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Fate of antibiotics and antibiotic resistance genes during conventional and additional treatment technologies in wastewater treatment plants

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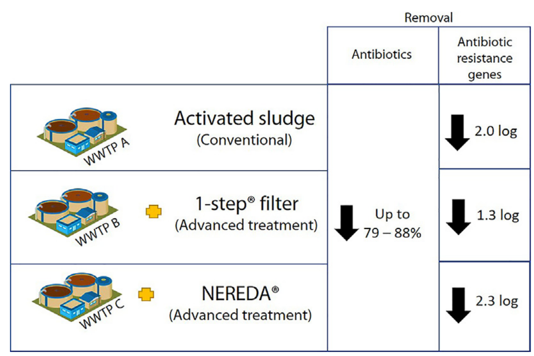
HIGHLIGHTS

- Good removal (79-88%) of total antibiotics was observed at all WWTPs.
- NEREDA® (2.3 log) and conventional WWTP (2.0 log) are more effective for log reduction of ARG than 1-STEP® filter (1.3 log).
- The tested additional treatment technologies did not fully remove antibiotics and ARGs from the waterphase.
- ARGs were detected in sludge, and the sludge is an important reservoir for ARGs.

GRAPHICAL ABSTRACT



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ABSTRACT

Information on the removal of antibiotics and ARGs in full-scale WWTPs (with or without additional treatment technology) is limited. However, it is important to understand the efficiency of full-scale treatment technologies in removing antibiotics and ARGs under a variety of conditions relevant for practice to reduce their environmental spreading. Therefore, this study was performed to evaluate the removal of antibiotics and ARGs in a conventional wastewater treatment plant (WWTP A) and two full-scale combined with additional treatment technologies. WWTP B, a conventional activated sludge treatment followed by an activated carbon filtration step (1-STEP® filter) as a final treatment step. WWTP C, a treatment plant using aerobic granular sludge (NEREDA®) as an alternative to activated sludge treatment. Water and sludge were collected and analysed for 52 antibiotics from four target antibiotic groups (macrolides, sulfonamides, quinolones, tetracyclines) and four target ARGs (*ermB*, *sul 1*, *sul 2* and *tetW*) and integrase gene class 1 (*intI1*). Despite the high removal percentages (79–88%) of the total load of antibiotics in all WWTPs, some antibiotics were detected in the various effluents. Additional treatment technology (WWTP C) showed antibiotics removal up to 99% (tetracyclines). For ARGs, WWTP C reduced 2.3 log followed by WWTP A with 2.0 log, and WWTP B with 1.3 log. This shows that full-scale WWTP with an additional treatment technology are promising solutions for reducing emissions of antibiotics and ARGs from wastewater treatment plants. However, total removal of the antibiotics and ARGS cannot be achieved for all types of antibiotics and ARGs. In addition, the ARGs were more abundant in the sludge compared to the wastewater effluent suggesting that sludge is an important reservoir representing a source for later

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ARG emissions upon reuse, i.e. as fertilizer in agriculture or as resource for bioplastics or bioflocculants. These aspects require further research.

1. Introduction

Antibiotics inhibit the growth of microorganisms and have been used widely since the 1930s to combat infectious diseases (Gallo et al., 1995; Chee-Sanford et al., 2009). Only a fraction of these antibiotics are completely metabolized within bodies of humans and animals, and 20% to 90% is generally excreted as parent compound or metabolite through urine and feces (Jelic et al., 2015).

As a result, antibiotics are widely present in our domestic sewage waters and enter WWTPs that have only limited capacities to remove these compounds, thus they end up in the environment. Even though antibiotics are detected at low concentrations (ng/L to μg/L scale) in the environment, these antibiotics can persist there for a long time (Kolář et al., 2001). This persistence depends not only on the characteristic of the respective antibiotics, but also on environmental conditions such as oxygen and other electron acceptors and donors, and light. As a result, some antibiotics are readily degraded, while others are not. Their presence in the environment can lead to the emergence and prevalence of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) (Andersson and Hughes, 2012). Antibiotic resistance develops naturally. However, use, misuse and inappropriate antibiotic prescriptions has accelerated the occurrence of ARB and ARGs in the environment (Kraemer et al., 2019), especially in wastewater (Sharma et al., 2016; Karkman et al., 2018; Pazda et al., 2019). WHO has listed antibiotic resistance as one of the world threats since 2014 (WHO, 2014).

Conventional WWTPs are designed to remove high concentrations of total organic carbon, and nutrients such as nitrates, and phosphates (Pronk et al., 2015; de Kreuk et al., 2010) but not specifically designed to remove micropollutants, including antibiotics and ARGs (Novo et al., 2013; Pal et al., 2015). In a conventional WWTP, activated sludge is the most common treatment technology for the biological treatment of wastewater (Samer, 2015). Two tanks are needed for activated sludge; one for aeration of biological reactions and for settling of the sludge.

The removal of antibiotics and ARGs varies (1–2 log) in conventional WWTPs (Pallares-Vega et al., 2019). Some studies showed an increased relative concentration of antibiotics and some ARGs after WWTP treatment (Pärnänen et al., 2019). Others reported a decrease in the prevalence of antibiotics and relative concentrations of ARGs after WWTP treatment (Liao et al., 2016; Pärnänen et al., 2019; Gao et al., 2012; Czekalski et al., 2012; Kulkarni et al., 2017). Even when a significant removal in the wastewater effluent was found, antibiotics were still detected in the receiving (surface) water body, ranging from 2 ng/L to 25 mg/L (Singh et al., 2019).

Therefore, additional treatment technologies may provide a promising contribution to reduce antibiotics and ARGs before effluent is discharged to the environment. Various additional treatment technologies have shown to remove antibiotics and/or ARGs from wastewater, e.g., physical treatment processes (Sun et al., 2019), disinfection (Shen et al., 2020; Khorsandi et al., 2019), advanced oxidation processes (Collivignarelli et al., 2018; Zhuang et al., 2015), as well as aerobic granular sludge (AGS) (Mihciokur and Oguz, 2016).

Activated carbon (AC) is a treatment technology that is based on adsorption and filter material, and can act as a carrier matrix for biomass. It is known to remove dissolved compounds, suspended matters, nitrogen, and phosphates (Hung et al., 2005). AC is a form of an amorphous carbonaceous material showing a high specific surface area (1000 m^2/g) (Tadda et al., 2016) microporous structure, and large pore volume (Choi et al., 2005). Therefore, due to its high adsorptive capacities, AC has shown potential in removing color/odor/taste (Matsui et al., 2015; Huang et al., 2019b), disinfection by-products (Gopal et al., 2007), micropollutants (Kårelid et al., 2017; Choi et al., 2005), antibiotics (Yu et al., 2016; Pachauri et al., 2009) and ARB (Ravasi et al., 2019) and ARGs (Sun et al., 2019). AC was also applied as a post-treatment to solar photo fenton treatment to remove antibiotics from wastewater (Michael et al., 2019).

AGS has been introduced to overcome the drawbacks in activated sludge, (Wilén et al., 2018). AGS is known for the excellent settling ability, simultaneous removal of organic matter and nitrogen, high biomass concentration, and a good ability to withstand the high organic load. AGS contain granules comprised of self-immobilized cells and does not depend on material support for biofilm growth. It retains a large number of microorganisms, at the same time permitting rapid bioconversion of many compounds and improving the performance and stability of the reactor (Bassin, 2018). In addition, AGS is a compact (using only one tank for settling and aeration) and cost-effective wastewater treatment (Nancharaiah and Reddy, 2017). Due to these characteristics, AGS gain more attention to be implemented in the wastewater treatment. Some full-scale AGS have recently been implemented to treat municipal and industrial wastewater (Pronk et al., 2015; van der Roest et al., 2011). As this is a relatively new technology, limited data are available on the removal of antibiotics in full-scale plants (Wang et al., 2019) and ARB&Gs.

We hypothesize that additional treatment technologies have the potential to improve the removal of antibiotics and ARGs at current WWTPs. Most studies that compare different treatment technologies have been carried out at lab-scale (Sousa et al., 2017; Zheng et al., 2017; Karaolia et al., 2018; Guo et al., 2017). However, limited research on antibiotics and ARGs removal has been performed in full-scale WWTPs, and studies so far focused on the comparison between conventional and additional treatment technology, e.g. membrane nanofiltration and reverse osmosis in China (Lan et al., 2019), parallel membrane bioreactors in China (Li et al., 2019) and four separate treatments based on UV irradiation in Spain (Rodríguez-Chueca et al., 2019).

Therefore, this study evaluates the removal of antibiotics and ARGs in full-scale WWTPs, with and without additional treatment technologies. A conventional WWTP operated with activated sludge is chosen as a control, receiving domestic wastewater for comparison purposes. In addition, two full-scale WWTPs with different additional treatment technologies were studies, a 1-STEP® filter (based on AC), and a NEREDA® technology (based on AGS). Grab samples were collected for two months and analysed for 52 antibiotics from 4 groups (macrolides, sulfonamides, quinolones, tetracyclines), 4 ARGs (*ermB*, *sul 1*, *sul 2* and *tetW*) and integrase gene class 1 (*intI1*).

2. Material and methods

2.1. WWTPs

Three Dutch WWTPs were selected to study the removal of antibiotics, and ARGs; one conventional WWTP (WWTP A) and two WWTPs with additional treatment technologies (WWTP B and WWTP C). WWTP A employs conventional steps, such as a primary treatment (grit removal), a reactor with activated sludge, a sludge settling tank, and finally sand filtration.

WWTP B consists of a conventional activated sludge treatment followed by an AC filtration step (1-STEP® filter). The 1-STEP® filter is in operation since August 2012 and consists of a vertical, compact fixed bed activated carbon filter, combining filtration, denitrification, coagulation, flocculation, and adsorption in one single treatment unit (Bechger et al., 2009; Bechger et al., 2013). It is in use as final treatment step (as a tertiary treatment) to improve the nitrogen and phosphorus removal and the final effluent quality meets the requirements of the European Water Framework Directive 2000/60/EC (Council Directive, 1991).

WWTP C employs a NEREDA® technology, which is based on AGS as an alternative to activated sludge (as a secondary treatment). The NEREDA® technology is in operation since 2011 and developed in the Netherlands (van der Roest et al., 2011). The granules have a robust structure of aerobic granular biomass, with dense, compact, large particles (0.2–2 mm) with a high specific gravity (van der Roest et al., 2011). The granules consist of different microorganisms, including phosphate accumulating organisms, nitrifiers, denitrifiers, and glycogen accumulating organisms, which allows several processes simultaneously (Giesen et al., 2013). Due to the growth in dense grains, there are different oxygen levels within the grain, allowing different organisms to be active.

The selected treatment technologies have been reported in different parameters such as chemical oxygen demand (COD), phosphorus in the 1-STEP® filter (Scherrenberg et al., 2012) aerobic granulation (Li et al., 2014; Pronk et al., 2015), and microorganisms analysis (Liu et al., 2016; Świątczak and Cydzik-Kwiatkowska, 2018) in AGS.

Process flow diagrams and sampling points of each WWTP are shown in Fig. 1. WWTP A has the capacity to treat 22,000 person equivalents (p.e.) with an average treated volume of 2860 m^3/d, WWTP B treats 200,000 p.e. with an average treated volume of 26,000 m^3/d and WWTP C is designed for 59,000 p.e and an average treated volume of 8000 m^3/d. Details for the three WWTPs are shown in Table S1.

2.2. Sample collection and pre-treatment

Collection of grab samples (water and sludge samples) of the WWTPs was carried out during the winter season (February and March 2017) at 3 sampling points (WWTP A) and 4 sampling points (WWTP B and WWTP C), which are shown in Fig. 1. The sampling was performed between 9:00–12:00 (after the daily morning peak hour), two times each month in duplicate at all sampling points. Average air temperatures during sampling were between 8 °C and 11 °C (data from the WWTPs), respectively.

Water samples ($n = 12$ per WWTP A, $n = 16$ per WWTP B and C) were taken at the outfall of the tank. A bucket was dipped into a tank for water sample collection, and the samples were stored in 1 L sterile glass bottles. On the sampling day, the water was measured for pH, water temperature and dissolved oxygen (DO) by using an HQ40D portable meter (Hach, Germany). The water samples were divided into two parts at different storage: for physicochemical and DNA filtration, the samples were stored at 4 °C and processed within 48 h. For antibiotics analyses, the samples were stored at −20 °C until processed. All sludge samples ($n = 8$ per WWTP) were collected in duplicate. For sludge collection, a bucket was dipped at the influent and at the sludge return flow (WWTP A) or via a sludge tap (WWTP B and C). The sludge samples stored in a 50 mL tube at −20 °C before DNA extraction.

2.3. Chemical analysis

2.3.1. Physicochemical parameters

During sampling, temperature, pH, and DO were measured using a probe (Hach, USA). Conductivity (in μS/cm) was measured in the laboratory by a conductivity probe (Hach, USA). Other analyses, total phosphate (TP), nitrogen (ammonium (NH_4-N), nitrate (NO_3-N) nitrite (NO_2-N)), and COD, were determined using Hach kits in laboratory (USA; LCK 349, LCK 304, LCK 339, LCK 342, and LCK 1414 respectively). Total suspended solids (TSS) analyses were performed by filtering 200 mL of raw samples through a glass filter (Whatman, England) and placed at 105 °C overnight. After weighing, the same samples were incubated at 550 °C for 2 h for volatile suspended solids (VSS). All procedures of analyzing nutrients and COD were performed according to the international standards of the American Public Health Association (APHA, 2005).

2.3.2. Solid phase extraction (SPE) and LC-MS/MS

SPE purification and concentration, and LC-MS/MS procedures were performed as previously described by Sabri et al. (2020). This analysis was performed for only water samples and not for sludge samples. The target antibiotic groups (macrolides, sulfonamides, quinolones, tetracyclines) and their chemical characteristics are shown in Table S3. No

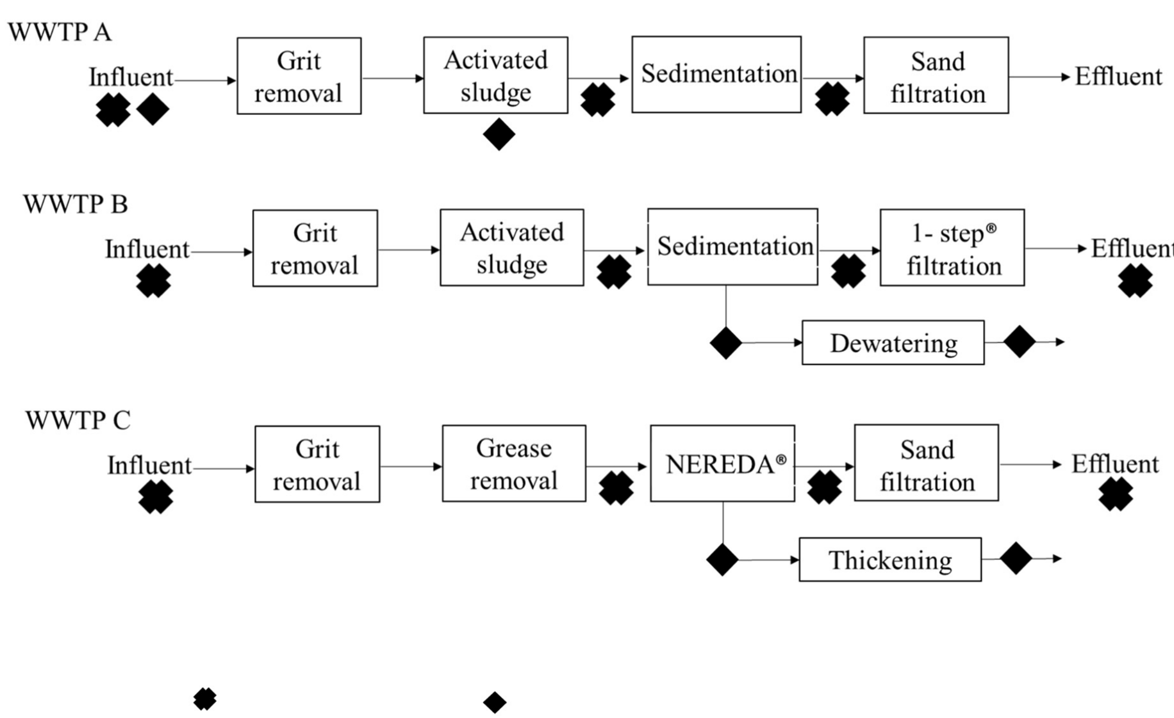


Fig. 1. Schematic flow diagram of the WWTPs, with the sampling points for three wastewater treatment plants. ✖ Water sample point. ◆ Sludge sample point.

significant difference was found after some samples at influent and effluent were tested for triplicate (data not shown). Therefore, the analyses were performed in a single measurement. In short, 10 mL of water sample of each sampling point was processed by using a Strata X Polymeric Reversed Phase SPE column (Phenomenex, USA). First, the SPE column was equilibrated by washing with 5 mL MeOH, followed by 5 mL EDTA-McIlvain buffer 0.1 M; pH 4.0. Then, the sample was loaded onto the SPE column and washed with 5 mL Milli-Q water. After this, the sample was eluted with 5 mL MeOH and dried under a nitrogen evaporator with a temperature of 40 °C. Finally, the dried sample extracts were dissolved in 500 μl MeOH: Milli-Q water (20:80). The extracts were analysed by LC-MS/MS (Sciex, USA, QTRAP 6500), equipped with a BEH C18 Waters Acquity column (Waters Corporation, USA, 100* 2.1 mm), at a column temperature of 40 °C. Data were analysed by using MultiQuant software (Sciex, version 3.0.2). The limit of detection ranged from 5 to >1000 ng/L (depending on the antibiotics). Details for each compound is presented in Table S4. For quality control, a known amount (100 μg/L) and internal standard of each compound were spiked to every sample. The recovery percentage of the spiked compound in the sample ranged from 70 to 120%.

2.4. DNA extraction and quantitative PCR (qPCR)

Molecular analyses were performed as previously described by Sabri et al. (2020). Briefly, the DNA extraction of 100 mL samples was filtered onto a membrane filter with a 0.2 μm pore size (Millipore, USA) and stored at −20 °C until further use. DNA was extracted by using the PowerWater kit (MoBio Laboratories, USA) for water samples and PowerSoil kit (MoBio Laboratories, USA) for sludge samples, following the manufacturer's protocols. Four ARGs of interest were selected: *ermB*, *sul1*, *sul2* and *tetW*, as well as 16S rRNA and *intI1*. These ARGs were chosen based on antibiotics that have been frequently used and detected in water (Ye et al., 2007). Each ARG corresponding to the respective antibiotic such as macrolides are corresponding to *ermB*, sulfonamides are corresponding to *sul1* and *sul2*, and tetracyclines are corresponding to *tetW*. Meanwhile, *intI1* was proposed by Gillings et al. (2015) as a good marker for environmental pollution since it is commonly linked to genes conferring resistance to antibiotics and rapid change in response to environmental pressures.

A PCR master mix was prepared, depending on the gene of interest. The master mix contained, as described in detail in Sabri et al. (2020), water, precision blue (Biorad, USA), a probe (if needed), the forward and reverse primer (Eurogentec, Belgium), and super mix (SYBR-Green or IQ (Bio-Rad, USA)). The primers are presented in Table S5. Negative control and a calibration curve were also included. The data were processed by Bio-Rad-CFX manager (Version 3.1).

2.5. Calculations and statistical analysis

Linear regression, *t*-test and correlation analyses were conducted to examine the influence of specific parameters on the performance of the WWTPs in removing antibiotics and ARGs. All statistical analyses were performed on the R platform (Version 3.5.2). The difference was considered statistically significant at $p < 0.05$.

The removal percentage of antibiotics and ARGs was calculated by comparing the total concentrations between influent and effluent, or before and after the respective additional treatment technology. The calculations for antibiotics (Eq. (1)) and ARGs (Eq. (2)) were used as below:

$$Removal\ percentage = \left(\frac{Influent\ concentration}{Effluent\ concentration}\right) \times 100\% \tag{1}$$

$$Log\ removal = Log_{ARGs} before\ treatment\ technology - Log_{ARGs}\ after\ treatment\ technology \tag{2}$$

3. Results and discussions

3.1. Performance of the studied WWTPs

The general performance of the studied WWTPs was identified by measuring removal of suspended solids, COD and nutrients. All measured parameters (DO, pH, water temperature, conductivity, TP, NH_4-N, NO_3-N, NO_2-N, COD, TSS, and VSS) met the effluent regulatory targets of the European Water Framework Directive (Council Directive, 1991). The increase of NO_3-N is the result of production through nitrification and associated with the decrease of NH_4-N. The removal percentages are summarized in Table 1. Detailed data for each parameter is given in Table S2(a) and (b). More information is given in the Text S1.

Dissolved organic compounds, nutrients, and WWTP operating parameters can affect the concentration of antibiotics and ARGs in a WWTP and its effluent. In our study, we found a good correlation (Pearson correlation, $r = 0.4$ to 0.8, $p < 0.05$) between NH_4-N and all antibiotics groups (only in February) and ARGs (in both sampling months) (Fig. S1). Huang et al. (2019c) observed that *sul2* was positively correlated with COD and NH_4-N, while other ARGs (*ermB*, *intI1*, *sul1* and *sul2*) were positively correlated with pH. However, in this study we did not observe significant correlation between ARGs and water temperature, DO and COD. Literature also described that WWTP operating parameters such as hydraulic retention time could enhance the antibiotics removal ability, e.g. a better removal of antibiotics was observed at a higher longer hydraulic retention time with AGS (Liao et al., 2019). We did not study the correlation between dissolved organic compounds, nutrients, and WWTP operating parameters in-depth, due to the limited number of WWTPs involved. Furthermore, a full-scale WWTP is also affected by unpredictable conditions such as sudden heavy rain, unpredictable antibiotic consumption at certain days that affect the removal performance of a WWTP. Therefore, this warrants more research to study this further.

3.2. Occurrence and distribution of antibiotics in three WWTPs

The presence of antibiotics in the influent and effluent of the WWTPs in conventional and in additional treatment technologies WWTPs was investigated. In general, 8 out of 52 antibiotics were detected in the influent of all 3 WWTPs in our sampling campaigns of February and March 2017 (Fig. 2). These antibiotics are norfloxacin (NOR), ciprofloxacin (CIP), levofloxacin/ofloxacin (LEV), sulfamethoxazole (SMX), trimethoprim (TRI), sulfadiazine (SF), sulfapyridine (SP) and tetracycline (TET). The antibiotics chlortetracycline (CTC), doxycycline (DC), flumequine (FLU), oxytetracycline (OTC), and lincomycin (LIN) were found occasionally in the WWTPs. The other analysed antibiotics (Table S4) were all below the quantification limit.

The total antibiotics concentration ranged from 3000 ng/L to 6000 ng/L in the influent of all WWTPs for both months. The quinolones were the most abundant antibiotics found in all influents, with an average concentration of 2300 ng/L (WWTP A), 1900 ng/L (WWTP B) and 1600 ng/L (WWTP C). Two antibiotics showed a high variation between the sampling moments in two months; Norfloxacin was measured 18 times lower in February (80 ng/L) compared to March (1400 ng/L) in WWTP A, and doxycycline was measured 20 times higher in February (80 ng/L) compared to March (1500 ng/L) in WWTP C.

Table 1
Removal (%) for selected parameters in three studied WWTPs. Data are mean value ± standard deviation ($n = 12$ per WWTP A, $n = 16$ per WWTP B and C).

	COD (mg/L)	NH_4-N (mg/L)	NO_3-N (mg/L)	TP (mg/L)	TSS (g/L)	VSS (g/L)
WWTP A	74 ± 31	97 ± 0	−511 ± 359	96 ± 0	100 ± 0	99 ± 3
WWTP B	73 ± 3	100 ± 0	−5 ± 5	99 ± 1	100 ± 0	99 ± 1
WWTP C	55 ± 27	98 ± 2	−525 ± 139	97 ± 1	97 ± 3	95 ± 4

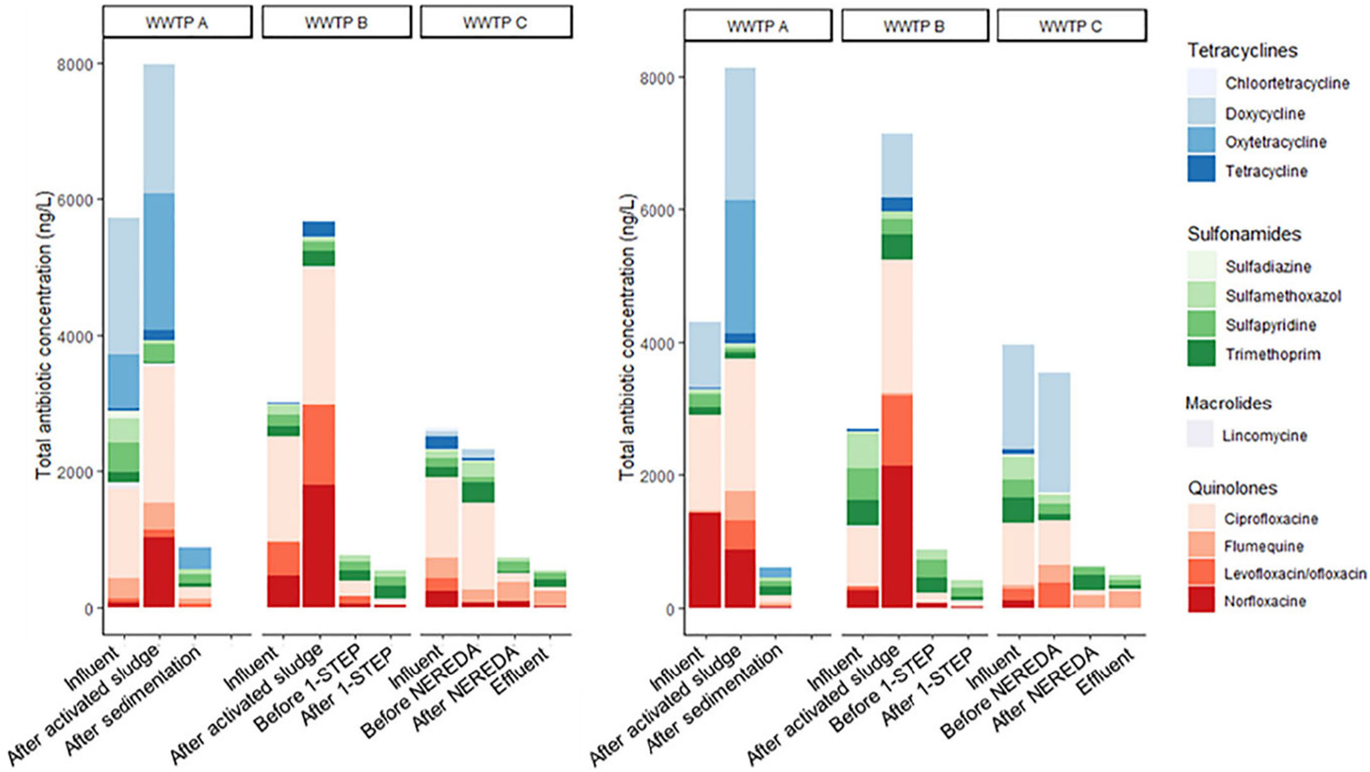


Fig. 2. Antibiotic concentrations (ng/L) in the water of the WWTPs in (a) February 2017 (b) March 2017.

An average of 3000 ng/L antibiotics was detected in the influent of all WWTPs, with the quinolones as most abundant antibiotics. This is in the same range as reported in winter time from other studies, e.g., 1000 ng/L (Huang et al., 2019a) to 3300 ng/L (Zheng et al., 2019) in Chinese wastewater influent, and can reach up to 6000 ng/L in Czech Republic (Golovko et al., 2014). It is known that more antibiotics are prescribed during winter periods, as a result of increased respiratory tract infections (Ferech et al., 2006; Werner et al., 2011). This is also in line with a study of Diwan et al. (2013) who showed that higher concentrations of quinolones were measured in the winter. Van Boeckel et al. (2014) reported that the consumption of antibiotics is highest between January and March in the northern hemisphere. For example, antibiotics prescription in the USA is 24.5% higher in the winter than in the summer (Suda et al., 2014), and 32% in Israel (Dagan et al., 2008). This high consumption of quinolones in winter also is shown in various countries in Europe (Adriaenssens et al., 2011). This elevated use in winter might explain the relatively high concentrations detected in the wastewater influent since our data were collected in the winter with maximum levels of antibiotics. As a consequence, this made it possible to study their removal in different treatments in a WWTP.

Along the WWTP treatments, a similar decreasing trend from influent to effluent was observed for all WWTPs in February and March. The only exception was the elevated concentration in the activated sludge tank in WWTP A and WWTP B, where higher concentrations of quinolones (LEV, CIP, and NOR) and of oxytetracycline were detected.

For the WWTP effluent concentrations, 5 of the 52 analysed antibiotics were detected in all WWTP effluents. These detected antibiotics are CIP, SMX, SP, SF, and TRI in which sulfonamides (SMX, SF, SP) and TRI were consistently detected in all WWTPs for both sampling months. Average concentrations of the sum of these sulfonamides and TRI were 230 ng/L (WWTP A), 370 ng/L (WWTP B), and 260 ng/L (WWTP C). A few antibiotics (TET, LEV, NOR, FLU, LIN) were found occasionally in only one of the WWTPs or in only one sampling campaign. The removal efficiencies of the total of antibiotics were 83–85% (WWTP A), 82–84% (WWTP B), and 82–88% (WWTP C), based on concentrations of the detected antibiotics.

Sulfonamides and quinolones were detected in the effluent of all studied WWTPs. Poor removal of sulfonamides in WWTPs was also observed by Marx et al. (2015) and Bengtsson-Palme and Larsson (2016). Sulfonamides group are not easy to degrade, have a low potential to volatilize, are very hydrophilic (K_{ow} <1) and are highly mobile in the sand and groundwater infiltration systems (log sorption-distribution coefficients (K_d) < 2) (Wegst-Uhrich et al., 2014; Kolpin et al., 2002). Therefore, sulfonamides are easily transferred into the aquatic environment, which can explain their high reported occurrence in the water phase (Xu et al., 2007; Jiang et al., 2018).

3.3. *Antibiotics removal within conventional and additional treatment technologies*

The efficiency of the removal of antibiotics in the WWTPs with additional treatment technologies (WWTP B and C) and without (WWTP A) was evaluated by comparing the water before and after the individual treatment processes. All the treatments in the respective WWTPs showed removal in the total load of antibiotics, regardless of conventional or additional treatment technology, although differences within the treatment steps in WWTPs were observed.

First, two conventional treatment steps were evaluated; activated sludge in WWTP A and WWTP B were compared. Concentrations of some antibiotics were significantly increased in concentration in the water phase after the activated sludge process, while others decreased in concentration ($p < 0.05$). For example, WWTP A showed a 177% increase of tetracyclines and 66% of quinolones, whereas 55% of sulfonamides and 74% of macrolides were removed. In WWTP B, the tetracyclines increased with 1650%, quinolones with 200%, whereas 29% of sulfonamides were removed. No removal of macrolides was observed.

In the activated sludge treatment steps of WWTP A and WWTP B, the concentration of quinolones and tetracyclines increased. The increase of both antibiotics in water are most likely due to quinolones and tetracylines being released from hydrolyzed organic waste fractions that enter the activated sludge and water phase with the influent. This is followed by a redistribution over the water and sludge phase by sorption processes, as also shown by another study Jia et al. (2012). We observed that the TSS at this treatment step was higher than in the other sampling points within the WWTP (Table S2), indicating that the amount of antibiotics attached to the particles in the wastewater could have been higher ($p < 0.05$). This is supported by the high correlation between TSS content and antibiotic concentration (Pearson correlation 0.95 in February and 0.96 in March). This indicates that the higher the particle concentration in the wastewater, the higher concentration of the antibiotics. CIP and TET are multivalent zwitterions with strong dipole and exhibited significant sorption capacity onto suspended solids and sludge in previous research (Polesel et al., 2015). Quinolone sorption is high ($\log K_d > 3$) and it adsorbs to sludge surfaces through electrostatic interactions (Golet et al., 2003). Concentrations up to 18.4 mg/kg have been measured in sludge (Jia et al., 2012) and up to 2.4 mg/kg of dry weight (Golet et al., 2003).

Other treatment steps of conventional treatment technology, such as the settling tank and subsequent sand filtration, were present in WWTP A and WWTP C. In the sedimentation tank, average removal fractions were respectively 94% for tetracyclines, 5% for sulphonamides and 94% for quinolones. In the sand filtration, the average removal of tetracyclines was 100%, 20% for sulphonamides, and 16% for quinolones. Macrolide concentrations increased after both the sedimentation tank and sand filtration.

The main removal mechanism in the sedimentation tank is the sorption of antibiotics on the colloidal matter, followed by removal in the coagulation/flocculation/sedimentation process (Adams et al., 2002; Shah, 2008). Xing and Sun (2009) showed that this resulted in 87% antibiotics removal after sedimentation and suggested this as an effective removal step to treat wastewater of antibiotics and pharmaceutical manufacturers. Low removal percentage in the sand filtration in WWTP C, which only removed 0.3% (quinolones) to 0.4% (sulfonamides), showed that antibiotics are largely unaffected by sand filtration. This is consistent with Rooklidge (2004), who also indicated that sand filtration removed <4% of sulfonamide and demonstrated limited mobility of lincomycin, trimethoprim, and tylosin within the sand filter. This is indicating that sand filter possesses low sorption properties and has a high persistence of microorganisms not adapted to biodegradation of these specific antibiotics (Ternes et al., 2002).

For the additional treatment technologies studied, the 1-STEP® filter removed 19% of sulfonamides, and 65% of quinolones, and produced concentrations of macrolides increased with 113%. No tetracyclines were detected before 1-STEP®. The present activated carbon removes antibiotics by physico-chemical adsorption onto the activated carbon and by the biofilm on the activated carbon (Ahmed, 2017; Östman et al., 2019). Activated carbon removes effectively hydrophobic compounds with a $\log K_{ow} > 4$, for example tetracyclines, and quinolones (Grandclément et al., 2017; NCBI, 2018; Raevsky et al., 2009). The removal observed in this study is lower than reported in other studies, although those were lab-scale studies (Choi et al., 2008; Zhang et al., 2016b). In a full-scale WWTP, the lifetime of the AC, the saturation level of the AC with other organic compounds, the water flow, hydraulic retention times, and the concentration of antibiotics in the influent will influence the removal percentage.

Meanwhile, NEREDA® removed 100% of tetracyclines, 36% of sulfonamides, 84% of macrolides, and 74% of quinolones. No increase of antibiotics was observed within the treatment, indicating little accumulation and consequent desorption within the treatment. This can be explained by the relatively high microbial activity of aerobic granules (Wang et al., 2019). The bacteria produce compact granules compared to flocs in conventional activated sludge and these granules settle faster in the wastewater (Forster, 2019). The aerobic granules are formed by bacteria that produce extracellular polymeric substances (EPS) and are stabilized by slow growing microorganisms (Świątczak and Cydzik-Kwiatkowska, 2018). EPS influence the surface properties of biomass and increase the sorption of organic pollutants (Kang et al., 2018; Schmidt et al., 2012). Xu et al. (2013) reported that protein in EPS interact and bind with sulfamethazine by hydrophobic interaction, contributed to the stability of the complex, and improve the efficient removal of sulfamethazine by harvesting the sludge EPS. This is also supported by Pi et al. (2019), and these authors reported that the chemisorption and hydrophobic interaction of tryptophan and tyrosine during the binding process to EPS and sulfonamides played an important role in adsorption capacity. The removal percentage in our NEREDA® technology is in the same range as other studies of AGS (Kang et al., 2018; Wang et al., 2019; Wang et al., 2018). Not only AGS has a promising step to remove antibiotics from wastewater, but also anaerobic granular and flocculent sludge showed a high removal of micropollutants (Butkovskyi et al., 2017).

When we compare 1-STEP® filter and NEREDA®, 1-STEP® filter removed 30% - 52%, meanwhile NEREDA® removed from 68% - 82%. The location of the treatment step may contribute to the removal difference. However, we did not observe any significant difference between the locations ($p > 0.05$). The 1-STEP® filter is located after activated sludge and acts as a polishing step of the treatment. This contributes to a low load of antibiotics in the system, when compared to NEREDA® which is located after the primary treatment. This indicates that the location of the treatment technology (either in the secondary treatment or as a polishing treatment) did not affect the removal percentage of antibiotics. The difference of the antibiotic concentration can be the results of other factors, such as concentration of the influent and WWTP operating parameters.

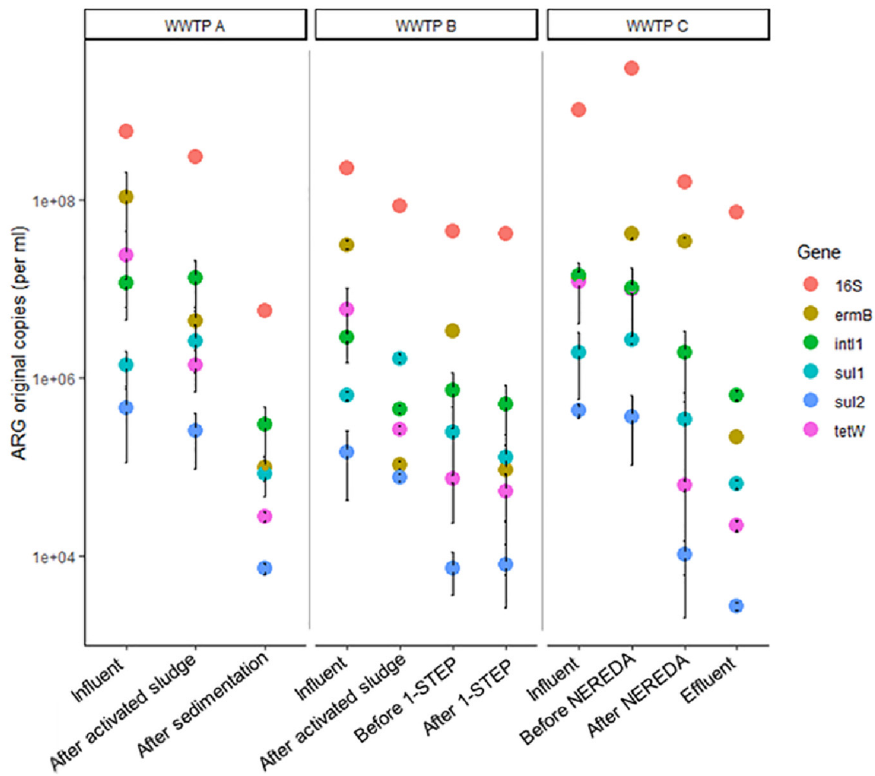
3.4. Occurrence and distribution of ARGs over the treatment phases in the three WWTPs

In this study, the 16S rRNA gene, the *intI1* gene, and four ARGs (*ermB*, *sul1*, *sul2*, and *tetW*) were detected at all sampling points in all WWTPs (Fig. 3 and Table S7), in water and in sludge samples. This illustrates the prevalence of ARGs along the phases of WWTPs in water and sludge.

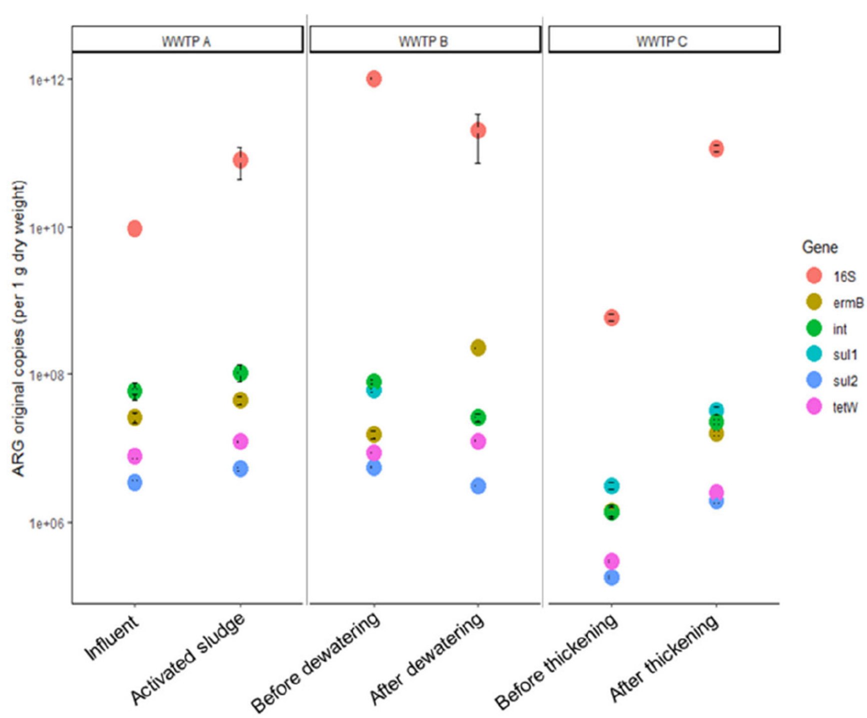
Generally, the absolute abundance of ARGs in the influent ranged from 4.6×10^5 to 1.1×10^8 copies/mL in WWTP A, 1.5×10^5 to 3.1×10^7 copies/mL in WWTP B and 4.3×10^5 to 1.4×10^7 copies/mL in WWTP C respectively. The most abundant ARG in the influent of all WWTPs was *ermB* with a range of 3.7×10^7 to 2.5×10^8 copies/mL in WWTP A, 2.1×10^5 to 7.9×10^7 copies/mL in WWTP B and 5.6×10^7 to 2.3×10^8 copies/mL in WWTP C. The second most abundant ARG was *intI1*, followed by *tetW*, *sul1*, and *sul2*. The relative abundance of the ARGs to the 16 s rRNA gene shows a decreasing trend for all ARGs, except for *intI1*, which is stable in the treatment transect from the influent to the effluent (Fig. S2).

In sludge samples, ARGs were detected before, and after dewatering at 3.5×10^6 to 8.3×10^{10} copies/g dry weight in WWTP A, 3.1×10^6 to 1.0×10^{12} copies/g dry weight in WWTP B, and 1.8×10^5 to 1.2×10^{11} copies/dry weight in WWTP C. *Sul1* and *intI1* were the most abundant in each treatment unit of all WWTPs, followed by *ermB*, *tetW* and *sul2*. ARGs concentration increased in WWTP A and C when compared before and after the dewatering system.

Unlike the antibiotics, the total amount of ARGs did not accumulate in the water phase in the activated sludge tank in WWTP A. We observed an average removal of 0.44 log from the water phase and -in parallel- we observed a slight increase (0.34 log) in the sludge phase. This was expected, as the sludge or sediments are known as hot spots of high bacterial density, activities and biofilm formations (Heß et al., 2018). This accumulated ARGs from the water phase to attach to the sludge since the majority of bacteria are known to live in association with surfaces (Davey and O'Toole, 2000). It has been shown that reduction of microbial biomass might correlate with the reduction of ARGs in the water phase and lead to an equivalent accumulation in the sludge



(a)



(b)

Fig. 3. Concentrations of antibiotic resistance genes in the (a) water and (b) sludge at different sampling points in three wastewater treatment plants. Error bars indicate the standard deviation of the respective gene data set (duplicate).

phase (Zhang et al., 2018). The precise mechanism behind this is not fully clear. As a result, ARGs will accumulate in the sludge and also in the sediment and soil (Chen et al., 2019; Peng et al., 2018).

Along the WWTP, all ARGs except *sul1* and *intI1* showed a decreasing trend. *Sul1* and *intI1* increased slightly but not significantly after some steps. *Sul1* increased with 0.26 ± 0.20 log in WWTP A after grit removal and activated sludge treatment, and 0.12 ± 0.22 log after activated sludge in WWTP B. *intI1* increased 0.20 ± 0.14 log at the activated sludge in WWTP A.

ARGs were present in the effluent ranging from 2.7×10^3 (*sul2*) to 6.3×10^5 (*intI1*). *intI1* was the most abundant gene in the effluents of all WWTPs. This is followed by *ermB*, *sul1*, *tetW*, and *sul2* in WWTP A, *sul1*, *ermB*, *tetW*, and *sul2* in WWTP B and *ermB*, *sul1*, *tetW* and *sul2* in WWTP C. Since a major amount of the ARGs ends up in the sludge phase, lower ARGs concentrations were detected at the effluent as compared to the influent. All WWTPs significantly ($P < 0.05$) reduced the total ARGs (copies/mL) from the influent to the effluent. A similar range of reduction about 1–3 log removal was observed in China (Lee et al., 2017; Chen and Zhang, 2013), 2.4 to 4.6 log removal in Michigan (Munir et al., 2011) and <2 log removal in Italy (Fiorentino et al., 2019). Our study showed that *ermB* was the most removed gene in all three WWTPs, as also reported by Rafraf et al. (2016). *ErmB* genes have been found mainly in gram-positive bacteria (Gupta et al., 2003), and it has been shown before that gram-positive bacteria were removed from influent to effluent (Forster et al., 2002).

In the effluent, *intI1* was the most detected gene in all three WWTPs. This was also observed by Narciso-da-Rocha et al. (2014), who suggested that *intI1* is stable in wastewater. Furthermore, ARGs and the *intI1* gene were not efficiently reduced during wastewater treatment (Rafraf et al., 2016). We also observed low log removal of *sul1* (0.60–1.63 log) in the three WWTPs, and a similar log removal (0.9–1.9 log) was observed by Chen and Zhang (2013). The limited removal of both *sul1* and *intI1* ($r = 0.81$) and *sul2* and *intI* ($r = 0.93$) were strongly correlated ($p < 0.05$), as shown by others, as *sul1* is one of the backbone genes of the 39-conserved segments in *intI1* (Muziasari et al., 2014; Partridge et al., 2002).

Through the sorption of ARG-carrying bacteria, sludge has the potential to act as ARGs reservoir and mitigate the spread of antibiotic resistance in the environment through effluents (Munir et al., 2011). This situation can also increase the exposure risks, especially in countries applying WWTP sludge for agricultural purposes, or producing and using products made from WWTP sludge materials.

In our study, the antibiotics and ARGs showed different patterns of reduction in the investigated treatments. In WWTP A, the total amount of antibiotics increased after the activated sludge treatment, while the concentration of ARGs decreased. The ARGs were removed in all treatment steps in the WWTP. There are inconsistencies in the literature in determining the correlation between antibiotics and ARGs. Some studies reported there is a correlation between presence and removal of antibiotics and ARGs (Rodriguez-Mozaz et al., 2015; Wu et al., 2010), and some studies showed no or partial correlations (Gao et al., 2012; Xu et al., 2015). In this study, we did not find such correlations either. This is maybe due to the different environments and pollution levels associated to the three different full-scale wastewater treatment systems. Therefore, further and more extensive studies for a multitude of full-scale WWTPs should be performed in order to provide a better insight into the absence or presence of generic correlations between the removal from effluents and the accumulations into sludges of antibiotics, ARB, and ARGs.

Overall, all WWTPs reduced ARGs significantly ($P < 0.05$), with respectively 2.0, 1.3, and 2.3 log ARGs for WWTP A, B, and C (Fig. 4 and Table S8). The highest removal was found for *ermB*, respectively 2.92, 2.22, and 3.11 log for WWTP A, B, and C. Finally, *sul1* and *intI1* were least removed in all WWTPs, with <1.4 log removal.

3.5. ARGs removal in conventional and additional treatment technologies

The efficiency of conventional and additional treatment technologies in removing ARGs was evaluated by comparing their presence in the water and sludge at different stages. First, two conventional treatments were evaluated, activated sludge in WWTP A and WWTP B.

Unlike antibiotics, ARGs did decrease significantly after the activated sludge process. In WWTP A, all ARGs decreased (except *sul1* and *intI1*),

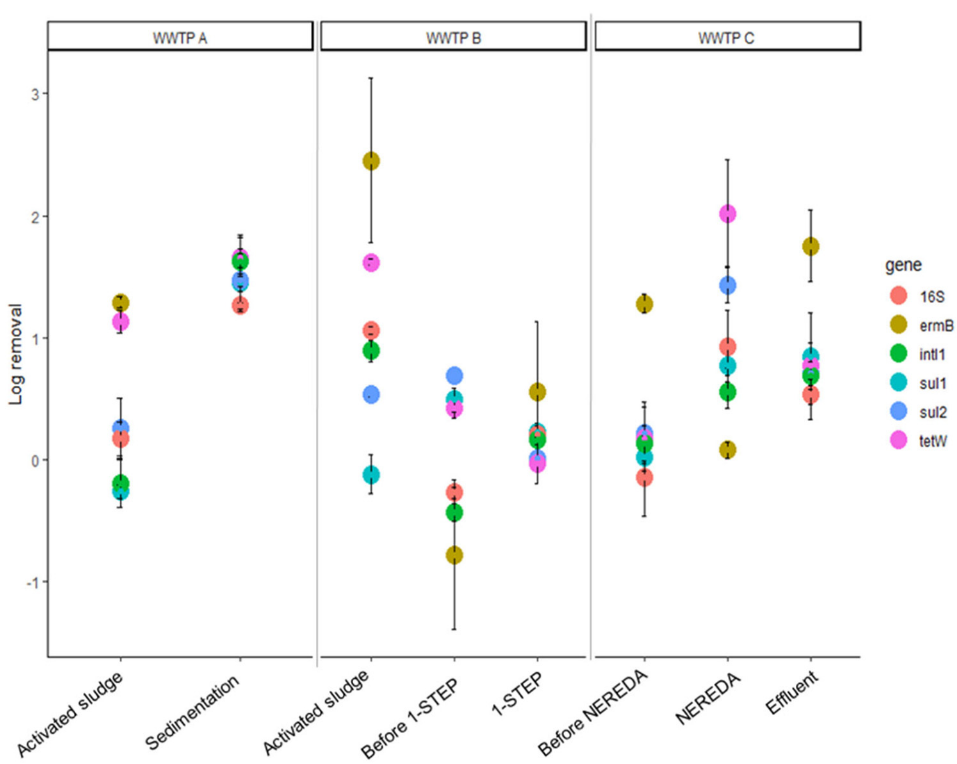


Fig. 4. Log removal of ARGs in the water at different sampling points in the three studied WWTPs. Error bars indicate the standard error of the respective gene data set (duplicate).

ranging from 0.26 log (*sul2*) to 1.29 log (*ermB*). *Sul1* increased 0.26 log while *intI1* increased 0.20 log. In WWTP B, conventional treatment removed 0.53 log *sul2* to 2.45 *ermB*, whereas *sul1* increased 0.12 log.

In the sedimentation tank, the average removal for *ermB* was 1.63 log, 1.45 log for *sul1*, 1.47 log for *sul2*, 1.66 log for *tetW*, and 1.26 log for *intI1*. For sand filtration, average removal for *ermB* was 1.75 log, 0.84 log for *sul1*, 0.71 log for *sul2*, 0.77 log for *tetW*, and 0.53 log for *intI1*. Interestingly, the sludge settling (sedimentation) tank of WWTP A showed the highest log removal in the water phase among the studied treatments. This implies that the sedimented sludge contains a large amount of the ARGs, as also observed by others (Nnadozie et al., 2017; Lee et al., 2017; Su et al., 2018). The final treatment in WWTP A, the sand filtration, also decreased the concentrations of ARGs. Similar removal in conventional WWTPs was shown by Hu et al. (2018), e.g. *sul1*, *sul2*, and *intI1* were removed in the flocculation, sedimentation, and sand filtration tank.

The 1-STEP®-filter removed −0.03 log (*tetW*) to 0.55 log (*ermB*). Overall, 1-STEP® showed the least removal of ARGs. Its activated carbon filter is known for removing organic contaminants, natural organic matter, humic and fulvic acids, and biodegradable compounds. However, poor removal in this study indicated that the ARGs did not adsorb onto the activated carbon may be due to the majority of genes being present in viable bacterial and non-adhering cells, that were not removed from the water phase during filter passage.

The additional treatment technology NEREDA® removed 0.08 log (*ermB*) to 2.02 log (*tetW*) and showed the second highest log removal of ARGs. The granules in the NEREDA® retain organic waste fractions and bacteria in close proximity to each other, thus allowing interactions to occur, including cell-cell communication, and the formation of synergistic microbial consortia (Flemming and Wingender, 2010). Furthermore, the excellent settling properties result in high biomass concentrations (Liu et al., 2003). These properties result in the accumulation of the ARGs within the NEREDA® granules, those granules sink at the bottom and reduce the concentration ARGs in the water. The capture mechanism of ARGs either in solution, bound to suspended solids, or as present of in free bacterial cells by NEREDA granules, is yet to be defined.

The total ARGs in our study were increased in the sludge with an average of 1.26 log in WWTP C. The NEREDA® granules consist of EPS-producing bacteria such as bacteria belonging to the order Xanthomonadales, Sphingomonadales, and family of Rhizobiales (Hyphomicrobiaceae) (Świątczak and Cydzik-Kwiatkowska, 2018). EPS also has the potential to control the lateral transfer of ARGs. This may result in an accumulation of ARGs and indicates that sludge can represent a sink for resistant bacteria and might become an important reservoir for the ARGs (Zhang et al., 2016a).

This study, however, is subject to two critical points; the sampling method (grab sampling) and the limited duration of the sampling campaign (two months). Grab sampling may help in determining the presence of the compounds of interests; however, it captures the concentration of antibiotics and ARGs at a specific time. Furthermore, the data only represent two months in winter, and the result might differ in different seasons throughout the year. However, our approach provides data and insights on the performance of WWTPs with additional treatment technologies (in this study, 1-STEP® and NEREDA®) in removing antibiotics and ARGs. Future research could, for instance, perform studies during a longer time (e.g., 1 year) by using composite sampling. Such research could contribute to better understanding the performance of WWTPs with additional treatment technology over time. Improvement or upgrading of the treatment technology can then be more specifically proposed. Furthermore, only 1 WWTP with advanced treatment options per type of treatment was available for this study, basically because the number of full-scale installations with these additional treatments in the Netherlands is limited, due to their innovative character. As a result, the results might be affected by local sewage parameters. Results from additional WWTPs with similar treatments are therefore needed.

3.6. Implications on public health, water industry, and regulations

Clean water as a source for drinking water is increasingly becoming limited due to climate change, urbanization, and growing populations in the world. Therefore, wastewater reuse is considered as an alternative to tackle this problem (Angelakis et al., 2018). However, the increasing presence of antibiotics, ARB, and their associated ARGs in water are of concern (Hong et al., 2013). Water pollution has been listed as one of the top three concerns in water industry, together with climate change and political instability, from a survey conducted by American Water Works Association (AWWA, 2019). However, there are currently no legal regulations or guidelines that define the permitted levels of antibiotics or antibiotic resistance determinants that are allowed into the environment (Pazda et al., 2019).

This study shows that antibiotics and ARGs are present in WWTP effluent, even with additional treatment technologies. Such technologies can induce the mitigation of antibiotic and ARGs emissions to a limited extent. Here, we show that the removal efficiency of additional activated carbon and AGS differs. Therefore, techniques for advanced treatment should be chosen carefully, depending on the micropollutants targeted in a specific situation. For example, AC is not recommended when there is recreational water downstream and ARGs removal is needed. If limited human exposure to ARG is intended, AC only modestly increases ARG removal according to our results, and is thus insufficient. The wastewater macro- and micropollutants (antibiotics and ARGs included) have a high impact on public health if the removal is insufficient and discharged effluents are directly or indirectly reused water for irrigation, washing and drinking water preparation (Helmecke et al., 2020).

4. Conclusion

In this study, the removal of antibiotics and ARGs were studied in water and sludge of three WWTPs with (1-STEP® filter and NEREDA®) and without additional treatment technologies. Total concentrations of 3000 ng/L antibiotics were found in the influent and decreased over the different treatment steps to <1000 ng/L in the effluent. Tetracyclines and quinolones concentrations were elevated in the water after the activated sludge treatment step. This shows that these compounds were able to adsorb and desorb in the sludge, with the activated sludge acting as a reservoir for quinolones. Generally, good removal (79–88%) of total antibiotics were observed at all WWTPs. However, sulfonamides and quinolones were still present in the effluent in all three WWTPs, with or without additional treatment technologies.

All WWTPs showed 1–2 log removal for the analysed ARGs from influent to effluent. Of the measured ARGs, *ermB* was most abundant in the influent, and the most removed ARG in the three WWTPs. WWTPs with or without additional treatment technologies were able to reduce antibiotics with a similar efficiency, although ARGs were best removed in NEREDA®, followed by conventional treatment. This is the first study of removing antibiotics and ARGs in NEREDA®. The 1-STEP® filter decreased the concentrations of ARG with up to 0.5 log extra on top of the reduction in the conventional part of the plant. When looking at specific treatments, the sedimentation tank showed the highest log removal of ARGs. In the activated sludge, a relatively higher concentration of ARGs was detected compared to other treatment steps, suggesting that sludge is an important reservoir and transmission point for the ARGs. This study demonstrates that in most cases, WWTP with additional treatment technologies have the potential to provide a higher removal of both antibiotics and ARGs compared to conventional WWTP. Further research is needed to identify and optimize the most suitable treatment technology, and further reduce spreading of antibiotics and ARGs via WWTPs into the environment.

CRediT authorship contribution statement

N.A. Sabri: Conceptualization, Data curation, Visualization, Writing - original draft. **S. van Holst:** Investigation, Data curation, Writing - review & editing. **H. Schmitt:** Conceptualization, Data curation, Visualization, Writing - review & editing. **B.M. van der Zaan:** Conceptualization, Data curation, Visualization, Funding acquisition, Writing - review & editing. **H.W. Gerritsen:** Validation, Writing - review & editing. **H.H.M. Rijnaarts:** Supervision, Writing - review & editing. **A.A.M. Langenhoff:** Conceptualization, Data curation, Visualization, Funding acquisition, Supervision, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.140199.

References

Adams, C., Wang, Y.Y., Loftin, K.K., Meyer, M.M., 2002. Removal of antibiotics from surface and distilled water in conventional water treatment processes. J. Environ. Eng. 128 (3), 253–260. https://doi.org/10.1061/(ASCE)0733-9372(2002)128:3(253).

Adriaenssens, N., Coenen, S., Versporten, A., Muller, A., Minalu, G., Faes, C., Vankerckhoven, V., Aerts, M., Hens, N., Molenberghs, G., Goossens, H., 2011. European Surveillance of Antimicrobial Consumption (ESAC): outpatient quinolone use in Europe (1997–2009). J. Antimicrob. Chemother. 66 (suppl_6), vi47–vi56. https://doi.org/10.1093/jac/dkr457.

Ahmed, M.J., 2017. Adsorption of quinolone, tetracycline, and penicillin antibiotics from aqueous solution using activated carbons: review. Environ. Toxicol. Pharmacol. 50, 1–10. https://doi.org/10.1016/j.etap.2017.01.004.

Andersson, D.I., Hughes, D., 2012. Evolution of antibiotic resistance at non-lethal drug concentrations. Drug Resist. Updat. 15 (3), 162–172. https://doi.org/10.1016/j.drup.2012.03.005.

Angelakis, A.N., Asano, T., Bahri, A., Jimenez, B.E., Tchobanoglous, G., 2018. Water reuse: from ancient to modern times and the future. Frontiers in Environmental Science 6 (26). https://doi.org/10.3389/fenvs.2018.00026.

APHA, 2005. In: American Public Health Association (Ed.), Standard Methods for the Examination of Water and Wastewater, 21 ed. Washington, DC.

AWWA, 2019. State of the Water Industry Report.

Bassin, J.P., 2018. Aerobic granular sludge technology. In: Dezotti, Márcia, Lippel, Geraldo, Bassin, João Paulo (Eds.), Advanced Biological Processes for Wastewater Treatment: Emerging, Consolidated Technologies and Introduction to Molecular Techniques. Springer International Publishing, Cham, pp. 75–142.

Bechger, M., Bult, B., van Dalen, R., den Elzen, J.J.M., Jonge, J.d., Koreman, E., Rijs, G.B.J., Uijterlinde, C.A., van Veldhuizen, H.M., Vente, D.d., 2009. 1-Step® Filter Als Effluent Polishings Techniek. STOWA.

Bechger, M., van der Oost, R.O., Rijs, G., Schellekens, M., Bult, B., Flameling, T., Scherrenberg, S., Uijterlinde, C., 2013. Monitoring 1-STEP® Filter Horstermeer. STOWA.

Bengtsson-Palme, J., Larsson, D.G.J., 2016. Concentrations of antibiotics predicted to select for resistant bacteria: proposed limits for environmental regulation. Environ. Int. 86, 140–149. https://doi.org/10.1016/j.envint.2015.10.015.

Butkovskyi, A., Sevenou, L., Meulepas, R.J.W., Hernandez Leal, L., Zeeman, G., Rijnaarts, H.H.M., 2017. Micropollutant removal from black water and grey water sludge in a UASB-GAC reactor. Water Sci. Technol. 77 (4), 1137–1148. https://doi.org/10.2166/wst.2017.640.

Chee-Sanford, J.C., Mackie, R.I., Koike, S., Krapac, I.G., Lin, Y.-F., Yannarell, A.C., Maxwell, S., Aminov, R.I., 2009. Fate and transport of antibiotic residues and antibiotic resistance genes following land application of manure waste. J. Environ. Qual. 38 (3), 1086–1108. https://doi.org/10.2134/jeq2008.0128.

Chen, H., Zhang, M., 2013. Effects of advanced treatment systems on the removal of antibiotic resistance genes in wastewater treatment plants from Hangzhou, China. Environmental Science & Technology 47 (15), 8157–8163. https://doi.org/10.1021/es401091y.

Chen, H., Jing, L., Yao, Z., Meng, F., Teng, Y., 2019. Prevalence, source and risk of antibiotic resistance genes in the sediments of Lake Tai (China) deciphered by metagenomic assembly: a comparison with other global lakes. Environ. Int. 127, 267–275. https://doi.org/10.1016/j.envint.2019.03.048.

Choi, K.J., Kim, S.G., Kim, C.W., Kim, S.H., 2005. Effects of activated carbon types and service life on removal of endocrine disrupting chemicals: amitrol, nonylphenol, and bisphenol-a. Chemosphere 58 (11), 1535–1545. https://doi.org/10.1016/j.chemosphere.2004.11.080.

Choi, K.J., Kim, S.G., Kim, S.H., 2008. Removal of antibiotics by coagulation and granular activated carbon filtration. J. Hazard. Mater. 151 (1), 38–43. https://doi.org/10.1016/j.jhazmat.2007.05.059.

Collivignarelli, M.C., Abbà, A., Benigna, I., Sorlini, S., Torretta, V., 2018. Overview of the main disinfection processes for wastewater and drinking water treatment plants. Sustainability 10 (1), 86.

Council Directive, 1991. Council Directive 91/271/EEC of 21 May 1991 Concerning Urban Waste-Water Treatment.

Czekalski, N., Berthold, T., Caucci, S., Egli, A., Buergmann, H., 2012. Increased levels of multiresistant bacteria and resistance genes after wastewater treatment and their dissemination into Lake Geneva, Switzerland. Front. Microbiol. 3. https://doi.org/10.3389/fmicb.2012.00106.

Dagan, R., Barkai, G., Givon-Lavi, N., Sharf, A.Z., Vardy, D., Cohen, T., Lipsitch, M., Greenberg, D., 2008. Seasonality of antibiotic-resistant streptococcus pneumoniae that causes acute otitis media: a clue for an antibiotic-restriction policy? J. Infect. Dis. 197 (8), 1094–1102. https://doi.org/10.1086/528995.

Davey, M.E., O'Toole, G.A., 2000. Microbial biofilms: from ecology to molecular genetics. Microbiology and molecular biology reviews: MMBR 64 (4), 847–867. https://doi.org/10.1128/mmbr.64.4.847-867.2000.

Diwan, V., Stålsby Lundborg, C., Tamhankar, A.J., 2013. Seasonal and temporal variation in release of antibiotics in hospital wastewater: estimation using continuous and grab sampling. PLoS One 8 (7), e68715. https://doi.org/10.1371/journal.pone.0068715.

Ferech, M., Coenen, S., Malhotra-Kumar, S., Dvorakova, K., Hendrickx, E., Suetens, C., Goossens, H., 2006. European surveillance of antimicrobial consumption (ESAC): outpatient antibiotic use in Europe. J. Antimicrob. Chemother. 58 (2), 401–407. https://doi.org/10.1093/jac/dkl188.

Fiorentino, A., Di Cesare, A., Eckert, E.M., Rizzo, L., Fontaneto, D., Yang, Y., Corno, G., 2019. Impact of industrial wastewater on the dynamics of antibiotic resistance genes in a full-scale urban wastewater treatment plant. Sci. Total Environ. 646, 1204–1210. https://doi.org/10.1016/j.scitotenv.2018.07.370.

Flemming, H.-C., Wingender, J., 2010. The biofilm matrix. Nat. Rev. Microbiol. 8, 623. https://doi.org/10.1038/nrmicro2415.

Forster, R., 2019. *Mark van Loosdrecht – Professor at Delft University of Technology*. International water association 2015 [cited 29/12/2019 2019]. Available from. http://www.thesourcemagazine.org/mark-van-loosdrecht-professor-at-delft-university-of-technology/.

Forster, S., Snape, J.R., Lappin-Scott, H.M., Porter, J., 2002. Simultaneous fluorescent gram staining and activity assessment of activated sludge Bacteria. Appl. Environ. Microbiol. 68 (10), 4772–4779. https://doi.org/10.1128/aem.68.10.4772-4779.2002.

Gallo, G.G., Lancini, G., Parenti, F., 1995. Antibiotics: A Multidisciplinary Approach. Springer, US.

Gao, P., Munir, M., Xagoraraki, I., 2012. Correlation of tetracycline and sulfonamide antibiotics with corresponding resistance genes and resistant bacteria in a conventional municipal wastewater treatment plant. Sci. Total Environ. 421–422, 173–183. https://doi.org/10.1016/j.scitotenv.2012.01.061.

Giesen, A., de Bruin, L.M.M., Niermans, R.P., van der Roest, H.F., 2013. Advancements in the application of aerobic granular biomass technology for sustainable treatment of wastewater. Water Practice and Technology 8 (1), 47–54. https://doi.org/10.2166/wpt.2013.007.

Gillings, M.R., Gaze, W.H., Pruden, A., Smalla, K., Tiedje, J.M., Zhu, Y.G., 2015. Using the class 1 integron-integrase gene as a proxy for anthropogenic pollution. International Society for Microbial Ecology 9 (6), 1269–1279. https://doi.org/10.1038/ismej.2014.226.

Golet, E.M., Xifra, I., Siegrist, H., Alder, A.C., Giger, W., 2003. Environmental exposure assessment of fluoroquinolone antibacterial agents from sewage to soil. Environmental Science & Technology 37 (15), 3243–3249. https://doi.org/10.1021/es0264448.

Golovko, O., Kumar, V., Fedorova, G., Randak, T., Grabic, R., 2014. Seasonal changes in antibiotics, antidepressants/psychiatric drugs, antihistamines and lipid regulators in a wastewater treatment plant. Chemosphere 111, 418–426. https://doi.org/10.1016/j.chemosphere.2014.03.132.

Gopal, K., Tripathy, S.S., Bersillon, J.L., Dubey, S.P., 2007. Chlorination byproducts, their toxicodynamics and removal from drinking water. Journal of Hazardous Materials 140 (1–2), 1–6. https://doi.org/10.1016/j.jhazmat.2006.10.063.

Grandclément, C., Seyssiecq, I., Piram, A., Wong-Wah-Chung, P., Vanot, G., Tiliacos, N., Roche, N., Doumenq, P., 2017. From the conventional biological wastewater treatment to hybrid processes, the evaluation of organic micropollutant removal: a review. Water Res. 111, 297–317. https://doi.org/10.1016/j.watres.2017.01.005.

Guo, C., Wang, K., Hou, S., Wan, L., Lv, J., Zhang, Y., Qu, X., Chen, S., Xu, J., 2017. H2O2 and/or TiO2 photocatalysis under UV irradiation for the removal of antibiotic resistant bacteria and their antibiotic resistance genes. J. Hazard. Mater. 323 (Part B), 710–718. https://doi.org/10.1016/j.jhazmat.2016.10.041.

Gupta, A., Vlamakis, H., Shoemaker, N., Salyers, A.A., 2003. A new Bacteroides conjugative transposon that carries an ermB gene. Appl. Environ. Microbiol. 69 (11), 6455–6463. https://doi.org/10.1128/AEM.69.11.6455-6463.2003.

Helmecke, M., Fries, E., Schulte, C., 2020. Regulating water reuse for agricultural irrigation: risks related to organic micro-contaminants. Environ. Sci. Eur. 32 (1), 4. https://doi.org/10.1186/s12302-019-0283-0.

Heß, S., Berendonk, T., Kneis, D., 2018. Antibiotic resistant bacteria and resistance genes in the bottom sediment of a small stream and the potential impact of remobilization. FEMS Microbiol. Ecol. 94. https://doi.org/10.1093/femsec/fiy128.

Hong, P.-Y., Al-Jassim, N., Ansari, M.I., Mackie, R.I., 2013. Environmental and public health implications of water reuse: antibiotics, antibiotic resistant Bacteria, and antibiotic resistance genes. Antibiotics (Basel, Switzerland) 2 (3), 367–399. https://doi.org/10.3390/antibiotics2030367.

Hu, Y., Jiang, L., Zhang, T., Jin, L., Han, Q., Zhang, D., Lin, K., Cui, C., 2018. Occurrence and removal of sulfonamide antibiotics and antibiotic resistance genes in conventional and advanced drinking water treatment processes. J. Hazard. Mater. 360, 364–372. https://doi.org/10.1016/j.jhazmat.2018.08.012.

Huang, H., Zeng, S., Dong, X., Li, D., Zhang, Y., He, M., Du, P., 2019a. Diverse and abundant antibiotics and antibiotic resistance genes in an urban water system. J. Environ. Manag. 231, 494–503. https://doi.org/10.1016/j.jenvman.2018.10.051.

Huang, X., Lu, Q., Hao, H., Wei, Q., Shi, B., Yu, J., Wang, C., Wang, Y., 2019b. Evaluation of the treatability of various odor compounds by powdered activated carbon. Water Res. 156, 414–424. https://doi.org/10.1016/j.watres.2019.03.043.

Huang, Z., Zhao, W., Xu, T., Zheng, B., Yin, D., 2019c. Occurrence and distribution of antibiotic resistance genes in the water and sediments of Qingcaosha Reservoir, Shanghai, China. Environ. Sci. Eur. 31 (1), 81. https://doi.org/10.1186/s12302-019-0265-2.

Hung, Y.-T., Lo, H.H., Wang, L.K., Taricska, J.R., Li, K.H., 2005. Granular activated carbon adsorption. In: Wang, Lawrence K., Hung, Yung-Tse, Shammas, Nazih K. (Eds.), Physicochemical Treatment Processes. Humana Press, Totowa, NJ, pp. 573–633.

Jelic, A., Rodriguez-Mozaz, S., Barceló, D., Gutierrez, O., 2015. Impact of in-sewer transformation on 43 pharmaceuticals in a pressurized sewer under anaerobic conditions. Water Res. 68, 98–108. https://doi.org/10.1016/j.watres.2014.09.033.

Jia, A., Wan, Y., Xiao, Y., Hu, J., 2012. Occurrence and fate of quinolone and fluoroquinolone antibiotics in a municipal sewage treatment plant. Water Res. 46 (2), 387–394. https://doi.org/10.1016/j.watres.2011.10.055.

Jiang, Y., Xu, C., Wu, X., Chen, Y., Han, W., Gin, K.Y.-H., He, Y., 2018. Occurrence, seasonal variation and risk assessment of antibiotics in Qingcaosha reservoir. Water 10 (2), 115. https://doi.org/10.3390/w10020115.

Kang, A.J., Brown, A.K., Wong, C.S., Huang, Z., Yuan, Q., 2018. Variation in bacterial community structure of aerobic granular and suspended activated sludge in the presence of the antibiotic sulfamethoxazole. Bioresour. Technol. 261, 322–328. https://doi.org/10.1016/j.biortech.2018.04.054.

Karaolia, P., Michael-Kordatou, I., Hapeshi, E., Drosou, C., Bertakis, Y., Christofilos, D., Armatas, G.S., Sygellou, L., Schwartz, T., Xekoukoulotakis, N.P., Fatta-Kassinos, D., 2018. Removal of antibiotics, antibiotic-resistant bacteria and their associated genes by graphene-based TiO2 composite photocatalysts under solar radiation in urban wastewaters. Appl. Catal. B Environ. 224, 810–824. https://doi.org/10.1016/j.apcatb.2017.11.020.

Kårelid, V., Larsson, G., Björlenius, B., 2017. Pilot-scale removal of pharmaceuticals in municipal wastewater: comparison of granular and powdered activated carbon treatment at three wastewater treatment plants. J. Environ. Manag. 193, 491–502. https://doi.org/10.1016/j.jenvman.2017.02.042.

Karkman, A., Do, T.T., Walsh, F., Virta, M.P.J., 2018. Antibiotic-resistance genes in waste water. Trends Microbiol. 26 (3), 220–228. https://doi.org/10.1016/j.tim.2017.09.005.

Khorsandi, H., Teymori, M., Aghapour, A.A., Jafari, S.J., Taghipour, S., Bargeshadi, R., 2019. Photodegradation of ceftriaxone in aqueous solution by using UVC and UVC/H2O2 oxidation processes. Appl Water Sci 9 (4), 81. https://doi.org/10.1007/s13201-019-0964-2.

Kolář, M., Urbánek, K., Látal, T., 2001. Antibiotic selective pressure and development of bacterial resistance. Int. J. Antimicrob. Agents 17 (5), 357–363. https://doi.org/10.1016/S0924-8579(01)00317-X.

Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., Buxton, H.T., 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999−2000: a National Reconnaissance. Environmental Science & Technology 36 (6), 1202–1211. https://doi.org/10.1021/es011055j.

Kraemer, S.A., Ramachandran, A., Perron, G.G., 2019. Antibiotic pollution in the environment: from microbial ecology to public policy. Microorganisms 7 (6), 180. https://doi.org/10.3390/microorganisms7060180.

de Kreuk, M.K., Kishida, N., Tsuneda, S., van Loosdrecht, M.C.M., 2010. Behavior of polymeric substrates in an aerobic granular sludge system. Water Res. 44 (20), 5929–5938. https://doi.org/10.1016/j.watres.2010.07.033.

Kulkarni, P., Olson, N.D., Raspanti, G.A., Rosenberg Goldstein, R.E., Gibbs, S.G., Sapkota, A., Sapkota, A.R., 2017. Antibiotic concentrations decrease during wastewater treatment but persist at low levels in reclaimed water. Int. J. Environ. Res. Public Health 14 (6), 668. https://doi.org/10.3390/ijerph14060668.

Lan, L., Kong, X., Sun, H., Li, C., Liu, D., 2019. High removal efficiency of antibiotic resistance genes in swine wastewater via nanofiltration and reverse osmosis processes. J. Environ. Manag. 231, 439–445. https://doi.org/10.1016/j.jenvman.2018.10.073.

Lee, J., Jeon, J.H., Shin, J., Jang, H.M., Kim, S., Song, M.S., Kim, Y.M., 2017. Quantitative and qualitative changes in antibiotic resistance genes after passing through treatment processes in municipal wastewater treatment plants. Sci. Total Environ. 605–606, 906–914. https://doi.org/10.1016/j.scitotenv.2017.06.250.

Li, J., Ding, L.-B., Cai, A., Huang, G.-X., Horn, H., 2014. Aerobic sludge granulation in a full-scale sequencing batch reactor. Biomed. Res. Int. 2014, 12. https://doi.org/10.1155/2014/268789.

Li, B., Qiu, Y., Li, J., Liang, P., Huang, X., 2019. Removal of antibiotic resistance genes in four full-scale membrane bioreactors. Sci. Total Environ. 653, 112–119. https://doi.org/10.1016/j.scitotenv.2018.10.305.

Liao, X., Li, B., Zou, R., Dai, Y., Xie, S., Yuan, B., 2016. Biodegradation of antibiotic ciprofloxacin: pathways, influential factors, and bacterial community structure. Environ. Sci. Pollut. Res. 23 (8), 7911–7918. https://doi.org/10.1007/s11356-016-6054-1.

Liao, J., Liu, C., Liu, L., Li, J., Fan, H., Ye, J., Zeng, Z., 2019. Influence of hydraulic retention time on behavior of antibiotics and antibiotic resistance genes in aerobic granular reactor treating biogas slurry. Frontiers of Environmental Science & Engineering 13 (3), 31. https://doi.org/10.1007/s11783-019-1115-6.

Liu, Y., Yang, S.-F., Xu, H., Woon, K.-H., Lin, Y.-M., Tay, J.-H., 2003. Biosorption kinetics of cadmium(II) on aerobic granular sludge. Process Biochem. 38 (7), 997–1001. https://doi.org/10.1016/S0032-9592(02)00225-X.

Liu, J., Li, J., Tao, Y., Sellamuthu, B., Walsh, R., 2016. Analysis of bacterial, fungal and archaeal populations from a municipal wastewater treatment plant developing an innovative aerobic granular sludge process. World J. Microbiol. Biotechnol. 33 (1), 14. https://doi.org/10.1007/s11274-016-2179-0.

Marx, C., Günther, N., Schubert, S., Oertel, R., Ahnert, M., Krebs, P., Kuehn, V., 2015. Mass flow of antibiotics in a wastewater treatment plant focusing on removal variations due to operational parameters. Sci. Total Environ. 538, 779–788. https://doi.org/10.1016/j.scitotenv.2015.08.112.

Matsui, Y., Nakao, S., Sakamoto, A., Taniguchi, T., Pan, L., Matsushita, T., Shirasaki, N., 2015. Adsorption capacities of activated carbons for geosmin and 2-methylisoborneol vary with activated carbon particle size: effects of adsorbent and adsorbate characteristics. Water Res. 85, 95–102. https://doi.org/10.1016/j.watres.2015.08.017.

Michael, S.G., Michael-Kordatou, I., Beretsou, V.G., Jäger, T., Michael, C., Schwartz, T., Fatta-Kassinos, D., 2019. Solar photo-Fenton oxidation followed by adsorption on activated carbon for the minimisation of antibiotic resistance determinants and toxicity present in urban wastewater. Appl. Catal. B Environ. 244, 871–880. https://doi.org/10.1016/j.apcatb.2018.12.030.

Mihciokur, H., Oguz, M., 2016. Removal of oxytetracycline and determining its biosorption properties on aerobic granular sludge. Environ. Toxicol. Pharmacol. 46, 174–182. https://doi.org/10.1016/j.etap.2016.07.017.

Munir, M., Wong, K., Xagoraraki, I., 2011. Release of antibiotic resistant bacteria and genes in the effluent and biosolids of five wastewater utilities in Michigan. Water Res. 45 (2), 681–693. https://doi.org/10.1016/j.watres.2010.08.033.

Muziasari, W.I., Managaki, S., Pärnänen, K., Karkman, A., Lyra, C., Tamminen, M., Suzuki, S., Virta, M., 2014. Sulphonamide and trimethoprim resistance genes persist in sediments at Baltic Sea aquaculture farms but are not detected in the surrounding environment. PLoS One 9 (3), e92702. https://doi.org/10.1371/journal.pone.0092702.

Nancharaiah, Y.V., Reddy, G.K.K., 2017. Aerobic granular sludge technology: mechanisms of granulation and biotechnological applications. Bioresour. Technol. 247. https://doi.org/10.1016/j.biortech.2017.09.131.

Narciso-da-Rocha, C., Varela, A.R., Schwartz, T., Nunes, O.C., Manaia, C.M., 2014. blaTEM and vanA as indicator genes of antibiotic resistance contamination in a hospital–urban wastewater treatment plant system. Journal of Global Antimicrobial Resistance 2 (4), 309–315. https://doi.org/10.1016/j.jgar.2014.10.001.

NCBI, 2018. National Center for Biotechnology Information. PubChem Compound Database; CID=54675776 2018 [cited Dec 28, 2018 2018]. Available from. https://pubchem.ncbi.nlm.nih.gov/compound/54675776.

Nnadozie, C.F., Kumari, S., Bux, F., 2017. Status of pathogens, antibiotic resistance genes and antibiotic residues in wastewater treatment systems. Rev. Environ. Sci. Biotechnol. 16 (3), 491–515. https://doi.org/10.1007/s11157-017-9438-x.

Novo, A., André, S., Viana, P., Nunes, O.C., Manaia, C.M., 2013. Antibiotic resistance, antimicrobial residues and bacterial community composition in urban wastewater. Water Res. 47 (5), 1875–1887. https://doi.org/10.1016/j.watres.2013.01.010.

Östman, M., Björlenius, B., Fick, J., Tysklind, M., 2019. Effect of full-scale ozonation and pilot-scale granular activated carbon on the removal of biocides, antimycotics and antibiotics in a sewage treatment plant. Sci. Total Environ. 649, 1117–1123. https://doi.org/10.1016/j.scitotenv.2018.08.382.

Pachauri, P., Falwariya, R., Vyas, S., Maheshwari, M., Vyas, R., Gupta, A., 2009. Removal of amoxicillin in wastewater using adsorption by powdered and granular activated carbon and oxidation with hydrogen peroxide. Nature Environment and Pollution Technology 8, 481–488.

Pal, C., Bengtsson-Palme, J., Kristiansson, E., Larsson, D.G.J., 2015. Co-occurrence of resistance genes to antibiotics, biocides and metals reveals novel insights into their co-selection potential. BMC Genomics 16 (1), 964. https://doi.org/10.1186/s12864-015-2153-5.

Pallares-Vega, R., Blaak, H., van der Plaats, R., de Roda Husman, A.M., Hernandez Leal, L., van Loosdrecht, M.C.M., Weissbrodt, D.G., Schmitt, H., 2019. Determinants of presence and removal of antibiotic resistance genes during WWTP treatment: a cross-sectional study. Water Res. 161, 319–328. https://doi.org/10.1016/j.watres.2019.05.100.

Pärnänen, K.M.M., Narciso-da-Rocha, C., Kneis, D., Berendonk, T.U., Cacace, D., Do, T.T., Elpers, C., Fatta-Kassinos, D., Henriques, I., Jaeger, T., Karkman, A., Martinez, J.L., Michael, S.G., Michael-Kordatou, I., O'Sullivan, K., Rodriguez-Mozaz, S., Schwartz, T., Sheng, H., Sørum, H., Stedtfeld, R.D., Tiedje, J.M., Giustina, S.V.D., Walsh, F., Vaz-Moreira, I., Virta, M., Manaia, C.M., 2019. Antibiotic resistance in European wastewater treatment plants mirrors the pattern of clinical antibiotic resistance prevalence. Sci. Adv. 5 (3), eaau9124. https://doi.org/10.1126/sciadv.aau9124.

Partridge, S.R., Collis, C.M., Hall, R.M., 2002. Class 1 integron containing a new gene cassette, aadA10, associated with Tn1404 from R151. Antimicrob. Agents Chemother. 46. https://doi.org/10.1128/aac.46.8.2400-2408.2002.

Pazda, M., Kumirska, J., Stepnowski, P., Mulkiewicz, E., 2019. Antibiotic resistance genes identified in wastewater treatment plant systems – a review. Sci. Total Environ. 697, 134023. https://doi.org/10.1016/j.scitotenv.2019.134023.

Peng, S., Dolfing, J., Feng, Y., Wang, Y., Lin, X., 2018. Enrichment of the antibiotic resistance gene tet(L) in an alkaline soil fertilized with plant derived organic manure. Front. Microbiol. 9, 1140. https://doi.org/10.3389/fmicb.2018.01140.

Pi, S., Li, A., Cui, D., Su, Z., Feng, L., Ma, F., Yang, J., 2019. Biosorption behavior and mechanism of sulfonamide antibiotics in aqueous solution on extracellular polymeric substances extracted from Klebsiella sp. J1. Bioresour. Technol. 272, 346–350. https://doi.org/10.1016/j.biortech.2018.10.054.

Polesel, F., Lehnberg, K., Dott, W., Trapp, S., Thomas, K.V., Plósz, B.G., 2015. Factors influencing sorption of ciprofloxacin onto activated sludge: experimental assessment and modelling implications. Chemosphere 119, 105–111. https://doi.org/10.1016/j.chemosphere.2014.05.048.

Pronk, M., de Kreuk, M.K., de Bruin, B., Kamminga, P., Kleerebezem, R., van Loosdrecht, M.C.M., 2015. Full scale performance of the aerobic granular sludge process for sewage treatment. Water Res. 84, 207–217. https://doi.org/10.1016/j.watres.2015.07.011.

Raevsky, O.A., Perlovich, G.L., Kazachenko, V.P., Strakhova, N.N., Schaper, K.-J., 2009. Octanol/water partition coefficients of sulfonamides: experimental determination and calculation using physicochemical descriptors. J. Chem. Eng. Data 54 (11), 3121–3124. https://doi.org/10.1021/je900189v.

Rafraf, I.D., Lekunberri, I., Sànchez-Melsió, A., Aouni, M., Borrego, C.M., Balcázar, J.L., 2016. Abundance of antibiotic resistance genes in five municipal wastewater treatment plants in the Monastir governorate, Tunisia. Environ. Pollut. 219, 353–358. https://doi.org/10.1016/j.envpol.2016.10.062.

Ravasi, D., König, R., Principi, P., Perale, G., Demarta, A., 2019. Effect of powdered activated carbon as advanced step in wastewater treatments on antibiotic resistant microorganisms. Curr. Pharm. Biotechnol. 20 (1), 63–75. https://doi.org/10.2174/1389201020666190207095556.

Rodríguez-Chueca, J., Varella della Giustina, S., Rocha, J., Fernandes, T., Pablos, C., Encinas, Á., Barceló, D., Rodríguez-Mozaz, S., Manaia, C.M., Marugán, J., 2019. Assessment of full-scale tertiary wastewater treatment by UV-C based-AOPs: removal or persistence of antibiotics and antibiotic resistance genes? Sci. Total Environ. 652, 1051–1061. https://doi.org/10.1016/j.scitotenv.2018.10.223.

Rodriguez-Mozaz, S., S. Chamorro, E. Marti, B. Huerta, M. Gros, A. Sànchez-Melsió, C. M. Borrego, D. Barceló, and J. L. Balcázar. 2015. Occurrence of antibiotics and antibiotic resistance genes in hospital and urban wastewaters and their impact on the receiving river. Water Res. 69 (0): 234–242. doi:https://doi.org/10.1016/j.watres.2014.11.021.

van der Roest, H.F., de Bruin, L.M.M., Gademan, G., Coelho, F., 2011. Towards sustainable waste water treatment with Dutch Nereda® technology. Water Practice and Technology 6 (3), wpt2011059. https://doi.org/10.2166/wpt.2011.059.

Rooklidge, S.J., 2004. Environmental antimicrobial contamination from terraccumulation and diffuse pollution pathways. Sci. Total Environ. 325 (1), 1–13. https://doi.org/10.1016/j.scitotenv.2003.11.007.

Sabri, N.A., Schmitt, H., Van der Zaan, B., Gerritsen, H.W., Zuidema, T., Rijnaarts, H.H.M., Langenhoff, A.A.M., 2020. Prevalence of antibiotics and antibiotic resistance genes in a wastewater effluent-receiving river in the Netherlands. Journal of Environmental Chemical Engineering 8 (1), 102245. https://doi.org/10.1016/j.jece.2018.03.004.

Samer, M., 2015. Biological and chemical wastewater treatment processes. Wastewater Treatment Engineering. IntechOpen.

Scherrenberg, S.M., Neef, R., Menkveld, H.W.H., van Nieuwenhuijzen, A.F., van der Graaf, J.H.J.M., 2012. Investigating phosphorus limitation in a fixed bed filter with phosphorus and nitrogen profile measurements. Water Environment Research 84 (1), 25–33. https://doi.org/10.2175/106143011X13188633467076.

Schmidt, S., Winter, J., Gallert, C., 2012. Long-term effects of antibiotics on the elimination of chemical oxygen demand, nitrification, and viable Bacteria in laboratory-scale wastewater treatment plants. Arch. Environ. Contam. Toxicol. 63, 354.

Shah, A.D., 2008. Antibiotics in Water Treatment: The Role of Water Quality Conditions on their Fate and Removal during Chlorination and Nanofiltration. School of Civil and Environmental Engineering Georgia Institute of Technology, Georgia.

Sharma, V.K., Johnson, N., Cizmas, L., McDonald, T.J., Kim, H., 2016. A review of the influence of treatment strategies on antibiotic resistant bacteria and antibiotic resistance genes. Chemosphere 150, 702–714. https://doi.org/10.1016/j.chemosphere.2015.12.084.

Shen, L., Griffith, T.M., Nyangaresi, P.O., Qin, Y., Pang, X., Chen, G., Li, M., Lu, Y., Zhang, B., 2020. Efficacy of UVC-LED in water disinfection on Bacillus species with consideration of antibiotic resistance issue. J. Hazard. Mater. 386, 121968. https://doi.org/10.1016/j.jhazmat.2019.121968.

Singh, R., Singh, A.P., Kumar, S., Giri, B.S., Kim, K.-H., 2019. Antibiotic resistance in major rivers in the world: a systematic review on occurrence, emergence, and management strategies. J. Clean. Prod. 234, 1484–1505. https://doi.org/10.1016/j.jclepro.2019.06.243.

Sousa, J.M., Macedo, G., Pedrosa, M., Becerra-Castro, C., Castro-Silva, S., Pereira, M.F.R., Silva, A.M.T., Nunes, O.C., Manaia, C.M., 2017. Ozonation and UV254nm radiation for the removal of microorganisms and antibiotic resistance genes from urban wastewater. J. Hazard. Mater. 323, 434–441. https://doi.org/10.1016/j.jhazmat.2016.03.096.

Su, H.-C., Liu, Y.-S., Pan, C.-G., Chen, J., He, L.-Y., Ying, G.-G., 2018. Persistence of antibiotic resistance genes and bacterial community changes in drinking water treatment system: from drinking water source to tap water. Sci. Total Environ. 616–617, 453–461. https://doi.org/10.1016/j.scitotenv.2017.10.318.

Suda, K.J., Hicks, L.A., Roberts, R.M., Hunkler, R.J., Taylor, T.H., 2014. Trends and seasonal variation in outpatient antibiotic prescription rates in the United States, 2006 to 2010. Antimicrob. Agents Chemother. 58 (5), 2763–2766. https://doi.org/10.1128/AAC.02239-13.

Sun, L., Gao, C., He, N., Yang, B., Duan, X., Chen, T., 2019. The removal of antibiotic resistance genes in secondary effluent by the combined process of PAC-UF. J. Environ. Sci. Health A 54 (11), 1075–1082. https://doi.org/10.1080/10934529.2019.1631090.

Świątczak, P., Cydzik-Kwiatkowska, A., 2018. Performance and microbial characteristics of biomass in a full-scale aerobic granular sludge wastewater treatment plant. Environ. Sci. Pollut. Res. Int. 25 (2), 1655–1669. https://doi.org/10.1007/s11356-017-0615-9.

Tadda, M.A., Ahsan, A., Shitu, A., Elsergany, M., Thirugnanasambantham, A., Jose, B., Razzaque, M., Norsyahariati, N., 2016. A Review on Activated Carbon: Process, Application and Prospects. 2 pp. 7–13.

Ternes, T.A., Meisenheimer, M., McDowell, D., Sacher, F., Brauch, H.-J., Haist-Gulde, B., Preuss, G., Wilme, U., Zulei-Seibert, N., 2002. Removal of pharmaceuticals during drinking water treatment. Environmental Science & Technology 36 (17), 3855–3863. https://doi.org/10.1021/es015757k.

Van Boeckel, T.P., Gandra, S., Ashok, A., Caudron, Q., Grenfell, B.T., Levin, S.A., Laxminarayan, R., 2014. Global antibiotic consumption 2000 to 2010: an analysis of national pharmaceutical sales data. Lancet Infect. Dis. 14 (8), 742–750. https://doi.org/10.1016/S1473-3099(14)70780-7.

Wang, X., Chen, Z., Kang, J., Zhao, X., Shen, J., 2018. Removal of tetracycline by aerobic granular sludge and its bacterial community dynamics in SBR. RSC Adv. 8 (33), 18284–18293. https://doi.org/10.1039/C8RA01357H.

Wang, S., Ma, X., Wang, Y., Du, G., Tay, J.-H., Li, J., 2019. Piggery wastewater treatment by aerobic granular sludge: granulation process and antibiotics and antibiotic-resistant bacteria removal and transport. Bioresour. Technol. 273, 350–357. https://doi.org/10.1016/j.biortech.2018.11.023.

Wegst-Uhrich, S.R., Navarro, D.A., Zimmerman, L., Aga, D.S., 2014. Assessing antibiotic sorption in soil: a literature review and new case studies on sulfonamides and macrolides. Chemistry Central Journal 8 (1), 5. https://doi.org/10.1186/1752-153x-8-5.

Werner, N.L., Hecker, M.T., Sethi, A.K., Donskey, C.J., 2011. Unnecessary use of fluoroquinolone antibiotics in hospitalized patients. BMC Infect. Dis. 11 (1), 187. https://doi.org/10.1186/1471-2334-11-187.

WHO, 2014. Antimicrobial resistance: global report on surveillance 2014 (Switzerland).

Wilén, B.-M., Liébana, R., Persson, F., Modin, O., Hermansson, M., 2018. The mechanisms of granulation of activated sludge in wastewater treatment, its optimization, and impact on effluent quality. Appl. Microbiol. Biotechnol. 102 (12), 5005–5020. https://doi.org/10.1007/s00253-018-8990-9.

Wu, N., Qiao, M., Zhang, B., Cheng, W.-D., Zhu, Y.-G., 2010. Abundance and diversity of tetracycline resistance genes in soils adjacent to representative swine feedlots in China. Environmental Science & Technology 44 (18), 6933–6939. https://doi.org/10.1021/es1007802.

Xing, Z.-P., Sun, D.-Z., 2009. Treatment of antibiotic fermentation wastewater by combined polyferric sulfate coagulation, Fenton and sedimentation process. J. Hazard. Mater. 168 (2), 1264–1268. https://doi.org/10.1016/j.jhazmat.2009.03.008.

Xu, W.H., Zhang, G., Zou, S.C., Li, X.D., Liu, Y.C., 2007. Determination of selected antibiotics in the Victoria Harbour and the Pearl River, South China using high-performance liquid chromatography-electrospray ionization tandem mass spectrometry. Environ. Pollut. 145. https://doi.org/10.1016/j.envpol.2006.05.038.

Xu, J., Sheng, G.-P., Ma, Y., Wang, L.-F., Yu, H.-Q., 2013. Roles of extracellular polymeric substances (EPS) in the migration and removal of sulfamethazine in activated sludge system. Water Res. 47 (14), 5298–5306. https://doi.org/10.1016/j.watres.2013.06.009.

Xu, J., Xu, Y., Wang, H., Guo, C., Qiu, H., He, Y., Zhang, Y., Li, X., Meng, W., 2015. Occurrence of antibiotics and antibiotic resistance genes in a sewage treatment plant and its effluent-receiving river. Chemosphere 119, 1379–1385. https://doi.org/10.1016/j.chemosphere.2014.02.040.

Ye, Z., Weinberg, H.S., Meyer, M.T., 2007. Trace analysis of trimethoprim and sulfonamide, macrolide, quinolone, and tetracycline antibiotics in chlorinated drinking water using liquid chromatography electrospray tandem mass spectrometry. Anal. Chem. 79 (3), 1135–1144. https://doi.org/10.1021/ac060972a.

Yu, F., Li, Y., Han, S., Ma, J., 2016. Adsorptive removal of antibiotics from aqueous solution using carbon materials. Chemosphere 153, 365–385. https://doi.org/10.1016/j.chemosphere.2016.03.083.

Zhang, J., Chen, M., Sui, Q., Tong, J., Jiang, C., Lu, X., Zhang, Y., Wei, Y., 2016a. Impacts of addition of natural zeolite or a nitrification inhibitor on antibiotic resistance genes during sludge composting. Water Res. 91, 339–349. https://doi.org/10.1016/j.watres.2016.01.010.

Zhang, X., Guo, W., Ngo, H.H., Wen, H., Li, N., Wu, W., 2016b. Performance evaluation of powdered activated carbon for removing 28 types of antibiotics from water. J. Environ. Manag. 172, 193–200. https://doi.org/10.1016/j.jenvman.2016.02.038.

Zhang, J., Yang, M., Zhong, H., Liu, M., Sui, Q., Zheng, L., Tong, J., Wei, Y., 2018. Deciphering the factors influencing the discrepant fate of antibiotic resistance genes in sludge and water phases during municipal wastewater treatment. Bioresour. Technol. 265, 310–319. https://doi.org/10.1016/j.biortech.2018.06.021.

Zheng, J., Su, C., Zhou, J., Xu, L., Qian, Y., Chen, H., 2017. Effects and mechanisms of ultraviolet, chlorination, and ozone disinfection on antibiotic resistance genes in secondary effluents of municipal wastewater treatment plants. Chem. Eng. J. 317, 309–316. https://doi.org/10.1016/j.cej.2017.02.076.

Zheng, W., Wen, X., Zhang, B., Qiu, Y., 2019. Selective effect and elimination of antibiotics in membrane bioreactor of urban wastewater treatment plant. Sci. Total Environ. 646, 1293–1303. https://doi.org/10.1016/j.scitotenv.2018.07.400.

Zhuang, Y., Ren, H., Geng, J., Zhang, Y., Zhang, Y., Ding, L., Xu, K., 2015. Inactivation of antibiotic resistance genes in municipal wastewater by chlorination, ultraviolet, and ozonation disinfection. Environ. Sci. Pollut. Res. 22 (9), 7037–7044. https://doi.org/10.1007/s11356-014-3919-z.