ species i with $[SMX]_{all}$ species j . α_i and β_j represent the equilibrium distribution coefficients of $[FC]_{all}$ and $[SMX]_{all}$ species, respectively, and they can be calculated at different pH by the following equation (Eqs. (9)–(13)):

$$\alpha_1 = [HClO]/[FC]_{all} = [H^+]/([H^+] + 10^{-7.54}) \quad (9)$$

$$\alpha_2 = [ClO^-]/[FC]_{all} = 10^{-7.54}/([H^+] + 10^{-7.54}) \quad (10)$$

$$\beta_1 = [HSMX^+]/[SMX]_{all} = ([H^+]^2)/([H^+]^2 + [H^+]*10^{-1.8} + 10^{-1.8}*10^{-5.6}) \quad (11)$$

$$\beta_2 = [SMX]/[SMX]_{all} = ([H^+]*10^{-1.8})/([H^+]^2 + [H^+]*10^{-1.8} + 10^{-1.8}*10^{-5.6}) \quad (12)$$

$$\beta_3 = [SMX^-]/[SMX]_{all} = (10^{-1.8}*10^{-5.6})/([H^+]^2 + [H^+]*10^{-1.8} + 10^{-1.8}*10^{-5.6}) \quad (13)$$

The k_{ij} was calculated by L-M (Levenberg-Marquardt) regressions of experimental k_{app} according to the Eq. (8), using MATLAB. While there were six possible reactions from the Eq. (8), fitting of the experimental data of Fig. 2 (c) and Fig. 2 (f) showed that only following two reactions (Eqs. (14) – (15)) could reasonably fit the experimental data (a solid line), $R^2 \geq 0.974$ and $R^2 \geq 0.972$ in batch reactor and WDS, respectively. The reason for this may be that $[HSMX^+]$ and $[SMX]$ have little effect in the reaction system due to their extremely low mole fraction at pH 6.3 – 9.0 (Fig. S5). In addition, contribution of the individual reactions to the rate constant (k_{app})

is also shown in Fig. 2(c) and Fig. 2(f) (dashed lines). This result indicated that the HClO reacts faster with deprotonated SMX (SMX^-) than HClO^- (i.e., in batch reactor: $k_{13} = (2.23 \pm 0.07) \times 10^2 > k_{23} = (5.04 \pm 0.30) \times 10^1 \text{ M}^- \text{ s}^-$; in WDS: $k_{13} = (1.76 \pm 0.07) \times 10^2 \text{ M} > k_{23} = (4.06 \pm 0.62) \times 10^1 \text{ M}^- \text{ s}^-$). At pH 6.3 – 8.2, reaction (14) mainly contributed to the k_{app} . In contrast, the k_{app} at pH from 8.2 to 9.0 could be attributed to reaction (15). At nearly neutral pH, the comprehensive effect of reaction (14) and reaction (15) is the best, the subsequent experiments can thus be carried out at a nearly neutral pH.

3.1.3. Effect of flow rate

[Fig. 3]

As shown in Fig. 3, the change of flow rate had little effect on the SMX degradation. The degradation rate constants for SMX were 0.129, 0.190, 0.211 and 0.201 min^{-1} under the condition of 0.7, 1.0, 1.3 and 1.5 m/s, respectively (Table. S5). The possible reason for this difference is that flow rate could change the hydraulic conditions in WDS, thereby affecting mass transfer coefficient. The Reynolds number (Re) was used to evaluate this behavior, $Re = \rho v d / \mu$, where ρ , v , d and μ represent the density, flow rate, pipe diameter and viscosity coefficient of fluid ($0.895 \times 10^{-3} \text{ Pa} \cdot \text{s}$, 25°C), respectively. All Re more than 4300 (Table. S5), indicating that turbulent flow conditions exist in WDS. Mass transfer coefficient increased with increasing Re under turbulent flow conditions, ultimately improves mixing and this may make the rate of

SMX degradation enhanced when the flow rate from 0.7 to 1.0 m/s. However, when the flow rate from 1.0 to 1.5 m/s, the degradation rate hardly increased because the effect of mass transfer on degradation has reached the limit.

3.1.4. Effect of pipe materials

[Fig. 4]

As shown in Fig. 4, after 15min of reaction, the removal of SMX reached 100%, 87.8% and 94.4% in the PE, DI and SS, respectively. Table S6 summarized the calculated first-order rate constants, these data showed that the order of degradation rate was SS (0.358 min^{-1}) > DI (0.191 min^{-1}) > PE (0.139 min^{-1}). Metal ions and corroded metal might contribute to this phenomenon, DI were better protected because they have lined with a layer of cement, but SS is more prone to rust due to poor welding (Dong et al. 2017). Consequently, compared with DI and PE, the SS has the most metal ions and corroded metal. As discussed in the section 3.1.3, when the flow rate was 1.0 m/s, these experiments were conducted with turbulent flows ($Re > 4300$), consequently, the metal ions and corroded metal in solder joints were easily transferred to the water. The concentrations of total iron in the three pipes were detected, which was 0.8 mg/L in SS and less than 0.1mg/L in DI and PE. Fe^{2+} could be oxidized to Fe^{3+} by FC, and the process produced some free radicals that enhanced the SMX degradation (Dong et al. 2019, Perez-Moya et al. 2010). In addition, Fe^{3+} might be oxidized to higher valence iron in hypochlorite solution system, such as a way to prepare ferrate (VI) is to oxidize Fe^{3+} with sodium hypochlorite solution under alkaline condition (Li et al. 2005). The

higher valence iron has stronger oxidize ability and it could promote the degradation of SMX.

3.2. Toxicity assessment of SMX degradation

[Fig. 5]

As shown in Fig. 5, compared with the inhibition of bioluminescence rate of tap water close to 0, the presence of SMX can significantly increase the toxicity of water. In the first 10 min of reaction, with the degradation of SMX, the toxicity showed a downward trend. Within 10 to 90 minutes of the reaction, the toxicity showed an upward trend, indicating that the intermediate products of the reaction were also toxic. However, within 90 to 120 minutes of the reaction, the toxicity showed a downward trend until it was the same as that of tap water, which indicated that SMX could be completely degraded by FC within 2 hours, and the toxicity of chlorinated products was low.

3.3. Generation of ARGs in WDS

[Fig. 6]

The comparison of ARGs contents and relative abundance of water samples under different treatments are shown in Figure 6. In general, compared with group A, the number and relative abundance of *sul1*, *sul2* and *int11* genes in group B, C and D were significantly increased. However, the number of 16S rRNA did not change significantly, indicates that the total number of bacteria changed little. Previous studies have shown that the variation of ARGs relative abundance is mainly caused by two aspects: one is the change of host bacteria abundance and the other is the horizontal transfer of ARGs

in bacteria (Jia et al. 2015, Su et al. 2015), and the increase of ARGs in drinking water was the result of two factors (Jia et al. 2015). Therefore, the following analysis mainly consider these two aspects.

In group B, although the total number of bacteria decreased slightly, the number of *sul1* and *sul2* increased. This may have been caused SMX inhibited the growth of other bacteria and promoted the increase of antibiotic-resistant bacterial community. In addition, SMX as an environmental pressure, could promote the replication of *sul1* and *sul2*, which was the main reason for the increase of *sul1* and *sul2*. On the other hand, the increase of *sul1* and *sul2* was also caused by the transfer of ARGs (because the number of *int11* has increased). In group C, the increase of 16S rRNA genes number reflected the increase of total bacterial count, indicating that chlorination products had no inhibitory effect on total bacterial count. These results showed that even if SMX was chlorinated in water, its chlorination products would still lead to the production of resistance genes. The reason may be that chlorination cannot completely open the aromatic ring in SMX molecule, some products can only replace one H atom by the electrophilic reaction of chlorine to form Cl-SMX, which may also have the properties of sulfonamide antibiotics.

High concentration of FC (> 10mg/L) can effectively degrade the concentration of ARGs in the water environment (Zhang et al. 2015b), but the results of group D under the experimental conditions of low concentration of FC are opposite, and the same results were observed in the study of Jia (Jia et al. 2015) and Shi (Shi et al. 2013). The reason is that under the condition of high FC value, FC can directly kill bacteria, while

under the condition of low FC value, the growth of non-chlorine resistant bacteria is inhibited, and chlorine resistant bacteria can not only survive, but also increase the copy number and relative abundance of ARGs through gene replication. Our team has found that *Pseudomonas* and *Mycobacterium* are the main bacterium in the WDS (Zhang et al. 2018a), which has the function of chlorine resistance. *Pseudomonas* is the host bacteria of the trans ARGs (Zhang et al. 2019), and its growth and replication will lead to the change of its relative abundance and quantity, which will lead to the increase of the relative abundance and copy number of *sul1* and *sul2*. In addition, under the external pressure of active chlorine, the permeability of bacterial cell membrane changes, and the frequency of *intI1* binding and transferring ARGs increases. However, the probability of binding and transferring ARGs is small when the bacteria in pipe network are less than 10000 CFU/ml (Guo et al. 2015a), so the number and relative abundance of ARGs are also affected by the external pressure of active chlorine, but its contribution rate is low. ARGs in group C increased more than that in other groups, this suggests that the chlorinated products of SMX can promote the production of ARGs in WDS (DI), but what chlorination products caused the increase needs to be further studied.

4. Conclusion

(1) The degradation rate of SMX in batch reactor was faster than that in WDS, and the rate was positive correlation with the initial concentration of FC.

(2) Whether in batch reactor or WDS, the SMX chlorination could be described by the first or the second-order kinetics.

(3) When the pH value was from 6.3 to 9.0, the degradation efficiency of SMX first increased and then decreased, and the efficiency reached a maximum at nearly neutral pH. In addition, the fastest rate of SMX degradation also occurred at pH close to neutral, and the relationship between the second-order rate constants and pH values were explained by using the species-specific rate constants of FC with SMX.

(4) Under different pipe materials conditions, the SMX degradation rate followed the order: SS > DI > PE, and high flow rate led to the rate increased in DI, but the increase was limited.

(5) SMX could increase the toxicity of water initially, but the toxicity reduced after 2 hours chlorination.

(6) The chlorination products of SMX can significantly promote the production of ARGs in WDS (DI), but which chlorination products lead to the increase remains to be further studied.

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Figures

Figure 1

Degradation of SMX at different chlorine concentration in batch reactor (a) and WDS (c), and the first-order kinetics plot of SMX degradation in batch reactor (b) and WDS (d). Experimental conditions: ductile iron pipe, 25 $\times$, pH = 7.0 $\pm$ 0.2, flow rate = 1.0 m/s, initial SMX = 200 μ g/L.

Figure 2

Degradation of SMX at different pH in batch reactor (a) and WDS (d), and the first-order kinetics plot of SMX degradation in batch reactor (b) and WDS (e), and the second-order rate constants (k_{app} , $M^{-1}s^{-1}$) as a function of pH for the SMX chlorination by FC in batch reactor(c) and WDS(f). Experimental conditions: ductile iron pipe, 25 ϕ , flow rate = 1.0 m/s, initial free chlorine = 0.8 mg/L, initial SMX = 200 $\mu g/L$.

Figure 3

Degradation of SMX at different flow rates in WDS. Experimental conditions: ductile iron pipe, 25 ϕ , pH = 7.0 ± 0.2 , initial free chlorine = 0.8 mg/L, initial SMX = 200 μ g/L.

Figure 4

Degradation of SMX in different pipe materials (Polyethylene (PE), ductile iron (DI) and stainless-steel (SS)). Experimental conditions: 25 °C, pH = 7.0 ± 0.2, flow rate = 1.0 m/s, initial free chlorine = 0.8 mg/L, initial SMX = 200 µg/L.

Figure 5

Evolution of toxicity of SMX chlorination. Experimental conditions: ductile iron pipe, 25 ϕ , pH = 7.0 ± 0.2 , flow rate = 1.0 m/s, initial free chlorine = 0.8 mg/L, initial SMX = 200 μ g/L.

Figure 6

Gene copy numbers and relative abundance of antibiotic resistance genes in different treatment conditions.

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