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RESEARCH ARTICLE

Impacts of advanced treatment processes on elimination of antibiotic resistance genes in a municipal wastewater treatment plant

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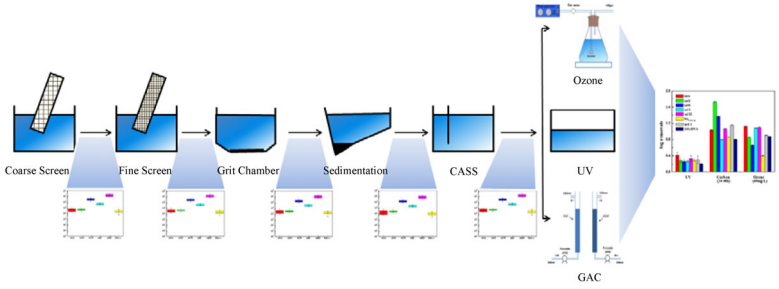
HIGHLIGHTS

- The distributions of ARGs were monitored in a WWTP in Harbin during six months.
- CASS had the best removal efficacy of ARGs compared to other processes in the WWTP.
- UV disinfection could effectively control the HGT.
- AGAC significantly remove ARGs and organics due to its high absorption capacity.
- Combination of ozone and AGAC significantly improve removal of ARGs and organics.

GRAPHIC ABSTRACT



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ABSTRACT

Antibiotic resistance genes (ARGs) pose a serious threat to public health. Wastewater treatment plants (WWTPs) are essential for controlling the release of ARGs into the environment. This study investigated ARG distribution at every step in the treatment process of a municipal WWTP located in Harbin for six consecutive months. Changes in ARG distribution involved in two advanced secondary effluent treatment processes, ozonation and granular activated carbon (GAC) adsorption, were analyzed. Biological treatment resulted in the highest ARG removal (0.76–1.94 log reduction), followed by ultraviolet (UV) disinfection (less than 0.5-log reduction). Primary treatment could not significantly remove ARGs. ARG removal efficiency increased with an increase in the ozone dose below 40 mg/L. However, amorphous GAC (AGAC) adsorption with a hydraulic retention time (HRT) of 1 h showed better removal of ARGs, total organic carbon (TOC), total nitrogen (TN), and total phosphorus (TP) than ozonation at a 60 mg/L dose. UV treatment could efficiently reduce the relative ARG abundance, despite presenting the lowest efficiency for the reduction of absolute ARG abundance compared with GAC and ozone treatments. The combination of ozone and AGAC can significantly improve the removal of ARGs, TOC, TN and TP. These results indicate that a treatment including biological processing, ozonation, and AGAC adsorption is a promising strategy for removing ARGs and refractory organic substances from sewage.

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1 Introduction

The overuse and misuse of antibiotics have become a worldwide problem. Antibiotics discharge cannot only chemically pollute the environment but also induce antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) (McKinney and Pruden, 2012). ARGs represent a significant threat because they are typically located in mobile genetic elements (such as plasmids and integrons), which disperse ARGs among microorganisms via horizontal gene transfer (HGT). If microbes, especially pathogens, acquire ARGs, they can seriously compromise

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the treatment of human and animal diseases (Zheng et al., 2017).

Wastewater treatment plants (WWTPs) are considered an important barrier to avoid the release of antibiotic resistance genes into surface waters. However, most conventional WWTPs have a limited capacity to remove antibiotic resistance genes from wastewater (Wen et al., 2016; Zheng et al., 2017). At the same time, the high microbial density and antibiotic residues of biological wastewaters can promote antibiotic-resistance determinants (Wen et al., 2016). An increasing number of clinically relevant ARB are being detected in WWTPs, and most ARB have multidrug resistance to different antimicrobial agents, such as aminoglycoside, quinolone (Osińska et al., 2016), erythromycin, and multi-targeting antibiotics (Szczepanowski et al., 2005). Higher amounts of ARB and ARGs have been detected in effluents and downstream of WWTPs than in natural waters (Rizzo et al., 2013). WWTPs, originally designed to protect the environment and public health, are considered an important reservoir of antibiotic resistance determinants, which cause a significant burden on the environment and human health (Rizzo et al., 2013; Sousa et al., 2017). Therefore, the use of post-treatments, such as oxidation and/or adsorption, should be considered to mitigate antibiotic resistance on site.

Potential processes include ultraviolet (UV) disinfection, which does not produce residues or by-products (McKinney and Pruden, 2012), and ozone oxidation, which can inactivate pathogens and remove organic micropollutants (Alexander et al., 2016). Both processes are commonly used in wastewater treatment and are effective in removing micro-pollutants. For instance, ozonation has been reported to efficiently degrade most micropollutants at a dose of 3–8 mg/L (Margot et al., 2013). Additionally, more than 90% removal efficiency of pharmaceuticals and metabolites was obtained using an ozonation dose of 1.08 gO_3/gDOC, whereas only a 33% removal efficiency of these pollutants was obtained using a UV dose of 2400 J/m^2 (Kovalova et al., 2013). Few researches have been conducted to analyze the removal of antibiotic resistance genes by different disinfection processes (Huang et al., 2011; Oh et al., 2014; Zheng et al., 2017). The wastewater disinfection process reduced overall ARB levels by 34%–75% using a 27 mJ/cm^2 UV dose at two South Korea WWTPs (Lee et al., 2017) and caused a 2.46 log reduction of tetracycline-resistance bacteria using 2 mg/L of ozone (Zheng et al., 2017). The removal efficiencies of ARGs by disinfection vary widely among different studies. Tetracycline resistance genes presented a 1.3 log reduction at a UV dose of 100 mJ/cm^2 in the research of McKinney and Pruden (2012), whereas no significant reduction was observed using the same UV dose in the study by Auerbach et al. (2007). Nevertheless, there are still DNA remnants in the form of extracellular genes, even after the deactivation of ARB by disinfection. If these extracellular genes are ARGs and remain intact, they still have a transmission capacity. Competent bacteria may acquire antibiotic resistance through these ARGs (Chang et al., 2017).

The use of activated carbon, a well-known purifying material, is technically and economically feasible for removing a broad spectrum of micropollutants from wastewater (Bonvin et al., 2016; Margot et al., 2013), as well as reducing effluent toxicity (Margot et al., 2013). However, the removal of ARGs by activated carbon has not been widely investigated. Some studies attempted to compare antibiotic removal from wastewaters through activated carbon adsorption and advanced oxidation treatment, but due to the difference of compound characteristics and treatment conditions, the removal efficiencies were not comparable (Margot et al., 2013; Altmann et al., 2014). A direct comparison of these processes for ARG removal from the WWTP effluent is needed.

Therefore, typical *tet* genes (*tetA*, *tetO*, *tetW*), *sul* genes (*sulI*, *sulII*), *bla* gene ($bla_{CTX\text{-}M}$), and the class 1 integrase gene *intI1* were monitored to assess the removal of ARGs in a municipal WWTP in Harbin, north-east of China. Laboratory-scale experiments of ozonation and activated carbon adsorption were also conducted to further treat the secondary effluent from the WWTP. The removal efficiency of ARGs under different advanced treatments were evaluated and compared. This study provides a feasible and efficient solution to eliminate ARGs from wastewater and reduce the environmental risks caused by them.

2 Materials and methods

2.1 Sampling campaigns

A WWTP in Harbin (China), with an average daily flow of 150,000 m^3/d and a catchment area of 110 km^2, was selected for the collection of wastewater samples. The WWTP employs a cyclic activated sludge system (CASS) to treat domestic sewage and approximately 30% of pharmaceutical and other industrial wastewaters. The main product manufactured by the pharmaceutical factory upstream of the WWTP in Harbin is penicillin sodium. UV disinfection is applied as the tertiary treatment with a dose of 20 mJ/cm^2. The sampling locations in each treatment process are shown in Fig. 1(a). The sampling campaigns lasted 6 months, from November to April. Following the "Discharge standards of pollutants for municipal wastewater treatment plant (GB18918-2002)," 24-h composite samples were collected at each sampling site to obtain average values. The wastewater was collected in sterile containers, and subsequent experiments were conducted in the laboratory. The CASS effluent samples (prior to the UV disinfection), collected in the April

sampling campaign, were used for laboratory-scale ozonation and activated carbon treatment experiments.

2.2 Laboratory experiments of tertiary treatment

2.2.1 Ozonation

Batch experiments were carried out in a 2 L reactor (Fig. 1 (b)). Ozone was generated from pure oxygen (99.9%) by an ozone generator (YT-018, China). An aerator at the bottom of the reactor was used to diffuse ozone into the water. The ozone concentration was measured using an ozone analyzer, and the ozone dose was controlled at 10, 20, 40, and 60 mg/L with 20 min of contact time.

2.2.2 Activated carbon adsorption

Two filter columns, each filled with a different type of activated carbon, were used in the ARG adsorption experiment. Coal-based granular activated carbon (GAC) and nutshell-based amorphous granular activated carbon (AGAC) were used (Fig. 1(c)). The physical properties of the activated carbon are listed in Table 1. The secondary effluent taken from the WWTP was fed into the column through the bottom at a flow rate of 0.6 L/h. The hydraulic retention time (HRT) remained 1 h. Two 24-h composite effluent samples were collected, respectively, at 48 h (24–48 h composite samples) and 96 h (72–96 h composite samples), and the refrigerated samples were subsequently used for DNA extraction.

2.3 DNA Extraction

Water samples were filtered through a 0.22 m sterile membrane (Millipore, USA) using a vacuum filtration apparatus. After its use, the filter membrane was cut into pieces, placed in sterile tubes, and stored at 20°C. Before DNA extraction, all filters were frozen using liquid nitrogen and cleaned by phosphate buffer saline (PBS). The supernatant was collected after 3 min centrifugation at 9000 r/min. Lysozyme (Omega, USA) was added for digestion at 30°C overnight, then the digested products were harvested at 9000 r/min for 5 min. The total DNA was extracted using Omega E.Z.N.A. Bacterial DNA Kit (Omega, USA), following the provided protocol. DNA purity and concentration were determined by 0.8% agarose gel electrophoresis and spectrophotometer analysis (Nano-Drop ND-2000C, Thermofisher, USA).

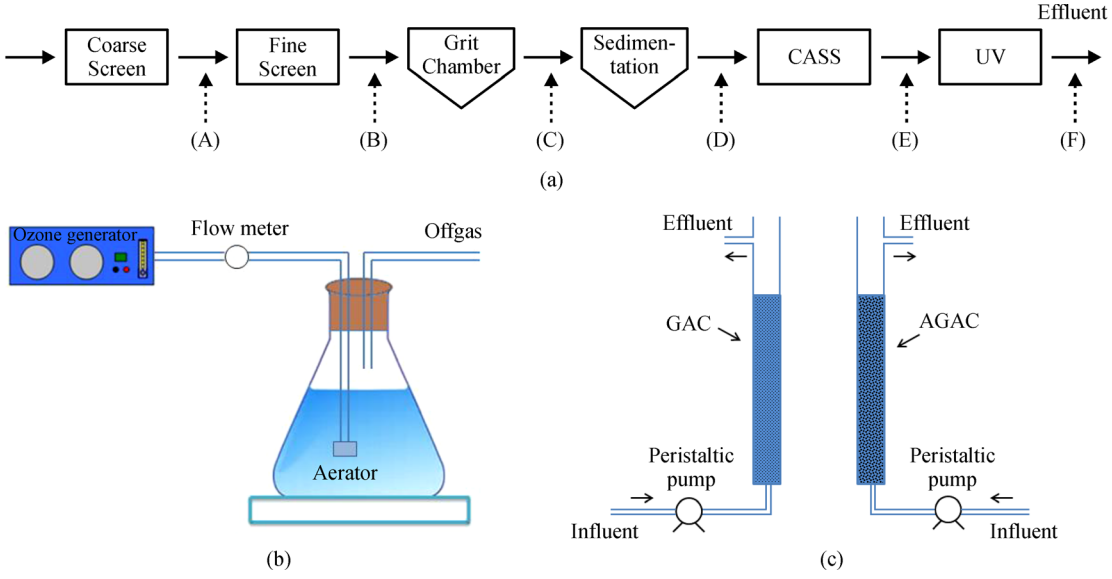

Fig. 1 (a) WWTP processes and sampling sites: (A) after the coarse screen, (B) after the fine screen, (C) after the grit chamber, (D) after the sedimentation tank, (E) after CASS, and (F) after UV disinfection. Configuration of post-treatments: (b) Ozone disinfection, (c) Granular activated carbon.

Table 1 Physical characteristics of the activated carbon used in this study

Specification	GAC (Coal-Based)	AGAC (Nutshell-Based)
Iodine number (mg/g)	⩾500–950	⩾900–1000
Specific surface area (m^2/g)	500–900	900–1100
Apparent density (g/cm^3)	0.45–0.58	0.35–0.45
Moisture content	⩽10%	⩽10%

2.4 DNA determination by quantitative real time PCR (qPCR)

Three *tet* genes (*tetA*, *tetO*, *tetW*), two *sul* genes (*sulI*, *sulII*), one *bla* gene (bla_{CTX-M}), *intI1*, and the 16S rRNA gene were selected for quantitative detection using SYBR Green I qPCR. Primers and amplification conditions are listed in Table S1. Qualitative PCR assays were conducted in a 50L reaction system containing 4 L of template DNA, 0.25 L of Takara ExTaq (5 U/L) (Takara, Japan), 1.0 L of each primer, 4.0 L of dNTP mixture, and 5.0 L of 10 × ExTaq buffer (Mg^{2+} plus). The experiments were conducted in duplicate to ensure reproducibility. The thermal cycling procedure included: initial denaturation at 95°C for 5 min, 35 cycles of 95°C for 45 s, annealing (according to the temperature listed in Table S1) for 45 s, 72°C for 60 s, and a final extension at 72°C for 5 min. Each assay contained a negative control. The PCR products were purified using the MiniBEST Agarose Gel DNA Extraction Kit Ver.4.0 (Takara).

Target genes were ligated to plasmids at 4°C overnight using pGEM-T Easy vectors (Promega, Beijing, China). Then, they were cloned into *Escherichia coli* DH5(Transgen Biotech). Plasmids carrying target genes were extracted and purified using Takara MiniBEST Plasmid Purification Kit Ver.4.0 (Takara) and determined by a spectrophotometer. A five- or six-point calibration curve (Ct value versus log value of initial target gene copy) was generated for qPCR by a 10-fold serial dilution of purified plasmid extracts with high R^2 values and efficiency. Based on the calibration curves, the Ct value of a test sample was used to calculate the gene copy, then normalized against volume of wastewater. With a final volume of 20 L, qPCRs were conducted using 10.0 L SYBR® Premix Ex *Taq*II (Tli RNaseH Plus) (Takara, Japan), 2.0 L DNA template, 0.8 L of each primer (10 M), 0.4 L ROX Reference Dye II (50×), and 6.0 L dH_2O. The temperature profile consisted of one cycle at 94°C for 30 s, 40 cycles including 95°C for 5 s, annealing temperature for 34 s, 95°C for 5 s, followed by a melt curve for specificity verification. All reactions were performed in triplicate and with negative control. The limit of detection for each assay was defined by the lowest Ct value determined in the PCR or in the extraction blanks (Czekalski et al., 2016).

2.5 Statistical analysis

Determination of the average value and standard deviation of the data was executed through the Microsoft Excel 2010 tool-pack (Microsoft, USA). OriginPro 8.0 (OriginLab, USA) was used to plot the variations of ARGs. Paired *t*-tests were conducted using SPSS® 20.0 (SPSS Inc. USA). The differences at $P < 0.05$ level were considered statistically significant. Non-metric multidimensional scaling (NMDS) was conducted using PRIMER 5 based on the Bray-Curtis distance.

3 Results and discussion

3.1 Distribution of ARGs in the WWTP

Figure 2 summarizes the absolute abundance (gene copies per milliliter of wastewater sample) of the six ARGs, *intI1*, and the 16S rRNA gene from each site during the six-month period.

In the influent, *sul* genes were the most dominant ARGs, representing 76.44%–94.01% of the total ARGs (ARG copies/total ARG copies) (Fig. S1). The absolute abundances of *sulI* and *sulII* were $10^{5.49}$–$10^{5.84}$ and $10^{6.88}$–$10^{7.53}$ copies/mL, respectively. The *tet* genes represented 5.86%–22.03% of the total ARGs. The absolute abundances of *tetA*, *tetO*, and *tetW* were $10^{4.18}$–$10^{5.05}$, $10^{4.35}$–$10^{4.91}$, and $10^{6.12}$–$10^{6.70}$ copies/mL, respectively. Comparatively, *bla* genes only accounted for 0.03%–0.25% of the total ARGs (bla_{CTX-M} ranged from $10^{3.72}$ to $10^{4.69}$ copies/mL) in the influent. Consistently, other studies also proved that sulfonamide resistance genes and tetracycline resistance genes were the most abundant ARGs in WWTPs (Gao et al., 2012b; Chen and Zhang, 2013). This is because sulfonamide and tetracycline are the most extensively used antibiotics in humans and animals (Gao et al., 2012a), leading to a wide distribution of *sul* and *tet* genes in WWTPs. *SulI* is commonly located in class 1 integrons, and *sulII* associates with large multi-resistance plasmids. Plasmid-associated genes are transmissible and can easily transfer though HGT, especially under the selective pressure of antibiotics (Luo et al., 2010), leading to the high abundance of *sulII* genes in the influent. The absolute abundance of *bla* genes were the lowest in the influent. Similar results were observed in other WWTPs from Harbin (Wen et al., 2016). The low abundance of *bla* genes in the WWTP may be justified by the low relative content of antibiotics in the wastewater, since-lactam antibiotics can be easily degraded and inactivated by hydrolysis (Sparbier et al., 2012; Wen et al., 2018).

The distribution of different ARGs in the influent fluctuated throughout the months (Fig. S2). The abundance of *sul* genes mostly decreased during the campaign. The abundance of *tet* genes initially increased, then decreased. The peak value was observed in January ($10^{6.71}$ copies/mL). The *bla* gene presented a down and up trend, and the nadir was found in February ($10^{3.72}$ copies/mL). The fluctuation of ARGs in the influent was mainly due to the considerable monthly variation of antibiotics in influent of the WWTP, such as sulfamerazine and tetracycline (shown as Table S2). The monthly variation of antibiotics can be attributed to either societal factors (such as production, consumption, and excretion) or environmental factors (such as solar irradiance, precipitation, and temperature) (Golovko et al., 2014; Zhai et al., 2016). In addition, other factors such as the presence of heavy metal in the wastewater could also create co-selection pressure on the distribution of ARGs (Stepanauskas et al., 2006).

3.2 ARG removal in different treatment units in the WWTP

3.2.1 ARG removal by primary treatments

Low ARG variation was observed during the primary processes (fine screen, grit chamber, and sedimentation) (Fig. 2 and 3). The Bray-Curtis similarity among A, B, C, and D was more than 90% (95.18% between A and B, 92.70% between B and C, and 93.20% between C and D) (Table S3). Only a small reduction in the ARG absolute abundance by primary processes was detected (0.17–0.5 orders of magnitude), which indicates that primary processes are not capable to effectively reduce ARGs. Additionally, there were almost no obvious variations in the relative abundance of ARGs after primary treatments (Fig. S3). The removal of ARGs in the primary treatment in the WWTP likely depend on the sedimentation of suspended particles where extracellular ARGs are attached (Börjesson et al., 2009; Mao et al., 2013).

3.2.2 ARG removal by biological treatment

The longest Bray-Curtis distance was between the influent and effluent of the CASS process (D and E) (Fig. 3). These results indicate that there was a big shift in ARG occurrence after the biological treatment (Bray-Curtis similarity was only 37.23%, as shown in Table S3). A remarkable decrease in ARGs can be observed after the biological treatment process (Fig. 2). The reduction in the absolute abundances of *tet* genes were the highest, with 1.19–1.80, 1.56–1.94, and 1.34–1.95 orders of magnitude for *tetA*, *tetO*, and *tetW*, respectively, followed by *sul* genes and *bla* genes. The reductions in *sulI*, *sulII*, and $bla_{CTX\text{-}M}$ were 0.88–1.41, 0.76–1.45, and 0.98–1.36 orders of

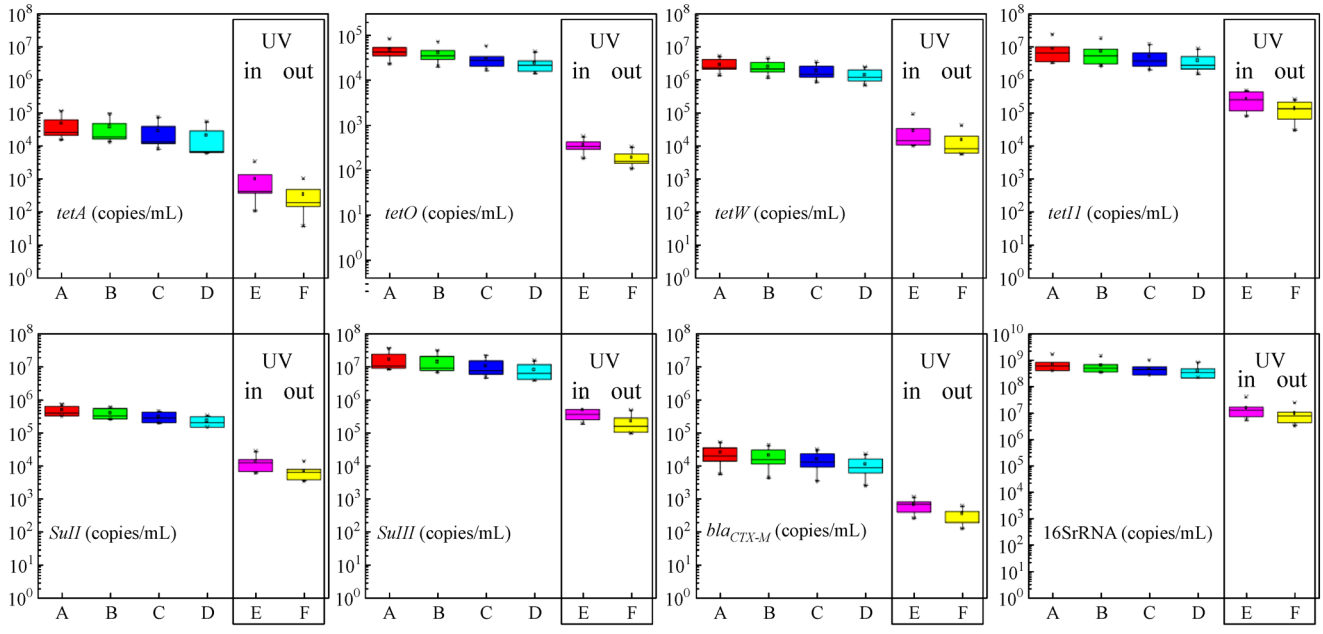

Fig. 2 Distribution of ARGs in the wastewater under different treatment processes (A, B, C, D, E, F represent the sampling locations after the coarse screen, the fine screen, the grit chamber, the sedimentation tank, CASS, and UV disinfection, respectively).

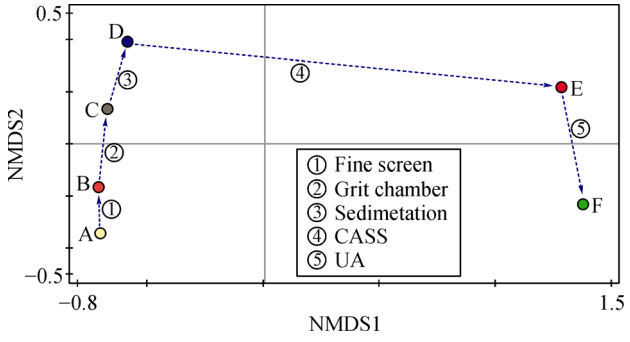

Fig. 3 Non-metric multidimensional scaling of ARG across the wastewater treatment process (A, B, C, D, E, F represent the sampling locations, ①, ②, ③, ④, and ⑤ represent the treatment processes).

magnitude, respectively. The 16S rRNA gene reduction reached 0.90–1.66 orders of magnitude. In case of biological treatment, cell-associated ARGs are closely related to suspended sludge, so the removal of suspended sludge contributed to the removal of intracellular ARGs (Zhang et al., 2017). The CASS process combines biological reaction and sedimentation. It adequately reduces ARG-harbouring microbes by separating sludge from water in the mixed liquor. After the ARG-harbouring microbes die, the remaining organic molecules contain extracellular ARGs, which can be removed via adsorption in the suspended sludge or degradation by microorganisms through the CASS process (Pei et al., 2007).

Though the absolute abundance of each ARG reduced after the biological treatment, the relative abundances of *sulI*, *sulII*, and $bla_{CTX\text{-}M}$ increased in the biological effluent (Fig. S3), indicating the enrichment of these ARG-harbouring microbes in the microbial community after biological treatment despite the good separation performance achieved by the CASS process. Integrons can transfer among bacteria in activated sludge with high microbial density and diversity. The association of integrons and plasmids simultaneously leads to an increased relative abundance of *intI1* in CASS and related ARGs (Gao et al., 2012b), such as *sulI*, which are structurally located in class 1 integrons. The relative abundance of *tet* genes decreased, revealing the competitive disadvantage of *tet* genes-harbouring bacteria in CASS (Zhang et al., 2017). Moreover, *tetA* and *tetO* associate into conjugative plasmids from an incompatible group, which cannot co-present in one cell simultaneously, thus limiting the spread of *tet* genes (Jones et al., 1992).

3.2.3 ARG removal by UV disinfection

In this study, there was no significant reduction of ARGs in the effluent before and after UV disinfection. The average reductions of *tetA*, *tetO*, *tetW*, *sulI*, *sulII*, $bla_{CTX\text{-}M}$, and *intI1* genes and the 16S rRNA gene were 0.41±0.09, 0.28±0.06, 0.27±0.04, 0.27±0.03, 0.33±0.08, 0.29±0.03, 0.30±0.10, and 0.2±0.02 orders of magnitude, respectively. Similar results were observed by Chen and Zhang's study (2013) in a full-scale WWTP, which showed a reduction of 0.3 orders of magnitude for *sul* and *intI1* genes and that of 0.5–0.7 orders of magnitude for *tet* genes. However, an ARG log reduction of 3–4 was achieved by UV of 200–400 mJ/cm^2 intensity in McKinney and Pruden's (2012) batch experiment. The main cause of the difference in removal efficiency was the intensity of UV. The UV intensity applied in WWTPs is generally less than 30 mJ/cm^2. In this research, it was 20 mJ/cm^2 (full-scale WWTP), which is far less than that used in McKinney and Pruden's (2012) experiment. In addition, the absorbance of UV light by organic substances in wastewater could impair UV disinfection. Further, photoreactivation was stronger in the WWTP compared with laboratory-scale experiments, leading to the low reduction of ARGs in the WWTP by UV treatment.

Though the UV treatment showed a limited reduction in absolute abundance of ARGs, unlike biological treatment, the relative abundance of target ARGs decreased (Fig. S3). Lin et al. (2016) also reported that UV irradiation reduced the transfer frequency of RP4 plasmid from 8.63×10^3 to 2.44×10^5 when UV intensity increased from 0 to 20 mJ/cm^2. Therefore, UV can effectively reduce the risk of ARG dispersion because of the reduction of microorganisms and plasmid transfer.

3.3 ARG removal by ozone disinfection

As shown in Fig. 4, ozone disinfection could reduce the absolute abundance of target ARGs and *intI1* in the secondary effluent. A 0.16–0.50 log removal of ARGs was observed under ozone dose of 10 mg/L (Fig. 4(a)). These results were attributed to the presence of organic substances, which consumed ozone, and to microorganisms protected from the ozone effect in flocs and particles

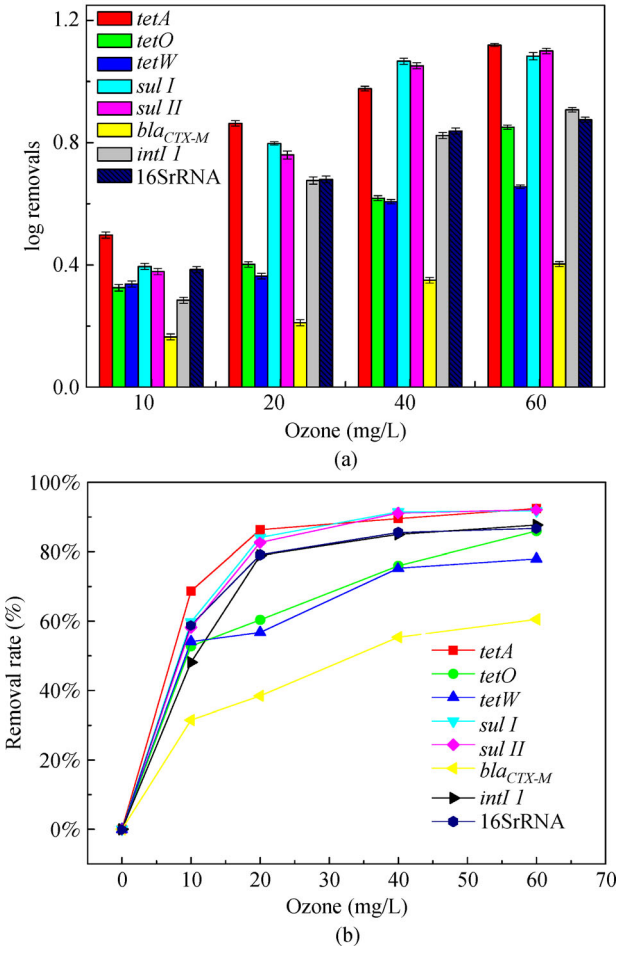

Fig. 4 Removal of ARGs by ozone disinfection.

(Czekalski et al., 2016). Dietrich et al. (2007) confirmed that soluble organic matter in bulk liquid phase rapidly consumes 99%–100% of the initial ozone (3 mg/L), resulting in the incapability of ozone to inactivate particles greater than 12 m. Therefore, high initial content of ozone is required to inactivate bacteria in bigger particles. In addition to that, high initial ozone concentration can increase the reaction of ozone to micropollutants as well (Hollender et al., 2009). The removal efficiency of ARGs rose sharply when the ozone dose increased from 0 to 10 mg/L, regardless of the consumption of organic matter (Fig. 4(b)). The log removal of ARGs increased with the increase of ozone concentration. However, the increase of removal efficiency slowed down when the ozone dose increased from 10 to 40 mg/L. An increased ozone concentration could further inactivate bacteria protected by particles and destroy cellular membrane through oxidation reactive, leading to bacterial lysis and death (Rojas-Valencia, 2011; Dodd, 2012). The intracellular ARGs leaked out of the cell as extracellular ARGs and were oxidized (Dodd, 2012). When the ozone dose further increased to 60 mg/L, the removal of most ARGs went up to 1 log. The removal efficiency of *tetA*, *tetO*, *tetW*, *sulI*, and *sulII* achieved 80%–90%. Removal of *intI1* and *16S rRNA* gene reached 87.6% and 86.7%, respectively. Therefore, although higher ozone doses could further reduce the absolute abundance of ARGs, the removal efficiency could hardly achieve 100% due to the protection of ARB by particles and/or flocs. The maximum removal efficiency of $bla_{CTX\text{-}M}$ was 60% in this experiment, which may be due to its low initial concentration in the secondary effluent (only $10^{2.13}$ copies/mL), resulting in insufficient reaction with ozone.

The progressive inactivation of ozone involved the alterations of membrane permeability, cell disintegration, and lysis reaction, leading to the leakage of cell constituents (Cataldo, 2006). Despite the effective reduction in absolute abundance of total ARGs, *intI1*, and *16S rRNA* gene by ozonation, the relative abundance of some ARGs, such as *tetO*, *tetW*, and $bla_{CTX\text{-}M}$, increased after ozone disinfection, while *tetA*, *sulI*, and *sulII*, decreased (Fig. S4). Though *tetA*, *sulI*, and *sulII* are associated with plasmid, ozone could directly damage plasmid DNA (Cataldo, 2006), inhibiting the HGT of plasmid. The ARG-harbouring bacteria which can cause sublethal damage can be resistant to ozone and accumulate in the surviving microbiota, resulting in ARG enrichment (Dodd, 2012; Alexander et al., 2016).

3.4 ARG removal by granular activated carbon

The abundance of ARGs in the secondary effluent and the effluent treated by GAC and AGAC were determined as shown in Fig. S5. The removal of ARGs by the two types of activated carbon is shown in Fig. 5. All assays demonstrated efficient removal of tetracycline resistance genes, followed by-lactam resistance genes, and sulfonamide resistance genes (in this order). Both GAC and AGAC had significant removal efficiency of *tetO* and *tetW*, up to 1.10–1.73 and 1.02–1.37 orders of magnitude, respectively. There was no obvious difference in the removal efficiency of $bla_{CTX\text{-}M}$ by GAC and AGAC ($P < 0.5$), which was of 0.53–0.85 orders of magnitude. The removal of *sul* genes and *intI1* by GAC was inconspicuous, at approximately 0.1 orders of magnitude. The removal of *sul* genes and *intI1* by AGAC achieved 0.8–1.07 and 1.15 orders of magnitude, respectively. Compared to GAC, the better removal efficiency of ARGs by AGAC was mainly due to its higher specific surface area (900–1000 m^2/g compared with 500–900 m^2/g (GAC)) and smaller porosity. The removal efficiency of the two types of granular activated carbon at 48 h was higher than that at 96 h because of the saturation with adsorption time.

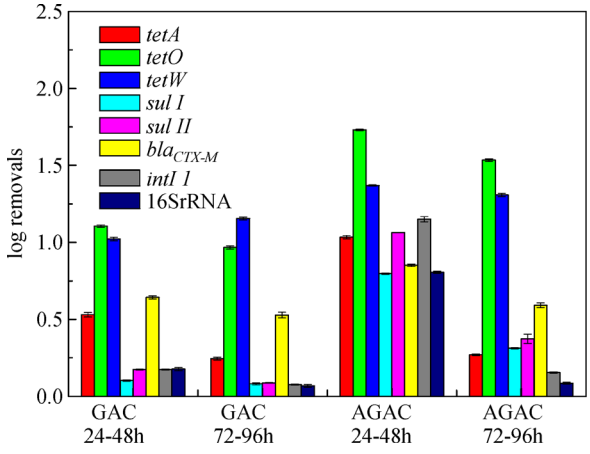

Fig. 5 Removal of ARGs by activated carbon treatments

Activated carbon has been widely used in wastewater treatment due to its high adsorption capacity in micropollutants removal (Altmann et al., 2014). The present research confirmed that AGAC can effectively mitigate ARGs. With prolonged operation time of activated carbon, backflushing is required. However, backflushing was not performed in this study due to the relatively short operating period. Therefore, the effects of backflushing on ARG removal need further investigation.

3.5 Comparison of ARG removal performance by different advanced treatment processes

To compare ARG elimination by different advanced wastewater treatments processes, the removal efficiency of ARGs by AGAC with 48 h of consecutive operation and ozone dose of 60 mg/L was selected. The result was compared with UV disinfection in the WWTP. As shown in Fig. 6, AGAC demonstrated higher ARG removal from secondary effluent, followed by ozonation. UV had the

minimum ARGs removal, which was less than 0.5 orders of magnitude during the research period.

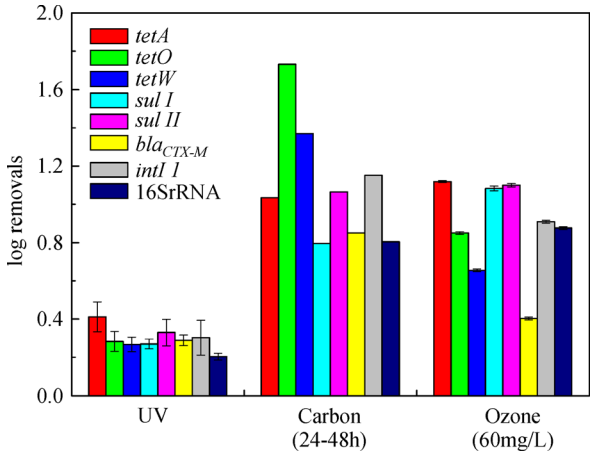

Fig. 6 Comparison of different advanced treatments for ARG removal.

Other wastewater quality parameters in ozonation and GAC experiments were also measured. GAC adsorption showed good removal of total organic carbon (TOC), total nitrogen (TN), and total phosphorus (TP), which reached 40%, 30%, and 20%, respectively. Ozonation cause only a slight reduction of these parameters because it transforms the pollutants instead of removing them. However, activated carbon can remove chemicals from wastewater by adsorption if high-temperature regeneration is used (Kovalova et al., 2013; Altmann et al., 2014). Moreover, the energy consumption of activated carbon adsorption is much lower than that of ozonation, 0.05 kWh/m^3 and 0.1–0.2 kWh/m^3 (with 5–10 mg/L ozone dose), respectively (Kovalova et al., 2013). However, when using 10 mg/L of ozone and 20 mg/L of activated carbon to upgrade the secondary effluent, the initial investment, operation, and annual cost of the activated carbon system were 386000, 2000, and 36717 CHF (Swiss francs) higher than for ozonation (Kovalova et al., 2013).

3.6 Combination of advanced treatments

Ozonation at a 60 mg/L ozone dose combined with AGAC was used to treat the secondary effluent according to the assay results. After the treatment, *tet* and *bla* genes were under the detection limit. Removal of *sulI* and *sulII* achieved 2.75 and 2.63 orders of magnitude. High *intI1* and *16S rRNA* gene removals were also detected, reaching 2.44 and 2.77 orders of magnitude, respectively. The higher removal efficiency was because ARG harbouring microorganisms were damaged by ozonation, whereas AGAC could further remove the dead and residual live cells by adsorption. In the combined process, the removal of TOC, TN, and TP increased to 65%, 54%, and 31%, respectively, due to the strong oxidative and absorption properties. The combination of ozone and GAC treatments has a synergetic effect on the removal of chemicals and ARGs in comparison with individual processes.

4 Conclusions

The distribution of *intI1*, the 16S rRNA gene, and six ARGs in the treatment processes of a WWTP were investigated during six consecutive months, as well as their elimination and deactivation by ozonation and AGAC adsorption. The fluctuation of ARGs in the influent throughout the study duration was mainly due to the monthly variation of antibiotic discharge. The biological treatment in the WWTP could significantly reduce the absolute abundance of ARGs, whereas little ARG removal was achieved by UV disinfection. Although ozone at a 60 mg/L dose could also reduce ARGs effectively, removal of TOC, TN, and TP was low. AGAC adsorption at 24–48 h of consecutive operation showed good removal of ARGs, TOC, TN, and TP. The combination of ozonation and AGAC adsorption significantly increased the elimination of ARGs, TOC, TN, and TP. Therefore, an advanced treatment process combining biological treatment, ozonation, and AGAC adsorption is a promising strategy to remove ARGs and refractory organic substances from municipal wastewater.

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