Inactivation of antibiotic resistance genes in municipal wastewater effluent by chlorination and sequential UV/chlorination disinfection

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HIGHLIGHTS

- Chlorine is more effective than UV irradiation in removing ARGs from MWTP effluent.
- The chlorination reaction followed the second-order reaction kinetic model.
- Higher NH3 – N contents result in lower removal of ARGs.
- FC is more effective than CC on the inactivation of ARGs.
- UV irradiation followed by chlorination shows high efficiency in removing ARGs.

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ABSTRACT

This study investigated disinfection methods including chlorination, ultraviolet (UV) irradiation and sequential UV/chlorination treatment on the inactivation of antibiotic resistance genes (ARGs). ARGs including sul1, tetX, tetG, intI1, and 16S rRNA genes in municipal wastewater treatment plant (MWTP) effluent were examined. The results indicated a positive correlation between the removal of ARGs and chlorine dosage ($p = 0.007$–0.014, $n = 6$), as well as contact time ($p = 0.0001$, $n = 10$). Greater free chlorine (FC) dosage leads to higher removal for all the genes and the maximum removal (1.30–1.49 logs) could be achieved at FC dosage of 30 mg L$^{-1}$. The transformation kinetic data for ARGs removal ($\log C_f / C$) followed the second-order reaction kinetic model with FC dosage ($R^2 = 0.6829$–0.9999) and contact time ($R^2 = 0.7353$–8624), respectively. Higher ammonia nitrogen (NH3–N) concentration was found to lead to lower removal of ARGs at the same chlorine dosage. When the applied Cl$_2$:NH$_3$–N ratio was over 7.6:1, a significant reduction of ARGs (1.20–1.49 logs) was achieved. By using single UV irradiation, the log removal values of tetX and 16Ss rRNA genes were 0.58 and 0.60, respectively, while other genes were 0.36–0.40 at a fluence of 249.5 mJ cm$^{-2}$, which was observed to be less effective than chlorination. With sequential UV/chlorination treatment, 0.006 to 0.31 log synergy values of target genes were observed under different operation parameters.

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1. Introduction

Nowadays, overuse of antibiotics leads to the emergence and spread of antibiotic resistance genes (ARGs) in the environment, as well as antibiotic-resistant bacteria (ARB) (Chen and Zhang, 2013; Laht et al., 2014), which may increase health risks to humans and animals (Kemper, 2008; Burch et al., 2014). ARGs have been recognized as the emerging environmental contaminants (Pruden et al., 2006). During the past few years, the occurrence of ARGs has been reported in different wastewater treatment plants (WWTPs) (Xu et al., 2015; Yang et al., 2014), river water (Jiang et al., 2013), soil (Su et al., 2014) and sediment (Chen et al., 2013). In particular, WWTPs are considered as one of the main ‘hotspots’ for ARGs spread into the environment (Michael et al., 2013; Rizzo et al., 2013a). Although ARGs can be partially removed through traditional wastewater treatment processes, there are still large numbers that survive in effluents because WWTPs are not designed for the removal of these emerging contaminants. ARGs residuals in effluents may be disseminated by horizontal gene transfer to the bacteria in the receiving water (Xu et al., 2015). Hence, controlling ARGs in effluents from WWTPs should be high on the environmentalists’ agenda in order to help reduce health risks.

Chlorine, the most widely used disinfectant in various countries, is an oxidant that acts by destroying nucleic acids and cell membranes of microorganisms (Anastasi et al., 2013). Previous studies showed that chlorination is a promising approach to effectively remove the ARB and ARGs in drinking water and wastewater (Koivunen and Heinonen-Tanski, 2005; Bekink and Nozaic, 2013). During the chlorination process, ammonia nitrogen (NH3–N) was proved to be an important parameter affecting chlorination disinfection efficiency because of
its rapid competition for free chlorine (FC) to form combined chlorine (CC), Li and Zhang (2013) have reported that an FC dosage over the breakpoint (the mass ratio of chlorine to NH\textsubscript{3}–N was 7.6:1) is required to show higher removal efficiency considering the presence of ammonia in wastewater. However, to date, very few studies of the effect of NH\textsubscript{3}–N have been conducted on the inactivation of ARGs by chlorination.

In comparison to chlorination, ultraviolet (UV) disinfection is also a commonly used disinfection method, which does not produce disinfection by-products (DBPs) at typical doses and does not leave a residue. UV disinfection can be directly absorbed by DNA and thus has the potential to impart ARGs damage (McKinney and Pruden, 2012). For example, Guo et al. (2013a) revealed that UV disinfection at a dose of 0.22 J cm\textsuperscript{-1} could eliminate antibiotic resistances to erythromycin and tetracycline in wastewater treatment effluents. Moreover, it has been reported that UV irradiation followed by chlorination could prevent microbial regrowth as well as generate a synergetic effect (Shang et al., 2007). Previous studies showed that sequential UV/chlorination was more effective in disinfecting reclaimed water (Wang et al., 2012) and inactivating microorganism in drinking water (Al-Gabr et al., 2007). Previous studies showed that sequential UV/chlorination treatments under laboratory conditions. The factors of chlorine dosage, contact time and UV doses were investigated, as well as the effect of NH\textsubscript{3}–N concentration on the removal of ARGs during the chlorination process. The objective of this study is to assess the potential of the disinfection process to inactivate ARGs in actual wastewater effluents.

2. Materials and methods

2.1. Chemicals and reagents

Sodium hypochlorite (NaClO, available chlorine ≥ 8%, XiLong Co., Ltd., China) was prepared for different doses of free chlorine stock solution. Ammonium chloride (NH\textsubscript{4}Cl, standard purity ≥ 99.5%, Nanjing Chemical Reagent Co., Ltd., China) was prepared for different doses of NH\textsubscript{3}–N. Sodium thiosulfate solution (Na\textsubscript{2}S\textsubscript{2}O\textsubscript{3}, 1.5%, w/w) was added as the terminator at the end of chlorination process. Chemicals used here were all analytically pure. Milli-Q water, with a resistivity of 18.2 M\textOmega\cdot cm, was produced from a Millipore purification system (Billerica, CA, USA). The mixed cellulose ester filter membrane (0.22 μm) was purchased from Xinya (Shanghai, China).

2.2. Wastewater samples

Samples were collected from the secondary effluent in a MWTP in Nanjing, China, which utilized activated sludge technology (CAST), a commonly activated sludge process used in removal of nitrogen and phosphorus as a type of sequencing batch reactor (SBR) (Demoulin et al., 2001). All samples were collected in roughly equal volumes per hour with composite samples and kept in sterile containers which were placed in ice and transported to the laboratory for immediate analysis. The pH of the secondary effluents was 7.0–7.2. The concentration of chemical oxygen demand (COD), total nitrogen (TN), NH\textsubscript{3}–N and suspended solid (SS) were 39 mg L\textsuperscript{-1}, 9.73 mg L\textsuperscript{-1}, 2.56 mg L\textsuperscript{-1} and 9 mg L\textsuperscript{-1}, respectively. The absorbance at 254 nm (UV\textsubscript{254}) of the secondary effluents was 0.093–0.15. The original gene copies of the wastewater samples were 5.49 × 10\textsuperscript{4} to 8.13 × 10\textsuperscript{4} copies mL\textsuperscript{-1} for sul\textsubscript{1}, 9.12 × 10\textsuperscript{3} to 1.38 × 10\textsuperscript{4} copies mL\textsuperscript{-1} for tet\textsubscript{X}, 6.31 × 10\textsuperscript{3} to 1.10 × 10\textsuperscript{4} copies mL\textsuperscript{-1} for tet\textsubscript{G}, 6.46 × 10\textsuperscript{3} to 9.33 × 10\textsuperscript{4} copies mL\textsuperscript{-1} for intI1 and 1.20 × 10\textsuperscript{5} to 1.95 × 10\textsuperscript{5} copies mL\textsuperscript{-1} for 16S rRNA genes.

2.3. Chlorine disinfection

2.3.1. Influences of the FC dosage and contact time

The chlorination experiments were carried out in 500 mL sterile beakers with magnetic stirers at 200 rpm to mix samples gently. NaClO was added to establish different doses of FC at 5 mg L\textsuperscript{-1}, 10 mg L\textsuperscript{-1}, 15 mg L\textsuperscript{-1}, 20 mg L\textsuperscript{-1}, 25 mg L\textsuperscript{-1} and 30 mg L\textsuperscript{-1}, respectively. Unless otherwise specified, the FC dosage was as Cl\textsubscript{2}. At the contact time of 30 min, the reaction was terminated by adding Na\textsubscript{2}S\textsubscript{2}O\textsubscript{3} solution. To investigate the effect of chlorination time on the removal of ARGs, at FC dosage of 15 mg L\textsuperscript{-1} and 30.0 mg L\textsuperscript{-1}, samples were collected at 5 min, 15 min, 30 min, 180 min, 300 min, 540 min, 780 min, 1020 min and 1200 min. Total chlorine (TC) and FC residual concentrations were immediately analyzed by DPD method (Environmental Protection Agency of China, 2002).

2.3.2. Influence of the NH\textsubscript{3}–N concentration

To investigate the influence of NH\textsubscript{3}–N concentration on the ARGs removal during chlorine disinfection, the NH\textsubscript{3}–N concentrations were set at 2.56 mg L\textsuperscript{-1}, 5 mg L\textsuperscript{-1} and 15 mg L\textsuperscript{-1}. The levels represent the typical concentration range for secondary effluent in WWTPs (Li and Zhang, 2013). In this study, 2.56 mg L\textsuperscript{-1} was the background concentration of the samples, while concentrations of 5 mg L\textsuperscript{-1} and 15 mg L\textsuperscript{-1} were achieved by adding suitable volumes of the NH\textsubscript{4}Cl stock solution into the wastewater samples. The samples were collected after 30 min reaction time with 30 mg L\textsuperscript{-1} FC dosage for ARGs detection. TC and FC residues were analyzed at 1 min, 2 min, 5 min, 10 min, 15 min and 30 min, respectively.

2.4. UV alone and sequential UV/chlorination treatment

Firstly, UV experiments were carried out in a cylinder Plexiglas reactor (310 mm height with radius of 450 mm). The reactor was equipped with a low-pressure mercury vapor 254 nm lamp (Model TUV 16 W T5 4P-SE, Philips) in a quartz sleeve, standing in the center of the installation. The fluence rate outer the sleeve was 9.85 mW cm\textsuperscript{-2} measured by an ultraviolet radiation meter equipped with a UV\textsubscript{254} detector (Beijing Normal University Optical Instrument Factory). An 1800 mL wastewater effluent sample was injected into the reactor and mixed gently with a magnetic stir bar at 300 rpm. Before initiating the experiment, the apparatus was allowed to warm up for 10 min and stir for approximately for 10 s to ensure stable UV irradiation fluence and a well-mixed solution. Compared to chlorination, UV disinfection has an obvious advantage of short contact time. In this study, the reaction time for UV treatment alone was from 0 to 60 s. The samples were withdrawn at 15 s, 30 s and 60 s, and the corresponding UV doses were 62.4 mJ cm\textsuperscript{-2}, 124.8 mJ cm\textsuperscript{-2} and 249.5 mJ cm\textsuperscript{-2}, respectively, calculating by multiplying the irradiation time for the intensity of UV lamp (Bolton and Linden, 2003; Rizzo et al., 2013b).

Secondly, in the following chlorination experiments, FC was added into the UV irradiated samples by doses of 5 mg L\textsuperscript{-1}, 15 mg L\textsuperscript{-1}, 25 mg L\textsuperscript{-1} and 30 mg L\textsuperscript{-1}, respectively. After chlorination duration of
30 min, a Na₂S₂O₃ solution was added to stop the chlorination for subsequent ARGs detection. All disinfection treatments of wastewater were conducted at room temperature (22 ± 2 °C) for statistical analysis. The synergy value of sequential UV/chlorination treatment was calculated by the following equation (Koivunen and Heinonen-Tanski, 2005):

\[ \text{Synergy} = \text{log reduction by combined UV/chlorination disinfection} - \text{log reduction by UV disinfection} - \text{log reduction by chlorine disinfection}. \]

2.5. Sample pretreatment and DNA extraction

2.5.1. Sample pretreatment and DNA extraction

After the disinfection treatment, 200 mL water samples were collected and concentrated by passing through the 0.22 μm mixed cellulose ester filter membranes; then, the filters were stored at −20 °C prior to DNA extraction and molecular analysis. DNA extraction was conducted using the Fast DNA™ Spin Kit for soil (MP Biomedicals, Santa Ana, CA), following the protocol of the manufacturer. The concentration and quality of DNA were determined by spectrophotometry (Biodropsis BD2000, Oriental Co., Ltd., Beijing, China) and agarose gel electrophoresis.

2.5.2. Quantitative polymerase chain reaction (qPCR)

qPCR (Applied Biosystems 7500 qPCR detection system, Life Technologies, USA) was applied to quantify the number of sul1, tetX, tetG, intI1 and 16S rRNA gene copies. Standard curves including 5 or 6 points should be generated in all qPCR runs. Plasmids carrying target genes were used to generate standard curves. The puriﬁcation of PCR products was conducted in 96-well plates with a 2 × power SYBR® Green PCR Master Mix (Life Technologies, USA), 0.16 μL of each primer (20 μM, listed in Table 1), a 2 μL template DNA (DNA extracts diluted near to 2 ng μL⁻¹) and 7.68 μL ddH₂O. The temperature procedure was as follows: 10 min at 95 °C, 40 cycles of 15 s at 95 °C and 1 min at annealing temperature, followed by a melt curve stage to verify speciﬁcity. Each reaction was run in triplicate. PCR efﬁciency of each gene ranged from 90% to 100%, with R² values more than 0.99 for all standard curves.

The removal of speciﬁc genes was calculated as follows.

\[ \text{Removal of speciﬁc gene} \ (j) = \left(1 - \frac{C_j}{C_i}\right) \times 100\% \]

Here, \( C_j \) indicates speciﬁc genes, which include sul1, tetX, tetG, intI1 and 16S rRNA genes. \( C_i \) indicates the gene copy number of speciﬁc gene \( j \) in the original wastewater effluent (copies mL⁻¹). \( C_i \) indicates the survival of the speciﬁc gene \( j \) after disinfection at a dosage of \( i \) (copies mL⁻¹).

2.6. Statistical analysis

Copy numbers were log-transformed prior to statistical analysis. All data are determined at least three times and expressed as mean values ± standard deviation (SD) and analyzed by one-way analysis of variance (ANOVA). The t-test was used to analyze the difference between two samples by SPSS statistics 22.0. Differences between means were considered significant at \( p < 0.05 \) for all tests used.

3. Results

3.1. Influence of chlorination on the removal of ARGs

The removals of target ARGs under different FC dosages are shown in Fig. 1. It was found that the removal of different ARGs and 16S rRNA genes have a similar changing pattern under different chlorination dosages. Specifically, the removal of ARGs rose slowly when adding chlorine from 5 mg L⁻¹ to 20 mg L⁻¹, and increased dramatically from the dosage of 20 mg L⁻¹ to 25 mg L⁻¹ (\( p = 0.007–0.014 < 0.05 \)). As expected, a greater FC dosage leads to higher removal efficiency for all the genes. The maximum removal could be achieved at an FC dosage of 30 mg L⁻¹, which was relatively low at 1.20 logs for sul1 removal, whereas other genes reached 1.30–1.49 logs removal.

In this study, the transformation kinetic data for ARG (log \( C_0 / C \)) was plotted with FC dosage. FC dosage of 20 mg L⁻¹ was a breakpoint in the reaction. When FC dosage was lower than 20 mg L⁻¹, the removal of ARGs reaction (log \( C_0 / C \)) followed second-order kinetic models. The kinetics constants \( k \) and \( R² \) values were summarized as following: sul1 \((k = 0.0157, R² = 0.9687), \) tetX \((k = 0.0156, R² = 0.9899), \) tetG \((k = 0.0871, R² = 0.6829), \) intI1 \((k = 0.0112, R² = 0.6499), \) 16S rRNA \((k = 0.0161, R² = 0.8297). \) When FC dosage increased from 20 mg L⁻¹ to 30 mg L⁻¹, the reaction followed the common second-order reaction kinetic model strictly. The corresponding \( k \) and \( R² \) were as following: sul1 \((k = 0.0895, R² = 0.9664), \) tetX \((k = 0.1170, R² = 0.9989), \) tetG \((k = 0.1315, R² = 0.9014), \) intI1 \((k = 0.1073, R² = 0.9999), \) 16S rRNA \((k = 0.1208, R² = 0.9350). \)

To inspect the influence of chlorination time on the removal of ARGs, the removal of genes was monitored at chlorine doses of 15 mg L⁻¹ and 30 mg L⁻¹. As shown in Fig. 2, the removal increased rapidly during the first 5 to 30 min, but increased slowly in the subsequent time. The transformation kinetic data for ARGs removal (log \( C_0 / C \)) with contact time also followed the second-order reaction kinetic model \((R² = 0.7353–8634). \) The FC and TC residual concentrations were monitored during the disinfection process as shown in Fig. 3. At a dosage of 30 mg L⁻¹, the FC and TC concentrations decreased dramatically during the first 15 min. After chlorination reaction with 15 min, the removal values of target genes were 0.99–1.32 logs, and the FC and TC residuals were 5.19 mg L⁻¹ and 8.87 mg L⁻¹, respectively. At the contact time of

| Table 1 | The primers used in qPCR. |
|-----------------|------------------|------------------|------------------|
| **Target genes** | **Sequences (5’-3’)** | **Annealing temperature (°C)** | **Amplicon size (bp)** | **Reference** |
| sul1 | F-W | CCGACCGGAAACATTGCCTGCAC | 65 | 163 | Pei et al. (2006) |
| | R-V | TGAATGTCGCCCGGCAAGTCCTG | 60 | 278 | LaPara et al. (2011) |
| tetX | F-W | AGCCTTACACGTGGCTTAAA | 60 | 134 | Auerbach et al. (2007) |
| | R-V | CCGGCGCGCGCGCGCGCGCGCG | 60 | 280 | LaPara et al. (2011) |
| tetG | F-W | CGACAGCAGCTGCCTGG | 65 | 202 | LaPara et al. (2011) |
| | R-V | CCYCGAGACGAGAACAGAACGAAG | 60 | 302 | LaPara et al. (2011) |
| intI1 | F-W | CCGACCGGAAACATTGCCTGCAC | 65 | 163 | Pei et al. (2006) |
| | R-V | CACAGCTGCTGCTGCCT | 60 | 278 | LaPara et al. (2011) |
| 16S rRNA genes | F-W | CCTACGCGAGCGCGGAGCAG | 65 | 163 | Pei et al. (2006) |
| | R-V | ATACGCGAGCGCGGAGCAG | 60 | 278 | LaPara et al. (2011) |
1200 min, the removal of the genes was 1.53–1.93 logs. Furthermore, the FC residuals and TC residuals were 0.27 mg L\(^{-1}\) and 2.50 mg L\(^{-1}\), respectively. Considering the initial 30 min was a fast and effective reaction and 30 mg L\(^{-1}\) contains lower TC residue, these two parameters were selected for our following studies.

3.2. Influence of NH\(_3\)–N concentration on the removal of ARGs

As shown in Fig. 4, NH\(_3\)–N played an important role in the ARGs removal during the chlorination process. Higher NH\(_3\)–N concentrations resulted in lower ARGs removal efficiencies by chlorination. The maximum log removal of target genes was 1.20–1.49 logs at NH\(_3\)–N concentration of 2.56 mg L\(^{-1}\), whereas 0.63–0.79 log and 0.03–0.10 log removal at NH\(_3\)–N concentrations of 5 mg L\(^{-1}\) and 15 mg L\(^{-1}\), respectively. Besides, among the target genes, the removal of tetX was the highest, while other genes stayed in a parallel level.

Moreover, as shown in Fig. 5, the TC residuals were 8.39 mg L\(^{-1}\), 17.25 mg L\(^{-1}\) and 26.52 mg L\(^{-1}\) under NH\(_3\)–N concentrations of 2.56 mg L\(^{-1}\), 5 mg L\(^{-1}\) and 15 mg L\(^{-1}\) after contact time of 30 min, respectively. The results showed that under the condition of the same FC dosages of 30 mg L\(^{-1}\), higher NH\(_3\)–N concentration can cause higher TC residuals.

3.3. Influence of UV and sequential UV/chlorination treatments on the removal of ARGs

While chlorination disinfection alone may have a good effect on ARGs removal, the problem of increased requirements for chlorine and DBPs is noteworthy. To address this limitation, we investigated UV treatment alone and the synergistic effect of sequential UV/chlorination on the removal of ARGs. As is shown in Fig. 6, higher UV dosage resulted in higher ARGs removal rate with UV dose changed from 62.4 mJ cm\(^{-2}\) to
Effect of NH3–N on chlorination disinfection to remove ARGs

During chlorination, NH3–N present in the wastewater plays a critical role in the removal of ARGs. Fig. 4 results show higher NH3–N concentration (15 mg L−1) leading to lower ARGs removal, which may attribute to its rapid competition for FC to form CC, such as monochloramine and dichloramine. According to breakpoint chlorination chemistry as Eqs. (1)–(3), when applied Cl2:NH3–N ratio (CNR) is less than 5:1 (by weight), monochloramine predominates. As CNR increases from 5:1 to 7.6:1, a breakpoint reaction occurs, reducing the TC residue level to a minimum, which can be demonstrated in Fig. 5. For a chlorine dose of 30 mg L−1, FC residue was the most prevalent (i.e., CNR = 11.72), but stayed at a higher level under 5 mg L−1 and 15 mg L−1 (i.e., CNR = 6.2, respectively). Lower NH3–N concentration resulted in higher ARGs removal (Fig. 4), due to FC and trichloramine start to appear as CNR goes above 7.6:1 (Qiang et al., 2006). One explanation states that before the breakpoint, NH3–N quickly reacted with FC and changed it to CC, and the reactivity of CC was much weaker than that of FC (Wang et al., 2007), indicating that FC is more effective than CC on the removal of ARB and ARG proportions were positively related to the COD and SS of the raw sewage, while negatively related to the corresponding variables in the effluent, as well as DO and temperature. In our experiment, SS (9 mg L−1) for effluent was quite low so that filtration is not needed before disinfection. Besides, the environmental pressure and microbial community shift may contribute to the effect of the removal of ARGs. The relative abundance of ARGs will decrease if more non-resistant bacteria survive in the environment of low-level chlorine, whereas a variety of ARGs may still be present in the effluent because chlorination might increase the copy number of plasmids in the cells of surviving bacteria (Shi et al., 2013).

The CT value (the product of initial NaClO concentration and contact time) is always used for calculating disinfectant dosages. For extracellular biomolecule, previous studies of chlorination have demonstrated that CT values over 180 mg (Cl2)·min L−1 could achieve fragmentation of isolated pETBlue plasmid DNA (Suquet et al., 2010). It was also reported that 2 log 16S rRNA genes reduction could be achieved at dosage of 1980 mg (Cl2)·min L−1 toward DNA extracted from E. coli (Van Aken and Lin, 2011). In this study, at a constant CT value of 450 mg (Cl2)·min L−1, 30 mg L−1 chlorine with 15 min contact time could achieve significant removal of ARGs, e.g., 0.99–1.32 logs reductions for sulI, tetX, tetG, intI1 and 16S rRNA genes, respectively, which was higher than that of 15 mg L−1 chlorine with 30 min contact time (0.19–0.27 log reduction). This result shows that tetracycline and sulfonamides resistance genes are more tolerant when exposed to lower concentration of chlorine with a longer contact time. Similar results have been reported by Huang et al. (2011), indicating that at a constant CT value of 50 mg (Cl2)·min L−1, the inactivation of total heterotrophic bacteria and ARB at an operation mode of 25 mg (Cl2) L−1 with 2 min of exposure time was significantly more effective than that of 2 mg (Cl2) L−1 for 25 min.

4.2. Effect of NH3–N on chlorination disinfection to remove ARGs

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Studies on the effect of chlorination can be traced back to 1970s, where chlorination was shown to influence the proportion of multiple antibiotic resistances in drinking water and wastewater (Huang et al., 2011). In this study, FC dosage of 30 mg L−1 was investigated to achieve the maximum ARGs removal. This result is consistent with a study demonstrating that more than 30 mg L−1 of chlorine was needed to remove over 90% of ARB and ARGs (Oh et al., 2014). Considering the removal and operating costs, a reaction time of 30 min, which is the normal treatment time in WWTPs (Wang et al., 2012), was chosen for a series of experiments. However, previous studies showed conflicting results about the effect of chlorination on ARB or ARG removal. For example, the percentage of tetracycline-resistant bacteria in sewage after chlorination showed a small increase according to the studies of Murray et al. (1984), but showed a decrease in the report by Armstrong et al. (1982). In addition, Huang et al. (2011) reported that even 10 mg L−1 Cl2 for 10 min can contribute to regrowth and reactivation of ARB in secondary effluents and can alter microbial community composition. Similarly, Shi et al. (2013) investigated short-term chlorination (2–4 h) as a way to promote the enrichment of ARB, ARGs and mobile genetic elements (MGEs) in drinking water. All these reports suggest that chlorination is an alternative disinfection method for removing ARB or ARG. Conflict results from literatures may be interpreted by different water properties and original ARG concentrations.

It has been generally accepted that wastewater quality and operational conditions affect the proportion change of ARGs including COD, NH3–N, SS, dissolved oxygen (DO) and temperature, and the effects are different among different ARGs. Du et al. (2014) analyzed the correlation between the fate of ARGs and water parameters in a MWTP and found that COD exhibited significant correlation with tetW (R = 0.636, p < 0.05), intI1 (R = 0.829, p < 0.01) and sulI1 (R = 0.832, p < 0.01), respectively. Yuan et al. (2014) indicated that most ARB and ARG proportions were positively related to the COD and SS of the raw sewage, while negatively related to the corresponding variables in the effluent, as well as DO and temperature. In our experiment, SS (9 mg L−1) for effluent was quite low so that filtration is not needed before disinfection. Besides, the environmental pressure and microbial community shift may contribute to the effect of the removal of ARGs. The relative abundance of ARGs will decrease if more non-resistant bacteria survive in the environment of low-level chlorine, whereas a variety of ARGs may still be present in the effluent because chlorination might increase the copy number of plasmids in the cells of surviving bacteria (Shi et al., 2013).

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4.2. Effect of NH3–N on chlorination disinfection to remove ARGs

During chlorination, NH3–N present in the wastewater plays a critical role in the removal of ARGs. Fig. 4 results show higher NH3–N concentration (15 mg L−1) leading to lower ARGs removal, which may attribute to its rapid competition for FC to form CC, such as monochloramine and dichloramine. According to breakpoint chlorination chemistry as Eqs. (1)–(3), when applied Cl2:NH3–N ratio (CNR) is less than 5:1 (by weight), monochloramine predominates. As CNR increases from 5:1 to 7.6:1, a breakpoint reaction occurs, reducing the TC residue level to a minimum, which can be demonstrated in Fig. 5. For a chlorine dose of 30 mg L−1, FC residue was the most prevalent under the NH3–N concentration of 2.56 mg L−1 (i.e., CNR = 11.72), but stayed at a higher level under 5 mg L−1 and 15 mg L−1 (i.e., CNR = 6.2, respectively). Lower NH3–N concentration resulted in higher ARGs removal (Fig. 4), due to FC and trichloramine start to appear as CNR goes above 7.6:1 (Qiang et al., 2006). One explanation states that before the breakpoint, NH3–N quickly reacted with FC and changed it to CC, and the reactivity of CC was much weaker than that of FC (Wang et al., 2007), indicating that FC is more effective than CC on the removal of ARGs (Dodd, 2012).

\[
\begin{align*}
\text{NH}_3 + \text{HOCI} & \rightarrow \text{NH}_2\text{Cl} + \text{H}_2\text{O} \\
\text{NH}_2\text{Cl} + \text{HOCI} & \rightarrow \text{NH}_2\text{Cl}_2 + \text{H}_2\text{O} \\
\text{NH}_2\text{Cl} + \text{HOCI} & \rightarrow \text{NCl}_3 + \text{H}_2\text{O}.
\end{align*}
\]
Thus, after the addition of chlorine, chlorine might be quickly consumed by NH$_3$–N to mainly generate monochloramine. In this experiment, the rapid ARGs removal stage was observed from an initial 5 min to 30 min because of the direct consumption of FC (Fig. 2). A small portion of FC might react with bacteria in the samples, depending on their respective reaction rate constant with FC contrasted to that of NH$_3$–N (Macauley et al., 2006).

Except for NH$_3$–N, other factors including pH, dissolved organic matter (DOM) can also affect chlorination disinfection. For example, Li and Zhang (2012) revealed that pH significantly affects the removal efficiency in chlorination ($p < 0.05$). Wang et al. (2007) found that DOM was an important factor involving the increasing genotoxicity during chlorination of wastewater. Further studies about how these factors work during ARGs chlorination disinfection process are needed.

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Fig. 6. Influences of UV alone, chlorination and sequential UV/chlorination treatment on the removal of ARGs. (A) sulI (B) tetX (C) tetG (D) intI1, and (E) 16S rRNA genes. The horizontal axis indicates three different UV dosages of 62.4, 124.8, and 249.5 mJ cm$^{-2}$, respectively.
### 4.3. Removal of ARGs by UV alone and sequential UV/chlorination

In the UV irradiation experiment, when the UV dosage was at 249.5 mJ cm\(^{-2}\), the maximum log reduction of tetX and 16S rRNA genes were 0.58 and 0.60, respectively, while other genes were 0.36–0.40 log. The results may be explained by the fact that UV light can penetrate the UV-transparent structures in the cell and primarily be absorbed by the nucleobases comprising DNA and RNA (Dodd, 2012). Various particular wavelengths toward different organisms can lead to different degrees of DNA damage (Chen et al., 2009). Previous studies have reported that at UV dosage of 200–400 mJ cm\(^{-2}\), ARGs in extracellular DNA or existing within ARB could be decreased by 3–4 logs (McKinney and Pruden, 2012). Guo et al. (2013a) revealed that UV disinfection at a dose of 5 mJ cm\(^{-2}\) could eliminate bacteria resistances to erythromycin and tetracycline in wastewater treatment effluents. However, Auerbach et al. (2007) determined that no significant reduction of tetR, tetG and tetQ occurs in effluent water at the UV dosage of 30 mJ cm\(^{-2}\) and 100 mJ cm\(^{-2}\). The results indicate various tolerance/sensitivity features of ARB or ARGs after UV treatment (Guo et al., 2013b).

Considering deficiencies in the chlorine or irradiation alone for disinfection, including incomplete inactivation of bacteria and increased requirements for chlorine, a sequential UV/chlorination treatment was investigated. It has been reported that UV irradiation followed by chlorination could prevent microbial regrowth as well as generate a synergetic effect (Shang et al., 2007). For example, Wang et al. (2012) revealed that the complete inactivation of heterotrophic plate count (HPC) in wastewater was accomplished by treatment with 15 mJ cm\(^{-2}\) UV followed by 1.6 mg L\(^{-1}\) chlorine and total bacteria count (TBC) were completely inactivated by sequential application of 40 mJ cm\(^{-2}\) UV and 2 mg L\(^{-1}\) chlorine. Al-Gabr et al. (2013) indicated that combined use of UV irradiation and chlorination was more effective than using either alone in inactivating microorganism in drinking water. In this study, the result showed that 0.31 log synergey values for 16S rRNA genes could be achieved at 62.4 mJ cm\(^{-2}\) UV irradiation followed by chlorination of 25 mg L\(^{-1}\) under a contact time of 30 min. For sequential UV/chlorination treatment, the synergy may be due to the decrease of the bioactivity affected by UV irradiation, causing the chlorine to react with the cells more readily. In addition, at the same level of removal efficiency, UV irradiation could reduce the demand dosages of chlorine and the possibility of DBPs formation. The combination of two disinfection methods was observed to be a promising method for ARGs removal. Further research should be conducted to verify these findings in a full-scale disinfection of wastewater because different wastewater characteristics may affect synergies achievable by sequential UV/chlorination disinfection methods. In summary, traditional single disinfection technologies including chlorination and UV irradiation are not efficient in the removal of ARGs especially under common dosage.

In terms of relationship among inactivation of different ARGs in chlorination and UV disinfection processes, it was found that the inactivation efficiency of tet genes were higher than sul genes, indicating tet genes were easier to remove than sul genes. The results can be explained by the research discovered that tetracycline-resistant bacteria were easier to remove than sulfonamide-resistant bacteria (Gao et al., 2012).

### 5. Conclusion

The disinfection process for ARGs (sul1, tetX, tetG, intI1, and 16S rRNA genes) in MWTP effluent by using chlorination, UV irradiation alone and sequential UV/chlorination treatment achieved different results. Chlorine, as a disinfectant, appeared more effective than UV irradiation, while NH\(_3\)-N proved to be an important factor in chlorination. When applied Cl\(_2\)-NH\(_3\)-N ratio was over 7.6:1, significant reduction of ARGs was achieved (1.20–1.49 logs). The combination of UV irradiation followed by chlorination showed higher removal efficiency than UV or chlorination alone. At the same level of removal efficiency, UV irradiation could reduce the demand dosages of chlorine and the possibility of the formation of DBPs. Further and systematic studies on the effect of conventional (e.g., chlorination and UV irradiation) and new alternative disinfection processes (e.g., advanced oxidation process) on the inactivation of ARB as well as the capacity to control ARGs spread into the environment are strongly recommended.

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### References


