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ASSESSMENT OF FULL-SCALE TERTIARY WASTEWATER TREATMENT BY UV-C BASED-AOPs: REMOVAL OR PERSISTENCE OF ANTIBIOTICS AND ANTIBIOTIC RESISTANCE GENES?

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ABSTRACT

This research reports for the first time the full-scale application of different homogeneous Advanced Oxidation Processes (AOPs) ($\text{H}_2\text{O}_2/\text{UV-C}$, $\text{PMS}/\text{UV-C}$ and $\text{PMS}/\text{Fe(II)}/\text{UV-C}$) for the removal of antibiotics (ABs) and antibiotic resistance genes (ARGs) from wastewater effluent at Estiviel wastewater treatment plant (WWTP) (Toledo, Spain).

AOPs based on the photolytic decomposition of H_2O_2 and peroxymonosulfate tested at low dosages (0.05 - 0.5 mM) and with very low UV-C contact time (4 - 18 s) demonstrated to be more efficient than UV-C radiation alone on the removal of the analyzed ABs. PMS (0.5 mM) combined with UV-C (7 s contact time) was the most efficient treatment in terms of AB removal: 7 out of 10 ABs detected in the wastewater were removed more efficiently than using the other oxidants. In terms of ARGs removal efficiency, $\text{H}_2\text{O}_2/\text{UV-C}$ seemed the most efficient treatment, although $\text{PMS}/\text{UV-C}$ and $\text{PMS}/\text{Fe(II)}/\text{UV-C}$ were supposed to generate higher concentrations of free radicals. The results show that treatments with the highest removal of ABs and ARGs did not coincide, which could be attributed to the competition between DNA and oxidants in the absorption of UV photons, reducing the direct photolysis of the DNA. Whereas the photolytic ABs removal is improved by the generation of hydroxyl and sulfate radicals, the opposite behavior occurs in the case of ARGs. These results suggest that a compromise between ABs and ARGs removal must be achieved in order to optimize wastewater treatment processes.

Keywords: *sulfate radicals; full-scale; WWTP; antibiotics; resistance genes; UV-C radiation.*

1. Introduction

Antibiotics (ABs), used in human and animal medicine in the treatment and prevention of bacterial infections, represent one of the most important discoveries in the history of medicine (Aminov, 2017). The rise of the human population and its activities have increased the antibiotics consumption up to by 35% between 2000 and 2010 (Van Boeckel et al., 2014). This has contributed to the dissemination of antibiotic residues, antibiotic resistant-bacteria (ARB) and antibiotic resistance genes (ARGs) in the environment, mainly in water bodies (Huerta et al., 2013; Marx et al., 2015; Rodríguez-Mozaz et al., 2015; Becker et al., 2016). Municipal wastewater treatment plants (WWTPs) are major receptors of AB residues, ARB and ARGs. However, conventional wastewater treatment is not able to completely remove these contaminants that consequently will be discharged in the recipient water bodies, and can persist in the environment (Golovko et al., 2014; Zhang et al., 2015; Berendonk et al., 2015; Manaia et al., 2016). The occurrence of these contaminants in the final treated wastewater may be related with the emergence and spread of multidrug-resistant pathogenic bacteria, representing a public health threat (French, 2010; Manaia, 2017).

The implementation of effective complementary treatment processes in WWTPs to remove those emerging contaminants and to avoid their discharge into the receiving water bodies is, thus, a priority. Options for complementary wastewater treatment include the Advanced Oxidation Processes (AOPs). AOPs involve *in situ* generation of highly reactive free radicals, such as hydroxyl ($\text{HO}\cdot$; HR-AOPs) or sulfate ($\text{SO}_4^{\cdot-}$; SR-AOPs), capable of removing a large group of organic chemicals and biological agents. SR-AOPs have attracted attention in the last years as an alternative to HR-AOPs treatments (Rodríguez-Chueca et al., 2017; Yang et al., 2017). Sulfate radicals processes are based on the addition of chemical oxidants as persulfate salts, such as $\text{Na}_2\text{S}_2\text{O}_8$,

$K_2S_2O_8$, and $KHSO_5$ (Wei et al., 2015). Sulfate radicals ($SO_4^{\bullet-}$) can be generated under different activation routes, such as UV, heat, high pH, and transition metal ions or hydroxyl radicals (Table 1). SR-AOPs have the potential to *in situ* decompose water organic contaminants (Shukla et al., 2010; Sharma et al., 2015), with important advantages, such as the safe handling and high solubility in water (Mora et al., 2009). In addition, although their reactivity is pH-dependent, high efficiencies are obtained at neutral pH (Rodríguez-Chueca et al., 2017). Direct photolysis has the potential to damage DNA by interacting with nitrogen bases, as long as the radiation permeates the cell wall and cytoplasm structures. The degree of DNA damage depends on the bacterial cell structure as well as on the overall conditions that may influence radiation absorption (Dodd, 2012). Therefore, in real wastewaters, where such variables are not easily controlled, photolysis efficiency can be potentiated by using UV-C driven AOPs, leading also to the generation of $\cdot OH$ radicals, i.e. through the photolysis of hydrogen peroxide (H_2O_2) when it is irradiated by photons of wavelengths lower than 300 nm. AOPs have clearly proved their efficiency on the removal of AB and ARG at lab scale. However their application on real wastewater matrices at full-scale is challenging and still an area to explore.

This work shows for the first time the comparison of the performance of different UV-C driven HR- and SR-AOPs on the removal of AB and ARG in a full-scale WWTP. The main goal of the study is to assess the feasibility of these treatments to be applied as tertiary treatment using conventional UV-C disinfection reactors already installed.

2. Materials and methods

2.1. Chemicals

Different chemical oxidation treatments (SR-AOPs and HR-AOPs) were carried out using oxidizing concentrations ranging from 0.05 to 0.5 mM, based on previous studies

(Rodríguez-Chueca et al., 2018a). Photolytic and catalytic activation of peroxymonosulfate (HSO_5^- , PMS) were performed. Potassium peroxymonosulfate ($2\text{KHSO}_5 \cdot \text{KHSO}_4 \cdot \text{K}_2\text{SO}_4$) was purchased from Sigma-Aldrich (St. Louis, MO, USA). For catalytic activation of PMS, iron (II) sulfate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) from Panreac (Barcelona, Spain) was used, always in the same molar ratio as PMS. Finally, H_2O_2 (30% w/w) was acquired from Panreac (Barcelona, Spain), for application in the H_2O_2 /UV-C treatments. All the reagents used were analytical grade. Milli-Q[®] water by Merck (Darmstadt, Germany) was used throughout the study.

Antibiotics standards and isotopically labeled compounds used as internal standards were of the highest purity available and were purchased from Sigma-Aldrich, except by azithromycin-d3, sulfamethoxazole-d4, and lincomycin-d3, purchased by Toronto Research Chemicals (Ontario, Canada). The individual standard solutions, as well as the isotopically labeled internal standard solutions were prepared according to Gros et al., 2012. Sulfadimethoxine-d6 and sulfadoxine-d3 were used as surrogate standards. HPLC grade methanol, acetonitrile, water (Lichrosolv[®]) and formic acid 98% were supplied by Merck (Darmstadt, Germany). Ammonium hydroxide and ethylene diamine tetraacetic acid disodium salt solution (Na_2EDTA) at 0.1 M were from Panreac (Barcelona, Spain).

2.2. Wastewater treatment plant and experimental set-up

This research was conducted at the Estiviel WWTP. This plant, operated by FCC Aqualia (Spain), is located in the city of Toledo, Spain. The plant was designed to treat an average flow of 36,000 m^3/day , to deliver services to a population equivalent of 270,000 inhabitants, but it is currently treating an average flow of 12,000 m^3/day . This facility includes tertiary treatment, producing an effluent used for irrigation of gardens surrounding the facility. Tertiary treatment comprises three steps: i) coagulation/flocculation/decantation; ii) rototilter; iii) UV-C radiation, using a

LBX400e UV-C reactor of 16 UV-C lamps (WEDECO ELR-30-1; 330 W). The reactor operates with a maximum emission peak of 254 nm, and it has a total volume of 140 L. Although the reactor is able to treat a maximum water flow rate of 270 m³/h, lower flow rates were used during the oxidation treatments, specifically 28, 75 or 114 m³/h. Therefore, different UV-C dosage (42 – 170 J/L) and contact time (4 – 18 s) were applied. Figure 1 shows the aerial image of the plant and the tertiary treatment scheme, and Table 2 shows the results corresponding to the physical-chemical characterization of inlet, primary and secondary wastewater effluents.

In order to apply the different AOP treatments, it was necessary to install a reagent's dosage system. This system was composed of two dosage pumps (Dostec AC; ITC Dosing Pumps) and two different feed tanks (with 0.3 M reagent's solutions). The reagents were dosed in the overflow after the rotofilter and before the UV-C reactor, where reagents were mixed with wastewater homogeneously.

Treatments were performed during May 2017, modifying the UV-C tertiary treatment (photolysis of H₂O₂ and PMS, and the catalytic activation of PMS with Fe(II) were assessed) and evaluating them in terms of removal efficiency of the target emerging pollutants. Table S1 (Supplementary Material) summarizes the experimental design matrix.

2.3. Analysis of antibiotics

Antibiotics (n = 53) (Table S2, supplementary material) belonging to 10 chemical classes were analyzed following the analytical procedure developed by Gros et al. (2012). The sampling process was carried out using amber glass bottles (1 L) pre-rinsed with ultrapure water. Before sampling, the bottle was rinsed three times with the water to be sampled. The samples were immediately processed in the WWTP laboratory. All

the water sampled were filtered using 1 μm glass fiber filter (Whatman) and 0.45 μm Polyvinylidene Fluoride (PVDF) filter (HVLP04700 Durapore, Millipore). Then, the sample was stabilized by adding 0.1% of Na_2EDTA , and adjusting the pH to 2.5 with HCl 0.1 M. Finally, 50 μL of the surrogate mix was added to each sample in order to have a concentration of 100 ng/L in the effluent wastewaters. After shaking the sample, and 20 minutes for stabilization, samples were pre-concentrated using solid phase extraction (SPE). Briefly, 200 mL of the samples were loaded on Oasis HLB (6 cm^3 , 200 mg) cartridges (Waters Corp. Mildford, MA, USA), which were previously conditioned with 5 mL methanol and 5 mL HPLC grade water. Dried cartridges were stored at -40°C and finally shipped with dry ice to ICRA laboratory. There, elution was done with 6 mL of pure methanol. The extracts were evaporated under a nitrogen stream and reconstituted with 1 mL of methanol–water (10:90 v/v). A volume of 10 μL of internal standard mix at 1 ng/L were added in the extracts for internal standard calibration. Chromatographic separation was carried out with an ultra-performance liquid chromatography (UPLC) system (Waters Corp. Mildford, MA, USA), using an Acquity HSS T3 column (50 mm $\times$ 2.1 mm i.d., 1.7 μm particle size) for the compounds analyzed under positive electrospray ionization (PI) and an Acquity BEH C18 column (50 mm $\times$ 2.1 mm i.d., 1.7 μm particle size) for the ones analyzed under negative electrospray ionization (NI), both from Waters Corporation. The UPLC instrument was coupled to 5500QqLit, triple quadrupole–linear ion trap mass spectrometer (5500 QTRAP, Applied Biosystems, Foster City, CA, USA) with a Turbo V ion spray source. Two MRM transitions per compound were recorded by using the Scheduled MRMTM algorithm and the data were acquired and processed using Analyst 2.1 software.

2.4. Analysis of antibiotic resistance genes

Wastewater samples were collected and transported refrigerated to the laboratory. A volume of 100 mL of each replicate sample was filtered through 0.22 μm polycarbonate Isopore[®] Membrane Filter by Merck (Darmstadt, Germany). Total DNA was extracted from the filtering membranes with the PowerWater DNA isolation kit (MOBIO Laboratories Inc., Carlsbad, CA) and the resulting DNA extracts were shipped at room temperature and then kept refrigerated at -20 °C until they were analyzed.

The 16S rRNA gene and a set of genes herein designated as ARGs were quantified using real-time quantitative PCR (qPCR). ARGs included genes encoding resistance to the antibiotic classes sulfonamides (*sul1* and *sul2*), quinolones (*qnrS*), β -lactams (*bla*_{TEM} and *bla*_{OXA-A}) and the class 1 integron integrase *intI1*. The abundance of the *intI1* was used as a proxy of acquired antibiotic resistance, while the other genes are commonly found in wastewater. The qPCR conditions used are indicated in the Table S3 (Supplementary Material). The DNA extracts were analyzed using the standard curve method as described in Brankatschk *et al.* 2012 in a StepOne[™] Real-Time PCR System (Life Technologies, Carlsbad, CA, USA). ARGs log removal were calculated according to Sousa *et al.*, (2017)

3. Results and discussion

3.1. HR- and SR-AOPs as tertiary treatments

3.1.1. Antibiotics removal

The efficiency in the removal of the ABs from the secondary effluent of the WWTP by means of different tertiary treatments based on UV-C was investigated. Ten out of 53 antibiotics were detected in the inlet of tertiary treatment (AZT, CLAR (macrolides group), CPFX, OFX (fluoroquinolones group), SMX, SFD, SFP (sulfonamides group), TMP (dihydrofolate reductase inhibitors group), MTZ (nitromidazole antibiotics

groups) and CLI (lincosamides group)) (Figure 2A). In spite of their high consumption, the compounds belonging to the classes of penicillin and tetracycline were not detected, probably due to the effective disappearance of these compounds in the conventional treatment of the WWTP through the hydrolysis of β -lactam ring of penicillin or the adsorption of tetracycline in the activated sludge (Graham et al., 2011; Rodríguez-Mozaz et al., 2015; Wang et al., 2018). The efficiency of UV-C radiation using two different flow rates (approximately 28 and 114 m³/h) was studied (Figure 2A). As expected, the lower the flow rate, the higher the removal efficiency, basically due to the higher UV-C contact time, except for CLAR, CPFX, SMX and MTZ, being this anomalous behavior probably consequence of fluctuations in the water composition. CPFX was almost totally removed in all cases (93 and 96% with 18 and 4 s of contact time respectively), whereas, for the rest of ABs, the removal efficiency was never higher than 50% (Figure 2a). Previous reports in the literature have explained the photoreactivity of fluoroquinolones (i.e. CPFX) based on their amino acid character and the donor and acceptor behaviour of their moieties (Bosca, 2015; Jaén-Gil et al., 2018). In general terms, these results are in agreement with those reported by Collado et al. (2014), who also investigated the elimination of pharmaceuticals in a full-scale wastewater treatment plant with a tertiary treatment based on UV.

Oxidation treatments based on hydroxyl and sulfate radicals were first applied using the lowest flow rate (28 m³/h) and a very low oxidant (H₂O₂, PMS) dosage of 0.2 mM, the same dosage of Fe (II) in the case of PMS catalytic activation. The results obtained are represented in Figure 3. Noticeably, the dosage of the oxidant added was not enough to remove ABs at a much higher extent than UV-C radiation alone. Only in the case of clindamycin (CLI) a clear improvement in the performance was observed: from the 7% removal by UV-C radiation alone till 34-72% removal obtained with the 3 AOPs tested,

suggesting a higher sensitivity of this compound to the oxidative degradation. Nevertheless, the photolysis of H_2O_2 was overall the most effective treatment, particularly for CLAR (24%), SMX (26%), SFP (41%) and MTZ (20%). These results can be explained by the 10 times higher reactivity of $HO^\bullet$ than that of the sulfate radicals (Sun et al., 2016). On the other hand, given the mechanisms involved, it was expected that the highest antibiotic degradation should be reached when using UV/Fe(II)/PMS, due to the synergistic effect of UV and Fe(II) in activating PMS. Two main reactions occur coupling PMS/Fe(II) (Eqs. 15 and 16). Fe(II) acts as electron acceptor, provoking the rupture of HO bond in the PMS structure (Eq. 15), while in parallel, the Fe(III) formed from the previous oxidation reaction now acts as an electron acceptor, further activating the PMS (Eq. 16) (Khan et al., 2013):

However the enhancement upon Fe(II) addition is not observed in all cases, such as for instance CLI, with a removal of 47% in presence of Fe(II), and 72% without it. However, this enhancement is observed for the antibiotic AZT, with efficiency of 7% (PMS/UV-C) and 36% (PMS/Fe(II)/UV-C).

With the aim of improving ABs removal and working in more realistic conditions, the reagents dosage was augmented 2.5-fold until 0.5 mM, and flow rate increased from 28 to 75 and 114 m^3/h , being the latest the usual capacity of the WWTP. These operating conditions improved the efficiency in the oxidation treatments (Figure 4A-J) although none of the photolytic oxidation treatments excelled over the others. As expected, in a similar way as for UV-C alone, the average efficiency removal was higher with lower flow rate (75 m^3/h , 7 s contact time) also for the tested AOPs. PMS/UV-C worked better

in the removal of AZT, CLI, OFX and TMP, while $\text{H}_2\text{O}_2/\text{UV-C}$ in the removal of CLAR, SFD and SFP. Finally, CPFEX, MTZ and SMX presented similar removal level using both photolytic treatments.

The addition of Fe(II) as catalyst did not always improve treatments efficiency. As it can be observed in Figures 4G-I, this treatment was the most suitable to remove the three sulfonamides detected in the WWTP, SMX, SFD and SFP, with percentages of 48, 95 and 72% respectively at $75 \text{ m}^3/\text{h}$. This system showed also high removal of MTZ, with an efficiency of 92% at the lowest flow rate (Figure 4E). Finally, in the case of CLI (Figure 4C), PMS/Fe(II)/UV-C system worked satisfactorily, with efficiencies higher than 95%, although at $75 \text{ m}^3/\text{h}$ it reached the same removal level than PMS/UV-C. However, the addition of Fe(II) seemed to work better when the flow rate was increased until $114 \text{ m}^3/\text{h}$, reaching almost total degradation (98%) in comparison with 55% of removal reached by PMS/UV-C system. With the exception of sulfonamides and metronidazole, ABs degradation was not improved with PMS/Fe(II)/UV-C treatment. Speciation of Fe as a function of pH could affect the catalytic activation of PMS. In the presence of oxygen at neutral pH, Fe(II) is subjected to fast conversion to Fe(III), that would be present as ferric (hydr)oxides. However, previous studies demonstrated that the efficiency of the PMS activation process is not so critically affected by this factor (Rodriguez-Chueca et al., 2017). Therefore, the main hypothesis to explain that PMS/Fe(II)/UV-C system did not work better than treatments in the absence of Fe(II) is related with the low UV-C contact time (4 and 7 s), making difficult the regeneration of Fe(II) to continue the free radicals generation cycle.

The activation of PMS using UV-C radiation was the most suitable treatment for the removal of 4 out of 10 compounds (AZT, OFX, TMP, and CLI). In the cases of AZT (Fig. 4A), CLI (Fig. 4C) and OFX (Fig. 4F) the total removal of these ABs was reached

by mean of this system at 75 m³/h. Although only 43% of TMP was removed with PMS/UV-C, this was the most efficient of all the studied treatments. The application of SR-AOPs and HR-AOPs to remove ABs in water has been successfully demonstrated by several authors (Khan et al., 2013; Yin et al., 2018a, 2018b; Feng et al., 2018; Ji et al., 2014; Ding et al., 2017; Matzek et al., 2017; Feng et al., 2015; Pi et al., 2014; Epold et al., 2015). However, in all those studies, the application of treatments was developed at lab-scale, using high-quality water matrix spiked with ABs. The present study constitutes a relevant proof of the feasibility of application of these processes under real conditions of water composition, with usual levels of DOM, bicarbonates, chlorides, microorganisms, turbidity, etc. of a wastewater. Besides, to our knowledge, there are no published information about the successful application of these treatments at full-scale with very low UV-C contact time. Further investigations should focus on the evaluation of the degradation routes of the antibiotics, looking for potential differences of the transformation products depending on the specific hydroxyl or sulfate radical process. In any case, from the experimental results we can conclude that the removal kinetics is strongly depending on the specific compound and also of the oxidant species and oxidative mechanism, as expected for second order reactions involving the attack of the radicals to the antibiotic species.

Table 3 compares the efficiency of all the applied treatments through the use of Electrical Energy per Order, defined as the number of kWh of electrical energy required to reduce the concentration of a pollutant by 1 order of magnitude (90%) (Bolton et al., 2001). The E_{EO} (kWh/m³ order) in continuous mode reactions can be calculated from the equation 17:

$$E_{EO} = \frac{P}{F \cdot \log \left(\frac{C_i}{C_f} \right)} \quad (17)$$

where P is the rated power (kW) of the AOP system, F is the flow rate, C_i and C_f are the initial and final pollutant concentrations (Bolton et al., 2001). As expected, the need of kWh to remove the ABs decreases in applied AOPs, regarding to the need in UV-C treatments. This behavior is similar for all the ABs analyzed. In addition, since the higher amount of oxidant mol per hour that is used, the lower the requirement of kWh, it is possible to increase the efficiency of the system without excessive energy costs. In general terms, the lowest E_{EO} in each antibiotic corresponds with SR-AOPs, meaning a higher efficiency than photolysis of hydrogen peroxide.

3.1.2. Antibiotic resistance genes removal

Figure 2B shows the logarithm transformed values of ARGs abundance, as well as the removal efficiency after different contact times. The bacterial inactivation achieved with only a few seconds of UV exposure was of up to five logs (Rodriguez-Chueca et al, 2018a). However, in terms of ARGs genes removal, the values were less than 1-log (equivalent to a 90% removal). This was observed also for the 16S rRNA gene, a measure of total bacterial DNA. Removal efficiencies of the 16S rRNA gene abundance were higher for longer contact times (18 s) (Figure 2B), reaching a value of 0.85 log-units. With lower contact times (4 s) the 16S rRNA gene removal was lower, of about 0.41 log-units. At these operating conditions (18 s), the log removal for the rest of genes was around 0.7-0.9 log and 0.25 log for the *bla*_{TEM} gene. These results are in agreement with previous studies in full-scale WWTPs that suggested that UV-C disinfection may have a limited action on ARGs, in part due to the UV-C radiation quenching by organic materials in wastewater (Lee et al. 2017).

After a first approach to assess the efficiency of UV-C radiation alone in the removal of ARGs, different AOPs were tested. Figure 5 shows the inlet and outlet abundance and removal efficiency of 16S rRNA gene and of each ARG, by photolysis of hydrogen

peroxide, photolysis of PMS and photocatalytic activation of PMS with Fe(II), using a dosage of reagents of 0.5 mM with two different UV-C contact times (4 and 7 s). Table 4 also shows the removal in prevalence values $\log$ (gene copy number/16S rRNA gene copy) and abundance $\log$ (gene copy number /mL) of genes in the inlet and outlet of tertiary treatment.

The abundance (gene copies/mL) of all ARGs decreased in ca. 0.60 $\log$ (7 s) and 0.21 $\log$ (4 s) (Table 4) when H₂O₂/UV-C tertiary treatment was applied. Unexpectedly, a lower efficiency was observed for PMS/UV-C (except with 4 seconds of contact time, 0.22 $\log$) and PMS/Fe(II)/UV-C gave rise to the lowest removal rate. Longer contact times between hydroxyl radical and both 16S rRNA gene ARGs, seemed to increase their removal rates, as observed in the different studied tertiary treatments with contact times of 7 s compared to 4 s. Contrary to what was expected, the increase of oxidant species in some cases reduced the ARGs removal efficiency, as it was observed for *bla*_{TEM} gene after treatment with PMS as oxidant. To overcome this limitation, the most feasible solution should be the increase of UV-C contact time to avoid the competition of DNA and oxidant species in the absorption of UV photons.

These results of ARGs removal were in agreement with 16S rRNA gene $\log$ removal, of 0.41 $\log$ (4 s) for UV-C; 0.61 $\log$ (7s) and 0.16 $\log$ (4s) for H₂O₂/UV-C; 0.41 $\log$ (7s) and 0.25 $\log$ (4 s) for PMS/UV-C; and 0.03 $\log$ (7 s) and 0.05 $\log$ (4 s) for PMS/Fe(II)/UV-C. Hence, H₂O₂/UV-C (7s) seemed to be the most effective treatment to remove 16S rRNA gene. As expected, it was observed a significant positive correlation between 16S rRNA gene and total ARGs abundance (Pearson r (28) = 0.944, p = 0.005)). A significant positive correlation between the concentration of sulfonamide ABs and their corresponding ARGs (*sul1* and *sul2*) was also observed in the inlet of wastewater effluent (Pearson r (28) = 0.821, p = 0.045)). The abundance of *int11* gene

was an indication of the abundance of genetic elements that may be associated with the incorporation of ARGs. Again, H₂O₂/UV-C (7 s) led to the highest removal rate (Table 4). Similar results were obtained for *sulI*, an indicator of anthropogenic pollution in water (Lu et al., 2015).

Despite PMS/UV-C and PMS/Fe(II)/UV-C are supposed to lead to a higher concentration of radicals compared with of H₂O₂/UV-C, H₂O₂/UV-C seemed the most effective treatment for ARGs removal. Hydroxyl radicals have shown to be effective against different microorganisms by leading to their lysis (Rodríguez-Chueca et al., 2014, 2018a). However, total DNA may maintain integrity, at least for some time, and therefore ARGs may still be targeted in the qPCR reaction and therefore be quantified. Zhang et al. 2016 reported that the addition of Fe(II) as catalysts at concentration values of 1 mg/L, resulted in better performances for ARGs removal than H₂O₂/UV-C alone. So, in agreement with Ferro et al. (2016), either the oxidant dose used may be low or the mechanism of action of hydroxyl radicals on DNA may not be strong enough. The effect on ARGs is expected to be mainly due to UV-C instead of hydroxyl radicals produced during UV/H₂O₂ process. In fact, removal rates of ARGs after PMS/UV-C (0.22 log, 4s) and H₂O₂/UV-C (0.21 log, 4s) were lower than those obtained after UV-C alone (0.41 log, 4 s) (Table 4). Hence, the other treatments based on increasing hydroxyl radical concentration led to lower ARGs removal rates. A possible explanation for these results is that DNA and oxidants compete for the absorption of UV photons, reducing the direct photolysis of the DNA. At a wavelength of 260 nm, the average extinction coefficient (ϵ) for double-stranded DNA is $0.020 (\mu\text{g/mL})^{-1} \text{ cm}^{-1}$ (Sambrook and Russell, 2001), whereas the value of ϵ at this wavelength for H₂O₂ is equivalent to $3.824 \times 10^{-4} (\mu\text{g/mL})^{-1} \text{ cm}^{-1}$. Therefore, although the specific absorption of H₂O₂ is around 50 times lower than that of DNA, its much higher concentration makes the global

absorption of UV photons significantly shifted to H_2O_2 in detriment of DNA. An important result of this study was that under the operating conditions used, the higher the removal of antibiotic compounds, the lower the removal of ARGs. Even though the absolute amounts of ARGs were considerably reduced, the amounts of ARGs discharged by the WWTP amounted to ca. 10^{11} copies/h in all cases (effluent flow rate x ARGs loading). These high and continuous loads in the environment raise public health concerns (Berendonk et al., 2015; Manaia et al., 2016).

To our knowledge, this is the first time that the efficiency of SR-AOPs applied at full-scale in a WWTP on the removal of ARGs was measured. Michael-Kordatou et al. (2018) summarize the most relevant reported works about the removal of ARB and ARGs by AOPs. A large number of authors have studied the efficiency of different AOPs, however, always in pilot or bench-scale conditions. Ferro et al. (2016 and 2017) reported that prolonged time of treatment (higher than 240 minutes) or high H_2O_2 doses (higher than 300 mg/L) were required for the efficient removal of ARGs using surrogate bacteria. The proposed operating conditions by Ferro et al. contrast with those applied in this study with a maximum contact time of 18 s. Yoon et al. (2017) reported the use of photolysis of hydrogen peroxide on the removal of different ARGs, and also concluding that the ARGs damage by H_2O_2 /UV-C treatment is similar to that produced by UV-C radiation, indicating a minor role of hydroxyl radicals for ARGs inactivation. In addition, a previously reported work (Rodríguez-Chueca et al., 2018b) under the same operating conditions to remove micropollutants detected in treated wastewater, proved the economic feasibility of the treatments.

4. Conclusions

This research successfully reported the application of different UV-C driven SR-AOPs at full scale in a real WWTP, using the UV-C tertiary treatment previously designed and installed in the facility for disinfection.

Our results show how AOPs in general improved the efficiency of UV-C on the removal of ABs. When applying the harshest operating conditions (0.5 mM dosage of oxidants and 7 s of UV-C contact time) 3 antibiotics (CPFX, MTZ and SMX) were similarly removed using PMS and H₂O₂ as oxidants, 4 antibiotics (AZT, CLI, OFX and TMP) were removed more efficiently using PMS and 3 (CLAR, SFD and SFP) using H₂O₂. Although PMS/Fe(II)/UV-C was expected to offer the highest efficiency, the addition of Fe(II) only improved the degradation of 4 antibiotics (SMX, SFD and SFP and MTZ) compared to the photolytic process.

In terms of ARGs removal, despite the fact that PMS/UV-C and PMS/Fe(II)/UV-C were supposed to lead to a higher concentration of free radicals the highest inactivation was actually obtained without adding any oxidant, being UV-C treatment the most efficient treatment followed by PMS/UV-C and H₂O₂/UV-C.

In general, it was observed that the higher antibiotics removal corresponded to lower ARGs removal values: The generation of hydroxyl and sulfate radicals, triggered by light absorption of the oxidants, actually reduces the availability of photons for photolytic degradation processes. In this scenario, the oxidative removal of ABs by the radicals is much more effective than photolytic process and thus their removal efficiency increases compared to UV-C treatment. In contrast, the direct photolysis of

the DNA (which seems to be more effective than oxidative removal) is reduced, and accordingly ARGs removal efficiency.

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Table 1. Main reactions occurring in the generation of sulfate radicals.

Oxidant	Reaction		Rate	Reference
Peroxydisulfate (PDS)	(1)	$HSO_5^- \xrightarrow{h\nu} SO_4^{\cdot-} + OH^-$		Anipsitakis and Dionysiou, 2004
	(2)	$Fe^{2+} + HSO_5^- \rightarrow Fe^{3+} + SO_4^{\cdot-} + OH^-$	$3.0 \cdot 10^4 \text{ M}^{-1} \cdot \text{s}^{-1}$	Rastogi et al., 2009
	(3)	$SO_4^{\cdot-} + OH^- \rightarrow SO_4^{2-} + OH^\cdot$	$(6.5 \pm 1.0) \cdot 10^7 \text{ M}^{-1} \cdot \text{s}^{-1}$	Ji et al., 2014
	(4)	$SO_4^{2-} + OH^\cdot \rightarrow SO_4^{\cdot-} + OH^-$	$1.18 \cdot 10^6 \text{ M}^{-1} \cdot \text{s}^{-1}$	Khan et al., 2013
	(5)	$Fe^{2+} + SO_4^{\cdot-} \rightarrow Fe^{3+} + SO_4^{2-}$	$3.0 \cdot 10^8 \text{ M}^{-1} \cdot \text{s}^{-1}$	Rastogi et al., 2009
	(6)	$Fe^{2+} + OH^\cdot \rightarrow Fe^{3+} + OH^-$	$4.3 \cdot 10^8 \text{ M}^{-1} \cdot \text{s}^{-1}$	Khan et al., 2013
	(7)	$SO_4^{\cdot-} + \text{Antibiotics} \rightarrow \text{products} + CO_2 + H_2O$		
	(8)	$OH^\cdot + \text{Antibiotics} \rightarrow \text{products} + CO_2 + H_2O$		
	(9)	$SO_4^{\cdot-} + OH^\cdot \rightarrow HSO_5^-$	$(0.95 \pm 0.08) \cdot 10^{10} \text{ M}^{-1} \cdot \text{s}^{-1}$	Klaning et al., 1991
Persulfate (PS)	(10)	$S_2O_8^{2-} \xrightarrow{h\nu} 2SO_4^{\cdot-}$		Antoniou et al., 2010
	(11)	$Fe^{2+} + S_2O_8^{2-} \rightarrow Fe^{3+} + SO_4^{\cdot-} + SO_4^{2-}$		Bennedsen et al., 2012
	(12)	$SO_4^{2-} + OH^\cdot \rightarrow SO_4^{\cdot-} + OH^-$	$1.18 \cdot 10^6 \text{ M}^{-1} \cdot \text{s}^{-1}$	Antoniou et al., 2010

	(13)	$SO_4^{\cdot-} + \textit{Antibiotics} \rightarrow \textit{products} + CO_2 + H_2O$		
	(14)	$OH^{\cdot} + \textit{Antibiotics} \rightarrow \textit{products} + CO_2 + H_2O$		
	(15)	$SO_4^{\cdot-} + SO_4^{\cdot-} \rightarrow S_2O_8^{2-}$	$(0.44 \pm 0.4) \cdot 10^8 \text{ M}^{-1} \cdot \text{s}^{-1}$	Huie and Clifton, 1993

Table 2. Physical-chemical characterization of wastewater in the WWTP (May 2017).

Parameter	Inlet	Outlet primary treatment	Outlet secondary treatment
pH	7.62 ± 0.07	7.51 ± 0.06	7.17 ± 0.04
Conductivity ($\mu\text{S}/\text{cm}$)	1330 ± 59	1290 ± 71	990 ± 44
Turbidity (NTU)	330 ± 60	170 ± 13	3 ± 0.46
Suspended solids (mg/L)	320 ± 80	120 ± 20	6 ± 3
BOD (mg/L)	340 ± 64	280 ± 53	8 ± 3
COD (mgO_2/L)	670 ± 160	510 ± 77	27 ± 3
Total Nitrogen (mg/L)	80 ± 2	71 ± 1	7 ± 1
Total Phosphorus (mg/L)	12 ± 2	9 ± 1	0.76 ± 0.14
Ammonia (mg/L)	63 ± 4	58 ± 2	0.32 ± 0.49
Nitrates (mg/L)	0.99 ± 0.02	0.85 ± 0.28	6 ± 1

Table 3. Electrical Energy per Order (E_{EO}) in the removal of MPs with the different UV-C treatments.

Compound	Treatment	E_{EO}			
		0 mol/h	5.6 mol/h	37.5 mol/h	57 mol/h
AZT	UV-C	1.44			
	H ₂ O ₂ /UV-C		1.40	0.37	5.88
	PMS/UV-C		5.61	0.02	0.06
	PMS/Fe(II)/UV-C		0.91	0.65	0.55
CLAR	UV-C	17.78			
	H ₂ O ₂ /UV-C		1.48	0.38	2.24
	PMS/UV-C		52.33	1.35	n.d.
	PMS/Fe(II)/UV-C		28.21	2.84	2.08
CPFX	UV-C	0.16			
	H ₂ O ₂ /UV-C		0.17	0.05	0.03
	PMS/UV-C		0.12	0.04	0.03
	PMS/Fe(II)/UV-C		0.11	0.04	0.03
OFX	UV-C	0.75			
	H ₂ O ₂ /UV-C		0.66	0.24	1.34
	PMS/UV-C		0.72	0.02	0.08
	PMS/Fe(II)/UV-C		2.09	3.78	n.d.
SMX	UV-C	5.03			
	H ₂ O ₂ /UV-C		1.36	2.30	0.18
	PMS/UV-C		1.81	2.00	0.63
	PMS/Fe(II)/UV-C		7.14	0.23	0.52

SFD	UV-C	1.13		
	H ₂ O ₂ /UV-C	1.74	0.16	0.42
	PMS/UV-C	7.05	0.47	0.34
	PMS/Fe(II)/UV-C	8.35	0.05	0.03
SFP	UV-C	1.75		
	H ₂ O ₂ /UV-C	0.78	0.26	7.65
	PMS/UV-C	2.36	0.58	1.03
	PMS/Fe(II)/UV-C	1.52	0.12	0.17
TMP	UV-C	1.17		
	H ₂ O ₂ /UV-C	0.99	0.74	2.46
	PMS/UV-C	2.21	0.28	0.22
	PMS/Fe(II)/UV-C	3.26	n.d.	n.d.
MTZ	UV-C	-6.61		
	H ₂ O ₂ /UV-C	1.90	0.45	1.48
	PMS/UV-C	4.96	0.47	9.91
	PMS/Fe(II)/UV-C	3.71	0.06	0.21
CLI	UV-C	5.58		
	H ₂ O ₂ /UV-C	0.99	0.30	0.53
	PMS/UV-C	0.32	0.02	0.13
	PMS/Fe(II)/UV-C	0.65	0.04	0.03

Table 4. Genes log removal after the application of UV-C, H₂O₂/UV-C, PMS/UV-C and PMS/Fe(II)/UV-C treatments (Flow rate = 114 m³/h; UV-C dosage = 42 J/L; UV-C contact time = 4 s; [Reagents] = 0.5 mM).

Gene	ARGs prevalence in Log(gene copy number /16S rRNA gene copy number)			Abundance in Log(gene copy number/mL)		
	UV-C (4 s)			UV-C (4 s)		
	Inlet	Outlet	Log removal	Inlet	Outlet	Log removal
16S rRNA				5.21 ± 0.11	4.80 ± 0.40	0.41
<i>intI1</i>	-1.70 ± 0.12	-1.73 ± 0.08	0.03	3.51 ± 0.12	3.07 ± 0.08	0.44
<i>sul1</i>	-1.97 ± 0.03	-2.04 ± 0.05	0.07	3.24 ± 0.12	2.75 ± 0.09	0.49
<i>sul2</i>	-1.82 ± 0.06	-1.80 ± 0.05	-0.03	3.39 ± 0.17	3.00 ± 0.12	0.39
<i>bla</i> _{OXA-A}	-2.95 ± 0.09	-3.00 ± 0.09	0.05	2.26 ± 0.18	1.80 ± 0.11	0.47
<i>bla</i> _{TEM}	-3.23 ± 0.07	-2.98 ± 0.07	-0.26	1.98 ± 0.05	1.82 ± 0.09	0.16
<i>qnrS</i>	-2.86 ± 0.14	-3.05 ± 0.10	0.19	2.35 ± 0.24	1.75 ± 0.13	0.60
Total *	-1.31	-1.38	0.07	5.24	4.83	0.41

* Total values calculated as the log10 of the sum of the six genes

Gene	ARGs prevalence in Log(gene copy number /16S rRNA gene copy number)						Abundance in Log(gene copy number/mL)					
	H ₂ O ₂ /UV-C (7 s)			H ₂ O ₂ /UV-C (4 s)			H ₂ O ₂ /UV-C (7 s)			H ₂ O ₂ /UV-C (4 s)		
	Inlet	Outlet	Removal	Inlet	Outlet	Log removal	Inlet	Outlet	Removal	Inlet	Outlet	Log removal
16S rRNA							5.26 ± 0.18	4.64 ± 0.22	0.61	5.04 ± 0.32	4.89 ± 0.24	0.16
<i>intI1</i>	-1.80 ± 0.18	-1.74 ± 0.17	-0.06	-1.72 ± 0.12	-1.98 ± 0.10	0.26	3.45 ± 0.18	2.90 ± 0.17	0.56	3.32 ± 0.12	2.91 ± 0.10	0.42
<i>sul1</i>	-1.99 ± 0.01	-2.03 ± 0.07	0.05	-1.86 ± 0.35	-2.21 ± 0.19	0.35	3.27 ± 0.19	2.61 ± 0.16	0.66	3.18 ± 0.13	2.68 ± 0.05	0.51
<i>sul2</i>	-1.94 ± 0.04	-1.90 ± 0.07	-0.04	-1.73 ± 0.33	-2.04 ± 0.17	0.31	3.31 ± 0.21	2.74 ± 0.16	0.57	3.31 ± 0.16	2.84 ± 0.07	0.47
<i>bla_{OXA-A}</i>	-3.00 ± 0.06	-3.11 ± 0.06	0.10	-2.86 ± 0.35	-3.19 ± 0.13	0.33	2.25 ± 0.21	1.68 ± 0.04	0.58	2.18 ± 0.15	1.70 ± 0.12	0.49
<i>bla_{TEM}</i>	-3.29 ± 0.19	-2.94 ± 0.24	-0.35	-3.11 ± 0.31	-3.10 ± 0.28	-0.01	1.97 ± 0.11	1.70 ± 0.05	0.27	1.94 ± 0.07	1.79 ± 0.09	0.15
<i>qnrS</i>	-3.11 ± 0.11	-3.21 ± 0.08	0.10	-3.18 ± 0.41	-3.29 ± 0.15	0.21	2.15 ± 0.23	1.57 ± 0.06	0.57	1.97 ± 0.09	1.60 ± 0.09	0.36
Total *	-1.41	-1.38	-0.03	-1.27	-1.56	0.29	5.30	4.70	0.60	5.16	4.95	0.21

* Total values calculated as the log10 of the sum of the six genes

Gene	ARGs prevalence in Log(gene copy number /16S rRNA gene copy number)						Abundance in Log(gene copy number/mL)					
	PMS/UV-C (7 s)			PMS/UV-C (4 s)			PMS/UV-C (7 s)			PMS/UV-C (4 s)		
	Inlet	Outlet	Removal	Inlet	Outlet	Log removal	Inlet	Outlet	Removal	Inlet	Outlet	Log removal
16S rRNA							5.27 ± 0.04	4.85 ± 0.10	0.41	5.44 ± 0.08	5.19 ± 0.20	0.25
<i>intI1</i>	-1.75 ± 0.03	-1.80 ± 0.03	0.04	-1.91 ± 0.21	-1.92 ± 0.23	0.02	3.51 ± 0.03	3.06 ± 0.03	0.45	3.54 ± 0.21	3.27 ± 0.23	0.27
<i>sul1</i>	-2.07 ± 0.06	-2.04 ± 0.02	-0.03	-1.85 ± 0.12	-1.77 ± 0.02	-0.07	3.20 ± 0.04	2.81 ± 0.10	0.39	3.59 ± 0.19	3.42 ± 0.18	0.18
<i>sul2</i>	-2.00 ± 0.06	-1.92 ± 0.04	-0.08	-2.08 ± 0.12	-1.86 ± 0.02	-0.21	3.27 ± 0.05	2.93 ± 0.09	0.34	3.37 ± 0.19	3.33 ± 0.18	0.04
<i>bla_{OXA-A}</i>	-3.00 ± 0.08	-2.91 ± 0.07	-0.09	-2.92 ± 0.09	-2.86 ± 0.04	-0.07	2.27 ± 0.06	1.95 ± 0.13	0.32	2.52 ± 0.16	2.33 ± 0.23	0.19
<i>bla_{TEM}</i>	-3.29 ± 0.08	-2.93 ± 0.16	-0.36	-3.54 ± 0.02	-3.30 ± 0.12	-0.23	1.97 ± 0.07	1.92 ± 0.07	0.05	1.91 ± 0.09	1.89 ± 0.08	0.02
<i>qnrS</i>	-3.18 ± 0.18	-2.99 ± 0.10	-0.18	-3.14 ± 0.13	-2.95 ± 0.11	-0.18	2.09 ± 0.18	1.86 ± 0.13	0.23	2.31 ± 0.19	2.24 ± 0.17	0.07
Total *	-1.40	-1.40	0	-1.42	-1.47	0.05	5.28	4.88	0.40	5.46	5.24	0.22
Gene	PMS/UV-C (7 s)			PMS/UV-C (4 s)			PMS/UV-C (7 s)			PMS/UV-C (4 s)		
<i>16S rRNA</i>							4.70 ± 0.28	4.68 ± 0.40	0.03	4.86 ± 0.11	4.81 ± 0.22	0.05
<i>intI1</i>	-2.12 ± 0.39	-2.27 ± 0.62	0.16	-2.13 ± 0.15	-2.07 ± 0.29	-13	2.59 ± 0.39	2.40 ± 0.62	0.18	2.73 ± 0.15	2.73 ± 0.29	0.00

<i>sul1</i>	-2.21 ± 0.21	-2.37 ± 0.31	0.16	-2.14 ± 0.03	-2.07 ± 0.10	-16	2.49 ± 0.47	2.31 ± 0.69	0.19	2.72 ± 0.12	2.74 ± 0.24	-0.01
<i>sul2</i>	-2.14 ± 0.10	-2.28 ± 0.19	0.14	-2.13 ± 0.03	-2.13 ± 0.07	24	2.56 ± 0.38	2.40 ± 0.58	0.16	2.73 ± 0.13	2.68 ± 0.27	0.06
<i>bla_{OXA-A}</i>	-3.04 ± 0.05	-3.07 ± 0.00	0.02	-3.28 ± 0.11	-3.13 ± 0.05	-41	1.95 ± 0.05	2.03 ± 0.02	-0.08	1.59 ± 0.06	1.96 ± 0.04	-0.37
<i>bla_{TEM}</i>	-2.99 ± 0.26	-3.00 ± 0.24	0.01	-3.14 ± 0.12	-3.12 ± 0.23	-4	1.71 ± 0.05	1.68 ± 0.07	0.03	1.72 ± 0.02	1.69 ± 0.05	0.04
<i>qnrS</i>	-3.29 ± 0.07	-3.24 ± 0.03	-0.05	-3.23 ± 0.15	-3.29 ± 0.09	-15	1.71 ± 0.07	1.86 ± 0.02	-0.15	1.56 ± 0.10	1.79 ± 0.09	-0.23
Total *	-1.68	-1.72	0.04	-1.61	-1.60	-0.01	4.78	4.82	-0.04	4.88	4.86	0.02

* Total values calculated as the log10 of the sum of the six genes

Figure captions

Figure 1. Aerial image of Estiviel WWTP (Spain) and diagram of tertiary treatment.

Figure 2. Results of the UV-C treatment at two different flow rates: 28 m³/h (170 J/L, 18 s) and 114 m³/h (42 J/L, 4 s). A) Antibiotics concentration (columns) and percentage of removal efficiency (lines). B) Antibiotic resistance genes (columns) and log removal values (lines).

Figure 3. Antibiotics concentration in the inlet and outlet of tertiary treatment after the application of UV-C, H₂O₂/UV-C, PMS/UV-C and PMS/Fe(II)/UV-C treatments (Flow rate = 28 m³/h; UV-C dosage = 170 J/L; UV-C contact time = 18 s; [Reagents] = 0.2 mM).

Figure 4. Antibiotics concentration (columns) in the inlet and outlet of tertiary treatment (H₂O₂/UV-C; PMS/UV-C; PMS/Fe(II)/UV-C), and percentage of removal efficiency (lines) at two different flow rates: 75 (64 J/L, 7 s) and 114 m³/h (42 J/L, 4 s). [Reagents] = 0.5 mM.

Figure 5. Antibiotic resistance genes (columns) in the inlet and outlet of tertiary treatment (H₂O₂/UV-C; PMS/UV-C; PMS/Fe(II)/UV-C), and log removal values (lines) at two different flow rates: 75 (64 J/L, 7 s) and 114 m³/h (42 J/L, 4 s). [Reagents] = 0.5 mM.

Highlights

- Wastewater treatment by Advanced Oxidation Processes studied at full-scale
- Removal of antibiotic residues and antibiotic resistance genes assessed
- Oxidants photolysis proved more efficient than UV-C alone for antibiotics removal
- Sulfate radical based processes did not improve the ARGs removal reached by UV-C
- Competition between DNA and oxidants for absorption of UV photons is hypothesized

Tertiary Treatment

Figure 1

Figure 2

Figure 3

Figure 4af

Figure 4g

Figure 5