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Review

The role of operating parameters and oxidative damage mechanisms of advanced chemical oxidation processes in the combat against antibiotic-resistant bacteria and resistance genes present in urban wastewater

I. Michael-Kordatou [a], P. Karaolia [a, b], D. Fatta-Kassinos [a, b, *]

[a] Nireas-International Water Research Centre, University of Cyprus, P.O. Box 20537, CY-1678, Nicosia, Cyprus
[b] Department of Civil and Environmental Engineering University of Cyprus, P.O. Box 20537, CY-1678, Nicosia, Cyprus

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ABSTRACT

An upsurge in the study of antibiotic resistance in the environment has been observed in the last decade. Nowadays, it is becoming increasingly clear that urban wastewater is a key source of antibiotic resistance determinants, i.e. antibiotic-resistant bacteria and antibiotic resistance genes (ARB&ARGs). Urban wastewater reuse has arisen as an important component of water resources management in the European Union and worldwide to address prolonged water scarcity issues. Especially, biological wastewater treatment processes (i.e. conventional activated sludge), which are widely applied in urban wastewater treatment plants, have been shown to provide an ideal environment for the evolution and spread of antibiotic resistance. The ability of advanced chemical oxidation processes (AOPs), e.g. light-driven oxidation in the presence of H_2O_2, ozonation, homogeneous and heterogeneous photocatalysis, to inactivate ARB and remove ARGs in wastewater effluents has not been yet evaluated through a systematic and integrated approach. Consequently, this review seeks to provide an extensive and critical appraisal on the assessment of the efficiency of these processes in inactivating ARB and removing ARGs in wastewater effluents, based on recent available scientific literature. It tries to elucidate how the key operating conditions may affect the process efficiency, while pinpointing potential areas for further research and major knowledge gaps which need to be addressed. Also, this review aims at shedding light on the main oxidative damage pathways involved in the inactivation of ARB and removal of ARGs by these processes. In general, the lack and/or heterogeneity of the available scientific data, as well as the different methodological approaches applied in the various studies, make difficult the accurate evaluation of the efficiency of the processes applied. Besides the operating conditions, the variable behavior observed by the various examined genetic constituents of the microbial community, may be directed by the process distinct oxidative damage mechanisms in place during the application of each treatment technology. For example, it was shown in various studies that the majority of cellular damage by advanced chemical oxidation may be on cell wall and membrane structures of the targeted bacteria, leaving the internal components of the cells relatively intact/able to repair damage. As a result, further in-depth mechanistic studies are required, to establish the optimum operating conditions under which oxidative mechanisms target internal cell components such as genetic material and ribosomal structures more intensively, thus conferring permanent damage and/or death and preventing potential post-treatment re-growth.

Main Abbreviations: **ARB**, Antibiotic-Resistant Bacteria; **ARGs**, Antibiotic Resistance Genes; **AOPs**, Advanced Chemical Oxidation Processes; **CAS**, Conventional Activated Sludge; **CLSI**, Clinical and Laboratory Standards Institute; **COD**, Chemical Oxygen Demand; **dE_fOM**, Dissolved Effluent Organic Matter; **DGGE**, Denaturing Gradient Gel Electrophoresis; **HGT**, Horizontal Gene Transfer; **MIC(s)**, Minimum Inhibitory Concentration(s); **MGEs**, Mobile Genetic Elements; **PCR**, Polymerase Chain Reaction; **PMA**, Propidium Monoazide; **ROS**, Reactive Oxygen Species; **RW**, Real Urban Wastewater Effluents; **SW**, Synthetic Urban Wastewater Effluents; **UN**, United Nations; **UWTPs**, Urban Wastewater Treatment Plants; **VBNC**, Viable But Not Cultivable; **WHO**, World Health Organisation.

* Corresponding author. Nireas-International Water Research Centre, University of Cyprus, P.O. Box 20537, CY-1678, Nicosia, Cyprus.
E-mail address: dfatta@ucy.ac.cy (D. Fatta-Kassinos).

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Contents

1. Current state of knowledge

► *General introduction and relevant terminology*

Despite the already diverse natural background of antibiotic-resistant bacteria and antibiotic resistance genes (ARB&ARGs), the intensive use and misuse of antibiotics are inextricably linked to the development and enrichment of an unexplored pool of ARB&ARGs not only in clinical settings but also in environmental compartments (Baquero et al., 2008; Berendonk et al., 2015). The development of antibiotic resistance traits amongst bacterial populations by horizontal gene transfer (HGT)-mediated processes which include conjugation, transduction, and natural transformation, as well as by vertical transfer (i.e., transfer of genetic material from parent cell to offspring) (Dodd, 2012), is considered to have been driven by the selective pressure of antibiotics used in therapeutic settings. The first identified incidence of antibiotic resistance occurred in 1930s to sulfonamides (Davies and Davies, 2010). Over the years, and continuing into the present, almost every known commensal and pathogenic bacterial species has acquired the ability to develop resistance to one or more antibiotics (Clatworthy et al., 2007).

The minimum inhibitory concentration (MIC) was determined as the ″*lowest concentration of the antibiotic that, under established in vitro conditions, inhibits visible growth of a target bacterial population*″ (CLSI, 2012; Andersson and Hughes, 2014), and thus ARB are defined as the bacteria that are able to visibly grow in agar media, in the presence of the determined MIC of the antibiotic of interest. The term ″ARGs″ refers to genes encoding resistance to antibiotics, and is the term used in the studies examined in this review. ARB possess unique features compared to antibiotics and ARGs as they have an ability to respond to stress, move and proliferate under specific conditions, among others. As bacterial communities are shaped by a complex array of environmental- and human-derived factors, it is difficult to predict the fate of ARB&ARGs that are released into the environment. Recent work shows that the function of these antibiotic resistance determinants in their natural habitats is likely to differentiate from their ″weapon-shield″ function when present in clinical settings (Martínez, 2008). This may be attributed to two functional characteristics: (i) the regulation of their expression in the pathogen is not equivalent to that in the original host, and (ii) after the dissemination of these antibiotic resistance determinants their integration in the novel metabolic host networks would be difficult, and their potential role will be resistance (Martínez, 2008). Bacteria incorporating genes harbouring resistance to fluoroquinolones (Cattoir et al., 2008; Poirel et al., 2005), macrolides (Roberts, 2011), sulfonamides (Heuer et al., 2011) and trimethoprim (Stoll et al., 2012) have been isolated from various environments. Moreover, the integron (*intI1*), a genetic platform able to capture and express gene cassettes, is a widely investigated element which has been found to mediate environmental ARGs dissemination. Also, mobile genetic elements (MGEs), such as those able to transfer genes between DNA molecules, i.e. mobile elements, for example insertion sequences, integrons and transposons, and those able to transfer between cells, for example, conjugative and mobilizable plasmids and integrative conjugative elements, play an important role in the evolution of bacterial genomes and the adaptation of microbial populations to specific environmental stress (Partridge, 2011).

The World Health Organisation (WHO) underlines that antibiotic resistance is complex and multidimensional, and the identification and surveillance of critical hotspots that can also foster resistance is neither coordinated nor harmonized, compromising the ability to assess and monitor the posed threat (WHO, 2014). Alert to this crisis, the May 2015 WHO Assembly adopted a global action plan consistent with the precautionary principle, which underscores the need for an effective ″One Health″ approach involving coherent and concerted multisectoral actions, to counter antibiotic resistance at diverse levels (WHO, 2015; EU, 2017). Recently, the UN acknowledged as imperative the need to tackle the impending multi-faceted issues related to antibiotic resistance at a political level by implementing national action plans encompassing long-term threat to human health and sustainable food production (UN, 2016). The EU has also set up the Joint Programming Initiative on antibiotic resistance (http://www.jpiamr.eu), which aims to better coordinate and align worldwide the research efforts on this topic.

Urban wastewater is regarded as one of the predominant anthropogenic sources of ARB&ARGs dissemination into the environment (Rizzo et al., 2013), and the consequences can be potentially compounded by the reuse of treated wastewater, a practice increasingly suggested as a way to address water scarcity in the EU and worldwide. Urban wastewater treatment plants (UWTPs) are also hotspots of clinically-derived ARB&ARGs, such as colistin resistance gene (*mcr-1*) and carbapenemase genes (bla_{TEM}, *CTX-M*, *CTX-M-32*, and *CMY-2*) in Gram-negative opportunistic pathogens (e.g. *Klebsiella pneumoniae*, *Acinetobacter baumannii*) (Hembach et al., 2017). It is widely accepted that the biological wastewater

treatment processes (i.e., conventional activated sludge [CAS]) currently applied in UWTPs create an environment potentially conducive to antibiotic resistance development, since human-commensal- and environmentally-relevant bacterial communities are in close contact, facilitating thus the potential generation and proliferation of new resistant strains via HGT. Commensal bacteria are those bacteria that live in or upon the host without causing disease. Mostly, this co-existence is of mutual benefit. However, many commensals can cause disease if they enter body sites that are normally sterile or when the host's immune defence is impaired (EFSA, 2011). On the other hand, environmentally-relevant bacteria are those which thrive in natural environments, and their diversity is much higher in such niche environments compared to the bacteria which are routinely isolated in the laboratory through conventional cultivation methods (Watanabe and Baker, 2000).

While ARGs in their environmental context may originally have had other primary functions aside from conferring resistance to antibiotics, these genes have now been recruited as resistance genes in pathogenic environmental bacteria. Furthermore, environmental bacteria harbour an as of yet unexplored pool of genes (Berglund, 2015). It was estimated that high loads of cultivable ARB (approx. 10^{12} per day) are continuously discharged to the receiving environment by a conventional UWTP (Vaz-Moreira et al., 2014). It is also likely that the acquisition of antibiotic resistance is triggered by other environmental microcontaminants, such as heavy metals and biocides, which have the ability to promote co-selection processes (i.e. the combined selection pressures exerted by two or more types of agents on a single organism), indirectly selecting for ARB&ARGs (Seiler and Berendonk, 2007). On the other hand, Bengtsson-Palme et al. (2016) reported no strong evidence for co-selection of biocide and metal resistance, along with antibiotic resistance, using a shotgun metagenomic sequencing of samples collected from different treatment steps in three Swedish UWTPs. Although antibiotic concentrations seldom or never reach minimum inhibitory concentrations (MICs) in urban wastewater (Michael et al., 2013a), recent evidence suggests that levels far below the MIC concentrations (sub-MIC) can potentially exert a selective pressure on bacterial populations (Andersson and Hughes, 2014; Lundström et al., 2016). While it is yet unclear how the microbial community is affected by the sub-MIC levels of antibiotics in complex environmentally-relevant matrices such as wastewater, caution is required as recent studies have revealed the *in vitro* capacity of sub-lethal concentrations of antibiotics to facilitate HGT and induce antibiotic resistance in bacterial communities (Gullberg et al., 2011). However, the current knowledge on the ability of sub-MIC concentrations to select ARB barely is sufficient and these findings cannot be regarded as concrete evidences of such cause-effect relationships. Manaia et al. (2016) reported that the potential of sub-MIC levels of antibiotics to select ARB can be limited given the complexity of the wastewater matrix in terms of dissolved effluent organic matter (dE_fOM), microbiota, and nutrients, which may reduce the bioavailability of antibiotic residues on target bacteria. It is also possible that various biotic and abiotic factors prevailing during wastewater treatment shape the bacterial communities in such a way that species carrying certain types of resistance genes increase in abundance by means of HGT processes even in the absence of any selection pressure from antibiotics (Rodríguez-Rojas et al., 2013). A novel high-throughput analysis was recently applied by Gatica et al. (2016) to acquire a comprehensive overview of integron gene cassette composition in wastewater effluents across Europe, with bla_{OXA} and bla_{GES} being the most prevalent genes in all effluents. In contrast to antibiotics, the concentration of which typically diminishes owing to dilution, degradation or transformation, ARB&ARGs have been shown to be capable of persisting and even disseminating in the receiving environment (Cantas et al., 2013; Berglund, 2015), including soil (Becerra-Castro et al., 2015), surface water (Schwartz et al., 2003; Zhang et al., 2009), and groundwater (Gallert et al., 2005). The presence of ARB in natural environmental ecosystems was also recently substantiated in a transnational river survey (2500 rkm) by Kittinger et al. (2016), who demonstrated the occurrence of waterborne *Pseudomonas* spp. conferring resistance to various antibiotics in the River Danube. More recently, Zhu et al. (2017) reported the continental-scale pollution of estuaries with a large range of different ARGs (18 estuaries over 4000 km of coastal China), with over 200 different resistance genes being detected. In addition, Lekunberri et al. (2017) quantified nine ARGs in bacterial, phage and plasmid DNA fractions in water samples collected downstream of the discharge point from an UWTP into the Ter River in Spain.

► *Advanced Chemical Oxidation Processes vs ARB&ARGs*

The crucial question that must be asked in this framework is ″***Are there disinfection processes capable of hindering genetic antibiotic resistance determinants abundance in parallel with the effective inactivation of ARB?***″. While the current state of knowledge evidences the need to improve the common treatment technologies currently applied in UWTPs for disinfection purposes (Pruden et al., 2013), such as chlorination and UV processes (Di Cesare et al., 2016), a big gap still exists with regard to the operating parameters that may influence the inactivation and the removal mechanisms of ARB&ARGs during the application of such processes. Moreover, the application of new disinfection technologies, such as advanced chemical oxidation processes (AOPs) (e.g. UV/H_2O_2, ozonation, homogeneous and heterogeneous photocatalysis), has gained attention during the last years for the inactivation of ARB and the removal of ARGs in urban wastewater; nonetheless the relatively scarce number of studies conducted, indicates knowledge is still lacking in the field.

In general, the extent to which the various AOPs inactivate ARB&ARGs is driven by various factors, such as the distinct mechanisms of each treatment technology and its operating conditions (e.g. disinfectant dose, contact time), as well as their target bacterial cell structure and their molecular characteristics (Bouki et al., 2013), which affect the bacterial susceptibility towards the specific treatment stress conditions applied. The ability to characterize the developed resistance in bacteria during disinfection and to elucidate the fundamental mechanisms is necessary to ensure that monitoring and diagnostic tools and methods remain updated. A challenging issue is that advanced chemical oxidation, despite being promising with regard to bacterial inactivation, may select for bacteria with higher capacity to resist stress conditions, thereby to also lead to the emergence and spread of ARB&ARGs (Rizzo et al., 2013). The operating conditions of these processes cause stressful oxidative conditions on the bacterial cells, damaging vital components such as cell walls and membranes, enzymes and DNA (Süss et al., 2009), thus selecting for resilient strains with higher capacity to resist oxidative stress conditions (e.g. enhanced defence mechanisms, HGT, or mutations). In some cases, AOPs have the potential to increase the relative abundance of ARB&ARGs, even in the absence of direct selection by a specific antibiotic compound (Rizzo et al., 2013). Moreover, bacterial populations may possess the ability to survive such treatment processes through the induction of defense and repair mechanisms (Alexander et al., 2016).

One important gap of knowledge is the general lack of protocols under which an advanced chemical oxidation process should be evaluated with respect to the inactivation of ARB and the removal of ARGs. For the selection of antibiotic resistance determinants as possible indicators to assess the status of antibiotic resistance in

environmental settings, criteria such as the clinical relevance, the background concentration, the prevalence in the environment, the association with mobile genetic elements (MGEs) and/or the potential to be acquired by any mode of HGT should be taken into consideration (Berendonk et al., 2015). The selection of these criteria was one of the most important focuses of the European COST Action DARE TD0803 (*Detecting evolutionary hotspots of antibiotic resistance in Europe*, 2009–2013, http://www.cost.eu/COST_Actions/essem/TD0803), followed by the NEREUS ES1403 COST Action (*New and emerging challenges and opportunities in wastewater reuse*, 2014–2018, http://www.nereus-cost.eu/).

► ***Methodological approaches for the identification and quantification of ARB&ARGs***

To date, there is a diversity of examination methods available that involve both phenotypic and genotypic identification and quantification of ARB&ARGs. Some are being used routinely in diagnostic laboratories under specific protocols and internationally acknowledged standards, while others are still under development and are currently solely used as research tools by the scientific community. Phenotypic, culture-dependent approaches include disc diffusion, agar dilution, broth dilution and microdilution, gradient strip, biochemical characterisation, mechanism-specific tests and other similar methods. Furthermore, cultivation methods, which were originally developed for the detection and enumeration of clinically-relevant pathogens, fail to detect viable but not cultivable (VBNC) bacteria, which might occur during disinfection stress (VBNC bacteria are mentioned in a later section). Nevertheless, the balance is tipping towards molecular genotypic methods (e.g. PCR [standard, real-time quantitative, digital], high-throughput diagnostic microarrays, metagenomics, whole-genome sequencing and immunological approaches (Manaia, 2016)) due to the depth of information they can provide in a short amount of time, as they do not strictly rely on bacterial cultivation. Considering the above, it has become apparent that there are no uniform protocols/methodologies of assessment of the efficiency of wastewater treatment processes among the scientific community with regard to the inactivation of ARB and the removal of ARGs, a fact that makes the comparison of the studies performed in this field, complicated. The reasons for the methodological discrepancy found among laboratories may be financial and practical, and may involve limited equipment availability at a given time, for the achievement of a specific research goal. Different studies may achieve the same goal, i.e. quantification of specific ARGs, through the use of different DNA extraction kits and/or through the use of different quantification equipment. Especially when it comes to disinfection processes which may be highly effective in a short timeframe, an appropriate methodology and equipment must be utilised in order to get apt and timely results. For example, the examination of ARGs during/after disinfection must include the use of long enough amplicons, which can easily be identified and quantified before and after treatment, in order to sufficiently capture the DNA breakage, alterations and mutations in the genetic material. For this reason, in order to have comparability among different studies from different laboratories, studies using the same amplicon length shall be compared between them, otherwise any comparisons may be hasted or biased. This may be also accompanied with information on the physiological state of the bacteria (injured but not dead), using approaches such as use of propidium monoazide (PMA), in conjunction with end-point polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) (Nocker et al., 2007). Also, immunological-based approaches can be utilised for the detection of UV-induced cyclobutane pyrimidine dimers and photoproducts in DNA from natural aquatic populations (Stephanie et al., 2011). Likewise, the utilised intra-laboratory experimental controls may differ, thus enabling variable accuracy/reproducibility of results within each laboratory and making inferences or associations between inter-laboratory observations inaccurate.

In this context and to overcome such methodological problems which can cause significant discrepancies among the produced studies so far, this review aims at focusing on the technological side of the issue, through gathering all the information available in the scientific literature, dealing with the application of AOPs for the minimization of ARB&ARGs abundance in wastewater effluents. An effort was made to perform a systematic and integrated evaluation of the ability of various AOPs to inactivate ARB and to remove ARGs in wastewater effluents under the various operating parameters examined; an investigation that according to the authors' knowledge, has not been performed so far. This review primarily seeks to provide an extensive critical appraisal on the assessment of the performance of these processes in inactivating ARB and removing ARGs in wastewater effluents, accompanied with the most recent available literature data, elucidating how the key operating conditions may affect the process efficiency and identifying major areas in need for further research. Thereafter, an overview is provided on the main oxidative damage pathways involved in the inactivation of ARB and the removal of ARGs by AOPs, in an effort to decipher the mechanisms that lie behind the observations noted in the discussed studies.

2. Operating parameters affecting the inactivation of ARB and the removal of ARGs during advanced chemical oxidation processes

The recent studies dealing with the inactivation of ARB and the removal of ARGs in urban wastewater effluents following advanced chemical oxidation treatment (i.e. H_2O_2-induced oxidation in the presence of UV or solar light, Fenton homogeneous photocatalysis, and TiO_2 heterogeneous photocatalysis) were extensively reviewed. Complementary information with respect to the experimental scale/operating conditions applied, the target ARB&ARGs, and the main findings presented in each study is provided (Table 1). In the following sections, special emphasis is placed on the key operating parameters and the challenges linked to the efficiency of each process concerning the inactivation of ARB and the removal of ARGs. The chemistry/process principles governing AOPs are well-known and are comprehensively documented in the scientific literature (Michael et al., 2013b), therefore this information is not provided in this review paper. Here it is noted that the terms ″ARB inactivation″ and ″ARGs removal″ refer to observations up to the limit of detection (LOD), as defined by each study. In addition, where available, the regrowth of ARB following advanced chemical oxidation is discussed.

In light of the findings reviewed, it is evident that AOPs constitute a challenging field of investigation, not only because of their capacity of inactivating ARB in wastewater, but also because upon their proper optimization, in terms of operating conditions, they could also offer a potential means of ARGs elimination. To date however, only little relevant synthesized knowledge is available. Furthermore, the scarcity of data and the different methodological approaches applied in each study do not allow for an easy, uniform and concrete comparison among the various studies performed, while only few studies exist, that provide a comprehensive and systematic investigation of the ways in which the operating conditions and the wastewater qualitative characteristics affect the inactivation of ARB and the removal of ARGs during the application of AOPs, and these are provided in this review. Taking into account that the level of reactive oxygen species (ROS) play a critical role in

Table 1
Inactivation of ARB and removal of ARGs in wastewater effluents through advanced chemical oxidation processes.

Treatment features	Target ARB	Target ARGs	Microbiological techniques used	Main findings *where available, quantitative information is provided*	Reference *given in chronological ascending order*
UV/H_2O_2, Solar/H_2O_2					
– **Pilot-scale** setup – Natural solar irradiation – 8.5 L of autoclaved CAS effluents spiked with multidrug-resistant *E. coli* strain (10^5 CFU mL^{-1}) – [H_2O_2] = 0.588, 1.470 and 2.205 mM – Q_{UV} = 5.93, 6.75 and 7.92 kJ L^{-1}	*E. coli* resistant to: – ampicillin – ciprofloxacin – tetracycline	___	Plate count method	– Complete inactivation (<LOD) was reached in the presence of all oxidant doses tested ([H_2O_2] = 0.588 mM/ Q_{UV} = 7.92 kJ L^{-1}/150 min; [H_2O_2] = 1.470 mM/ Q_{UV} = 6.75 kJ L^{-1}/120 min; [H_2O_2] = 2.205 mM/ Q_{UV} = 5.93 kJ L^{-1}/120 min). – The antibiotic resistance pattern was not affected (no variations in the inhibition zone diameters were observed).	Ferro et al. (2015a)
– **Pilot-scale** setup – Natural solar irradiation – 8.5 L of autoclaved CAS effluent spiked with multidrug resistant *E. coli* and *E. faecalis* strains (10^5 CFU mL^{-1}) and three pharmaceuticals at 100 μg L^{-1} (carbamazepine, flumequine and thiabendazole) – [H_2O_2] = 20 mg L^{-1}	*E. coli* and *E. faecalis* resistant to: – ampicillin – ciprofloxacin – ofloxacin – tetracycline	___	Plate count method	– The LOD of antibiotic-resistant *E. coli* was reached after 120 min (Q_{UV} = 6.29 kJ L^{-1}), while antibiotic-resistant *E. faecalis* was found to be more resistant as its LOD was achieved at a higher UV dose (Q_{UV} = 14.86 kJ L^{-1}) and treatment time (240 min).	Ferro et al. (2015b)
– **Bench-scale** setup – Natural solar irradiation – 250 mL of CAS effluents spiked with multidrug-resistant *E. coli* strain (10^6 CFU mL^{-1}) – [H_2O_2] = 10, 20 and 50 mg L^{-1} – Q_{UV} = 8, 18 and 30 kJ L^{-1}	*E. coli* resistant to: – ampicillin – ciprofloxacin – tetracycline	___	Plate count method	– Complete inactivation (<LOD) was reached in the presence of all oxidant doses tested within 90 min ([H_2O_2] = 10 mg L^{-1}/ Q_{UV} = 30 kJ L^{-1}; [H_2O_2] = 20 mg L^{-1}/ Q_{UV} = 380 kJ L^{-1}; [H_2O_2] = 50 mg L^{-1}/ Q_{UV} = 8 kJ L^{-1}). – The antibiotic resistance pattern was not affected. – Bacterial regrowth was observed 24 h after treatment (0.3×10^2 CFU mL^{-1}).	Fiorentino et al. (2015)
– **Bench-scale** setup – UV irradiation (250 W, λ = 320–450 nm) – 500 mL of CAS effluents with indigenous antibiotic-resistant *E. coli* strains (7×10^1 CFU mL^{-1}) – [H_2O_2] = 20 mg L^{-1} – UV_{dose} = 0–2.5×10^4 μW s cm^{-2}	*E. coli* resistant to: – ampicillin – ciprofloxacin – tetracycline	– bla_{TEM} – *qnrS* – *tetW* – 16S rRNA	Plate count method DNA extraction with DNeasy® Blood & Tissue Kit, iQ5 qPCR (Biorad-Laboratories, Milano, Italy)	– Complete inactivation of antibiotic-resistant *E. coli* and decrease of ARGs in intracellular DNA was achieved within 90 min of treatment. – UV/H_2O_2 process was not effective in achieving complete elimination of ARGs. The process did not affect the copies number of bla_{TEM} and poorly affected the copies number of *qnrS* (4.3×10^4 copies mL^{-1}) after 240 min of treatment. *tetW* (1.1×10^1 copies mL^{-1}) was significantly decreased after 240 min.	Ferro et al. (2016)
– **Bench-scale** setup – UV irradiation (250 W, λ = 320–450 nm) – 500 mL of sterile DNA-free water spiked with	*E. coli* resistant to: – ampicillin – ciprofloxacin – tetracycline	– 16S rRNA – bla_{TEM} – *qnrS* – *tetW*	Plate count method DNA extraction with DNeasy® Blood & Tissue Kit, iQ5 qPCR (Biorad-Laboratories, Milano, Italy)	– The LOD was reached after 240 min of treatment. – bla_{TEM} gene was still present in total DNA after 300 min (2.8×10^6 copies mL^{-1}), while no effect was observed	Ferro et al. (2017)

Table 1 (*continued*)

Treatment features	Target ARB	Target ARGs	Microbiological techniques used	Main findings *where available, quantitative information is provided*	Reference *given in chronological ascending order*
multidrug-resistant *E. coli* strains (10^5 CFU mL^{-1}) – $[H_2O_2] = 20$ mg L^{-1} – $UV_{dose} = 0–2.5 \times 10^4$ μW s cm^{-2}				in DNA extracted from cell cultures (3.8×10^8 copies mL^{-1} after 90 min). – *qnrS* and *tetW* genes were likely to be absent in total DNA and in DNA extracted from cell cultures, or present at very low concentrations, that make them not detectable through the qPCR assay.	
– **Bench-scale** setup – UV irradiation (16 W, λ = 254 nm) – 1800 mL of CAS effluents – $[H_2O_2]$ = 0.005, 0.01, 0.05, 0.15 moL L^{-1}	___	– *sul1* – *tetG* – *tetW* – *intI1* – 16S rRNA	Fast DNA Spin kit, Applied Biosystems 7500 qPCR detection system (Life Technologies, USA).	– The process ($[H_2O_2]$ = 0.01 moL L^{-1}/30 min) performed at pH 3.5 resulted in higher removal of 16S rRNA (2.63 log), *intI1* (2.98 log), sul1 (2.84 log), *tetX* (3.48 log), and *tetG* (3.05 log) compared to that observed at the inherent pH of the wastewater, i.e. 16S rRNA (1.90 log), *intI1* (2.04 log), *sul1* (1.55 log), *tetX* (2.32 log), and *tetG* (1.73 log). – When $[H_2O_2]$> 0.01 moL L^{-1}, the removal was 1 log lower than that for 0.01 moL L^{-1}.	Zhang et al. (2016)
Ozonation					
– **Pilot-scale** setup – CAS effluents – $[O_3] = 0.5–11$ mg L^{-1}	*E. coli*, and *Enterococcus*, resistant to various categories of antibiotics	___	Membrane filtration	– A positive selection of sensitive isolates of *E. coli* (except cefalosporins-resistant isolates) was observed. – Among *Enterococcus*, isolates sensitive to fluoroquinolones (ciprofloxacin, levofloxacin, moxifloxacin), as well as to gentamycin, erythromycin, chloramphenicol, and tetracycline were favoured.	Luczkiewicz et al. (2011)
– **Bench-scale** setup – Synthetic wastewater – $[O_3] = 3, 5, 7, 10$ mg L^{-1}	*E. coli* DH5α	Multi-resistance gene (pB10)	Plate count method Nucleobond DNA kit PC100, Eco real-time PCR System (Illumina, SD, USA)	– ARB&ARGs were inactivated/removed more than 90% at 3 mg L^{-1} of ozone concentration. – The addition of persulfate and peroxymonosulfate reduced the contact time to achieve a 2-log inactivation/removal of ARB&ARGs.	Oh et al. (2014)
– **Pilot-scale** setup – CAS effluents – O_3 dose = 0.73 mg O_3/mg DOC (contact time = 20 min)	*E. coli*, *Enterococcus*, and *Staphylococcus* resistant to: – ampicillin – chloramphenicol – ciprofloxacin – erythromycin – vancomycin	___	Membrane filtration and Plate count method	– An increase of antibiotic-resistant *E. coli* (16% increase) and *Staphylococcus* (5.5% increase) was observed, whereas a reverse behavior occurred in the case of antibiotic-resistant *Enterococcus* (25.4% reduction). – Ozonation followed by filtration (either sand filtration or granulated activated charcoal adsorption or a combination of both techniques) led to a decrease of 0.8–1.1 log units in the percentage of antibiotic-resistant *E. coli*, *Enterococcus* and *Staphylococcus*.	Lüddeke et al. (2015)

(*continued on next page*)

Table 1 (*continued*)

Treatment features	Target ARB	Target ARGs	Microbiological techniques used	Main findings *where available, quantitative information is provided*	Reference *given in chronological ascending order*
– **Bench-scale** setup – 6 L of CAS effluents – $[O_3] = 27–177.6$ mg L^{-1}	___	– *sul1* – *tetG* – *intI1* – 16S rRNA	Fast DNA Spin kit, Applied Biosystems 7500 qPCR detection system (Life Technologies, USA).	– The 16S rRNA was more efficiently removed than ARGs (16S rDNA > *tetG* > *sul1*>*intI1*). – At $[O_3] = 177.6$ mg L^{-1} the reduction of ARGs was in the range of 1.68–2.55. – The relative abundance of ARGs (normalized to 16S rDNA) increased during ozonation.	Zhuang et al. (2015)
– **Full-scale** ozonation system – CAS effluents – O_3 dose = 0.9 g of O_3/g DOC	Antibiotic-resistant *Enterococcus*, *P. aeruginosa*, *Staphylococcus*, and Enterobacteria	– *vanA* – bla_{VIM} – *ermB* – *ampC*	Plate count method Fast DNA Spin kit, SYBR Green qPCR, Illumina MiSeq system (Genome Analytics, Helmholtz Centre for Infection Research, Germany)	– Inactivation of ARB: *Enterococcus* (98% reduction), *Staphylococcus* (83% reduction), *P. aeruginosa* (minor alterations of their abundance in the ozonated samples). – Reduction of taxonomic gene markers: *Enterococcus* (98.9%), *Staphylococcus* (78.1%), *P. aeruginosa* (60.2%), and Enterobacteriaceae (69.8%). – Reduction of ARGs: *ermB* (99% reduction), *ampC* (9% reduction), *vanA* (4-fold increase), and bla_{VIM} (7-fold increase).	Alexander et al. (2016)
– **Bench-scale** setup – 2 L of CAS effluents spiked with erythromycin at 100 μg L^{-1} – $[O_3] = 0.1–0.5$ mg L^{-1}	*E. coli* resistant to: – erythromycin	___	Membrane filtration	– The complete inactivation of the erythromycin-resistant *E. coli* was achieved within 15 min at $[O_3] = 0.3$ mg L^{-1}.	Michael-Kordatou et al. (2017)
– **Bench-scale** setup – CAS effluents – Photocatalytic ozonation (TiO_2-coated glass Raschig rings, irradiation was provided by two 10 W LEDs [λ = 382 nm]) – $[O_3] = 50$ g N m^{-3}	Total heterotrophs, *Enterococcus*, and Enterobacteria, resistant to: – ciprofloxacin – gentamicin – meropenem	– *sul1* – *qnrS* – bla_{TEM} – *intI1* – 16S rRNA	Plate count method PowerWater® DNA Isolation kit (MO BIO Laboratories, Inc., USA), qPCR	– Reduction of ARB: *Enterococcus* and Enterobacteria (from 10^5 to 10^6 CFU/100 mL to 10^1 CFU/100 mL), total heterotrophs (from 10^5 to 10^6 CFU/100 mL to 10^2 CFU/100 mL). – All ARGs were significantly reduced after treatment. – After storage of the treated effluents, reactivation ARB, 16S rRNA and *intI1* was observed, whereas bla_{TEM}, *qnrS* and *sul1* were successfully reduced to levels below/close to the threshold of quantification.	Moreira et al. (2016)
– **Bench-scale** setup – Solution spiked with tetracycline at 10 mg L^{-1} – $[O_3] = 3$ and 7 mg L^{-1}	*E. coli* DH5α containing the antibiotic resistance plasmid pB10, which has multiple resistance to a number of antibiotics.	___	Plate count method	– With the presence of ppb level of residual tetracycline after ozone oxidation, higher facilitation of gene transfer was observed.	Oh et al. (2016)
– **Bench-scale** setup – 1 L of synthetic[a] and CAS wastewater effluents – $[O_3] = 50$ g N m^{-3}	Antibiotic-resistant Enterobacteria, total heterotrophs, and *Enterococcus*	– *sul1* – *qnrS* – bla_{TEM} – *vanA* – *intI1* – 16S rRNA	Membrane filtration PowerWater® DNA Isolation kit (MO BIO Laboratories, Inc., USA), qPCR (StepOneTM real-time PCR, Life Technologies, USA)	– The inactivation of ARB and the removal ARGs was lower in CAS effluents compared to synthetic wastewater. – Reduction of ARB&ARGs: ARB (4 log), 16S rRNA (2.1 log), *intI1* (2 log), *vanA* and *sul1* (<LOD), *qnrS* and bla_{TEM} (removal to levels close to the LOQ). – All ARB&ARGs, except *qnrS*, reached pre-treatment levels after 3 days of storage of the ozonated samples.	Sousa et al. (2017)

Table 1 (*continued*)

Treatment features	Target ARB	Target ARGs	Microbiological techniques used	Main findings *where available, quantitative information is provided*	Reference *given in chronological ascending order*
Fenton homogeneous photocatalysis					
– **Pilot-scale** setup – Natural solar irradiation – 250 L of CAS effluents spiked with ofloxacin and trimethoprim at 100 μg L^{-1} – $[Fe^{2+}]$ = 5 mg L^{-1}, $[H_2O_2]$ = 75 mg L^{-1}, pH = 6	*Enterococcus* resistant to: – ofloxacin – trimethoprim	___	Membrane filtration	– Complete inactivation of ofloxacin- and trimethoprim- resistant *Enterococcus* after 180 min of solar photo-Fenton treatment. – Ofloxacin resistance was almost double of that of trimethoprim.	Michael et al. (2012)
– **Pilot-scale** setup – Natural solar irradiation – 60 L of CAS effluents spiked with clarithromycin and sulfamethoxazole at 100 μg L^{-1} – $[Fe^{2+}]$ = 5 mg L^{-1}, $[H_2O_2]$ = 50 mg L^{-1}, pH = 4	*Enterococcus* resistant to: – clarithromycin – sulfamethoxazole	___	Membrane filtration	– The process resulted in a decrease in the prevalence of clarithromycin and sulfamethoxazole-resistant *Enterococcus* as treatment time increased. – A 5-log reduction was achieved after 120 min, with the prevalence of sulfamethoxazole-resistant *Enterococcus* being 10 times higher than those harbouring resistance to clarithromycin.	Karaolia et al. (2014)
– **Pilot-scale** setup – Natural solar irradiation – 8.5 L of autoclaved CAS effluents spiked with multidrug-resistant *E. coli* strain (10^5 CFU mL^{-1}) – $[Fe^{2+}]$ = 0.09, 0.179, 0.358 mM, $[H_2O_2]$ = 0.588, 1.470 and 2.205 mM, pH = 8.72	*E. coli* resistant to: – ampicillin – ciprofloxacin – tetracycline	___	Plate count method	– Complete inactivation (<LOD) was reached in 240 min of treatment with $[Fe^{2+}]/[H_2O_2]$ = 0.090/0.294 mM at Q_{UV} = 15.34 kJ L^{-1}. – No effect on antibiotic resistance pattern exhibited by the survived colonies.	Ferro et al. (2015a)
– **Bench-scale** setup – Natural solar irradiation – 250 mL of CAS effluents spiked with multidrug-resistant *E. coli* strain (10^6 CFU mL^{-1}) – $[Fe^{2+}/H_2O_2]$ = 5:10, 10:20, 20:40 mg L^{-1}, pH = 8.00 −8.92	*E. coli* resistant to: – ampicillin – ciprofloxacin – tetracycline	___	Plate count method	– All antibiotic-resistant *E. coli* were completely inactivated (LOD) with Fe^{2+}/H_2O_2 = 5:10 mg L^{-1} at 15 −23 kJ L^{-1}. – The process did not affect the profile of antibiotic resistance of survived colonies.	Fiorentino et al. (2015)
– **Bench-scale** setup – Waste iron shavings from machining processes were used as a metallic iron – Natural solar irradiation – 300 mL of CAS effluent – $[Fe^{2+}]$ = 10 M, $[H_2O_2]$ = 0.023 M, pH = 3	Total coliforms, and *E. coli* resistant to: – ampicillin – ciprofloxacin – gentamicin – tetracycline – chloramphenicol	___	Plate count method	– Total elimination of the examined ARB within 60 min.	Mackuľak et al. (2015)
– **Pilot-scale** setup – Natural solar irradiation – 60 L of MBR effluent spiked with erythromycin, clarithromycin and sulfamethoxazole at100 μg L^{-1} – $[Fe^{2+}]$ = 5 mg L^{-1}, $[H_2O_2]$ = 50 mg L^{-1}, pH = 2.8	*E. coli*, *P. aeruginosa* and *Klebsiella* spp. resistant to: – erythromycin – clarithromycin – sulfamethoxazole	– *ermB* – *sul1* – *mecA* – *ampC* – *Enc* – *ecfX*	Membrane filtration PowerWater® DNA Isolation kit (MO BIO Laboratories, Inc., USA), Bio-Rad CFX 96 TouchTM real-time PCR detection system	– A complete inactivation of all ARB after 240 min was observed, while a low level of *P. aeruginosa* repair (2 CFU 100 mL^{-1}) occurred 24 h after treatment. – Total DNA concentration was reduced by 97%, however the *Enterococcus*-specific gene markers (3.9log10 CE 100 ng^{-1} DNA), as well as *sul1* and *ermB* were still present after treatment (1.56 and 1.53log10 CE 100 ng^{-1} DNA, respectively).	Karaolia et al. (2017)

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Table 1 (*continued*)

Treatment features	Target ARB	Target ARGs	Microbiological techniques used	Main findings *where available, quantitative information is provided*	Reference *given in chronological ascending order*
– **Bench-scale** setup – No irradiation (dark Fenton) – 500 mL of CAS effluent – [Fe^{2+}/H_2O_2] = 0.033, 0.05, 0.067, 0.1, 0.2 and 0.5, pH = 3 and 7	___	– *sul1* – *tetX* – *tetG* – *intI1* – 16S rRNA	Fast DNA Spin kit, Applied Biosystems 7500 qPCR detection system (Life Technologies, USA).	– ARGs removal increased considerably with increasing the Fe^{2+}/H_2O_2 molar ratio from 0.033 to 0.1. – At the optimum Fe^{2+}/H_2O_2 molar ratio (i.e. 0.1) the removals of *sul1*, *tetX*, *tetG*, *intI1* and 16S rRNA were found to be 3.98, 3.80, 2.57, 4.62, and 3.03 log, respectively. – At pH = 3, the removals of *sul1*, *tetX*, *tetG*, *intI1* and 16S rRNA were found to be 3.79, 3.79, 2.58, 3.67, and 2.76 log, respectively. – At pH = 7 the removals of *sul1*, *tetX*, *tetG*, *intI1* and 16S rRNA were found to be 3.19, 3.06, 2.26, 3.35, and 2.48, respectively.	Zhang et al. (2016)
TiO_2 heterogeneous photocatalysis					
– **Bench-scale** setup – UV-A irradiation (400 μW cm^{-2} and 800 μW cm^{-2}) – Degussa P25 TiO_2, [TiO_2] = 0.0625 and 0.125 mg mL^{-1}	Methicillin-resistant *S.aureus* [MRSA], Multi drug-resistant *Acinetobacter baumannii* [MDRAB], and vancomycin-resistant *Enterococcus faecalis* [VRE]	___	Plate count method	– The prevalence of ARB decreased exponentially with increasing TiO_2 dose (CIt = 1743–2565, 99% inactivation, where CIt = TiO_2 concentration × UV-A intensity × reaction time). – The results obtained using the strains of MDRAB and VRE suggested that the altered resistance to TiO_2 photocatalysis might be related to the acquisition of genetic material in the form of plasmids.	Tsai et al. (2010)
– **Bench-scale** setup – Degussa P25 TiO_2-coated crystallizing dish – UV-A/LEDs irradiation (3 W; λ = 365 nm; 6, 7 and 8 mW cm^{-2})	*E. coli* resistant to: – ampicillin – streptomycin	___	Plate count method	– For 3-log inactivation of antibiotic-resistant *E. coli*, the UV dose required increased from 688 mJ cm^{-2} to 870 mJ cm^{-2} with light intensity decreasing from 8 mW cm^{-2} to 6 mW cm^{-2}. – Periodic illumination affected the extent of inactivation. – With the same UV dose, the inactivation of ARB decreased with circle time[b] in the studied range, while it increased with duty circle[b].	Xiong and Hu (2013)
– **Bench-scale** setup – Three types of irradiation: (i) a wide spectrum 250 W lamp, (ii) the same lamp equipped with a filter to simulate solar radiation, and (iii) a 125 W black light fluorescent lamp. – 500 mL of CAS effluent – Degussa P25 TiO_2, [TiO_2] = 0.05–2 g L^{-1}	*E. coli* resistant to: – ciprofloxacin – cefuroxime – tetracycline – vancomycin	___	Plate count method	– Only solar simulated irradiation resulted in the total inactivation of ARB after 60 min ([TiO_2] = 0.05 g L^{-1}). – The optimum TiO_2 loading estimated by radiation absorption-scattering modeling was found to be 0.1 g L^{-1}.	Rizzo et al. (2014a)

Table 1 (*continued*)

Treatment features	Target ARB	Target ARGs	Microbiological techniques used	Main findings *where available, quantitative information is provided*	Reference *given in chronological ascending order*
– **Bench-scale** setup – Natural or simulated solar irradiation (250 W) – 500 mL of CAS effluent – N-doped TiO_2, $[TiO_2] = 0.025$ –0.5 g L^{-1}	*E. coli* resistant to: – ciprofloxacin – cefuroxime – tetracycline – vancomycin	___	Plate count method	– The higher inactivation rate (8.5×10^5 CFU 100 mL^{-1} min^{-1}, after 10 min) of antibiotic-resistant *E. coli* was observed at 0.2 g L^{-1} dose of N-doped TiO_2. – The total inactivation was achieved after 60 min of treatment. – Higher bacterial inactivation efficiencies were recorder for the doped-TiO_2 catalyst compared to commercially available TiO_2 powders (Millennium PC50 and PC100). – The process did not significantly affect resistance of *E. coli* strain to tetracycline and vancomycin as irradiation time increased, but a decreasing trend in resistance to ciprofloxacin and cefuroxime was observed.	Rizzo et al. (2014b)
– **Bench-scale** setup – Simulated solar irradiation (250 W) – 500 mL of CAS effluent – Degussa P25 TiO_2, $[TiO_2] = 0.05$ g L^{-1}	*Enterococcus* resistant to: – tetracycline	___	Membrane filtration	– The total inactivation (7 log) was achieved within 60 min of treatment. – ARB surviving treatment showed that TiO_2 photocatalysis did not affect the antibiotic resistance pattern after 45 min irradiation.	Rizzo et a. (2014c)
– **Bench-scale** setup – UV-A irradiation (9 W, $\lambda = 370$ nm, 320–400 W m^{-2}) – 200 mL of distilled water and autoclaved CAS effluents – Aeroxide P25 TiO_2, Immobilized TiO_2 stirred tank reactor	*E. coli* resistant to: – rifampicin – chloramphenicol	___	Plate count method	– ARB were reduced by 2.5 log within 180 min of treatment. – Recovery of the two strains of ARB back to their original numbers, after 24 h of post-treatment incubation was observed. – Gene pair conjugant numbers of antibiotic-resistant *E. coli* in distilled water showed a four-fold increase following 180 min treatment. A lower reduction in ARB numbers and gene pair conjugates was observed in CAS effluents compared to that observed in distilled water.	Dunlop et al. (2015)
– **Pilot-scale** setup – Natural solar irradiation – 8.5 L of autoclaved CAS effluents spiked with multidrug-resistant *E. coli* strain (10^5 CFU mL^{-1}) – Aeroxide P25 TiO_2, $[TiO_2] = 50$ and 100 mg L^{-1}	*E. coli* resistant to: – ampicillin – ciprofloxacin – tetracycline	___	Plate count method	– $[TiO_2]$ = 50 mg L^{-1}: inactivation down to the LOD was achieved in 150 min of solar treatment with a Q_{UV} of 7.88 kJ L^{-1}. – $[TiO_2]$ = 100 mg L^{-1}: inactivation down to the LOD was achieved in 180 min with a Q_{UV} of 9.94 kJ L^{-1}.	Ferro et al. (2015a)
– **Bench-scale** setup – Natural solar irradiation – Aeroxide P25 TiO_2 – Sunlight/TiO_2: $[TiO_2] = 100$ mg L^{-1} – Sunlight/TiO_2/H_2O_2: $[TiO_2$:$H_2O_2] = 10$:100 and 50:100 mg L^{-1}	*E. coli* resistant to: – ampicillin – ciprofloxacin – tetracycline	___	Plate count method	– Sunlight/TiO_2: inactivation down to the LOD was achieved in 150 min of treatment with a Q_{UV} of 20 kJ L^{-1}. – Sunlight/TiO_2/H_2O_2: inactivation down to the LOD was achieved in 60 min with a Q_{UV} of 3 kJ L^{-1} at TiO_2/H_2O_2 = 10:100 mg L^{-1}.	Fiorentino et al. (2015)

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Table 1 (*continued*)

Treatment features	Target ARB	Target ARGs	Microbiological techniques used	Main findings *where available, quantitative information is provided*	Reference *given in chronological ascending order*
– **Bench-scale** setup – Natural or simulated solar irradiation (150 W) – 300 mL of CAS effluents spiked with *K. pneumonia* – Mn-, Co- and binary Mn/Co–TiO_2	*K. pneumonia* resistant to: – ampicillin – cefaclor – sulfamethoxazol – tetracycline	– *tetA* – *tetM* – *sul1* – bla_{TEM} – *ampC*	Broth microdilution method DNA extraction using a phenol/chloroform/isoamyl alcohol protocol, PCR	– Photocatalysis with metal-doped titania inactivated rapidly antibiotic-resistant *K. pneumoniae* under simulated solar irradiation, yielding 6 log reduction after 30 min. Slower inactivation was observed under natural solar irradiation conditions (2 log). – Only *sul1* and *a*mpC remained in the reaction solution after Mn/Co–TiO_2 photocatalysis.	Venieri et al. (2016)

Symbols ► Q_{UV}: cumulative energy per unit of volume (kJ L^{-1}).
Abbreviations (given in alphabetical order) ► ARB: Antibiotic -Resistant Bacteria; **ARGs**: Antibiotic Resistance Genes; **CAS**: Conventional Activated Sludge; **CFU**: Colony Forming Units; **LEDs**: Light Emitting Diodes; **LOD**: Limit of Detection; **LOQ**: Limit of Quantification; **MBR**: Membrane Bioreactor; **MGEs**: Mobile Genetic Elements; **MIC**: Minimum Inhibitory Concentration; **qPCR**: Qualitative Polymerase Chain Reaction.
[a] The synthetic wastewater effluents used in the cited studies approximate the effluent organic matter generally found in CAS wastewater effluents.
[b] Circle time is defined as one circle ″on″ and ″off″ time, and it focuses on the study of pulse frequency and calculated as ″on + off″; while duty circle highlights the importance of ″on″ time ratio in one circle, it is calculated as ″on/(on + off) ″.

the efficiency of AOPs, mathematical and kinetic models and tools may be incorporated for predicting the optimum operating conditions, under specific physicochemical properties of the wastewater matrix, to maximise the inactivation/removal of ARB&ARGs. Moreover, the insufficient results and inconclusive evidence available in the scientific literature make it difficult to elucidate the role of each treatment process on the alteration of the resistance profile in residual bacterial communities surviving chemical oxidation, and on the potential bacterial reactivation and repair of induced damages.

Table 2 summarises the key points and challenges emerged from the studies that were reviewed for each technology with regard to their capacity to inactivate ARB and to remove ARGs in wastewater effluents.

Table 2
Interesting points and challenges emerged from the literature studies reviewed.

General Remarks	
The inactivation of ARB and the removal of ARGs by advanced chemical oxidation processes strongly depends on both **technological** and **microbiological** parameters: (i) **operating conditions** (e.g. disinfectant dose, contact time, wastewater composition), (ii) specific **oxidative damage mechanisms** which are in place during the application of each treatment technology, (iii) **type of bacterial species and genes** under study, and (iv) **antibiotic**(s) to which resistance is acquired.	
UV-/solar-based processes (UV/H_2O_2, solar/H_2O_2)	• ARB can be effectively inactivated (near the limit of detection) at low oxidant doses and contact time (~20–50 mg L^{-1} and <240 min, respectively) • Prolonged time of treatment (>240 min) or high oxidant doses (>300 mg L^{-1}) are required for the efficient removal of ARGs • Solar-driven H_2O_2 oxidation treatment reduces the bacterial regrowth compared to chlorination
Ozonation	• Optimization of ozone dose (specific ozone concentration/contact time) depends on the bacterial/gene type • Ozonation may increase the relative abundance of ARGs • Post-filtration of ozone-treated flows may lead to further inactivation of ARB and removal of ARGs • ARB can be reactivated after TiO_2-photocatalytic ozonation suggesting a transitory rather than permanent microbial inactivation
Homogeneous photocatalysis (Fe^{2+} or Fe^{3+}/H_2O_2)	• Increased Fe^{2+}/H_2O_2 molar ratios result in higher inactivation of ARB and removal of ARGs • The use of excessive oxidant concentration is not encouraged since the ″oxidant scavenging effect″ prevails and lower inactivation of ARB/removal of ARGs is achieved • The optimum oxidant concentration is ARGs-specific • Wastewater inherent pH can lead to an effective inactivation of ARB and removal of ARGs, however the inactivation/removal rate is usually much slower compared to that observed under acidic conditions • Solar photo-Fenton can lead to high removal of DNA (>95%) but other types of genetic parameters, such as taxon-specific markers and ARGs, seem to require prolonged time of treatment for their effective removal • Prolonged irradiation may be necessary to avert bacterial inactivation
Heterogeneous TiO_2 photocatalysis	• TiO_2 dose (expressed as function of catalyst concentration, UV type/intensity and reaction time) strongly affects the inactivation of ARB and the removal of ARGs • Optimization of the reactor optical thickness that interrelates the catalyst loading, the light source and the reactor geometry, with the TiO_2-driven disinfection efficiency is very crucial • Solar simulated irradiation is more effective in achieving a complete bacterial inactivation compared to natural solar irradiation conditions • Antibiotic-sensitive and ARB strains exhibit different sensitivity to the photocatalytic treatment under the same operating conditions • Metal-doped TiO_2 photocatalysis can lead to higher inactivation/removal of ARB&ARGs compared to non-doped TiO_2 treatment • TiO_2-immobilized configurations require higher reaction times (>180 min) for the minimization of ARB&ARGs compared to the TiO_2-slurry systems • Prolonged time of TiO_2 photocalytic treatment is required to avoid post-treatment recovery of bacterial populations

2.1. UV- and solar-based processes (UV/H_2O_2, solar/H_2O_2)

The superiority of the UV/H_2O_2 process over the conventional UV disinfection process for the inactivation of ARB in wastewater was clearly evidenced in the scientific literature. This is attributed to the *in situ* generation of highly reactive free hydroxyl radicals ($HO^{\bullet}$), which can significantly enhance the oxidation potential of the chemical system, resulting in changes in the bacterial cell structure. In the case of ARGs, prolonged time of UV/H_2O_2 treatment seems to be required for their effective removal.

► ***Prolonged time of UV or solar/H_2O_2 treatment is required for the efficient inactivation of ARB and the removal of ARGs***

The evaluation of the effect of a UV-driven oxidation induced by H_2O_2 ($[H_2O_2] = 20$ mg L^{-1}) on the potential of antibiotic resistance transfer through the inactivation of an antibiotic-resistant *E. coli* strain (ampicillin, ciprofloxacin, and tetracycline) and bla_{TEM} (DNA extract from an *E. coli* strain) has been recently examined by Ferro et al. (2017). Whilst the detection limit of antibiotic-resistant *E. coli* was reached after 240 min, this treatment duration seemed to be insufficient in removing bla_{TEM}, the latter being still present even if the treatment time was extended to 300 min. These results are in agreement with those reported in another study of Ferro et al. (2016) dealing with the inactivation of indigenous antibiotic-resistant *E. coli* and the removal of selected ARGs (bla_{TEM}, *qnrS*, and *tetW*) by UV/H_2O_2 process under the same experimental conditions applied in Ferro et al. (2017). It was observed that despite the complete inactivation of antibiotic-resistant *E. coli* and decrease of ARGs in intracellular DNA within 90 min of treatment, the UV/H_2O_2 process was not effective in achieving complete elimination of ARGs even after 240 min. Particularly, the occurrence of bla_{TEM} in total DNA (3.7×10^3 copies mL^{-1}) was not significantly affected after 240 min of treatment, while no differences were observed for *qnrS* between the untreated and the samples taken after treatment (4.3×10^4 copies mL^{-1}). On the other hand, *tetW* was significantly reduced after 240 min (1.1×10^1 copies mL^{-1}). UV/H_2O_2 performed under acidic conditions (pH 3.5), in the presence of higher oxidant dose ($[H_2O_2] = 0.01$ mol $L^{-1} = 340$ mg L^{-1}) than that applied in the previous studies, was demonstrated to be more effective in removing *sul1*, *tetX*, and *tetG* (2.8–3.5 logs) within 30 min, compared to the inherent pH of the wastewater (pH = 7.0, 1.55–2.32 logs) (Zhang et al., 2016). In addition, the removal of tetracycline genes was higher than that of sulfonamide genes, indicating that the former were less recalcitrant than the latter. This behavior is also consistent with the results of Gao et al. (2012). The stepwise damage induced by the UV irradiation starts at the bacterial cell wall and gradually proceeds to the inner cell components, eventually causing permanent lethal damage. Therefore, on the basis of the obtained findings, it can be inferred that the effective removal of ARGs requires longer times of treatment compared to ARB inactivation. Also, one important observation is that the most effective UV dose as reported in the examined studies was higher than the UV dose commonly applied in UWTPs, which is often less than 100 mJ cm^{-2}.

Limited research studies have been reported in the scientific literature surrounding the application of solar-driven oxidation in the presence of natural solar light and H_2O_2 for the inactivation of ARB and the removal of ARGs. The synergistic effect of H_2O_2 and solar irradiation for the inactivation of multidrug-resistant *E. coli* strains has been recently demonstrated by Ferro et al. (2015a). The optimum oxidant concentration was found to be 1.470 mM, where the bacterial population reached its limit of detection within 120 min of treatment and cumulative energy per unit of volume (Q_{UV}) equal to 6.75 kJ L^{-1}. These findings were well reinforced by a study conducted by Fiorentino et al. (2015) during the application of solar/H_2O_2 treatment ($[H_2O_2] = 50$ mg L^{-1}, $Q_{UV} = 8$ kJ L^{-1}, 90 min) for the inactivation of multidrug-resistant *E. coli* in wastewater effluents. However, in the latter study bacterial regrowth was observed 24 h after treatment. In Agulló-Barceló et al. (2013), the inactivation profile of indigenous *E. coli* in wastewater under solar irradiation was examined under the same H_2O_2 doses applied in Ferro et al. (2015a). Interestingly, it was observed that the limit of detection of the bacterial strains required 3 h of treatment, indicating the lowest sensitivity of the natural occurring *E. coli* to the oxidative stress conditions prevailing during the treatment than the multidrug-resistant *E. coli*. Moreover, in the study by Ferro et al. (2015b) it was found that the limit of detection of antibiotic-resistant *E. coli* was reached after 120 min of exposure to solar irradiation ($Q_{UV} = 6.29$ kJ L^{-1}), while *E. faecalis* exhibited more resistance to the treatment conditions applied as its complete inactivation was achieved at higher Q_{UV} values ($Q_{UV} = 14.86$ kJ L^{-1}) and prolonged treatment time (240 min).

► ***Can solar/H_2O_2 treatment reduce the bacterial regrowth compared to chlorination?***

A comparison between solar/H_2O_2 process and chlorination with respect to the inactivation of indigenous multidrug-resistant *E. coli* revealed that chlorination (1.0 mg Cl_2 L^{-1}) was more effective compared to the solar-driven oxidation process ($[H_2O_2] = 50$ mg L^{-1}) in completely inactivating bacterial population and this is illustrated by the fact that the time duration required for the total bacterial inactivation was six times higher in the case of solar/H_2O_2 process (90 min vs 15 min for solar/H_2O_2 and chlorination, respectively). On the other hand, chlorination was found to be less effective in minimizing *E. coli* regrowth potential (24 h vs 48 h for solar/H_2O_2 and chlorination, respectively) (Fiorentino et al., 2015). These differences may be explained by the different inactivation mechanisms of the two disinfection processes applied.

► ***Sulfate radical-based oxidation processes seem to be effective in the inactivation of ARB***

The potential of a UV-C-driven oxidation process induced by both free sulfate and hydroxyl radicals, using sodium persulfate as the oxidant, in inactivating erythromycin-resistant *E. coli* has been also demonstrated in the scientific literature (Michael-Kordatou et al., 2015b). Sulfate radicals ($SO_4^{\bullet-}$), whose production can be performed by the activation of persulfate anion ($S_2O_8^{2-}$), are characterized by a high reduction potential (2.6 V), slightly lower than that of $HO^{\bullet}$. According to the authors' knowledge, no literature studies exist on the mechanisms/pathways underlying the oxidative damage of ARB&ARGs by $SO_4^{\bullet-}$.

2.2. Ozonation

There is limited knowledge on the efficiency of ozonation to inactivate ARB and to remove ARGs, since the majority of the studies performed so far only covered the effect of ozone on cultivable bacterial populations (e.g. *E. coli*, *Enterococcus*, faecal coliforms, etc.) rather than on antibiotic-resistant strains (Blatchley et al., 2012; Ostoich et al., 2013). The literature findings available so far revealed that the process performance with respect to ARB&ARGs elimination is strongly depended on the susceptibility of the target bacterium/gene, ozone concentration (which is indirectly linked to the concentration of $HO^{\bullet}$ upon its rapid decomposition under alkaline conditions), and contact time.

► ***The optimization of ozonation (specific ozone dose/contact time) for the inactivation of ARB and the removal ARGs depends on the bacterial/gene type***

In a recent study by Alexander et al. (2016), the abundance of ARB (*Enterococcus*, *P. aeruginosa*, *Staphylococcus*, and *Enterobacteriaceae*) and their associated ARGs (*vanA*, bla_{VIM}, *ermB*, and *ampC*) in wastewater effluents subjected to ozonation (0.9 g O_3 per 1 g DOC) was evaluated. Overall, different levels of resistance of opportunistic bacteria against ozone oxidative stress were observed, indicating that the survival mechanisms induced to cope with the bactericidal effects of ozone strongly depend on the bacterial species. Among the tested bacteria, *Enterococcus* exhibited the highest susceptibility to ozone (98% reduction), followed by *Staphylococcus* (83% reduction), whereas *P. aeruginosa* demonstrated the highest tolerance towards ozone with only minor alterations of their abundance in the ozonated samples being recorded. In accordance to the relative abundance of ARB, taxonomic gene markers of *Enterococcus* and *Staphylococcus* were reduced by 98.9% and 78.1%, respectively, while the respective taxonomic gene markers for P. *aeruginosa* and *Enterobacteriaceae* were less affected by ozonation (60.2% and 69.8% reduction, respectively). The investigated ARGs demonstrated an even more diverse pattern. Ozonation resulted in a significant removal of *ermB*, but on the other hand, *vanA* and bla_{VIM} were shown to be quite robust towards ozone since their relative abundance increased within the surviving wastewater population, suggesting that their potential transfer was triggered by the oxidative stress induced during ozonation. A much more differentiated picture of ARGs removal was reported by Zhuang et al. (2015) during the bench-scale application of ozonation. Ozonation performed at elevated oxidant doses ($[O_3] = 27–178$ mg L^{-1}) led to a significant removal of 16S rDNA, while the reduction of *tetG*, *sul1* and *intI1* was less efficient, with their relative abundances (normalized to 16S rDNA) increased after ozonation.

Lüddeke et al. (2015) observed that ozonation (0.73 mg O_3/mg DOC; 20 min contact time) of wastewater collected after a flocculation/filtration step, led to an increased percentage of both antibiotic-resistant *E. coli* (16%) and *Staphylococcus* (5.5%), whereas a reverse behavior was observed in the case of antibiotic-resistant strains of *Enterococcus*, the latter being inactivated by 25.4%. The contradictory effect of ozone on *Enterococcus* compared to *E. coli* and *Staphylococcus* may be ascribed to the different endurance of species towards ozone and it may be connected to the shift of the abundance of the respective *Enterococcus*-species, i.e. among the *Enterococcus*-species, *E. faecium* or *E. faecalis* decreased remarkably from 49.1% to 26.3% during ozonation. In contrast to these results, Luczkiewicz et al. (2011) reported that the bacterial populations of ciprofloxacin- and erythromycin-resistant *E. coli* and *Enterococcus* were lower upon ozonation (reduction of about 4.1% and 20.7% for ciprofloxacin and erythromycin, respectively). Öncü et al. (2011) observed that the oxidative damage on the plasmid DNA of multi-resistant *E. coli* induced by ozone was more pronounced with increasing oxidant doses. In addition, ozonation of wastewater effluents resulted in complete inactivation of erythromycin-resistant *E. coli* within 15 min of contact time (Michael-Kordatou et al., 2017).

The different extent of ARB inactivation and ARGs removal during ozonation of synthetic and real urban wastewater effluents (SW and RW, respectively) was demonstrated by Sousa et al. (2017). Since the organic matter present in the SW (expressed as chemical oxygen demand [COD]) was a least six times higher than that of the RW ($[COD]_{SW} = 300$ mg L^{-1}; $[COD]_{RW} = 25–50$ mg L^{-1}), one would expect that the ozonation process would possess a lower disinfection efficiency in the SW, since both molecular ozone and the generated radicals could react with the high load of the organic matter rather than the bacterial cells. Nevertheless, a reverse effect was observed (i.e. lower bacterial inactivation values were observed in RW than in SW) and this behavior could be linked to the complexity of the dE_fOM in RW in conjunction to the high load of suspended solids.

The findings of these studies clearly underline the importance of properly optimizing the ozonation process (e.g. specific ozone dose and contact time) taking into consideration both the bacterial species and associated ARGs, as well as the wastewater physicochemical properties, in order to mitigate the spread of ARB&ARGs. Overall, more studies are needed to provide in depth understanding of the ozonation effect on the removal of antibiotic resistance determinants.

► ***What do we know concerning post-filtration of ozone-treated flows and its effect on the inactivation of ARB and the removal ARGs?***

Interestingly, ozonation followed by either sand filtration or granulated activated charcoal adsorption led to an additional decrease (0.8–1.1 log-units) in the proportion of antibiotic-resistant *E. coli*, *Enterococcus* and *Staphylococcus* against selected antibiotics (Lüddeke et al., 2015). However, it is not consensual that filtration processes can lead to an effective reduction of antibiotic resistance, since the prolonged hydraulic retention time in the filters may enhance the induction of HGT mechanisms. For instance, Alexander et al. (2016) reported that the post-treatment of ozonated wastewater using bio- and granular activated carbon-filters (with or without aeration), demonstrated limited reductions of antibiotic resistance and taxonomic gene markers of *Enterococcus* and *Staphylococcus* in the final outflow. According to Dodd (2012), the percentage of ARB may also increase, if temporarily retained microorganisms in a filter would incorporate free cellular contents (including DNA fragments) of lysed cells after ozonation. More studies are necessary to gain a deeper insight into the mode of action of ozone on the inactivation of ARB, as well as the complex interactions of filter material/structure with bacterial cells. Investigations concerning HGT mechanisms that might occur on the filters and the respective rates would help to understand the described observations.

► ***The potential risk of bacterial regrowth was demonstrated after 3-days storage of (photocatalytically)ozonated-treated wastewater***

The literature around the efficiency of immobilized photocatalytic ozonation to inactivate ARB and to remove ARGs is scarce, since its application for the removal of microcontaminants from complex aqueous matrices, such as wastewater effluents, suffers from relatively poor mass transfer (Mehrjouei et al., 2015). A study on the capacity of photocatalytic ozonation of urban wastewater implementing an innovative experimental setup consisting of TiO_2-coated glass Raschig rings and light emitting diodes (LEDs) for the inactivation of selected ARB and the removal of ARGs was recently performed by Moreira et al. (2016). The catalytic ozonation process was run under the optimum experimental conditions previously determined in Restivo et al. (2012). The synergistic effect of photocatalysis and ozonation led to a remarkable inactivation of ARB (ciprofloxacin, gentamicin, and meropenem), and removal of 16S rRNA, *intI1*, and specific ARGs (bla_{TEM}, *qnrS*, and *sul1*). Nonetheless, it was evidenced that after storage of the treated wastewater, ARB, 16S rRNA and *intI1* increased to values close to the pre-treatment levels, whereas bla_{TEM}, *qnrS* and *sul1* were successfully dropped down to levels below or close to the threshold of quantification. Similar findings were observed by Sousa et al. (2017) during the

assessment of the potential of ozonation to inactivate various ARB (*E. coli*, *Enterobacter cloacae*, *Achromobacter* sp., *Enterococcus faecalis*), as well as to remove 16S rRNA, *intI1* and ARGs (*vanA*, bla_{TEM}, *sul1* and *qnrS*). All bacterial populations and ARGs, except *qnrS*, reached pre-treatment levels after 3 days of storage of the ozonated samples, suggesting a transitory rather than permanent microbial inactivation. The findings of these studies clearly demonstrated that ARB in spite of their stress status, they still maintain viability, being able to regrow when the ozone stress is relieved. Therefore, the optimization of the ozone dose in conjunction with exposure time to the oxidant should be taken into consideration, in order to minimize the potential risk of regrowth. Moreover, in the case of photocatalytic ozonation, although the removal of ARGs was satisfactory, there are many engineering-related issues to be tackled, in order to avoid elevated cost associated with its full-scale application.

2.3. Homogeneous photocatalysis (Fe^{2+}or Fe^{3+}/H_2O_2)

A limited number of studies has dealt with the capacity of the Fenton process to inactivate ARB and to remove ARGs in wastewater and the operating parameters affecting the process efficiency. According to Table 1, the efficiency of the Fenton/photo-Fenton systems to inactivate ARB and to remove ARGs is primarily affected by H_2O_2 and Fe^{2+} concentrations, pH and reaction time.

► *Fenton reagent's concentration strongly affects the inactivation of ARB and the removal of ARGs*

A comprehensive study on the evaluation of the effect of various operating parameters such as Fe^{2+}/H_2O_2 molar ratios, H_2O_2 concentration, solution pH, and reaction time, on the Fenton process efficiency to remove 16S rRNA, *intI1* and selected ARGs (*sul1*, *tetX*, and *tetG*) present in wastewater effluents, was recently published by Zhang et al. (2016). It was found that the reagents' concentrations (expressed as Fe^{2+}/H_2O_2 molar ratio and H_2O_2 concentration) and solution pH were the most prominent parameters affecting the removal of the target antibiotic resistance determinants. Particularly, the removal increased considerably with increasing the Fe^{2+}/H_2O_2 molar ratio from 0.033 to 0.1, the latter being selected as the optimum one. Interestingly, the effect of the oxidant concentration on the removal of the target ARGs was found to be ARGs-specific (i.e. the maximum removal of ARGs occurred under different oxidant doses), while the known "oxidant scavenging effect" was found to occur only for *tetG* and *intI1*. It was observed that *tetG* and 16S rRNA were less susceptible to Fenton oxidation compared to the other ARGs, with *intI1* exhibiting the maximum reduction under the optimum Fe^{2+}/H_2O_2 molar ratio. In another study by Fiorentino et al. (2015) the performance of solar photo-Fenton under various Fe^{2+}/H_2O_2 molar ratio values on the inactivation of antibiotic-resistant *E. coli* strain (ampicillin, ciprofloxacin, and tetracycline) was investigated. The complete inactivation of all antibiotic-resistant *E. coli* was achieved under all examined Fe^{2+}/H_2O_2 ratios (5:10, 10:20, 20:40 mg L^{-1}), with a Q_{UV} ranging between 15 and 23 kJ L^{-1}, while the most rapid bacterial inactivation was observed for Fe^{2+}/H_2O_2 = 5:10 at 15 kJ L^{-1}.

► *Wastewater's inherent pH can lead to high ARGs removal*

Regarding pH, Zhang et al. (2016) reported that the maximum ARGs log reduction by Fenton process was achieved at pH 3 (2.58–3.79-log reduction). The potential prospect of broadening the process operation pH range up to mild neutral pH conditions (i.e. wastewater inherent pH, pH around 7) was also demonstrated in this study, since the removal of both ARGs and 16S rRNA was in the range of 2.26–3.35 logs, a little bit lower than the log reductions observed under acidic conditions. It is reasonable to suppose that this behavior can be attributed to the iron complexation by dE_fOM originally present in wastewater, resulting in the generation of Fe^{3+}-dE_fOM complexes, which can further induce Fenton oxidation reactions, as in the case of the application of the modified photo-Fenton process for the removal of chemical microcontaminants. Fiorentino et al. (2015) reported the complete inactivation of *E. coli* harbouring resistance to ampicillin, ciprofloxacin, and tetracycline under the inherent wastewater pH conditions (Fe^{2+}/H_2O_2 = 5:10; Q_{UV} = 15 kJ L^{-1}). The findings of Ferro et al. (2015a) have also shown the capacity of the solar photo-Fenton process ($[Fe^{2+}]/[H_2O_2]$ = 0.090/0.294 mM, Q_{UV} = 15.34 kJ L^{-1}, 4 h) to inactivate multi-drug resistant *E. coli* strains in urban wastewater under the inherent neutral pH of the wastewater. However, the inactivation rate was much slower compared to that observed under pH 4. The inactivation rate recorded in Ferro et al. (2015a) seemed to fit quite well with the results obtained by Agulló-Barceló et al. (2013) during the application of photo-Fenton (pH 3) for the inactivation of the inherent *E. coli* strains present in secondary treated wastewater effluents. An interesting observation though was that the tested strains did not lose their resistance to the examined antibiotics (ampicillin, ciprofloxacin, tetracycline, and vancomycin) during the process. Nonetheless, this does not necessarily imply that any alteration in antibiotic resistance occurred at all. On the contrary, no change took place in the specific bacterial cells randomly selected among those surviving to Fenton treatment at the given sampling time.

Michael et al. (2012) reported that *Enterococcus* harbouring resistance to ofloxacin and trimethoprim, were completely inactivated after 180 min of solar photo-Fenton process ($[Fe^{2+}]$ = 5 mg L^{-1}; $[H_2O_2]$ = 75 mg L^{-1}; pH 6) performed at a pilot scale, while it was also demonstrated that ofloxacin resistance was almost double of that of trimethoprim. The same approach has been followed in another pilot-scale study conducted by Karaolia et al. (2014), who revealed that solar-driven Fenton oxidation performed under the optimum experimental conditions ($[Fe^{3+}]$ = 5 mg L^{-1}; $[H_2O_2]$ = 50 mg L^{-1}; pH 4) exhibited a 5-log reduction in antibiotic resistance of *Enterococcus* towards sulfamethoxazole and clarithromycin in wastewater effluents. It was also observed that after 120 min of solar photo-Fenton treatment, there were 10 times more sulfamethoxazole-resistant *Enterococcus* compared to those harbouring resistance to clarithromycin, indicating the induction of strong tolerance mechanisms which favour the survival of the specific bacteria in sulfamethoxazole-containing environments. In addition, it was observed that the mixture of sulfamethoxazole and clarithromycin reduced the resistance percentage of *Enterococcus*, suggesting a lack of survival mechanisms of these bacteria against a combination of the two antibiotics, compared to each antibiotic individually.

From the aforementioned studies, it is still questionable whether the inactivation of ARB and the removal of ARGs during Fenton/photo-Fenton processes at pH 3 is induced by the radicals generated in the oxidation system, or just by pH trimming to the values in the acidic range. It was reported that low pH is effective in provoking a permanent damage to the bacteria, but DNA denaturation is assured by $HO^{\bullet}$, ensuring thus that plasmid information will not be spread among different bacterial species by horizontal-driven transmission (Mackuľak et al., 2015).

► *Prolonged time of solar photo-Fenton is required to provoke permanent bacterial damage and to completely remove ARGs and taxon-specific markers*

The findings of Karaolia et al. (2017) demonstrated the high

efficiency of the solar photo-Fenton process ($[Fe^{2+}]$ = 5 mg L^{-1}; $[H_2O_2]$ = 50 mg L^{-1}; pH 2.8) to inactivate antibiotic-tolerant bacteria (*Klebsiella* spp., *P. aeruginosa*, and *E. coli*), and to remove total DNA, selected ARGs (*ampC*, *sul1*, and *ermB*) and selected taxon-specific genetic markers (*Enc*, and *ecfX*). Among the three types of the investigated Gram-negative bacteria, *E. coli* and *Klebsiella* spp. were completely inactivated, whereas a repair of *P. aeruginosa* was observed, with 2 CFU 100 mL^{-1} growing on the selective media 24 h after solar photo-Fenton oxidation, indicating their tolerance to the experimental conditions applied in this study. Since bacteria may possess repair mechanisms, enabling them after repairing their injured structures to regrow on nutritive media, it may be assumed that prolonged irradiation doses may be necessary, in order to cause heavy and permanent damage to susceptible bacteria. Furthermore, the potential of the solar photo-Fenton oxidation process to effectively remove total DNA (97%), which may be found incorporated inside bacteria or freely suspended in wastewater, was revealed. On the other hand, it seems that other types of genetic parameters, such as taxon-specific markers and ARGs may require a different treatment approach of removal, since in the remaining total DNA (3%), the *Enterococcus*-specific gene markers (3.9log_{10} CE 100 ng^{-1} DNA), and the ARGs *sul1* and *ermB* (1.56 and 1.53log_{10} CE 100 ng^{-1} DNA, respectively) were still present in the treated samples.

2.4. Heterogeneous TiO_2 photocatalysis

To date there have been very few studies investigating the application of TiO_2-mediated photocatalysis, in both slurry and immobilized configurations, for the inactivation of ARB and the removal of ARGs in wastewater effluents (Tsai et al., 2010; Xiong and Hu, 2013; Rizzo et al., 2014a,b,c; Dunlop et al., 2015; Ferro et al., 2015a; Fiorentino et al., 2015; Venieri et al., 2016).

► *TiO_2 dose (synergistic effect of catalyst concentration, UV intensity and reaction time) strongly affects the inactivation of ARB and the removal of ARGs*

The effect of a UV-A-driven TiO_2-photocatalytic treatment on the inactivation of three ARB (methicillin-resistant *S. aureus* [MRSA], multi drug-resistant *Acinetobacter baumannii* [MDRAB], and vancomycin-resistant *E. faecalis* [VRE]) was examined by Tsai et al. (2010). For comparison purposes, various antibiotic-sensitive strains of bacteria were used as controls, i.e. *S. aureus* (MSSA), *A. baumannii* (MDSAB), *E. faecalis* (VSE), *E. coli*, and the bacteriophage MS2. The evaluation of the process efficiency comprised determination of the effect of the catalyst dose, UV-A intensity and reaction time on the bacterial cell concentration. The inactivation of the ARB examined in this study increased exponentially with increasing TiO_2 dose in the reactor (dose herein refers to the synergistic effect of the TiO_2 concentration [*C*], UV-A intensity [*I*] and reaction time [*t*], expressed as *CIt,* on the bacterial population). The target ARB required TiO_2 doses (*CIt*) in the range of 1743–2565 to achieve 99% inactivation, while the bacterial population declined exponentially with increasing reaction time, rendering thereby the reaction time as the most critical parameter governing the process efficiency with regard to microbial survival. Different bacterial inactivation efficiencies were recorded during treatment, with VRE being less susceptible to TiO_2 photocatalysis compared to MRSA and MDRAB. Also, among all the tested bacteria (both resistant and sensitive), MSSA and MRSA exhibited the same susceptibility against TiO_2 photocatalysis, while MDSAB and VRE were found to be significantly more resistant than MDRAB and VSE, respectively. This behavior indicates the different sensitivity of different resistant bacterial strains to the photocatalytic treatment under the same operating conditions.

Different ARB inactivation efficiencies of antibiotic-resistant *E. coli* under various irradiation types (UV-A, UV-C, simulated and natural solar light) were reported by Rizzo et al. (2014a), and the optimum TiO_2 concentration estimated through an irradiation absorption-scattering modeling approach was found to be 0.1 g L^{-1} under all irradiation conditions. The TiO_2-photocatalytic bacterial inactivation was found to be strongly correlated with the catalyst concentration, exhibiting the typical behavior usually observed in slurry photocatalytic reactors (Malato et al., 2009). Generally, a low catalyst concentration can result in a surface site limiting reaction and consequently in insufficient $HO^{\bullet}$ production, whereas a high TiO_2 dose above the optimum one, can reduce the transmittance of the light due to the increased turbidity. This was also observed in Ferro et al. (2015a), where the highest catalyst concentration ($[TiO_2]$ = 100 mg L^{-1}) did not improve the TiO_2 photocatalytic efficiency for the inactivation of multidrug-resistant *E. coli* but it rather required more energy accumulated per treated volume and treatment time. The findings of these studies clearly demonstrated the fundamental importance of optimizing the reactor optical thickness that interrelates the catalyst loading, the light source and the reactor geometry, with the disinfection efficiency. In addition, solar irradiation type affected in different ways the resistance of *E. coli* towards the target antibiotics (ciprofloxacin, cefuroxime, tetracycline, and vancomycin), with solar simulated irradiation being the most effective in achieving a complete bacterial inactivation ($[TiO_2]$ = 0.05 g L^{-1}, 60 min) compared to natural solar irradiation conditions (Rizzo et al., 2014a). This behavior was also observed by Rizzo et al. (2014c) during the inactivation of tetracycline-resistant *Enterococcus* by TiO_2 photocatalytic treatment ($[TiO_2]$ = 0.05 g L^{-1}) under simulated or natural solar light.

The effect of solar-driven TiO_2 photocatalysis on the survival of multidrug-resistant *E. coli* isolated from wastewater effluents (ampicillin, ciprofloxacin, and tetracycline) was investigated by Fiorentino et al. (2015). The complete bacterial inactivation was achieved within 150 min of solar treatment with 100 mg L^{-1} of TiO_2 and a Q_{UV} of 20 kJ L^{-1}, while the performance of the process was facilitated by the addition of H_2O_2, resulting in faster inactivation of *E. coli* (60 min) with a significantly lower cumulative energy (TiO_2/H_2O_2 = 10:100, Q_{UV} = 3 kJ L^{-1}). It is well known that the H_2O_2 addition in the TiO_2-photocatalytic system, facilitates the charge separation (e^-_{CB} - h^+_{VB}) on the semiconductor surface, promoting thus the $HO^{\bullet}$ production (Malato et al., 2009). The results achieved in this study are in a quite good agreement with those documented in other studies, where solar-driven TiO_2 photocatalytic process was applied for the inactivation of multidrug-resistant *E. coli* (Rizzo et al., 2014a; Ferro et al., 2015a), and *Enterococcus* (Rizzo et al., 2014c) strains in biologically treated urban wastewater.

► *The superiority of doped over non-doped TiO_2 treatment for the inactivation of ARB and the removal of ARGs has been demonstrated*

The potential of a metal-doped TiO_2 photocatalytic treatment in inactivating ARB and removing ARGs was also assessed by some research groups. Venieri et al. (2016) investigated the efficiency of manganese and cobalt-doped TiO_2 photocatalysis (Mn-, Co-, and binary Mn/Co-TiO_2) to inactivate antibiotic-resistant *K. pneumoniae* (ampicillin, cefaclor, sulfamethoxazole, and tetracycline) and to remove selected ARGs (*tetA*, *tetM*, *sul1*, bla_{TEM}, and *ampC*) in real wastewater under both simulated and natural solar irradiation. The superiority of the metal doped-catalysts on the bacterial inactivation compared to the non-doped TiO_2 was clearly demonstrated in this study, while experimental runs under simulated and natural solar irradiation conditions have shown a notable difference in the

reduction of bacterial population between the two types of solar light (6-log and 2-log reduction under simulated and natural solar irradiation, respectively). The interesting observation is that photocatalysis with the binary-dopant TiO_2 resulted in a significant decrease of the MIC values (especially in the case of cefaclor and tetracycline), which could be indicative of mutations and/or considerable lesions in the genetic material of surviving cells. The latter may be derived from the fact that additional reactive oxygen species (ROS) are generated during multiple-dopant TiO_2 photocatalytic processes, which affect not only the microbial inactivation rate, but also their overall response towards biocidal factors like antibiotics. Moreover, among the ARGs tested, only *sul1* and *ampC* remained in the reaction solution after Mn/Co-TiO_2 photocatalysis. In the study performed by Rizzo et al. (2014b), a higher photocatalytic inactivation of *E. coli* harbouring resistance against cefaclor was observed for the N-doped TiO_2 compared to commercially available non-doped TiO_2.

► ***TiO_2-immobilized configurations seem to require longer reaction times for the inactivation of ARB and the removal of ARGs***

From an engineering point of view, the post-separation and the necessity of regenerating slurry TiO_2 after treatment remains the major technical obstacle towards its full-scale application. In the study of Dunlop et al. (2015), the efficiency of TiO_2-immobilized photocatalytic configuration in the presence of UV-A irradiation to inactivate antibiotic-sensitive (K12) and antibiotic-resistant *E. coli* strains (J-53R and HT-99 conferring resistance to rifampicin and chloramphenicol, respectively), as well as the potential for conjugative gene transfer between antibiotic-resistant *E. coli* as a result of the oxidative stress, were examined. *E. coli* J-53R and HT-99 inactivated by 2.5 log within 180 min of treatment, while a complete inactivation of *E. coli* K12 was obtained. The applied treatment time (180 min) seemed to be insufficient in provoking a permanent oxidative damage to both strains of antibiotic-resistant *E. coli*. The recovery of injured antibiotic-resistant *E. coli* was confirmed by regrowth analysis, suggesting that both strains were less sensitive to the sub-lethal oxidative stress involved in TiO_2 photocatalysis compared to the antibiotic-sensitive strain. The findings of this study also revealed that the TiO_2-photocatalytic systems experienced substantial interferences by the constituents of dE_fOM originally present in wastewater effluents, which can inhibit the ARB inactivation/ARGs transfer, through multiple mechanisms, including ROS scavenging essentially "protecting" the bacteria from the biocidal agents, antagonistic adsorption reactions onto the catalyst surface and direct interferences with light penetration (Rincon and Pulgarin, 2004a,b; Michael-Kordatou et al., 2015a). In the study of Xiong and Hu (2013), where UV-A/LED TiO_2-coated system was applied for the inactivation of antibiotic-resistant *E. coli*, it was found that both the light intensity and periodic illumination mode (circle time vs duty time, please refer to Table 1) affected the disinfection capacity of the process. A higher inactivation rate of *E. coli* was observed under high light intensity values, while shorter circle periodic illumination time and higher pulse frequency led to a higher log-reduction, under the same UV dose, compared to that observed under continuous illumination conditions. Moreover, small duty circles favoured the bacterial inactivation (under the same UV dose, the inactivation of antibiotic-resistant *E. coli* at 0.25-duty circle periodic illumination exceeded that observed under continuous illumination).

Overall, the results of these studies clearly highlight the necessity of applying prolonged time of TiO_2 photocalytic treatment to avoid post-treatment recovery from sub-lethal injury and the highly undesirable transfer of ARGs amongst bacteria during wastewater treatment. Furthermore, the optimization of TiO_2 photocatalysis for the minimization of ARB&ARGs should take into consideration the qualitative characteristics of the matrix under investigation, which usually aid in the resistance of microbial population, requiring thus higher catalyst loadings for effective and accelerated inactivation rate. A pre-treatment step (e.g. membrane filtration, activated carbon adsorption) may be considered as a possible solution, in order to minimize the organic/inorganic content of the wastewater prior to TiO_2 photocatalytic application.

3. Main oxidative damage pathways involved in the inactivation of ARB and the removal of ARGs by advanced chemical oxidation processes

A series of events takes place in the presence of oxidative disinfectants on bacterial cells. This begins with the interaction of the disinfectant with the cell surface, followed by its penetration into the cell and action on specific target sites. The oxidant-cell component interactions taking place at the cell surface, i.e. on the cell membrane/cell wall (extracellularly) may induce important viability effects on the cell, although most disinfectants perform their most pertinent effects including cellular death, intracellularly. As there is very limited scientific evidence shedding light onto the main oxidative damage pathways on bacteria by AOPs on ARB&ARGs, this section provides an account on the main damage mechanisms involved in their inactivation/removal by the above-mentioned treatment processes, which are given in ***italics***. Table 3 presents the main oxidative damage pathways involved in the inactivation of ARB and the removal of their genetic constituents by the examined treatment processes, as well as their main physiological impacts on bacteria. It is noted that this Section provides a review of the relevant studies regarding the main oxidative pathways involved in the inactivation of ARB and the removal of ARGs. However, where these are not available, appropriate studies regarding the main oxidative pathways of removal of total bacteria are discussed.

3.1. UV- and solar-based processes (UV/H_2O_2, $solar/H_2O_2$)

The disinfecting action and the achievement of inactivation of ARB and the removal of ARGs by $solar/H_2O_2$ and UV/H_2O_2 processes may be credited to the combined action of UV or solar radiation with the enhanced production of radicals by H_2O_2 due to received radiation energy. It has been shown that ***UV radiation interacts with target moieties in bacterial cells by physical processes first, such as light absorption by certain chromophores*** such as the L-tryptophan, which subsequently leads to photochemical inactivation reactions inside the cell. The effect of UV radiation was shown to be exhibited according to Dodd (2012), through UV-A oxidative stress which is damaging to internal cell components, causing: (i) lipid peroxidation, (ii) DNA rupture, (iii) generating single strand breaks, and (iv) nucleic acid modifications which eventually lead to mutagenesis or lethal damage. As a result of ***the observed high absorption capacity by nucleic and amino acids which may lead to DNA modifications***, UV radiation is highly suited for reduction of ARGs (Eischeid et al., 2009). The pyrimidines (TMP, UMP and dCMP) are most susceptible to UV-induced damage due to three mechanisms of UV action on their structure according to Dodd (2012): (i) pyrimidine-pyrimidine dimerization where two pyrimidine bases are located next to each other on single stranded DNA, (ii) pyrimidine (6–4) pyrimidone coupling between two adjacent pyrimidines, and (iii) protein-DNA cross linking. Microorganisms receiving a sub-lethal dose of UV radiation may become resistant to induced oxidative stress, leading to partial recovery of damaged defence mechanisms and adaptations to oxidative stress.

Table 3
Main oxidative pathways of advanced chemical oxidation processes with regard to the inactivation of ARB and the removal of their genetic constituents - Corresponding impacts on the physiological bacterial cellular function.

Treatment process	Mechanisms of action	References
UV- and solar-based processes (UV/H_2O_2, $solar/H_2O_2$)	**1. Interaction of UV radiation with target moieties by physical processes, i.e. absorption by chromophores** • UV-A oxidative stress • Lipid peroxidation • DNA rupture • Single strand breaks • Nucleic acid modifications • Mutagenesis • Cell death	Eischeid et al. (2009) Dodd (2012)
	2. DNA modifications • Replication alterations • Growth limitations • Limitation of capacity of survival	Hoelzer and Michod (1991) Michod et al. (2008)
	3. Oxidative damage due to H_2O_2 exposure • High mutation rates • Growth defects • Cell death • Oxidation of loose ferrous iron inside the cell and further production of ROS through the initiation of intracellular Fenton oxidation reactions	Park et al. (2005) Bosshard et al. (2010) Mishra and Imlay (2012) Lee et al. (2015) Giannakis et al. (2016a,b)
Ozonation	**1. Reaction of O_3 with unsaturated bonds within the membrane-bound phospholipids and lipopolysaccharides** • Reactivity mainly towards N-terminal amino acids, free amines, aromatic and organosulfur side chains of amino acids in proteins found in the peptidoglycan layer and inside the cell	Pryor et al. (1991) Dodd (2012)
	2. Molecular ozone attack on organic molecules • Attack on carbon double bonds, aromatic rings and functional groups containing sulfur, phosphorus, nitrogen and oxygen	Oppenländer (2003) Ikehata et al. (2006)
	3. ROS attack on organic and inorganic compounds • Hydrogen abstraction • Radical-radical reactions • Electrophilic addition • Electron transfer reactions • Complete mineralization of organic compounds	Oppenländer (2003) Ikehata et al. (2006)
	4. Compromise of membrane selective permeability and structural integrity • Exposure of the cell interior to the external environmental conditions • Increased cell permeability • Leakage of cellular components to the outside	Hunt and Mariñas (1999) Cho et al. (2010)
	5. Damage of molecular O_3 and ROS on genetic material • Single strand breaks • Individual base damage producing altered plasmid conformations • Cross-linking of proteins	Ishizaki et al. (1987) Theruvathu et al. (2001) Öncü et al. (2011)
Homogeneous photocatalysis (Fe^{2+} or Fe^{3+}/H_2O_2)	**1. Release of cellular components and deformation of cell surface** • Loss of permeability • Cell swelling and rupture • Cell lysis	Diao et al. (2004)
	2. H_2O_2 ligands with cellular Fe traces found inside enzymes • Oxidation of catalytic iron atom of dehydratase clusters • Loss of precipitated Fe^{3+} • Enzyme inactivation • Repeated cycles of oxidation and release of iron leads to misinstallation of enzymes with zinc leading to progressive loss in enzymic function	Lee et al. (2004) Mishra and Imlay (2012)
	3. ROS oxidative damage inside bacterial cells • Hyperoxia • Disruption of amino acid biosynthesis pathways • Cellular dehydration	Kuo et al. (1987) Flint et al. (1993) Imlay (2003) Anjem and Imlay (2012)
	4. DNA damage by Fenton reactions • Oxidation of base and ribose moieties • Lesions on DNA • Mutagenesis	Hogg et al., 2005 Imlay (2015) Giannakis et al. (2016a,b)
Heterogeneous TiO_2 photocatalysis	**1. Produced ROS damage on bacteria** • Cell membrane/cell wall structure modifications • Lipid peroxidation • Protein and polysaccharide oxidation • Alteration of cell permeability • Conformational change of genetic material • Single DNA strand breaks	Sunada et al. (2003) Gogniat et al. (2006) Alrousan et al. (2009) Malato et al. (2009) Kambala and Naidu (2009) Dalrymple et al. (2010)

Table 3 (*continued*)

Treatment process	Mechanisms of action	References
	2. Bacterial adhesion onto TiO_2 particles • Rupture of outer membrane • Decomposition of cytoplasmic membrane • Photocatalytic killing	Dunford et al. (1997) Bandala and Bustos (2015) Shen et al. (2008) Öncü et al. (2011)

Regardless of the studies made on the effect of UV radiation on the quantification of extracellular ARGs through molecular analyses, only limited studies have been performed on the effects of UV radiation *in vivo* on bacterial DNA. These effects may include behavioural changes, changes in replication, growth and other survival mechanisms. In detail, Hoelzer and Michod (1991) investigated the effect of UV radiation on tryptophan- and methionine-prototrophic *Bacillus subtilis* cells, producing 20–40% reduction in the ability of the intracellular DNA ability to transform auxotrophic recipient bacteria to prototrophic ones. Moreover, limited work has been made on the ability of bacteria which have been UV-irradiated, to undergo light or dark repair, thus restoring activity of DNA. DNA repair may take place in ARB which were exposed to UV or within competent recipient cells which have received UV-damaged DNA from ARB donors. Remarkably, Michod et al. (2008) suggests that UV-induced damage in genetic material may bring about recombinational repair of damaged DNA, which may substantially benefit pathogenic bacteria through oxidative defense, potentially creating new infective strains.

Regrowth of inactivated bacterial cells has been observed in the presence of UV radiation. Bacterial exposure to UV was shown to cause reversible damage to cells, with a temporary disinfection effect (Lee et al., 2004). In a first instance, a 19-fold recovery of *Vibrio anguillarum and Aeromonas salmonicida* compared to residual bacterial levels after exposure to UV was achieved post-exposure (Liltved and Landfald, 2000). In another study by Oguma et al. (2001), it was observed that in *E. coli* and *Cryptosporidium parvum*, which were exposed to fluorescent light after 99.9% inactivation by UV irradiation, UV-induced pyrimidine dimers in the DNA were continuously repaired and their colony-forming ability recovered gradually. Interestingly, when these bacteria were kept in darkness after the UV inactivation, they showed neither repair of pyrimidine dimers nor recovery of colony-forming ability. In order to explain this phenomenon of regrowth, it was suggested by Lee et al. (2015) that the pyrimidine dimers in the DNA can be repaired under a range of 330–480 nm of wavelength through a light-induced enzymatic process (photoreactivation) or without light (dark repair). Thus, as suggested by the authors, as long as there is no accumulation of energy which is necessary to cause permanent effects on bacteria (Bosshard et al., 2010) or to throw them in a viable, but not cultivable state and dark repair, disinfected bacteria may potentially recover their ability of proliferation and result in regrowth (Giannakis et al., 2016a,b).

The damaging impact of H_2O_2 in exposed bacteria includes: (i) high mutation rates, (ii) growth defects, and (iii) death (Mishra and Imlay, 2012), while increased levels of environmental oxygen concentrations can exacerbate this impact. H_2O_2 has the ability to penetrate the lipid bilayer in the cell membrane, as it has a permeability coefficient similar to the one for water. As molecular oxygen in H_2O_2 cannot react with biomolecules, the reduced forms of it, namely ROS are the main damaging molecules to cell components. The resulting presence of ROS produces oxidation of loose ferrous iron inside the cell that is not protein-bound, producing the Fenton oxidation and further production of ROS (Park et al., 2005). According to Mishra and Imlay (2012), as the H_2O_2 reaction constants with iron centers are very high (1000-50,000 M^{-1} s^{-1}), even sub-micromolar concentrations of H_2O_2 can inactivate enzymes in a very short time. Therefore, the key is to keep H_2O_2 concentrations below harmful levels. Employing extremely active peroxidases and catalases accomplishes this, i.e. ROS scavenging enzymes that reduce and degrade H_2O_2 respectively, down to physiological levels inside cells, bringing about bacterial survival and repair in the presence of H_2O_2. ROS scavengers include β-carotene (singlet oxygen scavenger) and superoxide dismutase (SOD) which protect bacteria from radiation and oxidation.

3.2. Ozonation

The strong oxidation potential of both ozone and produced $HO^{\bullet}$ (2.07 and 2.8 V, respectively) indicates their high oxidative capacity (Ikehata et al., 2006). The difference between the two types of reaction taking place during ozonation is that molecular ozone reactions are selective, specifically attacking organic molecules having nucleophilic moieties such as carbon double bonds, aromatic rings and functional groups containing sulfur, phosphorus, nitrogen and oxygen while $HO^{\bullet}$ reactions are non-selective, ***attacking organic and inorganic compounds through hydrogen abstraction, radical-radical reactions***, ***electrophilic addition and electron transfer reactions***, ***eventually leading to complete mineralization of organic compounds*** (Oppenländer, 2003).

The action of O_3 ***against microorganisms for effective inactivation of ARB***, ***has been attributed to its reactivity with various organic functional groups***, such as amines, activated aromatics and reduced sulfur moieties, within the cellular membrane in Gram-positive bacteria and inside the cell wall/membrane in Gram-negative bacteria (Von Gunten, 2003). Initially, on the surface of the bacterial cell, ***O_3 reacts rapidly with unsaturated bonds within the membrane-bound phospholipids and lipopolysaccharides*** (Pryor et al., 1991). Moreover, O_3 was shown to be relatively unreactive towards amide groups of peptide-bound amino acids, making it reactive only towards N-terminal amino acids, free amines, aromatic and/or organosulfur side chains of tryptophan, tyrosine, histidine, lysine, methionine and cysteine in cellular proteins and peptidoglycan layer (Dodd, 2012).

After the oxidative damage on the cell wall/membrane which leads to cell inactivation, ***increased permeability occurs***, ***leading to leakage of cellular components to the outside of the cell*** (Dodd, 2012). In a study by Hunt and Mariñas (1999) using transmission electron microscopy (TEM), it was shown that *E. coli* cells entered a viable but not cultivable state once exposed to O_3, before the extensive degradation of cellular components, demonstrating that oxidation of the membrane is a crucial step to the lethal action of O_3. More precisely, the electron micrographs showed a similar cellular content of *E. coli* cells exposed to 0.0826 mg L^{-1} s^{-1} O_3 at pH 7.2 and *E. coli* cells in controls (no O_3), prior to cellular component leakage. Despite the similarity in cell content in O_3 exposed and non O_3-exposed cells, there was 5-log reduction in cultivable *E. coli* cells after exposure to O_3. Despite the remarkable damage induced on the cell membrane by O_3, in a study by Cho et al. (2010) it was shown that internal component damage is less intense when most damage takes place on the outer cell components. As a result, the inner molecular components such as ARGs may be less affected by such oxidants, as they may remain intact within cell debris after ARB loss of viability.

The effect of O_3 on genetic material has been shown to include strand breaks along with base damage producing altered plasmid DNA conformations (i.e. from circular to open linear), while proteins have been shown to be cross-linked (Ishizaki et al., 1987). The molecular action of O_3 on nucleic acids has been investigated by Ishizaki et al. (1984). The mononucleotides thymidine monophosphate (TMP) and deoxyguanosine monophosphate (dGMP) were shown to react faster with O_3 than deoxycytidine monophosphate (dCMP) and deoxyadenosine monophosphate (dAMP) at a neutral pH environment, making the first two mononucleotide reactions with O_3 a ***limiting factor in the extension of DNA*** and thus in cell structural modifications during O_3 treatment. The double strand DNA (dsDNA) reactivity with O_3 was shown to be lower compared the O_3 reaction rates on each individual nucleobase, a phenomenon which has been attributed to the protection of dsDNA reactive sites by H-bonding between the individual DNA strands (Theruvathu et al., 2001). In another study, Öncü et al. (2011) investigated the effectiveness of ozonation on antibiotic resistance determinants, i.e. reduction of plasmid DNA from a multi-resistant *E. coli* HB101 and the effect of plasmid DNA concentration on the treatment process effectiveness. It was shown by this study that as O_3 concentration increased (maximum O_3 concentration examined was 4.2 mg L^{-1}), the PCR band intensity of conformations other than the original supercoiled plasmid increased, indicating a change in plasmid structure and reduction in fragment size caused by O_3. A lower concentration of 0.9 mg L^{-1} was able to reduce band intensity at the lower plasmid concentrations examined.

Despite the important findings of the studies examining the potential of the ozonation process to inactivate ARB and to remove ARGs, there is still lack of research on the separation of the role of molecular O_3 action from the secondary reactions taking place in solution by the produced $HO^{\bullet}$, which are continuously generated during aqueous decomposition of O_3.

3.3. Homogeneous photocatalysis (Fe^{2+}or Fe^{3+}/H_2O_2)

The action of the solar Fenton process on the cell metabolism is dependent on the concentrations of iron and H_2O_2, which are used during the photocatalytic process and can penetrate cells. The mode of action of the Fenton reagent on *E. coli* bacteria was investigated by Diao et al. (2004). The authors examined scanning-electron microscope (SEM) images of Fenton-treated bacteria, showing that the reaction caused a ***release of cellular components and deformation of cell surface***. Such deformation may lead to: (i) loss of permeability, (ii) cell swelling and rupture, thus confirming the effect of ROS on cell surface which may lead to cell lysis.

H_2O_2 has the capacity to directly ligand and oxidize the catalytic iron atom of dehydratase clusters, precipitating Fe^{3+} loss and leading to enzyme inactivation. Surprisingly, it was shown that when H_2O_2 inactivates these enzymes *in vitro*, there is the potential of restoration of enzyme capacity through the supply of iron and a reductant. This has been attributed to the abstraction of a second electron by the ferryl radical ($[FeO]^{2+}$) converting the $HO^{\bullet}$ into HO^{-}, a harmless anion. Next, although there is temporary disablement of enzymes, a reparable species [3Fe-4S] is left behind (Mishra and Imlay, 2012). H_2O_2 was further shown to damage the Fe-S cluster protein synthesis system, responsible of Fe–S clusters to new apoenzymes, through the attack to nascent clusters on the scaffold protein IscU, which mediates the assembly of new clusters. This type of damage is moderated through the use of the OxyR system, an active site Cys residue containing transcription factor, which reacts rapidly with H_2O_2 for the induction of the Suf system, which also builds clusters and transfers them to proteins while avoiding H_2O_2 interference (Lee et al., 2004).

Excessive H_2O_2 above 200 nM is primarily detected by OxyR. This system is transformed into a disulphide-bonded form, which drives the transcription of various operons in the chromosome. OxyR drives the synthesis of KatG and Ahp in an effort to drive H_2O_2 levels back to harmless levels. Also, superoxide dismutase (SOD) is shown to act intracellularly during solar Fenton oxidation according to Carlioz and Touati (1986), in a first line of bacterial defense against ROS toxicity. The authors reported that SOD-deficient bacterial mutants of *E. coli* were not unable to grow unless their growth medium was supplemented with aromatic (tyrosine, tryptophan, phenylalanine), sulphurous (methionine, cysteine) or branched (leucine, isoleucine, valine) chain amino acids. It was thus suggested that produced ***ROS, especially O_2^- found inside the cell produce hyperoxia while disrupting amino acid biosynthesis pathways*** (Imlay, 2003). Moreover, O_2^- was shown to destroy the catalytic [4Fe–4S] cluster of the dihydroxy-acid dehydratase enzymes, leading to ***cellular dehydration*** (Kuo et al., 1987). Other enzymes found to be sensitive to O_2^- include aconitase A and B and fumarase A and B of the tricarboxylic acid cycle (Flint et al., 1993). Oxidation and release of the iron atoms found in mononuclear enzymes was shown to take place by O_2^-, although this damage is not significant during the beginning of contact. Repeated cycles of oxidation and release of these iron atoms inside cells leads to misinstallation of the enzyme with zinc, which is not as efficient catalytically as iron, leading to a progressive loss in enzymic function (Anjem and Imlay, 2012). Despite the proof of enzymic damage by iron, *E. coli* was shown to sustain mononuclear enzyme activity by replacing iron with manganese, increasing its levels during oxidative stress, and serving as a H_2O_2 resistant cofactor while being as active in intracellular catalytic actions as iron.

Mutagenesis is another impact of the solar Fenton process on bacteria, through DNA damage. $HO^{\bullet}$ has the potential of oxidation of base and ribose moieties in DNA, causing lesions. Certain bases such as guanine may receive an additional amount of damage compared to other bases, e.g. guanine. This effect is due to the low reduction potential allowing electrons to move to holes in near oxidized base radicals. A common guanine damage product is 8-hydroxyguanine, which has the ability to base pair with adenine, leading to the misleading of the mispair detection system, which is inherent in DNA polymerases (Hogg et al., 2005). Thymine oxidation produces non-coding lesions, which block polymerase progression leading to cell death and not mutagenesis.

In summary, it was shown that the solar Fenton oxidation is an effective process of bacterial disinfection, as various pathways of bacterial damage and inactivation are included in the available literature, including disruption of amino acid biosynthesis, cell surface deformation, enzyme inactivation, as well as cellular dehydration.

3.4. Heterogeneous TiO_2 photocatalysis

As has been previously mentioned, the main inactivation mechanism of microorganisms during AOPs application is the conferred ***cell damage produced by ROS***, which is able to modify and destroy the structure of the cell wall and cell membrane (Alrousan et al., 2009; Malato et al., 2009). Initial bacterial cell membrane damage is brought about in the outer lipopolysaccharide and peptidoglycan wall, followed by lipid peroxidation and protein and polysaccharide oxidation. These processes, which alter cell permeability, also bring about negative impacts on the regulatory function of cell membrane, as to its capacity for internal and external interchange (Dalrymple et al., 2010). This effect has been demonstrated through the leakage of K^+ ions by Kambala and Naidu (2009), who showed that cell death of *E. coli* cells takes place due to the photocatalytic treatment, while Gogniat et al.

(2006) showed that membrane permeability alterations followed after attachment of *E. coli* to TiO_2 particles during heterogeneous photocatalysis. In this study, it was observed that ***bacterial adhesion onto catalytic particles was positively associated to the photocatalytic disinfecting effect*** and with a reduction in loss of bacterial membrane integrity. According to Sunada et al. (2003), thin TiO_2 films caused outer membrane damage first, followed by decomposition of cytoplasmic membrane, leading to photocatalytic killing, despite an absence of visible peptidoglycan degradation.

Once external component damage and increased membrane permeability is achieved, further oxidative attack on the cell inner components, such as genetic material and vital cell structures is allowed leading to cell damage and inactivation (Bandala and Bustos, 2015). For example, treatment of plasmid DNA with TiO_2 was shown to produce conformation change in a study by Öncü et al. (2011), as PCR band intensity of smaller size fragments increased after treatment with TiO_2, compared to the original plasmid DNA size, with complete disappearance of the fragments after 75 min of treatment. Thus, it was suggested by the authors that single strand breaks took place on the supercoiled plasmid because of treatment, producing changes in plasmid structure, while smaller fragments underwent DNA degradation. Another study by Dunford et al. (1997) showed that TiO_2 photocatalysis produces strand breaks on DNA because of direct ROS oxidation of the genetic material, an observation which is in agreement with Shen et al. (2008), which showed that TiO_2 photocatalysis produces nicks on circular plasmid structure, as well as linear configurations of previously circular plasmid structures.

Despite the damage incurred by TiO_2 photocatalysis on bacterial cells leading to inactivation, cellular death and thus permanent removal of bacteria may not be achieved, due to reactivation of cells post-treatment, leading to their regrowth. The phenomenon of reactivation is observed to the fact that during photocatalytic processes, generated ROS have a far shorter half-life time than chlorine-based oxidants. Thus, when photocatalytic treatment is applied, the time required to reach complete disinfection of water but also to verify the durability of the photo-disinfection is vital for the success of the process (Rincón and Pulgarin, 2004a,b). In the study conducted by Rincón and Pulgarin (2004a,b), the regrowth of *E. coli* K12 cells was examined 60 h after suspended TiO_2 photocatalysis. The results of this study showed that *E. coli* continued their replication in the dark, reaching values of two-orders of magnitude higher than those at the beginning of the photocatalytic treatment. Even when cultivable *E. coli* bacteria which were enumerated in non-selective media were eventually not able to grow on the media, only 2 h in the dark were enough to reach a cell concentration of 2 logs demonstrating that some bacteria did not die at the end of the photocatalytic process, but were merely inactivated.

Whilst heterogeneous photocatalysis has great potential as a biocidal for pathogenic microorganisms including ARB when appropriate illumination treatment times are used, its exact damaging mechanisms on ARGs has not been investigated so far. Moreover, caution must be exercised when conducting photocatalytic disinfection, as organisms have the potential to recover from sub-lethal ROS dose stress, leading in repair and regrowth. Some studies in fact, have reported this phenomenon (Rincón and Pulgarin, 2004a,b; Dunlop et al., 2010), highlighting the need for complete microorganisms' inactivation and further in-depth analyses to ensure the prevention of further subsequent repair and regrowth.

4. Concluding remarks and open questions

Antibiotic resistance has been justifiably described by Salyers and Amabile-Cuevas (1997) as an ″***easy-to-get, hard-to-lose***″ phenomenon, since ARB can, and often do, work synergistically, by interacting with each other in a variety of mechanisms that enhance remarkably their collective capacity to transfer ARGs. A lack of knowledge regarding variation in the distribution and routes of dissemination of antibiotic resistance determinants in the environment is a key factor contributing to the ineffective mitigation of antibiotic resistance spread and transmission.

UWTPs represent a critical node for control of the global emergence and spread of antibiotic resistance in the environment. Over the last years, the research on AOPs with respect to the removal of antibiotic resistance determinants (i.e. ARB&ARGs) in wastewater has gained a new impetus. Even though the research focus has been recently diverted to this area and the importance of disinfection treatment systems for the mitigation of antibiotic resistance is unequivocally demonstrated, the current state of knowledge on the effect of advanced chemical oxidation on the minimization of ARB&ARGs in wastewater effluents is still fragmentary.

Both technological- and microbiological-based factors, including among others the process bactericidal mechanisms and its operating conditions (e.g. disinfectant dose, contact time, wastewater inorganic/organic composition, etc.), the target bacterial species and genes and the type of antibiotic(s) to which resistance is acquired, have been shown to strongly affect the extent to which advanced chemical oxidation processes inactivate ARB and remove ARGs in wastewater. The different experimental configurations, operating conditions and methodological approaches applied, the variety of both the target ARB&ARGs and the antibiotic(s) to which resistance is acquired, as well as the sparse data in relation to the qualitative characteristics of wastewater, do not allow for an accurate comparison among the various studies conducted in this field. Also, information on the DNA (in terms of plasmids, condensed or restricted DNA, PCR amplicons, etc.) is generally missing from the studies reviewed in this manuscript, making it thus difficult to perform a comparison among the studies evaluated and to further explain the different inactivation and removal levels of ARB&ARGs observed.

Ozonation upon its proper optimization with regard to ozone dose in conjunction with exposure time, was found to be effective in inactivating ARB and removing ARGs and eliminating the potential bacterial regrowth. Photocatalytic processes, such as homogeneous (Fenton) and heterogeneous (TiO_2) photocatalysis, have demonstrated a high efficiency in inactivating ARB and removing ARGs. However, prolonged time seems to be required for their complete elimination and to avoid post-treatment repairing oxidative damage and subsequent recovery of the bacterial species. In some cases, it was observed that AOPs may select for resistance during the course of treatment, a fact that may be attributed to a critical, but often underappreciated, feature of ARB&ARGs related to their stability and ability to adapt to oxidative stressful conditions.

One current open challenge is to identify and understand how the diverse chemical-, biological- and microbiological-derived components of wastewater effluents affect the efficiency of AOPs to inactivate ARB and to remove ARGs. For example, it has been acknowledged in the scientific literature that metals present in wastewater can induce co-selection pressures, thereby promoting the proliferation of ARB. This is of particular concern considering that anthropogenic levels of heavy metals are currently several orders of magnitude greater than the levels of antibiotics, and unlike antibiotics, metals are not subjected to degradation and can subsequently represent a long-standing selection pressure. The types and levels of metal contamination and specific patterns of antibiotic resistance suggest that several mechanisms underlie this

co-selection process. Also, it is yet unclear whether the metals used in homogeneous and heterogeneous photocatalytic applications, e.g. iron and TiO_2, may have such an effect on ARB&ARGs. Thus, it is apparent that the monitoring of metals and/or other physico-chemical parameters may decipher the correlation of such factors to co-selection of ARB&ARGs.

There is limited literature surrounding the post-treatment regrowth of ARB after AOPs and the mechanisms through which bacteria are able to repair their cellular damage and to regrow during the storage of the AOPs-treated effluents. Thus, from the technological point of view, it is important to make an in-depth investigation of how the operating conditions affect the bacterial regrowth and to optimise AOPs, in order to avoid any bacterial repair and regrowth phenomena.

Another aspect that lacks investigation is whether the transformation products of antibiotics or of other contaminants originating during advanced chemical oxidation can contribute towards antibiotic resistance development. AOPs may encourage the formation of new, unknown compounds that may preserve the same mode of action with that of the parent contaminant or possess their own antimicrobial activity, potentially contributing to the evolution of antibiotic resistance in bacterial strains in wastewater. However, the lack of commercial reference standards constitutes a major obstacle in verifying this.

As the disinfection toolbox grows, so must our knowledge of how to apply, combine and monitor the technologies available to achieve effective inactivation of ARB and removal of ARGs. Still, ***many questions remain open*** as to the mechanisms through which ARB are inactivated, survive, multiply, evolve during wastewater treatment by AOPs and the conditions and/or other indirect factors that may enhance or mitigate the occurrence of HGT mechanisms and selection of antibiotic resistance. *Can what we know about AOPs mechanisms help explain the fate of ARB&ARGs during the application of the specific AOP? How does aggregating all ARB&ARGs together when assessing the efficiency of AOPs hide important survival and antibiotic resistance trends in certain bacteria or in certain cellular mechanisms though these processes? What should the basis of a harmonized methodological framework to be utilised by the scientific community when assessing the efficiency of AOPs as to the inactivation of ARB and the removal of ARGs? Can AOPs optimization and upscaling provide a promising solution for the mitigation of ARB&ARGs in urban wastewater? Should the operating conditions of AOPs be harsh to ensure permanent inactivation of ARB or should be mild in order to avoid any selection of ARB during treatment? How can we achieve a compromise? Is there any difference on the behavior of ARB and of non-resistant bacteria during the application of AOPs? Which ARB&ARGs should be monitored during AOPs? What actions can be taken by UWTPs in the light of the current knowledge?*

As a conclusion, the establishment of universal methods and of course their harmonisation, and extensive data gathering, are a prerequisite for a more accurate and continuous monitoring of the ongoing global spread of antibiotic resistance in the environment, especially in countries where wastewater reuse practices are being implemented. This is needed in order to support reliable and advanced risk assessment evaluations, to determine the impact that wastewater treatment processes, including AOPs, have on antibiotic resistance proliferation.

The implementation of the precautionary principle is necessary at this point, where no adequate information is available regarding the abovementioned questions. In line with the precautionary principle and also the One-Health approach, and considering the fact that resistance occurs not only in bacterial populations but also in other microorganisms, such as fungi, viruses and parasites to antimicrobials (WHO, 2014), as an outlook, we suggest that the research must be diverted in general on antimicrobial and not only on antibiotics resistance.

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References

Agulló-Barceló, M., Polo-López, M., Lucena, F., Jofre, J., Fernández-Ibáñez, P., 2013. Solar advanced oxidation processes as disinfection tertiary treatments for real wastewater: implications for water reclamation. Appl. Catal. B Environ. 136, 341–350.

Alexander, J., Knopp, G., Dötsch, A., Wieland, A., Schwartz, T., 2016. Ozone treatment of conditioned wastewater selects antibiotic resistance genes, opportunistic bacteria, and induce strong population shifts. Sci. Total Environ. 559, 103–112.

Alrousan, D.M.A., Dunlop, P.S.M., McMurray, T.A., Byrne, J.A., 2009. Photocatalytic inactivation of *E. coli* in surface water using immobilised nanoparticle TiO_2 films. Water Res. 43, 47–54.

Andersson, D.I., Hughes, D., 2014. Microbiological effects of sublethal levels of antibiotics. Nat. Rev. Microbiol. 12 (7), 465–478.

Anjem, A., Imlay, J.A., 2012. Mononuclear iron enzymes are primary targets of hydrogen peroxide stress. J. Biol. Chem. 287, 15544–15556.

Bandala, E.R., Bustos, E., 2015. In: Hernández-Ramírez, A., Medina-Ramírez, I. (Eds.), Photocatalytic Semiconductors. Springer International Publishing, Cham, pp. 255–278.

Baquero, F., Martínez, J., Cantón, R., 2008. Antibiotics and antibiotic resistance in water environments. Curr. Opin. Biotechnol. 19 (3), 260–265.

Becerra-Castro, C., Lopes, A.R., Vaz-Moreira, I., Silva, E.F., Manaia, C.M., Nunes, O.C., 2015. Wastewater reuse in irrigation: a microbiological perspective on implications in soil fertility and human and environmental health. Environ. Int. 75, 117–135.

Bengtsson-Palme, J., Hammarén, R., Pal, C., Östman, M., Björlenius, B., Flach, C., Fick, J., Kristiansson, E., Tysklind, M., Larsson, D.J., 2016. Elucidating selection processes for antibiotic resistance in sewage treatment plants using metagenomics. Sci. Total Environ. 572, 697–712.

Berendonk, T.U., Manaia, C.M., Merlin, C., Fatta-Kassinos, D., Cytryn, E., Walsh, F., Bürgmann, H., Sørum, H., Norström, M., Pons, M., 2015. Tackling antibiotic resistance: the environmental framework. Nat. Rev. Microbiol. 13 (5), 310–317.

Berglund, B., 2015. Environmental dissemination of antibiotic resistance genes and correlation to anthropogenic contamination with antibiotics. Infect. Ecol. Epidemiol. 5, 28564.

Blatchley, E.R., Weng, S., Afifi, M.Z., Chiu, H., Reichlin, D.B., Jousset, S., Erhardt, R.S., 2012. Ozone and UV254 radiation for municipal wastewater disinfection. Water Environ. Res. 84 (11), 2017–2029.

Bosshard, F., Bucheli, M., Meur, Y., Egli, T., 2010. The respiratory chain is the cell's Achilles' heel during UVA inactivation in *Escherichia coli*. Microbiology 156 (7), 2006–2015.

Bouki, C., Venieri, D., Diamadopoulos, E., 2013. Detection and fate of antibiotic resistant bacteria in wastewater treatment plants: a review. Ecotoxicol. Environ. Saf. 91, 1–9.

Cantas, L., Shah, S.Q., Cavaco, L., Manaia, C., Walsh, F., Popowska, M., Garelick, H., Bürgmann, H., Sørum, H., 2013. A brief multi-disciplinary review on antimicrobial resistance in medicine and its linkage to the global environmental microbiota. Front. Microbiol. 10.

Carlioz, A., Touati, D., 1986. Isolation of superoxide dismutase mutants in Escherichia coli: is superoxide dismutase necessary for aerobic life? EMBO J. 5 (3), 623–630.

Cattoir, V., Poirel, L., Aubert, C., Soussy, C., Nordmann, P., 2008. Unexpected occurrence of plasmid-mediated quinolone resistance determinants in environmental *Aeromonas* spp. Emerg. Infect. Dis. 14 (2), 231–237.

Cho, M., Kim, J., Kim, J.Y., Yoon, J., Kim, J.H., 2010. Mechanisms of Escherichia coli inactivation by several disinfectants. Water Res. 44 (11), 3410–3418.

Clatworthy, A.E., Pierson, E., Hung, D.T., 2007. Targeting virulence: a new paradigm for antimicrobial therapy. Nat. Chem. Biol. 3, 541–548.

Clinical and Laboratory Standards Institute (CLSI), 2012. Methods for Dilution

Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically: Approved Standard-ninth Edition. www.clsi.org.
Dalrymple, O.K., Stefanakos, E., Trotz, M.A., Goswami, D.Y., 2010. A review of the mechanisms and modeling of photocatalytic disinfection. Appl. Catal. B Environ. 98 (1–2), 27–38.
Davies, J., Davies, D., 2010. Origins and evolution of antibiotic resistance. Microbiol. Mol. Biol. Rev. 74, 417–433.
Diao, H., Li, X., Gu, J., Shi, H., Xie, Z., 2004. Electron microscopic investigation of the bactericidal action of electrochemical disinfection in comparison with chlorination, ozonation and Fenton reaction. Process Biochem. 39 (11), 1421–1426.
Di Cesare, A., Fontaneto, D., Doppelbauer, J., Corno, G., 2016. Fitness and recovery of bacterial communities and antibiotic resistance genes in urban wastewaters exposed to classical disinfection treatments. Environ. Sci. Technol. 50, 10153–10161.
Dodd, M.C., 2012. Potential impacts of disinfection processes on elimination and deactivation of antibiotic resistance genes during water and wastewater treatment. J. Environ. Monit. 14 (7), 1754–1771.
Dunford, R., Salinaro, A., Cai, L., Serpone, N., Horikoshi, S., Hidaka, H., Knowland, J., 1997. Chemical oxidation and DNA damage catalysed by inorganic sunscreen ingredients. FEBS Lett. 418 (1–2), 87–90.
Dunlop, P.S.M., Sheeran, C.P., Byrne, J.A., McMahon, M.A.S., Boyle, M.A., McGuigan, K.G., 2010. Inactivation of clinically relevant pathogens by photocatalytic coatings. J. Photochem. Photobiol. A Chem. 216 (2–3), 303–310.
Dunlop, P., Ciavola, M., Rizzo, L., McDowell, D., Byrne, J., 2015. Effect of photocatalysis on the transfer of antibiotic resistance genes in urban wastewater. Catal. Today 240, 55–60.
Eischeid, A.C., Meyer, J.N., Linden, K.G., 2009. UV disinfection of adenoviruses: molecular indications of DNA damage efficiency. Appl. Environ. Microbiol. 75 (1), 23–28.
EFSA, 2011. EFSA approaches to risk assessment in the area of antimicrobial resistance, with an emphasis on commensal microorganisms. EFSA J. 9 (10), 196. https://doi.org/10.2903/j.efsa.2011.196.
EU, 2017. A European One Health Action Plan against Antimicrobial Resistance (AMR). https://ec.europa.eu/health/amr/sites/amr/files/amr_action_plan_2017_en.pdf.
Ferro, G., Fiorentino, A., Alferez, M.C., Polo-López, M.I., Rizzo, L., Fernández-Ibáñez, P., 2015a. Urban wastewater disinfection for agricultural reuse: effect of solar driven AOPs in the inactivation of a multidrug resistant *E. coli* strain. Appl. Catal. B Environ. 178, 65–73.
Ferro, G., Polo-Lopez, M.I., Martínez-Piernas, A.B., Fernández-Ibáñez, P., Agüera, A., Rizzo, L., 2015b. Cross-contamination of residual emerging contaminants and antibiotic resistant bacteria in lettuce crops and soil irrigated with wastewater treated by sunlight/H_2O_2. Environ. Sci. Technol. 49 (18), 11096–11104.
Ferro, G., Guarino, F., Castiglione, S., Rizzo, L., 2016. Antibiotic resistance spread potential in urban wastewater effluents disinfected by UV/H_2O_2 process. Sci. Total Environ. 560, 29–35.
Ferro, G., Guarino, F., Cicatelli, A., Rizzo, L., 2017. β-lactams resistance gene quantification in an antibiotic resistant Escherichia coli water suspension treated by advanced oxidation with UV/H_2O_2. J. Hazard. Mater. 323 (Part A), 426–433.
Fiorentino, A., Ferro, G., Alferez, M.C., Polo-López, M.I., Fernández-Ibañez, P., Rizzo, L., 2015. Inactivation and regrowth of multidrug resistant bacteria in urban wastewater after disinfection by solar-driven and chlorination processes. J. Photochem. Photobiol. B Biol. 148, 43–50.
Flint, D.H., Tuminello, J.F., Emptage, M.H., 1993. The inactivation of Fe-S cluster containing hydrolyases by superoxide. J. Biol. Chem. 268 (30), 22369–22376.
Gallert, C., Fund, K., Winter, J., 2005. Antibiotic resistance of bacteria in raw and biologically treated sewage and in groundwater below leaking sewers. Appl. Microbiol. Biotechnol. 69, 106–112.
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, 173–183.
Gatica, J., Tripathi, V., Green, S., Manaia, C.M., Berendonk, T., Cacace, D., Merlin, C., Kreuzinger, N., Schwartz, T., Fatta-Kassinos, D., Rizzo, L., 2016. High throughput analysis of integron gene cassettes in wastewater environments. Environ. Sci. Technol. 50, 11825–11836.
Giannakis, S., Polo López, M.I., Spuhler, D., Sánchez Pérez, J.A., Fernández Ibáñez, P., Pulgarin, C., 2016a. Solar disinfection is an augmentable, in situ-generated photo-Fenton reaction-Part 1: a review of the mechanisms and the fundamental aspects of the process. Appl. Catal. B Environ. 199, 199–223.
Giannakis, S., Voumard, M., Grandjean, D., Magnet, A., De Alencastro, L.F., Pulgarin, C., 2016b. Micropollutant degradation, bacterial inactivation and regrowth risk in wastewater effluents: influence of the secondary (pre)treatment on the efficiency of Advanced Oxidation Processes. Water Res. 102, 505–515.
Gogniat, G., Thyssen, M., Denis, M., Pulgarin, C., Dukan, S., 2006. The bactericidal effect of TiO_2 photocatalysis involves adsorption onto catalyst and the loss of membrane integrity. FEMS Microbiol. Lett. 258 (1), 18–24.
Gullberg, E., Cao, S., Berg, O.G., Ilbäck, C., Sandegren, L., Hughes, D., Andersson, D.I., 2011. Selection of resistant bacteria at very low antibiotic concentrations. PLoS Pathog. 7 (7), e1002158.
Hembach, N., Schmid, F., Alexander, J., Hiller, C., Rogall, E.T., Schwartz, T., 2017. Occurrence of the *mcr-1* colistin resistance gene and other clinically relevant antibiotic resistance genes in microbial populations at different municipal wastewater treatment plants in Germany. Front. Microbiol. 8, 1–11.
Heuer, H., Solehati, Q., Zimmerling, U., Kleineidam, K., Schloter, M., Muller, T., Focks, A., Thiele-Bruhn, S., Smalla, K., 2011. Accumulation of sulfonamide resistance genes in arable soils due to repeated application of manure containing sulfadiazine. Appl. Environ. Microbiol. 77 (7), 2527–2530.
Hoelzer, M.A., Michod, R.E., 1991. DNA repair and the evolution of transformation in Bacillus subtilis. III. Sex with damaged DNA. Genetics 128 (2), 215–223.
Hogg, M., Wallace, S.S., Doublié, S., 2005. Bumps in the road: how replicative DNA polymerases see DNA damage. Curr. Opin. Struct. Biol. 15 (1), 86–93.
Hunt, N.K., Mariñas, B.J., 1999. Inactivation of Escherichia coli with ozone: chemical and inactivation kinetics. Water Res. 33 (11), 2633–2641.
Ikehata, K., Jodeiri Naghashkar, N., Gamal El-Din, M., 2006. Degradation of aqueous pharmaceuticals by ozonation and advanced oxidation processes: a review. Ozone Sci. Eng. 28 (6), 353–414.
Imlay, J.A., 2003. Pathways of oxidative damage. Annu. Rev. Microbiol. 57 (1), 395–418.
Imlay, J.A., 2015. Diagnosing oxidative stress in bacteria: not as easy as you might think. Curr. Opin. Microbiol. 24, 124–131.
Ishizaki, K., Sawadaishi, K., Miura, K., Shinriki, N., 1987. Effect of ozone on plasmid DNA of Escherichia coli in situ. Water Res. 21 (7), 823–827.
Ishizaki, K., Shinriki, N., Ueda, T., 1984. Degradation of nucleic acids with ozone. V. Mechanism of action of ozone on deoxyribonucleoside 5′-monophosphates. Chem. Pharm. Bull. 32 (9), 3601–3606.
Kambala, V.S.R., Naidu, R., 2009. Disinfection studies on TiO_2 thin films prepared by a Sol-gel method. J. Biomed. Nanotechnol. 5 (1), 121–129.
Karaolia, P., Michael, I., García-Fernández, I., Agüera, A., Malato, S., Fernandez-Ibanez, P., Fatta-Kassinos, D., 2014. Reduction of clarithromycin and sulfamethoxazole-resistant Enterococcus by pilot-scale solar-driven Fenton oxidation. Sci. Total Environ. 468, 19–27.
Karaolia, P., Michael-Kordatou, I., Hapeshi, E., Alexander, J., Schwartz, T., Fatta-Kassinos, D., 2017. Investigation of the potential of a Membrane BioReactor followed by solar Fenton oxidation to remove antibiotic-related micropollutants. Chem. Eng. J. 310 (Part 2), 491–502.
Kittinger, C., Lipp, M., Baumert, R., Folli, B., Koraimann, G., Toplitsch, D., Liebmann, A., Grisold, A.J., Farnleitner, A.H., Kirschner, A., 2016. Antibiotic resistance patterns of *Pseudomonas* spp. isolated from the River Danube. Front. Microbiol. 7.
Kuo, C.F., Mashino, T., Fridovich, I., 1987. alpha, beta-Dihydroxyisovalerate dehydratase. A superoxide-sensitive enzyme. J. Biol. Chem. 262 (10), 4724–4727.
Lee, J.H., Yeo, W.S., Roe, J.H., 2004. Induction of the sufA operon encoding Fe-S assembly proteins by superoxide generators and hydrogen peroxide: involvement of OxyR, IHF and an unidentified oxidant-responsive factor. Mol. Microbiol. 51 (6), 1745–1755.
Lee, O.M., Kim, H.Y., Park, W., Kim, T.H., Yu, S., 2015. A comparative study of disinfection efficiency and regrowth control of microorganism in secondary wastewater effluent using UV, ozone, and ionizing irradiation process. J. Hazard. Mater. 295, 201–208.
Lekunberri, I., Villagrasa, M., Balcázar, J.L., Borrego, C.M., 2017. Contribution of bacteriophage and plasmid DNA to the mobilization of antibiotic resistance genes in a river receiving treated wastewater discharges. Sci. Total Environ. 601, 206–209.
Liltved, H., Landfald, B., 2000. Effects of high intensity light on ultraviolet-irradiated and non-irradiated fish pathogenic bacteria. Water Res. 34 (2), 481–486.
Luczkiewicz, A., Jankowska, K., Bray, R., Kulbat, E., Quant, B., Sokolowska, A., Olanczuk-Neyman, K., 2011. Antimicrobial resistance of fecal indicators in disinfected wastewater. Water Sci. Technol. 64 (12), 2352–2361.
Lüddeke, F., Heß, S., Gallert, C., Winter, J., Guede, H., Loeffler, H., 2015. Removal of total and antibiotic resistant bacteria in advanced wastewater treatment by ozonation in combination with different filtering techniques. Water Res. 69, 243–251.
Lundström, S.V., Östman, M., Bengtsson-Palme, J., Rutgersson, C., Thoudal, M., Sircar, T., Larsson, D.J., 2016. Minimal selective concentrations of tetracycline in complex aquatic bacterial biofilms. Sci. Total Environ. 553, 587–595.
Mackuľak, T., Nagyová, K., Faberová, M., Grabic, R., Koba, O., Gál, M., Birošová, L., 2015. Utilization of Fenton-like reaction for antibiotics and resistant bacteria elimination in different parts of WWTP. Environ. Toxicol. Pharmacol. 40 (2), 492–497.
Malato, S., Fernández-Ibáñez, P., Maldonado, M.I., Blanco, J., Gernjak, W., 2009. Decontamination and disinfection of water by solar photocatalysis: recent overview and trends. Catal. Today 147 (1), 1–59.
Manaia, C.M., Macedo, G., Fatta-Kassinos, D., Nunes, O.C., 2016. Antibiotic resistance in urban aquatic environments: can it be controlled? Appl. Microbiol. Biotechnol. 100 (4), 1543–1557.
Manaia, C.M., 2016. Assessing the risk of antibiotic resistance transmission from the environment to humans: non-direct proportionality between abundance and risk. Trends Microbiol. 25, 173–181.
Martínez, J.L., 2008. Antibiotics and antibiotic resistance genes in natural environments. Sci. (New York, N. Y.) 321 (5887), 365–367.
Mehrjouei, M., Müller, S., Möller, D., 2015. A review on photocatalytic ozonation used for the treatment of water and wastewater. Chem. Eng. J. 263, 209–219.
Michael, I., Hapeshi, E., Michael, C., Varela, A., Kyriakou, S., Manaia, C., Fatta-Kassinos, D., 2012. Solar photo-Fenton process on the abatement of antibiotics at a pilot scale: degradation kinetics, ecotoxicity and phytotoxicity assessment and removal of antibiotic resistant enterococci. Water Res. 46 (17), 5621–5634.
Michael, I., Rizzo, L., McArdell, C., Manaia, C., Merlin, C., Schwartz, T., Dagot, C., Fatta-Kassinos, D., 2013a. Urban wastewater treatment plants as hotspots for the

release of antibiotics in the environment: a review. Water Res. 47 (3), 957–995.
Michael, I., Frontistis, Z., Fatta-Kassinos, D., 2013b. Removal of pharmaceuticals from environmentally relevant matrices by advanced oxidation processes (AOPs). Compr. Anal. Chem. 62, 345–407.
Michael-Kordatou, I., Andreou, R., Iacovou, M., Frontistis, Z., Hapeshi, E., Michael, C., Fatta-Kassinos, D., 2017. On the capacity of ozonation to remove antimicrobial compounds, resistant bacteria and toxicity from urban wastewater effluents. J. Hazard. Mater. 323 (Part A), 414–425.
Michael-Kordatou, I., Iacovou, M., Frontistis, Z., Hapeshi, E., Dionysiou, D., Fatta-Kassinos, D., 2015b. Erythromycin oxidation and ERY-resistant Escherichia coli inactivation in urban wastewater by sulfate radical-based oxidation process under UV-C irradiation. Water Res. 85, 346–358.
Michael-Kordatou, I., Michael, C., Duan, X., He, X., Dionysiou, D., Mills, M., Fatta-Kassinos, D., 2015a. Dissolved effluent organic matter: characteristics and potential implications in wastewater treatment and reuse applications. Water Res. 77, 213–248.
Michod, R.E., Bernstein, H., Nedelcu, A.M., 2008. Adaptive value of sex in microbial pathogens. Infect. Genet. Evol. J. Mol. Epidemiol. Evol. Genet. Infect. Dis. 8 (3), 267–285.
Mishra, S., Imlay, J., 2012. Why do bacteria use so many enzymes to scavenge hydrogen peroxide? Archives Biochem. Biophys. 525 (2), 145–160.
Moreira, N.F., Sousa, J.M., Macedo, G., Ribeiro, A.R., Barreiros, L., Pedrosa, M., Faria, J.L., Pereira, M.F.R., Castro-Silva, S., Segundo, M.A., 2016. Photocatalytic ozonation of urban wastewater and surface water using immobilized TiO_2 with LEDs: Micropollutants, antibiotic resistance genes and estrogenic activity. Water Res. 94, 10–22.
Nocker, A., Sossa-Fernandez, P., Burr, M.D., Camper, A.K., 2007. Use of propidium monoazide for live/dead distinction in microbial ecology. Appl. Environ. Microbiol. 73, 5111–5117.
Oguma, K., Katayama, H., Mitani, H., Morita, S., Hirata, T., Ohgaki, S., 2001. Determination of pyrimidine dimers in *Escherichia coli* and *Cryptosporidium parvum* during UV light inactivation, photoreactivation, and dark repair. Appl. Environ. Microbiol. 67 (10), 4630–4637.
Oh, J., Salcedo, D.E., Medriano, C.A., Kim, S., 2014. Comparison of different disinfection processes in the effective removal of antibiotic-resistant bacteria and genes. J. Environ. Sci. 26 (6), 1238–1242.
Oh, J., Medriano, C.A., Kim, S., 2016. The effect of tetracycline in the antibiotic resistance gene transfer before and after ozone disinfection. Desalination Water Treat. 57, 646–650.
Öncü, N.B., Mencelogluu, Y.Z., Balcioğlu, I., 2011. Comparison of the effectiveness of chlorine, ozone, and photocatalytic disinfection in reducing the risk of antibiotic resistance pollution. J. Adv. Oxid. Technol. 14 (2), 196–203.
Oppenländer, T., 2003. Photochemical Purification of Water and Air: Advanced Oxidation Processes (AOPs) - Principles, Reaction Mechanisms, Reactor Concepts. John Wiley and Sons.
Ostoich, M., Serena, F., Falletti, L., Fantoni, A., 2013. Control of dangerous substances in discharges and microbiological abatement: European framework and a case study of an ozone disinfection system. Water Sci. Technol. 67 (6), 1238–1246.
Park, S., You, X., Imlay, J.A., 2005. Substantial DNA damage from submicromolar intracellular hydrogen peroxide detected in Hpx- mutants of Escherichia coli. Proc. Natl. Acad. Sci. U. S. A. 102 (26), 9317–9322.
Partridge, S.R., 2011. Analysis of antibiotic resistance regions in Gram-negative bacteria. FEMS Microbiol. Rev. 35, 820–855.
Poirel, L., Rodriguez-Martinez, J.M., Mammeri, H., Liard, A., Nordmann, P., 2005. Origin of plasmid-mediated quinolone resistance determinant QnrA. Antimicrob. Agents Chemother. 49 (8), 3523–3525.
Pruden, A., Larsson, D.J., Amézquita, A., Collignon, P., Brandt, K.K., Graham, D.W., Lazorchak, J.M., Suzuki, S., Silley, P., Snape, J.R., 2013. Management options for reducing the release of antibiotics and antibiotic resistance genes to the environment. Environ. Health Perspect. 121 (8), 878 (Online).
Pryor, W.A., Das, B., Church, D.F., 1991. The ozonation of unsaturated fatty acids: aldehydes and hydrogen peroxide as products and possible mediators of ozone toxicity. Chem. Res. Toxicol. 4 (3), 341–348.
Restivo, J., Órfão, J.J.M., Armenise, S., Garcia-Bordejé, E., Pereira, M.F.R., 2012. Catalytic ozonation of metolachlor under continuous operation using nanocarbon materials grown on a ceramic monolith. J. Hazard. Mater. 239–240, 249–256.
Rincon, A., Pulgarin, C., 2004. Effect of pH, inorganic ions, organic matter and H_2O_2 on *E. coli* K12 photocatalytic inactivation by TiO2: implications in solar water disinfection. Appl. Catal. B Environ. 51 (4), 283–302.
Rincón, A.G., Pulgarin, C., 2004. Bactericidal action of illuminated TiO_2 on pure *Escherichia coli* and natural bacterial consortia: post-irradiation events in the dark and assessment of the effective disinfection time. Appl. Catal. B Environ. 49 (2), 99–112.
Rizzo, L., Manaia, C., Merlin, C., Schwartz, T., Dagot, C., Ploy, M., Michael, I., Fatta-Kassinos, D., 2013. Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: a review. Sci. total Environ. 447, 345–360.
Rizzo, L., Della Sala, A., Fiorentino, A., Puma, G.L., 2014a. Disinfection of urban wastewater by solar driven and UV lamp-TiO_2 photocatalysis: effect on a multidrug resistant Escherichia coli strain. Water Res. 53, 145–152.
Rizzo, L., Sannino, D., Vaiano, V., Sacco, O., Scarpa, A., Pietrogiacomi, D., 2014b. Effect of solar simulated N-doped TiO_2 photocatalysis on the inactivation and antibiotic resistance of an *E. coli* strain in biologically treated urban wastewater. Appl. Catal. B Environ. 144, 369–378.
Rizzo, L., Ferro, G., Manaia, C., 2014c. Wastewater Disinfection by Solar Heterogeneous Photocatalysis: Effect on Tetracycline Resistant/sensitive Enterococcus Strains. Press, Available online 18 02 2014.
Roberts, M.C., 2011. Environmental macrolide-lincosamide-streptogramin and tetracycline resistant bacteria. Front. Microbiol. 2, 40.
Rodríguez-Rojas, A., Rodríguez-Beltrán, J., Couce, A., Blázquez, J., 2013. Antibiotics and antibiotic resistance: a bitter fight against evolution. Int. J. Med. Microbiol. 303 (6–7), 293–297.
Salyers, A.A., Amabile-Cuevas, C.F., 1997. Why are antibiotic resistance genes so resistant to elimination? Antimicrob. Agents Chemother. 41 (11), 2321–2325.
Schwartz, T., Kohnen, W., Jansen, B., Obst, U., 2003. Detection of antibiotic-resistant bacteria and their resistance genes in wastewater, surface water, and drinking water biofilms. FEMS Microbiol. Ecol. 43 (3), 325–335.
Seiler, C., Berendonk, T.U., 2007. Role and prevalence of antibiosis and the related resistance genes in the environment. Heavy Metal Driven Co-selection of Antibiotic Resistance in Soil and Water Bodies Impacted by Agriculture and Aquaculture, vol. 101.
Shen, X.C., Zhang, Z.L., Zhou, B., Peng, J., Xie, M., Zhang, M., Pang, D.W., 2008. Visible light-induced plasmid DNA damage catalyzed by a CdSe/ZnS-photosensitized nano-TiO_2 film. Environ. Sci. Technol. 42 (14), 5049–5054.
Sousa, J.M., Macedo, G., Pedrosa, M., Becerra-Castro, C., Castro-Silva, S., Pereira, M.F.R., Silva, A.M., Nunes, O.C., Manaia, C.M., 2017. Ozonation and UV 254 nm radiation for the removal of microorganisms and antibiotic resistance genes from urban wastewater. J. Hazard. Mater. 323 (Part A), 434–441.
Stephanie, K., Ursula, O., Thomas, S., 2011. Immunological detection of UV induced cyclobutane pyrimidine dimers and (6-4) photoproducts in DNA from reference bacteria and natural aquatic populations. J. Microbiol. Methods 84, 435–441.
Stoll, C., Sidhu, J.P.S., Tiehm, A., Toze, S., 2012. Prevalence of clinically relevant antibiotic resistance genes in surface water samples collected from Germany and Australia. Environ. Sci. Technol. 46 (17), 9716–9726.
Sunada, K., Watanabe, T., Hashimoto, K., 2003. Studies on photokilling of bacteria on TiO_2 thin film. J. Photochem. Photobiol. A Chem. 156 (1–3), 227–233.
Süss, J., Volz, S., Obst, U., Schwartz, T., 2009. Application of a molecular biology concept for the detection of DNA damage and repair during UV disinfection. Water Res. 43 (15), 3705–3716.
Theruvathu, J.A., Flyunt, R., Aravindakumar, C.T., von Sonntag, C., 2001. Rate constants of ozone reactions with DNA, its constituents and related compounds. J. Chem. Soc. Perkin Trans. 2 (3), 269–274.
Tsai, T., Chang, H., Chang, K., Liu, Y., Tseng, C., 2010. A comparative study of the bactericidal effect of photocatalytic oxidation by TiO_2 on antibiotic-resistant and antibiotic-sensitive bacteria. J. Chem. Technol. Biotechnol. 85 (12), 1642–1653.
UN, 2016. Draft Political Declaration of the High-level Meeting of the General Assembly on Antimicrobial Resistance. http://go.nature.com/2e3bMdF.
Vaz-Moreira, I., Nunes, O.C., Manaia, C.M., 2014. Bacterial diversity and antibiotic resistance in water habitats: searching the links with the human microbiome. FEMS Microbiol. Rev. 38 (4), 761–778.
Venieri, D., Gounaki, I., Bikouvaraki, M., Binas, V., Zachopoulos, A., Kiriakidis, G., Mantzavinos, D., 2016. Solar photocatalysis as disinfection technique: inactivation of *Klebsiella pneumoniae* in sewage and investigation of changes in antibiotic resistance profile. J. Environ. Manag. https://doi.org/10.1016/j.jenvman.2016.06.009.
Von Gunten, U., 2003. Ozonation of drinking water: part II. Disinfection and by-product formation in presence of bromide, iodide or chlorine. Water Res. 37 (7), 1469–1487.
Watanabe, K., Baker, P.W., 2000. Environmentally relevant microorganisms. J. Biosci. Bioeng. 89, 1–11.
WHO, 2014. Antimicrobial Resistance Global Report on Surveillance. http://apps.who.int/iris/bitstream/10665/112642/1/9789241564748_eng.pdf?ua1/41.
WHO, 2015. Global Action Plan on Antimicrobial Resistance. http://www.who.int/antimicrobial-resistance/global-action-plan/en.
Xiong, P., Hu, J., 2013. Inactivation/reactivation of antibiotic-resistant bacteria by a novel UVA/LED/TiO_2 system. Water Res. 47 (13), 4547–4555.
Zhang, X., Zhang, T., Fang, H.H., 2009. Antibiotic resistance genes in water environment. Appl. Microbiol. Biotechnol. 82 (3), 397–414.
Zhang, Y., Zhuang, Y., Geng, J., Ren, H., Xu, K., Ding, L., 2016. Reduction of antibiotic resistance genes in municipal wastewater effluent by advanced oxidation processes. Sci. Total Environ. 550, 184–191.
Zhu, Y.G., Zhao, Y., Li, B., Huang, C.L., Zhang, S.Y., Yu, S., Chen, Y.S., Zhang, T., Gillings, M.R., Su, J.Q., 2017. Continental-scale pollution of estuaries with antibiotic resistance genes. Nat. Microbiol. 2, 16270.
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.