Review

Does light-based tertiary treatment prevent the spread of antibiotic resistance genes? Performance, regrowth and future direction

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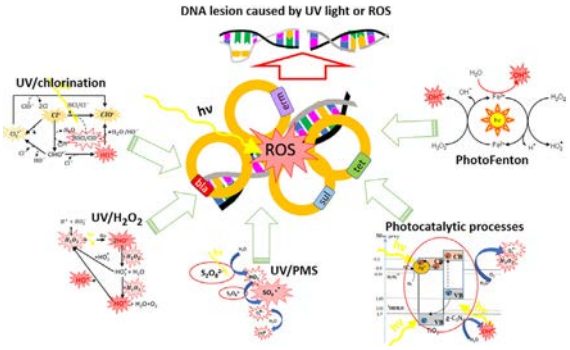
HIGHLIGHTS

- Light-based treatment is not able to fully remove ARGs and avert bacterial regrowth.
- UV light and simulated sunlight accelerate HGT, visible light does not affect on it.
- ROS may enhance the irreversible destruction of bacterial DNA reducing ARGs spread.
- Hybrid action of light, O_3 and/or H_2O_2 prevents bacterial regrowth.
- Photocatalytic and PhotoFenton processes led to highest ARGs removal.

GRAPHICAL ABSTRACT



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ABSTRACT

The common occurrence of antibiotic-resistance genes (ARGs) originating from pathogenic and facultative pathogenic bacteria pose a high risk to aquatic environments. Low removal of ARGs in conventional wastewater treatment processes and horizontal dissemination of resistance genes between environmental bacteria and human pathogens have made antibiotic resistance evolution a complex global health issue. The phenomenon of regrowth of bacteria after disinfection raised some concerns regarding the long-lasting safety of treated waters. Despite the inactivation of living antibiotic-resistant bacteria (ARB), the possibility of transferring intact and liberated DNA containing ARGs remains. A step in this direction would be to apply new types of disinfection methods addressing this issue in detail, such as light-based advanced oxidation, that potentially enhance the effect of direct light interaction with DNA. This study is devoted to comprehensively and critically review the current state-of-art for light-driven disinfection. The main focus of the article is to provide an insight into the different photochemical disinfection methods currently being studied worldwide with respect to ARGs removal as an alternative to conventional methods. The systematic comparison of UV/chlorination, UV/H_2O_2, sulfate radical based-AOPs, photocatalytic processes and photoFenton considering their mode of action on molecular level, operational parameters of the processes, and overall efficiency of removal of ARGs is presented. An in-depth discussion of different light-dependent inactivation pathways, influence of DBP and

Abbreviations: AMR, antimicrobial resistance; AOPs, advanced oxidation processes; ARB, antibiotic-resistant bacteria; ARGs, antibiotic-resistance genes; BER, base excision repair; CAT, catalase; CEC, contaminants of emerging concern; Ct, disinfectant concentration times treatment time; DBPs, disinfection by-products; DOM, dissolved organic matter; eARGs, extracellular ARGs (free eARGs); eDNA, extracellular DNA; GO, graphene oxide; HD, hydrothermal treatment; HGT, horizontal gene transfer; iARGs, intracellular ARGs; iDNA, intracellular DNA; LOQ, Limit of quantification; MGEs, mobile genetic elements; NER, nucleotide excision repair; NrGO, nitrogen-doped reduced graphene oxide; PH, photocatalytic treatment; PMS, peroxymonosulfate; PS, persulfate; rGO, reduced graphene oxide; RCR, reactive chlorine species; ROS, reactive oxygen species; SOD, superoxide dismutase; SR-AOPs, sulfate radicals AOPs; UV, ultraviolet rays/light; UV/Cl_2, UV and chlorination as concomitant processes; UV → Cl_2, UV and chlorination are applied as consecutive processes; UVDE, UV-damage endonuclease; VBNC, viable, but not culturable status; VGT, vertical gene transfer; Vis, visible light; WWTPs, wastewater treatment plants.

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Photoactivation of persulfate
Tertiary treatment
Secondary effluent

DOM on ARG removal and the potential bacterial regrowth after treatment is presented. Based on presented revision the risk of ARG transfer from reactivated bacteria has been evaluated, leading to a future direction for research addressing the challenges of light-based disinfection technologies.

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1. Antimicrobial resistance

Since the early 2000s, much attention has been given to the increase in antimicrobial resistance (AMR) due to an ever increasing range of infections caused by health relevant bacteria, which have become resistant to antibacterial drugs commonly used in medical treatment (Text S1) (Amarasiri et al., 2020; Asif et al., 2017; Harkins et al., 2017; McKenna, 2013; WHO, 2016). The widespread use of antibiotics in medicine and veterinary results in their continuous direct and indirect release into the environment and the development of antibiotic resistance in bacterial populations (ARB) and antibiotic resistance genes (ARGs) (Hashmi et al., 2017; Karaolia et al., 2017, 2018; WHO, 2016). Some of the ARB, even not pathogenic, can transfer their resistant genes to human pathogens (WHO, 2016).

Despite the high risk related to the presence of antibiotics in wastewater, there is a lack of discharge guidelines or standards for antimicrobial drug monitoring and the components are excluded from EU environmental regulations. Since there are currently no limits set for antibiotics in water bodies and the biological wastewater treatment has limited capacity to remove them, AMR is spreading dramatically. The problem of antibiotic pollution and antibiotic resistance is being noticed by international institutions (e.g. the World Health Organization (WHO), Food and Agriculture Organization of the United Nations (FAO), the World Organization for Animal Health (OIE), European Commission). The WHO has recognized AMR threats and several actions have been taken (Fig. S1). Based on that in 2015, a Global Action Plan (GAP) was created by the WHO, the FAO and the OIE, to implement pertinent policies and plans to prevent, control, and monitor AMR (Shallcross and Davies, 2014). Since particular role of AMR in pollution waterbodies has been highlighted it was more and more clear that wastewater treatment technologies (including disinfection) need to be revised to more advanced technologies (European Commission, 2017, 2019; WHO et al., 2020). Regardless of efforts, ARB and ARG occurrence is only in the monitoring phase and there are no legal conditions or requirements so far. In a recent European Parliament discussion, a clear link was drawn between pharmaceutical residues in wastewater and antimicrobial resistance. Therefore, new quality standards for environmentally harmful subsidies such as pharmaceuticals, antibiotic resistant bacteria, endocrine disrupters could be expected in the revised Urban Waste Water Treatment Directive. This should be the aim of EU water policy strategy for the next years (still under consultation, planned to be accepted by the EU Commission in the first quarter of 2022) (European Commission, 2021a,b).

1.1. ARGs occurrence and dissemination

Due to providing promising conditions for the survival and proliferation of ARB and dissemination of ARGs in secondary wastewater treatment both of them have been included as microbial contaminants to the group of contaminants of emerging concern (CEC) (Karaolia et al., 2017, 2018; Reichert et al., 2021). The most common ARGs detected in wastewater treatment plants (WWTPs) are genes coding the resistance against β lactams, quinolones, sulfonamides, tetracyclines, macrolides (Hu et al., 2019; Paruch et al., 2021; Reichert et al., 2021). While, intI1 (Integrase1 gene) associated with mobile genetic elements is considered as a promising indicator for abundance and elimination of ARGs monitoring (Zheng et al., 2020). Table S1 presents the most relevant ARGs.

During the last few years, more attention was paid to eliminate the spread of ARB and ARGs in WWTPs, which are reservoirs of microbes coming along with resistance genes (Ferro et al., 2016, 2017; Zieliński et al., 2021). Hospitals, nursing homes, slaughterhouses, urban sewer systems, but also agricultural activities are considered as a source of ARGs. ARGs were also detected in surface water, in sediments, and biofilms within the aquatic environment (Brown et al., 2019; Hao et al., 2019; Reichert et al., 2021). Several ARGs against tetracyclines (*tetW*, *tetM*, *tetO*, *tetT*, *tetQ*, *tetB*) and sulfonamides (*sul1*, *sul2*, and *sul3*) were detected in 6 aquaculture fish farm sediments, some of them were detected in river near the farm (Panpan Gao et al., 2012). Tetracycline resistance genes (*tetA*, *tetC*, *tetH* and *tetM*) were also detected in the sediments near a fish farm where antibiotic usage was stopped for six years earlier (Tamminen et al., 2011). The long living sulfonamide resistant bacteria can remain stable in the aqueous environment for 5 or 10 years (Panpan Gao et al., 2012). It was proved that during several decades ARGs can accumulate in the soil. Certain ARGs, namely *tetQ* and bla_{TEM} showed around 15 times higher abundance in 2008 than in the 1970s (Knapp et al., 2010).

Due to the fact of the high abundance in conventionally treated effluent, WWTPs are often reorganized as hotspots of ARGs. The absolute abundance of ARGs in WWTP influents from 10^6 to 10^9 copies/cm^3 is detected, while after primary and secondary treatment the abundance decreases by 1 log to3 log units, respectively (J. Li et al., 2016; Pallares Vega et al., 2019; Zheng et al., 2020). The abundance of intI1 and 16S rRNA was 3 5 orders of magnitude higher than ARGs. However, the ARGs abundance in second ary effluent after membrane bioreactor (MBR) treatment is 3 4 log unit lower than that in conventional activated sludge (CAS) treatment effluent (Iakovides et al., 2021). The minor correlation between the microbial oc currence of ARGs and the detection of antibiotics as a chemical contami nant was confirmed (Pin Gao et al., 2012). Many studies showed that disinfection could play an important role in removing the spread of ARB and ARGs at WWTPs (Rodríguez Chueca et al., 2019; Wang et al., 2020; Yuan et al., 2015; Zarei Baygi and Smith, 2021; Y. Zhang et al., 2015; C.S. Zhou et al., 2020; Zhuang et al., 2015). Limited efficiency of antibiotics as well as ARB and ARGs removal from wastewater during conventional treatment in WWTPs is well known. As a result, detection of ARB and ARGs in downstream aquatic systems receiving WWTP effluents is quite common (Brown et al., 2019; Reichert et al., 2021). The abundances of ARB and ARGs depend on several factors, such as the water quality and sea sonal parameters (Reichert et al., 2021), as well as presence of aquatic con taminants. Especially heavy metals, particles and preservatives (like sodium nitrite, sodium benzoate, and triclocarban), are often discussed since they can act as carriers (co or cross participated in ARGs occurrence promoting horizontal gene transfer (HGT)) (Cen et al., 2020; Li et al., 2021). Discharge of WWTP effluent containing residual suspended solids to rivers contributes in an increase of ARGs abundance in river sediments (Brown et al., 2020). As shown by Brown et al. (2019) the impact of partic ulate matter originated from WWTPs is particularly strong for the abun dances of *ermB*, bla_{TEM}, and *tetM* genes.

The widespread dissemination of ARGs can be cycled and potentially amplified by vertical gene transfer (VGT, i.e., by cell division) and HGT (Dodd, 2012; Li et al., 2021; Michael Kordatou et al., 2018). The devel opment of resistance can be intrinsic, acquired via gene transfer, or de veloped by spontaneous mutations (de novo) (Sharma et al., 2016). HGT is claimed as the main dissemination way of ARGs in the natural envi ronment (Dodd, 2012; Dunlop et al., 2015; Karaolia et al., 2018; Yin et al., 2021; Yoon et al., 2017). Conjugation by plasmids, natural trans formation by extracellular DNA, and transduction by bacteriophages allow genetic material to be transferred from a resistance donor to a re sistance recipient (Text S2, Fig. S2) (Dodd, 2012; Dunlop et al., 2015; Li et al., 2021; Michael Kordatou et al., 2018). In the aquatic environment, mobile genetic elements (MGEs) play a significant role in promoting HGT, consequently favoring the acquisition and spread of ARGs (Li et al., 2021; Rodríguez Beltrán et al., 2020). ARGs are often located on MGEs (such as transposons, plasmids, and integrons) which facilitate the dissemination among taxonomically unrelated species (Li et al., 2021). ARGs disseminate in the environment, not only due to the dis charge of treated municipal wastewater, but also sewage sludge and livestock manure recycling in agriculture which contained large num bers of class 1 integrons (Gaze et al., 2011).

It is worth to mention that the main goal of WWTPs is to remove organic matter and nutrients and for that they were designed. Therefore, WWTPs are not efficient for removal of micropollutants (e.g. antibiotic residues), ARB, ARGs and MGEs. In the biological treatment high nutrient concentra tions in WWTPs are crucial to achieve a successful wastewater purification. On the other hand, it supports HGT and proliferation leading to spread of ARGs in WWTPs. An optimal environment to stimulate HGT was found dur ing aerobic and anaerobic processes (Korzeniewska and Harnisz, 2018). All environments were the bacterial density is very high like sewage and acti vated sludge, are considered as HGT hotspots due to non limited access to MGEs with ARGs (Korzeniewska and Harnisz, 2018). As a result of confirm ing accumulation and dispersal of ARGs in surface biofilms from sewer pipes, sewer system was identified as HGT hotshot as well (Auguet et al., 2017).

2. Antibiotic resistance removal by conventional disinfection

Conventional disinfection (UV, chlorination and ozonation) is often ap plied for municipal water, wastewater as well as water reuse treatment, pro viding pathogen inactivation. Several countries have established regulations for UV drinking water disinfection. From a legal standpoint, ef fluents from wastewater treatment plants must comply with all quality stan dards established by national, regional, and local laws for discharge into surface water or all reclaimed wastewater reuse regulations. As it was dis used above, unfortunately, despite identification of AMR issue, currently, there are no guidelines or legal regulations with regard to antimicrobial re sistance. In addition, a minimum UV dose for wastewater disinfection is not regulated while only 40 mJ/cm^2 for disinfection of drinking water is re quired. Low dose of UV and research on the ARGs removal from drinking water matrix suggests that similar disinfection conditions are not satisfac tory in the case of wastewater. As can be seen in Text S3, conventional UV disinfection is not capable to eliminate or reduce AMR spread. More over, the low effectivity can be explained because those systems have been designed for bacteria removal not for DNA or ARGs removal (thus, ARB are easier removed). As it was mentioned, UV treated cells can regain viability through light and dark repair, which may not possess a long lasting inactivation effect (Goosen and Moolenaar, 2008; Wang et al., 2021; Weigel et al., 2017). The regrowth of bacteria can be an issue, espe cially in the receiving water, where HGT may occur, contributing to the spread of antibiotic resistance. Furthermore, it has to be considered that conjugative transfer frequency is accelerated by UV light (up to 100 fold) or simulated sunlight (2 10 fold), while visible light does not affect HGT (Chen et al., 2019). This finding can explain the low efficiency of conven tional UV disinfection toward ARGs. UV irradiation induce forceful stimula tion, resulting in higher oxidative stress, superior gene expression and increasing frequency of ARG conjugative transfer (Chen et al., 2019). It should be noted, that not only conventional UV disinfection has limited ef ficiency toward ARGs, but also other disinfection methods such as ozona tion and chlorination. Ozonation and chlorination can remove completely ARGs, but only under conditions (in terms of oxidant/disinfectant concen tration, time) that are unrealistic from the operational point of view (Text S4 and Text S5).

Having this in mind, a high interest should be to limit the release of ARGs and ARB to the environment and ultimately to reduce the risk of their spread. Such reduction effect can be potentially achieved by improved disinfection. A step in this direction can be the application of new types of disinfection methods addressing this issue in detail, such as light based ad vanced oxidation processes (AOPs).

3. ARGs removal enhanced by UV/VIS and additional oxidants

The potential of light has been in the focus of researchers and the con text of AOPs. In principle, the oxidation in the AOPs occurs due to the onsite formation of Reactive Oxygen Species (ROS). In AOPs, hydroxyl radicals (HO•) are considered as a main ROS responsible for oxidizing (due to their non selective nature and high oxidation potential (E^0_{NHE} = 2.73 V (von Sonntag, 2006)). However, the formation of all ROS (HO•, superoxide radical anion ($O_2^{\bullet -}$), hydroperoxyl radical (HO_2•), and hydrogen peroxide (H_2O_2)) requires some external energy, for instance, radiation. Among typ ical light based AOPs, we can find UV/H_2O_2, UV/TiO_2, UV/O_3, photo Fenton (Cowie et al., 2020; Giannakis et al., 2018b; Ozores Diez et al., 2020). Such methods potentially enhance the effect of direct reaction of light with DNA by the production of ROS. The common occurrence of clin ically relevant ARGs in the aquatic environment as well as the horizontal dissemination of resistance between environmental bacteria and human pathogens demonstrate that the AMR issue is relevant for the human public health and from the environmental point of view. ROS plays an important role in photo driven AOPs for inactivating ARB and ARGs. The chemical mechanism of action of HO•, H_2O_2, singlet oxygen ($^1O_2$), hole (h^+), elec tron (e^-), and other ROS, can be identified and proved experimentally by ROS scavengers (Text S6) or be verified by using spin trapping electron

paramagnetic resonance (EPR). Light based disinfection technologies (UVC, UVA photolysis, photo Fenton, photo/H_2O_2, photocatalysis, photo/chlorination, sulfate radical based AOPs and photo ozonation) can lead to bacteria inactivation by different mechanisms that are discussed in detail in this work.

Considering, that conventional wastewater disinfection methods such as ozonation, chlorination, and UV irradiation do not provide satisfactory results, advanced tertiary treatment is strongly recommended, and light based methods seem to be an interesting option. Thus, the interest is devoted to photochemical technologies such as light based advanced oxidation, that potentially enhance the effect of direct light interaction with DNA by ROS action. The aim of the study is to review the existing literature concerning light based disinfection methods in a critical and comprehensive way. Here, for the first time, light based AOPs used for disinfection were discussed deeply on the molecular level. The study encompasses light/chlorination, UV/H_2O_2, light/PMS, photocatalytic processes, and photo Fenton oxidation and their effect on the elimination of ARGs and ARB from wastewater. Eventually, the efficiency of the methods is discussed in the context of new potential regulations concerning wastewater quality. To the best of our knowledge, the available review papers were not devoted to the biology molecular point of view. In the review written by Michael Kordatou et al. (2018) a critical assessment of the advanced chemical oxidation processes in terms of their efficiency in removing ARB and ARGs can be found. However, Michael Kordatou and co workers were mainly focused on operational conditions or AOPs efficiency, while the mechanism of action or discussion was provided from a chemical oxidation point of view. As was discussed above, the ARM gained extraordinary attention, which led to an increased number of research papers concerning ARGs removal. Here, the most recent available literature data has been reviewed and the discussion mostly covers the new finding. Contrary to other review studies this paper presents the systematic comparison of the light based disinfection methods, considering their mode of action on the molecular level, operational parameters of the processes, light source, the scale of experiments (laboratory scale, pilot, or full scale), and overall efficiency of ARGs removal from effluents. The review has been carried out with a technological (engineering) approach, clarifying the applicability of these methods. Besides, the biological point of view allowed to discuss results with regard to the maintenance of the microbial quality of effluent discharged to surface water. According to our knowledge, this is the first review paper that tackles the problem of elimination of ARGs and ARB by light based in such detail. In distinction from the existing papers, the present research provides novelty with respect to an in depth discussion of different light dependent inactivation pathways, the influence of DBP and DOM on ARGs removal, and the regrowth potential of the bacteria conveying ARGs after light AOPs treatment. Finally, we propose a future research direction to overcome the challenges of light based disinfection technologies.

3.1. Coupling of UV and chlorination

Although both UV radiation and chlorination belong to the conventional disinfection methods, their combination is not classified in such a manner. One of the main disadvantages of chlorination is the formation of harmful DBPs. Compared to that, Malley et al. reported that UV dose below 400 mJ/cm^2 does not initiate the formation of DBPs (Malley et al., 1996). To mitigate the DBP problem, UV radiation was recommended to reduce the chlorine dose used for disinfection (X. Zhang et al., 2015). There are two approaches of combining UV irradiation and chlorination (i) UV and chlorination are applied as consecutive processes (UV → Cl_2), or (ii) UV and chlorination are executed as concomitant processes (UV/chlorination).

In consecutive processes (UV → Cl_2) the ROS are not generated, the disinfection is firstly based on the UV action, and then free chlorine oxidation. It was shown that UV followed by chlorination caused 0.31 log synergy values for 16S rRNA genes that was achieved after irradiation of 62.4 mJ/cm^2 followed by *Ct* value equals 750 mg Cl_2 min/dm^3 (Y. Zhang et al., 2015). By looking at the individual targets (*sul1*, *tetX*, *tetG*, *intI1*), the removal efficiencies were higher for tetracycline (*tet*) genes than for sulfonamide (*sul*) genes. Nevertheless, the observed synergy could be explained by the fact that UV radiation disrupts the structure of the DNA and facilitates the reaction of chlorine with components of cells. That means that comparable inactivation effect can be achieved with a lower chlorine dose, and at the same time the formation of DBPs is lower compared to disinfection only by chlorination.

Destiani and Templeton tested the sequential UV → chlorination for removal of diverse ARGs (*tetA*, bla_{TEM1}, *sul1*, *mphA*) as well as selected ARB (*Escherichia coli*, *Pseudomonas aeruginosa* and *Pseudomonas luteola*) (Destiani and Templeton, 2019). Here, UV disinfection followed by chlorination resulted in more than 2 log inactivation of all tested ARGs when *Ct* equals 48 mgCl_2 min/dm^3 (200 mJ/cm^2) showing a significant synergistic effect. Higher inactivation was observed for lactamase resistance gene, while the lowest for sulfonamide resistance gene. It was suspected, that in all cases UV decreased the bioactivity of the cells and made them more susceptible to the oxidation of the chlorine, which agreed with the hypothesis of Y. Zhang et al. (2015).

During oxidation by UV/chlorination, not only hydroxyl radicals are formed, but also reactive chlorine species (RCS) such as Cl•, $Cl_2^{\bullet -}$, ClO• (T. Zhang et al., 2019). The generation of various reactive species may result in an efficient abatement of different pollutants, not only micropollutants but also ARGs and ARB. When treated water is exposed simultaneously to chlorine and UV radiation, the photolysis of chlorine occurs that results in the formation of HO• and Cl• that partly can transform to reactive chlorine species (RCS) such as $Cl_2^{\bullet -}$ and ClO• (Fig. 1, Text S7). Pathways and mechanisms of simultaneous enhancing the inactivation by the combination of UVC and chlorine are presented in Fig. 1. The RCS and HO• generated during UV/Chlorination lead to inactivation of bacteria by impairing membrane permeability, resulting in the leakage of cellular constituents (ATP, DNA, proteins, etc., (1)) (Xu et al., 2018). What is more, free available chlorine (FAC), hypochlorous acid (HOCl), and hypochlorite ion (OCl^-)) have a much higher reactivity toward purines and pyrimidines and nucleic acids than NH_2Cl or ClO_2 (Dodd, 2012). Due to the possibility of penetration through the cell membrane, HOCl and OCl^- may further oxidize nucleic acids and other inner cellular components, leading to lesions of mRNA and DNA (2), and oxidation of ATP (3) (Dodd, 2012; Xu et al., 2018). Also, HO• and light lead to DNA lesions (2).

T. Zhang et al. (2019) compared the efficiency of AOP UV/chlorination with the individual processes. Complete bacterial inactivation during UV treatment was achieved after 5 min, whereas combining the UV treatment (200 μW/cm^2) with chlorine decreased this time to 1 min (T. Zhang et al., 2019). 20 min of disinfection by UV/chlorination showed a higher gene degradation rate than chlorination alone. Comparison of UV, chlorination, and UV/chlorination suggested no clear influence of HO• on ARGs degradation (T. Zhang et al., 2019). The beneficial effect of UV/chlorination was attributed to the formation of reactive chlorine species (Cl•, $Cl_2^{\bullet -}$ and ClO•) formed during contact with UV and chlorine (T. Zhang et al., 2019). The information concerning the type of radicals playing the key role in ARGs removal is ambiguous. Compared to the aforementioned study of T. Zhang et al. (2019), Liu and Hu (2020) also showed a synergic effect during AOP UV/Cl_2, but they explained it by formation of ROS, especially hydroxyl radicals, claiming that the contribution of RCS was negligible. Also, in the study of Chuang et al. (2017) formation of hydroxyl radicals was found to be more than 5 times higher than concentration of Cl•, indicating that hydroxyl radicals were main oxidizing agents.

In the study of Phattarapattamawong et al. (2021) it was noticed that with insufficient chlorine dose (0.5 2 mg/dm^3), UV/chlorination might be less efficient than UV itself (21,672 mJ/cm^2). This can result from the fact, that UV causes formation of RCS, but their amount is too low to observe the ARGs degradation effect (Phattarapattamawong et al., 2021). When 20 mg/dm^3 of chlorine was used for disinfection during chlorination and the UV/chlorination ARB were completely inactivated (>7.3 log), while the UV irradiation could not achieve the complete disinfection

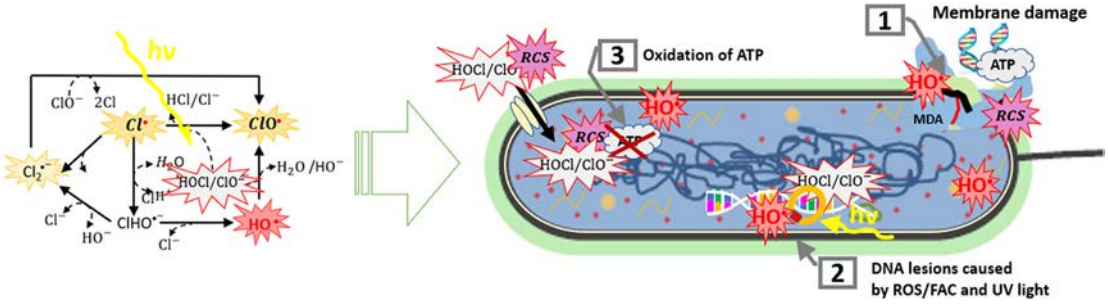

Fig. 1. Pathways and mechanisms of simultaneously enhancing the inactivation by the combination of UVC and chlorine.

(Phattarapattamawong et al., 2021). At the same time, the abatement of ARGs (tet_M and bla_{TEM}) was lower in the same conditions (Table 1) (Phattarapattamawong et al., 2021). The kinetic study revealed, that in the case of tet_M contribution of HO• was estimated as 48% with similar ac tion of UV/chlorination. During bla_{TEM} inactivation, HO• and UV/chlorina tion contributed in 19% and 80%, respectively. The effect of reactive chlorine species on ARGs removal was minor (Phattarapattamawong et al., 2021).

Overall, the bacterial inactivation can be achieved by conventional methods, such as UV radiation or chlorination, and the combination of both. But when ARGs removal is considered, the disinfection effect strongly depends on the bacteria host conveying ARGs. Moreover, as it was shown, the ARGs removal has to be evaluated with respect to HGT. Wang et al. (2020) demonstrated that when only UV was applied, no conjugation trans fer of RP4 plasmid was observed, but the addition of non lethal dose of chlorine (0.5 mg/dm^3) increased the mobility of the plasmid. When UV dis infection (>4 mJ/cm^2) was supported by at least 1 mgCl_2/dm^3, the risk of RP4 plasmid conjugation was significantly reduced. That emphasized that UV/chlorination can be a tool to control horizontal gene transfer and con tribute in minimizing of ARGs spread. As can be seen in Table 1, when UVC as a preliminary step is considered before chlorination more than 200 mJ/cm^2 has been required. The chorine dose showed to be less rele vant as UV dose. Application of 2 mgCl_2/dm^3 and 30 mg HClO/dm^3 re sulted in similar ARGs removal (*sul1* 2.2 log and 2 log decrease, respectively). While, UV/chlorination required much higher UV fluence (320 mJ/cm^2, with 2 mgFAC/dm^3) or higher Cl_2 dose (20 mgCl_2/dm^3, 240 mJ/cm^2) to obtain around 3 log ARGs removal. Beware, that low dose of UV may lead to bacteria regeneration after inactivation, either by dark repair or photoactivation. Moreover, due to the change in permeabil ity of cell membranes, bacteria competence to take up DNA or plasmids in vitro increases, the HGT between microbial populations in which ARG is involved can be expected. Despite that, compared to direct UV or chlorine alone, UV/chlorination favors generation RCS that can react with genes more directly than HO• limiting the risk of HGT. However, the operational condition makes this process unreasonable from economical perspective.

3.2. UV/H_2O_2

The efficiency of UV/H_2O_2 was tested mainly with regards to the elim ination of micropollutants from water (Kwon et al., 2020; Ngumba et al., 2020; Rodríguez Chueca et al., 2019). However, due to the formation of po tent hydroxyl radicals (Text S6), the potential of the method was also tested in the context of disinfection (Miranda et al., 2016; Sharpless et al., 2003; Zeng et al., 2020; Zhao et al., 2021).

Mechanisms of intracellular and extracellular inactivation via UV/H_2O_2 disinfection are presented in Fig. 2. In biological systems, UV radiation is di rectly absorbed by DNA (Bolton and Cotton, 2008; Hockberger, 2002; Jungfer et al., 2007), and due to the structural changes that it causes, UV ra diation inhibits replication (Goosen and Moolenaar, 2008; Mullenders, 2018; Pullerits et al., 2020) and thus can lead to damage of ARGs located on plasmid DNA. H_2O_2 naturally occurs in bacteria (at 0.68 μg/dm^3), while, the excess at concentration 17 μg/dm^3 is already cytotoxic (Feng et al., 2020). H_2O_2 acts on bacteria in two ways, directly by extracellular ox idation of the polyunsaturated phospholipids in the membrane that leads to an increase of its permeability (1), or indirectly via diffusion into the cell leading to disruption of the steady state concentration of intracellular H_2O_2 (2) (Feng et al., 2020; Uhl and Dukan, 2016). The excess of H_2O_2 in a cell, may lead to an extra radical internal reaction. The internal Fenton re action can be initiated by the H_2O_2 action with iron released from the Fe/S clusters (3), whereas from H_2O_2, additional HO• can be produced via elec tron transfer from ATP (4) (Feng et al., 2020). When the UV disinfection is enhanced by the addition of H_2O_2, the mode of action is therefore both in ternal (DNA lesions caused by ROS and UV light (5)), as well as external (membrane damage by H_2O_2 and HO• (1)), what eventually improve the disinfection capacity (Beretsou et al., 2020; Feng et al., 2020). Table 2 pre sents UVC/H_2O_2 treatment application for ARGs removal.

UVC/H_2O_2 treatment was applied for ARGs removal from secondary ef fluent with pH adjustment in the range of 2 to 9 to investigate the process parameter influence (Zhang et al., 2016). It was found that removal of se lected ARGs (*sul1*, *tetG*, *tetX*) and *intl1*, 16S rRNA followed the first order ki netics (Zhang et al., 2016). Under optimal experimental conditions (pH 3.5, 340 mg H_2O_2/dm^3, 2.57 μmol photons s/dm^3), the removal of 2.83, 3.48, 3.05, 2.98 and 2.63 log units for sul1, *tetX*, *tetG*, *intl1*, and 16S rRNA genes, respectively was observed. On the other hand, when the 10 times higher concentration of H_2O_2 was applied, the removal decreased by at least 1 log. This could be explained by scavenging effect, which occurs when H_2O_2 excess acts as a HO• trapper. Compared to UVC disinfection, UVC/H_2O_2 was more efficient in the removal of ARGs (ARGs removal of 2.63 3.48 logs and 0.8 1.21 logs, respectively). The applicability of UVA/H_2O_2 process for ARGs removal from secondary effluent was verified as well (Ferro et al., 2016). The combination of UVA with 20 mg H_2O_2/dm^3 resulted in the successful inactivation of coliforms *Escherichia coli* and antibiotic resistant *Escherichia coli*. Despite 240 min of treatment, ARGs (*qnrS*, bla_{TEM}, *tetW*) were still present in the treated wastewater with abun dance almost similar to the initial one (Ferro et al., 2016). No significant difference was found in the total DNA removal (5.1×10^4 copies/cm^3 de creased to 4.3×10^4 copies/cm^3) (Ferro et al., 2016). Therefore, overall it was concluded that as remaining ARGs can still be transferred to bacteria present in the receiving waters, they can contribute to the spread of antibi otic resistance. Further studies of the Ferro group confirmed that the UVA/ H_2O_2 process led to total inactivation of antibiotic resistant *Escherichia coli* after 240 min (with 20 mg H_2O_2/dm^3, using UVA light) (Ferro et al., 2017). While the abundance of the antibiotic resistant gene bla_{TEM} was mostly un changed during 300 min of treatment (2.7×10^6 copies/cm^3 in total DNA) (Ferro et al., 2017).

UVC/H_2O_2 was tested with respect to *ampC* and *mecA* genes present in *Pseudomonas aeruginosa*, and methicillin resistant *Staphylococcus aureus* (MRSA), respectively (Guo et al., 2017). When UV/H_2O_2 treatment (12 mJ/cm^2) was applied for ARB inactivation, a similar reduction of MRSA (2.5 3.7 log) compared to *Pseudomonas aeruginosa* (2.5 3.6 log) was achieved for all tested H_2O_2 concentrations (from 0 to 3.4 g H_2O_2/ dm^3). For comparison, in the same study 480 min long dark treatment with H_2O_2 (3.4 g H_2O_2/dm^3) caused reduction of 2.3 log on and 2.2 log of MRSA and *Pseudomonas aeruginosa*, respectively. The improvement in

Table 1
The operational condition of UV/chlorination disinfection applied for ARGs removal. Removal efficiency is provided as log unit removal.

Methods	Molecular target	ARGs removal efficiency	Light source	Operational parameters			Ref.
				Reaction time	Operational conditions	Reactor parameters	
UVC → chlorination	*sul1* *tetX* *tetG* *intI1* 16S rRNA	Max. removal by 249.5 mJ/cm^2 [HClO] = 30 mg/dm^3 ARGs 1.7–2.2 log intI1 1.75 log 16S rRNA 1.75 log	LP Hg-lamp 254 nm (16 W) UV fluence: 62.4 mJ/cm^2–249.5 mJ/cm^2	UV exposition: up to 60 s Chlorination: 30 min	Matrix: wastewater Disinfectant: sodium hypochlorite Concentrations: 5–30 mg/dm^3	Cylinder Plexiglas reactor (310 mm height with radius of 450 mm) equipped with a LP Hg-lamp in a quartz sleeve	(X. Zhang et al., 2015; Y. Zhang et al., 2015)
UVC → chlorination	*tetA* bla_{TEM1} *sul1* *mph(A)* MAR *E. coli* (NCTC 13400) MAR *P. aeruginosa* (NCTC 13437)	Maximal removal by 200 mJ/cm^2 [Cl_2] = 2 mg/dm^3 *tetA* 2.5 log *mph(A)* 2.7 log *sul1* 2.2 log bla_{TEM1} 3.1 log	LP Hg-lamp 254 nm (n.d) UV fluence: 50 mJ/cm^2–200 mJ/cm^2	UV exposition: 6, 12, 24 min Chlorination: 15 min	Matrix: drinking water, bacterial suspension in PBS Disinfectant: chlorine Concentration: 1–2 mg/dm^3	Bench-scale UV collimated beam apparatus with a LP UV lamp placed over a 100 cm^3 glass beaker (6.5 cm diameter)	(Destiani and Templeton, 2019)
UVC → chlorination	*sul1*	[FAC] = 20 mg/dm^3 *sul1* removal 24 mJ/cm^2–0.35 log 72 mJ/cm^2–0.38 log 144 mJ/cm^2–0.65 log 288 mJ/cm^2–1.05 log	LP Hg-lamp 254 nm (10 W) UV fluence: up to 432 mJ/cm^2	UV exposition: up to 90 min Chlorination: 5–60 min	Matrix: bacterial cultures Disinfectant: sodium hypochlorite Concentration: 5–100 mg/dm^3	Bench-scale collimated beam apparatus with a LP Hg lamp, placed over on Petri dishes	(Liu and Hu, 2020)
UVC/chlorination	*sul1*	[FAC] = 20 mg/dm^3 *sul1* removal 24 mJ/cm^2–0.4 log 72 mJ/cm^2–0.9 log 144 mJ/cm^2–1 log 288 mJ/cm^2–1.5 log	LP Hg-lamp 254 nm (10 W) UV fluence: up to 432 mJ/cm^2	UV exposition: up to 90 min Chlorination: 5–60 min	Matrix: bacterial cultures Disinfectant: sodium hypochlorite Concentration: 5–100 mg/dm^3	Bench-scale collimated beam apparatus with a LP Hg lamp, placed over on Petri dishes	(Liu and Hu, 2020)
UVC/chlorination	*sul1* *intI1* MAR *Pseudomonas* HLS-6	After 20 min *sul1* > 3.5 log *intI1* > 4.0 log After 1 min sterilization of HLS-6 by 4 log	LP Hg- lamp 254 nm (n.d.) UV fluence: 720 mJ/cm^2 UV irradiance: 0.2 mW/cm^2	60 min	Matrix: drinking water with bacterial suspension Disinfectant: sodium hypochlorite Concentration: 20 mg Cl_2/dm^3	UV lamp placed over Petri dishes	(T. Zhang et al., 2019)
UVC/chlorination	*tetM* bla_{TEM} Tetracycline resistant bacteria (TRB) Amoxicillin resistant bacteria (AmRB)	Maximal removal by [Cl_2] = 20 mg/dm^3 *tetM* 3.20 log bla_{TEM} 3.36 log Bacterial inactivation TRB > 7.15 log AmRB > 7.34 log	3× LP Hg-UV lamps 254 nm (16 W) UV fluence: 21,672 mJ/cm^2 UV irradiance: 9.03 mW/cm^2	40 min	Matrix: bacterial culture Disinfectant: chlorine Concentration: 0.5–20 mg/dm^3	3 LP UV lamps with quart sleeves (dia. × L = 4 × 20 cm) were installed 15 cm above the water surface (300 cm^3 of sample solution in a 500 cm^3 wide mouth glass container)	(Phattarapattamawong et al., 2021)
UVC/chlorination	RP4 plasmids ARB, including *Morganella morganii* (*tetB*, *sul2*, *aacC2*) *Enterococcus faecalis* (*tetA*, *tetB*, *strB*)	ARB inactivation 1.5 log better compared to UV alone 8 mJ/cm^2, [FAC] = 2 mg/dm^3: efficient prevention of photoactivation 320 mJ/cm^2, [FAC] = 2 mg/dm^3 1.5–3 log ARGs removal 320 mJ/cm^2, [FAC] = 1 mg/dm^3	LP Hg-UV lamp 254 nm (n.d) UV fluence: 1 mJ/cm^2–32 mJ/cm^2	10 min	Matrix: wastewater (WWTP effluent) Disinfectant: sodium hypochlorite Concentration: 1–2 mg/L	Collimated beam UV lamp over Petri dish containing 10 cm^3 of microorganisms suspension, with stirring	(Wang et al., 2020)

Table 1 (*continued*)

Methods	Molecular target	ARGs removal efficiency	Light source	Operational parameters			Ref.
				Reaction time	Operational conditions	Reactor parameters	
		0.5–2 log ARGs removal					

MAR – Multiple antibiotic resistant, FAC – free active chlorine.

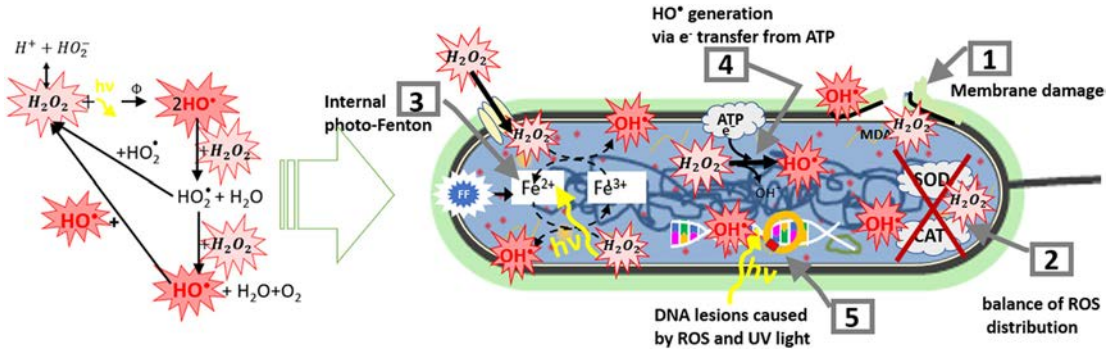

Fig. 2. Intracellular and extracellular inactivation via UV/H_2O_2 disinfection.

ampC and *mecA* removal was observed when UV/H_2O_2 was applied (1.3 and 2.2 log units, respectively (60 mJ/cm^2, 3.4 g H_2O_2/dm^3)) compared to exposure to only UVC with the same condition. Similar log removals of the targets in the UVC process were achieved only when UV fluence was doubled (120 mJ/cm^2). The disinfection with only H_2O_2 (3.4 g H_2O_2/dm^3), after 480 min resulted in 0.90 and 0.85 log reduction of *ampC* and *mecA*, respectively (Guo et al., 2017). Consistent results were delivered by the same group in the further study on UVC and UVC/H_2O_2 treatment of *ampR* gene encoded in pUC19 plasmid, present in an eARG (extracellular ARGs) and iARG (intracellular ARGs) form (*Escherichia coli* as a host) (Yoon et al., 2018). For conditions typical during water disinfection (UVC fluence of 40 mJ/cm^2) only 1.0 log elimination of the transforming activity of iARG and eARG was achieved with UV, and 1.0 log and 1.3 log for iARG and eARG during UVC/H_2O_2 (10 mg H_2O_2/dm^3) treatment, respectively. To achieve complete elimination of transforming activity (4 log unit reduction), a UVC fluence of 150 mJ/cm^2 (125 mJ/cm^2 and (10 mg H_2O_2/dm^3) for UVC/H_2O_2 treatment of eARG) would be necessary (Yoon et al., 2018).

The application of enormous UV fluence of 24,000 mJ/cm^2 with 40 mg H_2O_2/dm^3 resulted in 99% of DNA removal, and bla_{OXA}, bla_{TEM}, $bla_{CTX\ M}$, *qnrS*, and *tetM* completely reduction (*sul*1 was on the limit of quantification). While, incomplete removal of 16 s rRNA would also suggest applying a higher concentration of H_2O_2 to achieve improved removal (Beretsou et al., 2020). The study showed that the application of 50 mg/dm^3 of H_2O_2, already decreased the efficiency of antibiotics abatement. Interestingly, with a concentration of 5 mg H_2O_2/dm^3 and 14,040 mJ/cm^2 the abundance of 16S rRNA after UVC/H_2O_2 treatment of wastewater decreased by 2.4 log units and all β lactams ARGs ($bla_{OXA\ A}$, bla_{SHV}, bla_{TEM}) and *qnrS* were removed completely (>3log units) (Michael et al., 2020). In case of *tetM* and *sul1* and *sul2* only the removal of around 2 log units was observed (Michael et al., 2020). In the same study the exposure to sunlight was tested additionally. Under the fluence of 46,800 mJ/cm^2 (sunlight/H_2O_2) enhanced by 30 mg H_2O_2/dm^3 16S rRNA abundance decreased only by 0.8 log, whereas after 300 min of treatment, the average removal for ARGs was around 0.7 log (Michael et al., 2020). UVC/H_2O_2 and UVC/persulfate demonstrated a similar removal rate of the $bla_{KPC\ 3}$ antibiotic resistance gene at the initial stage (0.14 mJ/cm^2 with 34 mg H_2O_2/dm^3) (Serna Galvis et al., 2020). However, after 600 s (1.38 mJ/cm^2) all processes (including UVC) caused the elimination of $bla_{KPC\ 3}$, that resulted in genetic material damage that inhibit to its transfer to another bacteria.

Surprisingly, higher ARGs removal during UVC than during UVC/H_2O_2 was observed for 4 s contact time (40 mJ/cm^2, 17 mg H_2O_2/dm^3) (Rodríguez Chueca et al., 2019). Total log removal of all investigated ARGs (*sul1*, *sul2*, $bla_{OXA\ A}$, bla_{TEM}, *qnrS*) and *intl1* was calculated as 0.41 and 0.21 log for UVC and UVC/H_2O_2, respectively (Rodríguez Chueca et al., 2019). However, an increase in contact time to 7 s resulted in overall 0.6 log ARGs removal (the most resistant bla_{TEM} demonstrated 0.27 log removal) (Rodríguez Chueca et al., 2019). At the same time, the UVC/H_2O_2 process led to higher antibiotics abatement compared to the UVC process. The authors of the study suggested a competition between these two types of water contaminants for UV photons. It seemed that since antibiotics cannot be removed by UV light in the process of direct photolysis, ARGs are the main target. HO• radicals formed during UVC/H_2O_2 reacts easier with antibiotics than with DNA, and that explains the reverse tendency. Overall that means, that a selection of a proper oxidation method, as well as operational conditions, has to be a compromise between antibiotics and ARGs removal. Therefore, during the optimization of wastewater treatment for antibiotics and ARB/ARGs removal a trade off between UV fluence and adjusted H_2O_2 dose is necessary.

Moreover, it is known, that during micropollutant removal as well as disinfection process the efficiency strongly depends on the chemical composition of water. The presence of dissolved organic matter may have a negative impact on UVC penetration and light absorption by bacterial cells. Water components (effluent organic matter (EfOM), nitrite, bicarbonate and carbonate ions) can also cause the scavenging effect of formed radicals (Grant and Hofmann, 2016). In respect to ARGs removal, the water matrix seems not to be a limiting factor (Guo et al., 2017; Serna Galvis et al., 2020), and there is a potential to apply such oxidation methods for more complex matrices, such as hospital wastewater. As discussed in the previous paragraphs, the disinfection with UVA/H_2O_2 does not provide satisfactory results in removal of ARGs from wastewater. As it was shown, the UV dose required for ARGs removal in UV/H_2O_2 is much higher that commonly used (40 mJ/cm^2), therefore this method cannot be directly implemented to real conditions in WWTPs. It was also reported that even at the concentration higher than 25 mg H_2O_2/dm^3 dose combination UVC/H_2O_2 only slightly influenced the microbial reduction, compared to exposure to UVC radiation only. Interestingly, rapid bacterial inactivation (*E. coli*, *E. faecalis*, and *S. enteritidis*) was observed in the first stage for UVC and UVC/H_2O_2 process, proving that the main inactivation mechanism came

Table 2
The operational condition of UVC/H_2O_2 disinfection applied for ARGs removal. Removal efficiency for a proper time treatment is provided as log unit removal or reaction constant k′.

Methods	Molecular target	ARGs removal efficiency	Light source	Operational parameters			Ref.
				Reaction time	Operational conditions	Reactor parameters	
UVC/H_2O_2	*sul1* *tetG* *tetX* *intI1* 16S rRNA	$[H_2O_2]$ = 0.01 mol/dm^3 $t_{UV/H2O2}$ = 30 min Reduction of all ARGs by 2.8–3.5 log at pH 3.5 1.55–2.32 log at pH 7 k'_{sul1} = 0.0316 1/h, k'_{tetX} = 0.0258 1/h k'_{tetG} = 0.0364 1/h, k'_{intI1} = 0.0250 1/h, $k'_{16SrRNA}$ = 0.04711/h	LP Hg-UV lamp 254 nm (16 W) UV fluence/UV intensity n.a.	Up to 60 min	Matrix: CAS effluents $[H_2O_2]$ = 0.005–0.15 mol/dm^3	Plexiglas cylinder reactor (height 310 mm and radius 450 mm) with a lamp in a quartz sleeve	(Zhang et al., 2016)
UVA/H_2O_2	bla_{TEM} *qnrS* *tetW* 16S rRNA	$t_{UV/H2O2}$ = 240 min - Not effect on the abundance of bla_{TEM} - Poor effect on the abundance of *qnrS* (4.3 × 10^4 copies/cm^3) - Significant decrease of abundance of *tetW* (1.1 × 10^1 copies/cm^3)	Wide spectrum lamp with a UV filter - emission in the range of 320–450 nm (250 W) UV fluence: up to 25 mJ/cm^2	Up to 240 min	Matrix: CAS effluents with indigenous antibiotic-resistant *E. coli* strains $[H_2O_2]$ = 20 mg/dm^3	2.2 dm^3 cylindrical glass reactor (13.0 cm in diameter) filled with 500 cm^3 wastewater (5.0 cm water height) Light source placed 40 cm above water surface	(Ferro et al., 2016)
UVA/H_2O_2	bla_{TEM} *qnrS* *tetW* 16S rRNA	$t_{UV/H2O2}$ = 90 min No effect in DNA extracted from cell cultures (3.8 × 10^8 copies/cm^3) $t_{UV/H2O2}$ = 300 min no effect on the abundance of bla_{TEM} in total DNA after 300 min (2.8 × 10^6 copies/cm^3)	Wide spectrum lamp with a UV filter - emission in the range of 320–450 nm (250 W) UV fluence: up to 25 mJ/cm^2	Up to 300 min	Matrix: Sterile DNA-free water spiked with multidrug-resistant *E. coli* strain $[H_2O_2]$ = 20 mg/dm^3	2.2 dm^3 cylindrical glass reactor (13.0 cm in diameter) filled with 500 cm^3 wastewater (5.0 cm water height) Light source placed 40 cm above water surface	(Ferro et al., 2017)
UVC/H_2O_2	*mecA*, *ampC* Within methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*	Fluence of 120 mJ/cm^2 Various $[H_2O_2]$ ARGs reduction: - ampC ~2.3–2.9 log - mec A ~1.4–2.7 log Fluence of 12 mJ/cm^2, various $[H_2O_2]$ ARB reduction: - MRSA: 2.5–3.7 log - *P. aeruginosa* 2.5–3.6 log	LP Hg-UV lamp 254 nm (300 W, 800 W) UV fluence: up to 120 mJ/cm^2	Up to 480 min	Matrix: PBS solution (pH = 7.4) NW from drinking water source (pH = 7.2) $[H_2O_2]$ = 10–100 mmol/dm^3	Photochemical reactor (XPA-7, Nanjing Xujiang Machinery Factory, Nanjing, China) equipped with Hg lamps	(Guo et al., 2017)
UVC/H_2O_2	amp^R, kan^R Plasmid-encoded (intracellular in *E. coli*, extracellular)	ARG reduction of 2 log: - In phosphate buffered solutions (pH 7) e-ARG at 22 mJ/cm^2 i-ARG at 65–73 mJ/cm^2 - In wastewater e-ARG at 50 mJ/cm^2 i-ARG 60–75 mJ/cm^2	LP Hg-UV lamp 254 nm (n.a.) UV fluence: up to 130 mJ/cm^2	n.a.	Matrix: Wastewater effluent spiked with pUC4k plasmid or *E. coli* (prepared with phosphate solutions) Phosphate buffered solutions of pUC4k plasmid or *E. coli* $[H_2O_2]$ = 10 mg/dm^3	Bench-scale quasi-collimated beam system equipped with a light source Placed over Petri dish (diameter 9 cm) with sample solutions (120 cm^3)	(Yoon et al., 2017)
UVC/H_2O_2	amp^R plasmid-encoded (intracellular in *E. coli*, extracellular)	Reduction of transforming activity of a plasmid-encoded ARG: - Of ~1 log at fluence of 40 mJ/cm^2 - Of >4 log at fluence of >150 mJ/cm^2	LP Hg-UV lamp 254 nm (n.a.) UV fluence: up to 320 mJ/cm^2	n.a.	Matrix: Phosphate buffered solutions with pUC4k plasmid or *E. coli* $[H_2O_2]$ = 10 mg/dm^3	Bench-scale quasi-collimated beam system equipped with a light source Placed over Petri dish (diameter 9 cm) with sample solutions (120 cm^3)	(Yoon et al., 2018)
UVC/H_2O_2	*sul1* *sul2* *qnrS* bla_{TEM} $bla_{OXA\ A}$ *intI1* 16S rRNA	$[H_2O_2]$ = 0.5 mM $t_{UV/H2O2}$ = 4 s Reduction of: - 16S rRNA by 0.16 log - All targets[a] by 0.21 log $t_{UV/H2O2}$ = 7 s	16 × LP Hg-UV lamps 254 nm (330 W) UV fluence: up to 40 mJ/cm^2 Energy density:	4–18 s	Matrix: CAS effluent $[H_2O_2]$ = 0.05–0.5 mM	Reactor equipped with 16 UV-C lamps of 140 dm^3 volume	(Rodríguez-Chueca et al., 2019)

Table 2 (*continued*)

Methods	Molecular target	ARGs removal efficiency	Light source	Operational parameters			Ref.
				Reaction time	Operational conditions	Reactor parameters	
		Reduction of: - 16S rRNA by 0.61 log - All targets[a] by 0.60 log	42–170 J/dm^3				
UVC/H_2O_2	bla_{OXA} bla_{TEM} $bla_{CTX\ M}$ *qnrS* *sul1* *tetM* 16S rRNA Faecal coliforms, *Enterococcus* spp., *Pseudomonas aeruginosa*[a]	[H_2O_2] = 40 mg/dm^3 Fluence 0.7 J/cm^2 Reduction of: - 16S rRNA by 1 log - Individual ARGs by ~1.5 log unit *sul1* not removed even after 24 J/cm^2	LP Hg-UV lamp 254 nm (9 W) UV fluence: 700–24,000 mJ/cm^2	n.a.	Matrix: CAS effluent [H_2O_2] = 0–50 mg/dm^3	Bench-scale cylindrical reaction vessel with a total capacity of 600 cm^3 with a light source placed in the center of the reactor	(Beretsou et al., 2020)
UVC/H_2O_2	bla_{TEM} $bla_{OXA\ A}$ bla_{SHV} $bla_{CTX\ M}$ *mecA* *sul1* *sul2* *qnrS* *vanA* *tetM* 16 s rRNA	[H_2O_2] = 5 mg/dm^3 0.8 kJ/dm^3 Reduction of: - 16S rRNA by 2.4 log - *sul*1, *sul*2, *tet*M, *blaOXA-A* and *blaTEM* by 2.0–3.7 log - *qnrS* and *bla*SHV below limit of quantification	3 × LP Hg-UV lamps 254 nm (230 W) UV fluence: up to 14,040 mJ/cm^2 UV irradiance: 2.6 mW/cm^2	90 min	Matrix: Urban WW Saline solution [H_2O_2] = 0.5–30 mg/dm^3	Recirculating batch mode pilot-scale reactor V 6.21 dm^3 (working volume 80 dm^3)	(Michael et al., 2020)
UVC/H_2O_2	*Klebsiella pneumoniae* resistant to carbapenem antibiotics (CR-Kp) *blaKPC-3* gen	Inactivation by 6 log: - In deionized water after 60 s - In wastewater after 180 s	LP Hg-UV lamp 254 nm (8 W) UV fluence: 1.38 mJ/cm^2 UV irradiance: 2.3 × 10^{3} mW/cm^2	Up to 600 s	Matrix: CAS effluent Deionized water inoculated with CR-Kp bacteria [H_2O_2] = 1 mM	A beaker with 150 cm^3 of experimental solution placed 8.5 cm away from UV lamp	(Serna-Galvis et al., 2020)

[a] Total heterotrophs resistant to trimethoprim, ofloxacin and erythromycin.

from the effect of UVC radiation rather than from the damages caused by HO• generated through H_2O_2 photolysis (Sánchez Montes et al., 2020). On the other hand, in the study of Zhao et al. (2021) in which UV/H_2O_2 dis infection was tested with respect to *E. coli* and *S. aureus*, UVC enhanced by H_2O_2 delivered stronger disinfection effect than the single UVC. Such result suggests that the type of the bacteria plays the key role in the overall effi ciency of UVC/H_2O_2 as a disinfection technique. The disinfection effect on the plasmid encoded antibiotic resistance genes (*ampR* and *kanR* on pUC4K plasmid) are located extracellularly (eARG) and within *Escherichia coli* (iARG) showed importance of HO• radicals (Yoon et al., 2017). iARGs degradation rates were lower than for eARGs, due to the protective effect of cell components against UVC (Yoon et al., 2017). Also, the effect of HO• radicals on iARGs was negligible, most likely for the same reason. In contrast, the damage rates for eARGs were ~1.5 fold higher in UVC/H_2O_2 compared to in UV treatment. The effect of HO• radicals on eARGs was also illustrated during electrophoresis, by showing the changes in the structural integrity of extracellular plasmid. In general, even if the kinetics of ARGs degradation by UVC and UVC/H_2O_2 are comparable, the effect of formed HO• radicals in UVC/H_2O_2 process is rather limited and it can play a role only in the removal of eARG.

3.3. Photo activated sulfate radical based AOPs

In recent years, increasing attention has been paid to the new genera tion of AOPs (persulfate AOPs or sulfate radicals AOPs (SR AOPs)) which mode of action is based on sulfate radicals ($SO_4^{\bullet -}$). These radicals have strong oxidative properties with a redox potential in a range of 2.5 3.1 V (Guan et al., 2011), that is similar to the redox potential of HO• radicals. $SO_4^{\bullet -}$ has a much longer half life time (30 40 μs) than HO• (<1 μs), which makes sulfate radicals a promising oxidant and disinfectant (Wang et al., 2019). Photoactivation of persulfate (PS) or peroxymonosulfate (PMS) by UVC light promotes the generation of $SO_4^{\bullet -}$ and HO• as well as $O_2^{\bullet -}$ and $^1O_2$ (Zhang et al., 2021). Due to the formation of ROS with $SO_4^{\bullet -}$ inherence, the SR AOPs were successfully applied for micropollutants removal from water as well as for disinfection (Duan et al., 2020; Rodríguez Chueca et al., 2019; Serna Galvis et al., 2020; Zeng et al., 2020; C.S. Zhou et al., 2020).

$SO_4^{\bullet -}$ radicals are the main oxidants in the disinfection mechanism of sulfate radical photo AOPs. At the same time, $O_2^{\bullet -}$ and HO• are also in volved in bacteria inactivation (Wang et al., 2019). The UV/PMS and UV/PS or visible light/PS treatment cause inactivation via destruction of the cell membrane (1), enzymes, (2) and genetic materials (3) (Fig. 3). The disinfection process firstly begins from oxidative lipid peroxidation in the cell membrane (1), leading to disruption of membrane permeability and inhibition of the normal metabolism. $SO_4^{\bullet -}$ radicals easily permeate through the cell membrane and further react with intracellular components (Wang et al., 2019). $S_2O_8^{2-}$ light activation generates ROS that enhance the damage of the cell membrane (Liu et al., 2020; Wang et al., 2019; Xiao et al., 2019). Simultaneously, action of ROS and $SO_4^{\bullet -}$ improves the inac tivation. Additionally, ROS concentration raises due to intra reaction in the presence of naturally occurring iron (4), which leads to the induction of antioxidant enzymes (CAT and SOD, (2)) as well as DNA damage (3) and finally to cell death (Liu et al., 2020; Wang et al., 2019). Meanwhile, the DNA damage is also caused by light (3).

Although the SR AOPs have gained the special interest of many scien tists due to their high effectivity in micropollutant degradation or disinfec tion, only a few publications addressed ARGs removal via photoactivation of PS or PMS by light (Table 3).

The first attempt of UVC/PMS application for the removal of ARGs (*sul1*, *sul2*, *qnrS*, bla_{TEM}, $bla_{OXA\ A}$) as well as general bacterial gene marker 16S rRNA and mobile genetic elements class 1 integrons (*intI1*) has been done in a full scale WWTP using a conventional UVC

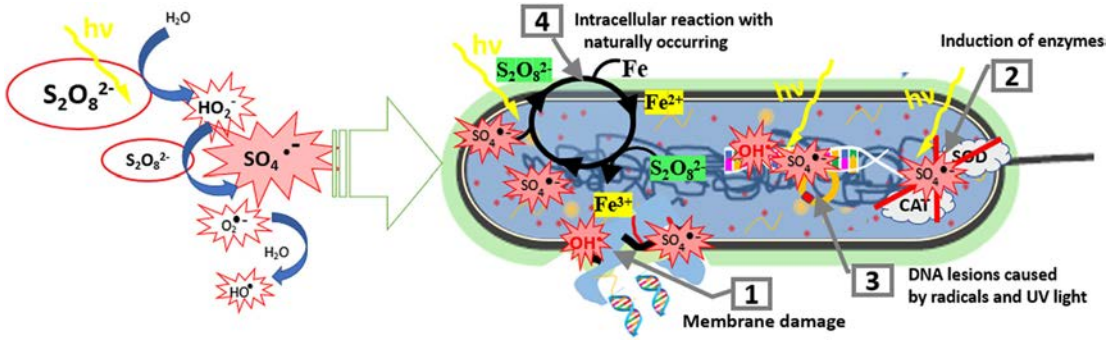

Fig. 3. Sulfate radicals photo-AOPs disinfection mechanism based on Liu et al. (2020), Wang et al. (2019) and Xiao et al. (2019).

disinfection reactor (Rodríguez Chueca et al., 2019). Despite the assumption that SR AOPs generate more radicals than the UVC/H_2O_2 process, the observed ARG removal during PMS/UVC was similar or lower compared to UVC/H_2O_2 (0.40 log (7 s); 0.22 log (4 s), and 0.60 log (7 s); 0.21 log (4 s), respectively) (Rodríguez Chueca et al., 2019). In the same study, 4 s of UVC treatment caused 0.41 log removal of the target. It should be noted, that PMS and H_2O_2 have a similar absorption coefficient at 260 nm and both were applied in the concentration of 0.5 mM and exposed to 40 mJ/cm^2. Considering that DNA has a lower absorption coefficient compared to PMS and H_2O_2, the observed efficiencies of the process were lower than efficiency of the direct UVC action. This fact could be explained by the photon competition. Therefore, it can be concluded that during fist few seconds $SO_4^{\bullet -}$ and HO• are not responsible for ARGs removal, and only UVC lead to DNA damage. When PMS/Fe(II)/UVC was applied, no removal was observed (Rodríguez Chueca et al., 2019). When lower light intensity, but higher oxidant concentration was applied a complete *Klebsiella Pneumoniae* inactivation was achieved after 60 s by UVC, UVC/H_2O_2, and UVC/PS (PS and H_2O_2 at 1 mM, 0.14 mJ/cm^2) (Serna Galvis et al., 2020). However, that condition resulted only in 5%, 65%, and, 80% removal of $bla_{KPC\ 3}$ (UVC, UVC/H_2O_2, and UVC/PS, respectively). To achieve 98% of $bla_{KPC\ 3}$ removal treatment had to be prolonged to 300 s for UVC/H_2O_2 and UVC/PS and 600 s for UVC (Serna Galvis et al., 2020). In case of UVC/PS treatment with 240 mJ/cm^2 the highest removal efficiencies for *sul1*, *sul2*, *ermB*, *qnrS*, *tetO*, *intI1*, *intI2* were noted compared to UVC and PS alone (240 mJ/cm^2 with PS at 1 mM, for 10 min) (C.S. Zhou et al., 2020). However, the overall efficiency of UVC/PS (0.55 log removal) is still not satisfactory compared to others techniques (0.47 log for UVC and 0.33 log for PS) (C.S. Zhou et al., 2020). It was found that all treatments resulted in highest integrases removal compared to all targets (Table 3). The investigation of the ARGs removal mechanism did show that both $SO_4^{\bullet -}$ and HO• support the ARGs degradation and bacterial inactivation (C.S. Zhou et al., 2020). This is in opposite to the suggestion made by L. Zhang et al. (2020). Zhang with co workers, demonstrated that in novel sulfidated micron zero valent activated persulfate system (without light) the radical pathway ($SO_4^{\bullet -}$ and HO•) is the key factor when considering membrane lipid oxidation and removal of 16S rRNA and *tetB*. These contrary results show that in case of light disinfection treatment the most important is light intensity. In principle, the exposure to higher UV fluence resulted in ARG removal caused by UV induced DNA lesions. On the other hand, insufficient UV exposure triggered mainly the production of ROS that eventually led to ARG removal. All in all, the review of several studies discussing the application of photo activated sulfate radical based AOPs for disinfection purposes clearly indicates that it is still difficult to predict the inactivation levels of various kinds of ARGs during disinfection. However, none of the reported studies classified UVC/PS or UVC/PMS AOPs as an efficient disinfection method with respect to removal ARGs. All were considered as a method that can be applied to eventually minimize the spread of antibiotic resistance in natural environment.

3.4. Photocatalytic processes

Photocatalytic processes promote strong oxidation reactions by generating HO• and/or photogenerated holes (Fig. 4), which attack directly or through a series of oxidative chain reactions causing extensive damage to the biological resulting in cell death (Karaolia et al., 2017; Loeb et al., 2016).

The mechanism of photocatalytic based disinfection processes is presented in Fig. 4. Firstly, bacteria have to adhere to the photocatalysts, ROS production has to be triggered. During photocatalytic disinfection, the attack of ROS on microorganisms firstly occurs outside of the cell membrane. After entering the cell, ROS destroy the genetic material and inhibit the metabolic processes. The cell membrane is attacked by ROS causing co enzyme A damage, inhibition, reduction, or loss of cellular respiration activity, which may result in cell death (1) (Deng et al., 2020; Matsunaga et al., 1985). Furthermore, ROS oxidation of cell membranes, breaking down cell membranes and cell walls, resulting in intracellular macromolecules leakage of nucleic acids, proteins, cations, which finally lead to the death of bacterial cells (2) (Deng et al., 2020). Further, oxidation of macromolecular substances such as nucleic acids and proteins by ROS (after cell wall and cell membrane damage) (3) as well as photocatalyst penetration inside the cell (4), result in morphological and structural damage and cell death (Deng et al., 2020; Wu et al., 2010). UVC and UVB damage directly DNA (3), whereas UVA, and visible light lead to indirect DNA damage via generation of ROS (especially, $^1O_2$). These species are produced by photochemical reactions after the light absorption of photosensitizers, and there after attack lipids, enzymes (CAT, SOD), proteins, and DNA (5) (Schauen et al., 2007).

As photocatalytic processes can successfully be applied for water disinfection, similar effectivity for the removal of ARGs was expected. The effectivity of light driven photocatalytic ARGs removal is presented in Table 4.

The link between differences in photocatalytic disinfection activity under the same operating conditions and acquisition of genetic material in the form of plasmids was found (Tsai et al., 2010). Öncü with co workers proved that for low plasmid DNA concentration (6.4 μg/cm^3) 75 min of treatment was required to completely remove all plasmid DNA bands from the gel image (Öncü et al., 2011). Bearing in mind that ARGs are encoded on the plasmid, it was confirmed that the photocatalytic process is promising for ARG removal. After Tsai and co workers demonstrated that suspension of TiO_2 in the presence of UVA effectively reduced the antibiotic resistant microbes by 1 3 logs (Tsai et al., 2010), the photocatalytic processes became the most commonly investigated for ARG removal. Higher inactivation was observed in distilled water compared to effluent (Dunlop et al., 2015). The lower numbers of gene pair conjugants were caused by scavenging of ROS by organic and inorganic components of the effluent, resulting in reducing oxidative stress on the antibiotic resistant *Escherichia coli*. Longer treatment time was recommended to avoid post treatment recovery, which could minimize the highly unwanted transfer of ARGs among bacteria (Dunlop et al., 2015). Mostly, photocatalytic disinfection processes were applied for secondary effluents, but continuous

Table 3
The operational condition of UV-light sulfate radicals AOPs applied for ARGs removal. Removal efficiency for a proper time treatment is provided as log unit removal or percentages of removal.

Methods	Molecular target	ARGs removal efficiency	Light source	Operational parameters			Ref.
				Reaction time	Operational conditions	Reactor parameters	
UVC/PMS UVC/PMS/Fe (II)	*sul1*, *sul2*, *qnrS*, bla_{TEM}, $bla_{OXA\ A}$, intI1 16S rRNA,	$t_{PMS/UVC}$ = 4 s Reduction of: - Total ARGs by 0.22 log $t_{PMS/UVC}$ = 7 s Reduction of: - Total ARGs by 0.4 log $t_{PMS/Fe(II)/UVC}$ = 4 s Reduction of: - Total ARGs by 0.02 log $t_{PMS/UVC}$ = 7 s Reduction of: - Total ARGs by 0.04 log	LP Hg-lamp (254 nm) 16lamps 330 W UV fluence/ UV intensity n.a.	4 s; 7 s	Matrix: secondary wastewater effluent UVC dosage: 42 J/dm^3 (4 s, 114 m^3/h); 67 J/dm^3 (7 s, 75 m^3/h) [PMS] = 0.5 mM [Fe(II)] = 0.5 mM	LBX400e UV-C Volume:140 dm^3 Flow rates: 75 m^3/h;114 m^3/h	(Rodríguez-Chueca et al., 2019)
UVC/PS	$bla_{KPC\ 3}$ *Klebsiella pneumoniae*	$t_{PS/UVC}$ = 60 s 80% removal of $bla_{KPC\ 3}$ $t_{PS/UVC}$ = 300 s 98% removal of $bla_{KPC\ 3}$	LP Hg-lamp (254 nm) 8 W Photon fluence rate 2.19×10^{15} E/dm^3 s UV fluence: 0.138–1.38 mJ/cm^2 UV irradiance: 2.3×10^{3} mW/cm^2	60s; 300 s; 600 s	Matrix: autoclaved (2 h) distilled water; secondary wastewater effluent Both spiked with bacterial stock [PS] = 1 mM	Aluminum reflective box endowed with a UVC lamp. Beaker as a reactor placed at 8.5 cm from the lamp. Mixing by magnetic stiller Volume: 151 cm^3	(Serna-Galvis et al., 2020)
UVC/PS	*sul1*, *sul2*, *ermB*, *qnrS* *tetO*, intl1, intl2	The removal *sul1*: 0.38 log *sul2*: 0.24 log *ermB*: 0.78 log *tetO*: 0.25 log *qnrS*: 0.44 log intl1: 0.62 log intl2: 1.14 log	LP Hg-lamp (254 nm) 8 W UV fluence: 240 mJ/cm^2 UV irradiance: 0.4 mW/cm^2	10 min	Matrix: secondary effluent of a WWTP UVC dosage: 240 mJ/cm^2 [PS] = 1 mM (0.27 g PS/100 cm^3)	Beaker as a reactor placed below UV lamp with temp. control at 25 °C Volume: 100 cm^3	(C.S. Zhou et al., 2020)

UVA_{LED}/TiO_2 (suspension) was applied as pre treatment (before secondary treatment) as well (Cai and Hu, 2018). However, photocatalytic pre treatment improved *sul2* and *dfrV* genes removal in *Escherichia coli* host cells, but was not effective toward *sul1* (Cai and Hu, 2018). More details about the operational conditions of aforementioned studies can be found in Table 4.

The potential of various catalysts and various light sources for disinfection purposes have been investigated. Anyhow, application of UV light to this purpose is rather scarce. The results confirmed that under UV light condition, hybrid photocatalysts based on graphene oxide TiO_2 (Z. Zhou et al., 2020) and thin TiO_2 film (Guo et al., 2017) can be applied for ARG removal. Much higher e*ampC* and e*tetA* removal within the first 7 min was obtained for TiO_2 GO (not reduced) compared to reduced form TiO_2 rGO (4.32 log and 4.02, 1.57 log and 1.47 log, respectively (Table 4)) (Z. Zhou et al., 2020). When UVC/TiO_2^{film} was applied, 5.8 log of *mecA* reduction and 4.7 log of *ampC* reduction were achieved (120 mJ/cm^2) (Guo et al., 2017).

The light germicidal effect was ordered as follows UVC > UVB > UVA > visible rays, with the dose required for cell death increasing by orders of magnitude in the visible region (Hockberger, 2000, 2002; Reed, 2010). However, when photocatalytic processes are performed, the light disinfection effect should be supported by the action of ROS. The inactivation of three strains *Escherichia coli* (two donors: bacterial strain harbors a plasmid containing (i) *E. coli* DH5a (CTX) bla_{CTX} gene; (ii) *E. coli* DH5a (MCR mcr_1 gene) and one recipient strain (*E. coli* C600 (SM)) under different light sources in the presence of natural magnetic sphalerite were discussed by Yin et al. (2021). Photocatalytic disinfection (4 μW/cm^2, 50 mg PC/dm^3) showed that the despite photocatalysts under UVC condition the main inactivation pathway was due to UVC_{254nm} action. The strong UVC disinfection properties led to rapid inactivation (6 log for all strains), while no ROS infuence was observed (Yin et al., 2021). While when the photocatalytic process was conducted under UVA_{356nm} (100 mW/cm^2), visible light (solar simulator occupied by XBO lamp (λ < 300 nm, 60 mW/cm^2) bacteria entered the induction period firstly, but then due to ROS a 6 log and a 5 log reduction, respectively, was achieved (Yin et al., 2021). Surprisingly, when a LED lamp (λ < 400 nm, 60 mW/cm^2) was applied at the same condition (50 mg PC/dm^3), less than 2 log removal was detected mostly related to ROS action (much lower concentration) (Yin et al., 2021). Considering that conjugation can happen intercellularly, it was found that this process is promoted during the photocatalytic process with natural magnetic sphalerite under UVC_{254nm} and visible light (above 400 nm) in water systems. On the other hand, photocatalytic process under UVA_{356nm} and simulated sunlight (above 300 nm) did not clearly increase conjugative transfer (Yin et al., 2021). This finding, suggests that by applying UVA or light above 300 nm, strongly inhibit possibility of conjugative ARGs transfer.

In all discussed experiments, TiO_2 (in the suspended system (Cai and Hu, 2018; Öncü et al., 2011; Tsai et al., 2010) and in the immobilized system (Dunlop et al., 2015; Guo et al., 2017)) was applied under UV light. Compared to UVC/H_2O_2, UVC/PS and UVC/PMS treatment (discussed above), removal obtained by UV photocatalytic processes seems to be impressive. Although, it should be noted that the processes involving immobilized catalysts are much slower compared to processes with suspended catalysts. The higher efficiency of the suspended catalysts is

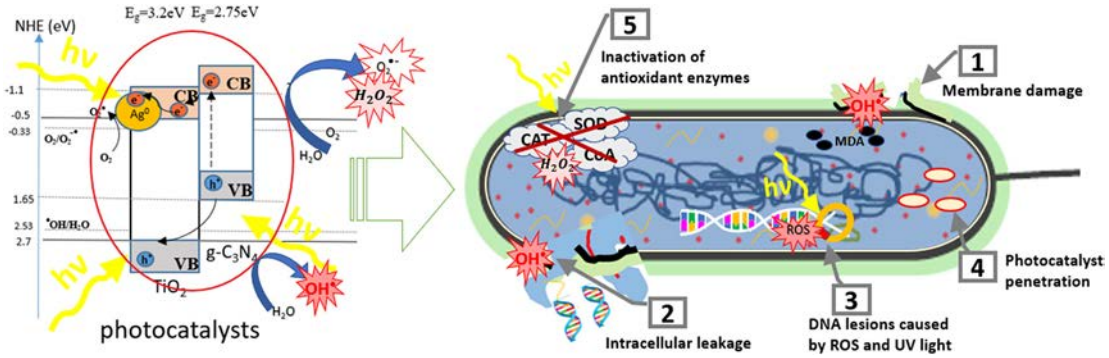

Fig. 4. Photocatalytic-based disinfection mechanism based on Deng et al. (2020).

related to its larger surface area compared to the immobilized alternative but related also to penetration of catalysts particles inside the cells. Unfor tunately, the photocatalytic treatment based on the suspended catalyst re quires separation of the used catalyst after the treatment. Furthermore, using UV light to induce the process is quite expensive. To address these two drawbacks, the attention of researchers shifted to the catalysts in the in corporated form, as well as using visible light to initiate the photocatalysis. TiO_2, the most frequently used catalysts, shows the highest efficiency under UV light. For its applicability under visible light some modifications are re quired. The ARGs removal under simulated or natural sunlight was investi gated with the application of several catalysts (manganese and cobalt doped TiO_2 photocatalysis (Mn , Co , and binary Mn/Co TiO_2) (Venieri et al., 2017), graphene based composite TiO_2 photocatalysts (TiO_2 GO (Moreira et al., 2018); TiO_2 rGO PH and TiO_2 rGO HD (Karaolia et al., 2018); Ag/TiO_2 GO (Z. Zhou et al., 2020)), hierarchical $Bi_2O_2CO_3$ micro spheres wrapped with nitrogen doped reduced graphene oxide (D. Li et al., 2020), photocatalyst graphitic carbon nitride (Ag/AgBr/g C_3N_4 (Yu et al., 2020)) and natural magnetic sphalerite (G. Li et al., 2020; Yin et al., 2021), Text S8).

The applicability of TiO_2 GO was widely investigated under visible light condition. Despite, high removal of e*ampC* and e*tetA* under UV/TiO_2 GO, Zhou and co workers did not recommend it for ARG removal under visible light. While modifications by silver nanoparticles enable TiO_2 GO to be suc cessfully applied for ARG removal (Z. Zhou et al., 2020). The process in which unreduced form of Ag/TiO_2 GO (STG) was used was much more ef ficient in the removal of ARGs compared to the process with Ag/TiO_2 rGO (STrG) (pH 7, *ampC* decreases 7.5/3.7 log; *tetA* decreases 7.3/3.6 logs for STG/STrG, respectively). STG catalyst was also more effective for iARGs and eARGs in the model solution, and for eARGs in secondary effluent (Z. Zhou et al., 2020). 35 min of STG/STGr treatment of model solution led to 5.39/1.12 log i*tetA* removal and 7.46/3.55 log for e*tetA* removal. In the same study, 60 min of treatment of secondary effluents led to 3.12/0.92 logs removal for e*tetA* for STG/STGr, respectively (Z. Zhou et al., 2020). The photoactivation of TiO_2 rGO (100 mg/dm^3) by simulated visible light for *Escherichia coli*, ARGs (*ampC*, *sul*, *ermB*), as well as enterococci (23S rRNA) gene and *Pseudomonas aeruginosa* (*ecfX*) was examined by Karaolia et al. (2018). 23S rRNA primer system was the most prevalent genetic se quence detected in all samples (Karaolia et al., 2018). *ecfX* was the most sensitive to photocatalytic disinfection (Table 4). In comparison to Vis/TiO_2, Vis/TiO_2 rGO was sufficient in *ampC* elimination (below detection limit). Removal of *sul1* and *ermB* showed to be more challenging for TiO_2 rGO PH and TiO_2 rGO HD treatments, in contrast to Vis/TiO_2 (Karaolia et al., 2018). These results are in contradiction to the study of Z. Zhou et al. (2020), where neither UV nor visible light are recommended in com bination with unreduced catalysts. Almost identical operational conditions were applied in both studies (the same catalysts dose, the same time of treatment), only lamp intensity was different (1000 W XBO (Karaolia et al., 2018), 300 W (Z. Zhou et al., 2020)). Again, the results demonstrated that the operational conditions related to light exposure (light intensity, lamp power, possible used cut off) are essential in light based disinfection.

Photocatalytic ozonation under UVA_{LED} lamps caused a significant re duction of *sul1*, *qnrS* and bla_{TEM} genes (Moreira et al., 2016). All target ARGs were below the LOQ after photocatalytic ozonation in secondary treated wastewater and surface water (Moreira et al., 2016). Abundances of 16S rRNA and intI1 were also reduced in effluents (4 log and 5 log, re spectively) as well as in surface water, proving that possible ARGs spread can be reduced by photocatalytic ozonation (Table 4).

The photocatalytic disinfection enhanced by additional H_2O_2 (up to 3.4 g H_2O_2/dm^3) any improvement in ARG reduction was observed (Guo et al., 2017). However, compared to H_2O_2 alone and UVC/H_2O_2, the TiO_2^{film}/UVC/H_2O_2 process led to increase *mecA* and *ampC* removal by 2.7 3.4 and 2.7 3.2 log, respectively (Guo et al., 2017).

As it was discussed above, the light properties are a crucial factor in light based disinfection since they directly affect the inactivation efficien cies and the severity of DNA damage. It is extremely important when the ac tion of visible light is considered. It was shown by Rizzo et al., that simulated irradiation is more effective in complete bacterial inactivation compared to natural solar irradiation when suspended TiO_2 (50 mg/dm^3) was used (Rizzo et al., 2014). But considering that UVC/B filter was applied for solar simulator ($|\lambda < 330$ nm) and the intensity of the lamp was 5 times higher than natural sunlight intensity, it was not surprising that higher in activation was observed. In case of photocatalytic processes triggered by sunlight (sunlight/TiO_2, sunlight/TiO_2/H_2O_2, sunlight/GO TiO_2 and sun light/H_2O_2 (200 mg/dm^3 of catalysts, 20 mg H_2O_2/dm^3 mg), none of the process was able to completely remove ARGs (Moreira et al., 2018). Addi tion of H_2O_2 accelerates sunlight/TiO_2 treatment. The highest efficiency was observed for sunlight/TiO_2/H_2O_2 and sunlight/H_2O_2 (removal of *sul1*, *qnrS*, *16S rRNA*, *intl1* by 1 log and $bla_{CTX\ M}$ by 3 log) (Moreira et al., 2018). Other types of treatment resulted in slight removal (below 1 log) (Table 4).

Those results show that photocatalytic disinfection process reduces the abundances of ARGs to certain extend, however not to the degree that could efficiently limit the spread of antibiotic resistance. No matter, what kind of light or catalysts is applied. Photocatalytic disinfection leads to not enough ROS generation to cross the barrier that cause total DNA damage and stop conjugative transfer of ARGs in the aquatic environment. Thus, to enhance the efficiency, an electron acceptor, such as H_2O_2 or O_3 in combination with a photocatalyst should be employed in further studies.

3.5. Photo Fenton

Due to the simplicity of the Fenton method, and relatively easy applica tion of the method for wastewater treatment in larger scale, Fenton method (H_2O_2/Fe^{2+}) has become the most popular AOP. However, the Fenton re action mechanism is quite complex and it involves radical reactions. To in crease the efficiency, the Fenton's reagent can be combined with UV Visible (λ = 300 600 nm) radiation (called the photo Fenton process, Fig. 5) (Rincón and Pulgarin, 2006). As a result of the catalytic decomposi tion, reactive hydroxyl radicals are formed. In 2006, for the first time, it was reported that the presence of Fe^{3+} accelerates the simulated sunlight

Table 4
Operational condition for semiconductor photocatalysis ARGs removal. Removal efficiency for a proper time treatment is provided as log unit removal.

Methods	System/precursor	Molecular target	ARGs removal efficiency	Light source	Operational parameters			Ref.
					Reaction time	Operational conditions	Reactor parameters	
UVA/TiO_2	Suspended system Precursor: P25, Evonik	Plasmid DNA HB101 *Escherichia coli*	$t_{UVA/TiO2}$ = 75 min reduction of - Low conc. (6 μg/cm^3) by 4 log - High conc. (12.8 μg/cm^3) by 0.6 log	UVA radiation Black-light blue (BLB, 370 nm) Lamp 125 W Photon flux: 6.5 × 10^{-6} quanta/s	Up to 75 min	Matrix: Milli-Q water spike with 80 μ dm^3 of plasmid DNA with concentration of 200 ng/μ dm^3 or 100 ng/μ dm^3 [PC] = 171 mg/dm^3	Reactor: borosilicate glass tubes, temperature control with mixing (40 rpm) Volume: 4 cm^3	(Öncü et al., 2011).
UVC/TiO_2^{film} UVC/TiO_2^{film}/H_2O_2	Immobilized system Immobilization on the quartz plate titanium (IV) Precursor: isopropoxide	*mecA*, *ampC* within methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*	Fluence of 120 mJ/cm^2 various [H_2O_2] -no effects on inactivation ARGs reduction: - *ampC* ~ 4.7 log - *mec A* ~ 5.5 log Fluence of 120 mJ/cm^2, iARG and eARG reduction: - *i-ampC* = 4.4 log - *e-ampC* = 2.6 log - *i-mec A* = 5.2 log -*e-mec A* = 3.3 log	LP Hg-UV lamp 254 nm (300 W, 800 W) Fluence: up to 120 mJ/cm^2	Up to 480 min	Matrix: PBS solution (pH = 7.4) NW from drinking water source (pH = 7.2) [PC] = n.a [H_2O_2] = 10–100 mM	Photochemical reactor (XPA-7, Nanjing Xujiang Machinery Factory, Nanjing, China) equipped with Hg lamps Volume: 50 cm^3	(Guo et al., 2017)
UV/TiO_2/GO graphene oxide UV/TiO_2/rGO reduced graphene oxide	Suspended system Precursor: P25	*e-ampC*, *e-tet*A	UV/TiO_2/GO//UV/TiO_2/rGO *t* = 7 min reduction: -*e-ampC* = 4.32 log // 1.57 log -*e-tet*A = 4.02 log // 1.47 log *t* = 35 min reduction above 99%	UV light UV Fluence/ UV Irradiance n.a.	35 min	Matrix: Buffered solution (pH range 5–9 [PC] = 100 mg/dm^3	n.a.	(Z. Zhou et al., 2020)
Vis/Ag-TiO_2/GO Vis/Ag-TiO_2/rGO	Suspended system Precursor: P25	*e-ampC*, *e-tet*A *i-ampC*, *i-tet*A	Model solution Vis/Ag-TiO_2/GO t = 5(i)/7(e) min reduction: -*i-ampC*/*e-ampC* = 0.62 log/6.20 log -*i-tet*A/*e-tet*A = 0.6 log/6.03 log *t* = 30(i)/35(e) min reduction above 99% Vis/Ag-TiO_2/rGO *t* = 5(i)/7(e) min reduction: -*i-ampC*/*e-ampC* = 0.51 log/0.37 log -*i-tet*A/*e-tet*A = 0.62 log/0.35 log	Simulated and natural sunlight XBO lamp with a 420 nm cut-off *300 W* Fluence/ Irradiance n.a.	35 min for model solution 60 min for effluents	Matrix: model solution, and secondary effluents from WWTP [PC] = 100 mg/dm^3	Reactor: n.a.	(Z. Zhou et al., 2020)

(continued on next page)

Table 4 (*continued*)

Methods	System/precursor	Molecular target	ARGs removal efficiency	Light source	Operational parameters			Ref.
					Reaction time	Operational conditions	Reactor parameters	
			t = 30(i)/35(e) min reduction above 90% Effluents t = 60 min. Vis/Ag-TiO_2/GO//Vis/Ag-TiO_2/rGO reduction: *-e-ampC/* = 3.31 log // 0.98 log *-e-tetA/* = 3.12 log // 0.92 log				
Vis/TiO_2 Vis/TiO_2-rGO-PH Vis/TiO_2-rGO-HD	Suspended system Precursor: P25, Evonik Aeroxide	*ampC*, *sul1*, *ermB*, *ecfX*, 23S rRNA for *Escherichia coli*	*E. coli*. All effective after 180 min Abundance after treatment (log_{10} CE/100 ng DNA) T = 60 min. Lowest values for Vis/TiO_2 *-ampC:* all treatment effective *-sul1:* 2.37–2.92log *-ermB*: 0.3–1.6log 23S rRNA:3.52–3.95log *-ecfX*: 0.55–1.14log (lowest for Vis/TiO_2-rGO-HD)	Simulated solar radiation-visible light Xe Lamp 1000 W, Fluence: 22,680 mJ/cm^2 UV irradiance: 6.3 mW/cm^2	60 min	Matrix: effluents after MBR from WWTP (pH 5.2–6.2) [PC] = 100 mg/dm^3	Reactor: Solar simulator Newport type 91,193, double-walled Pyrex glass batch reactor, stirred, aerated, Temp: 25 °C (controlled) Volume: 300 cm^3 (working vol.)	(Karaolia et al., 2018)
Vis/NRGO- $Bi_2O_2CO_3$ Vis/RGO-$Bi_2O_2CO_3$ Vis/$Bi_2O_2CO_3$ Vis/TiO_2	Suspended System microspheres	$ibla_{NDM-1}$ $ebla_{NDM-1}$	t = 120 min reduction: Vis/NRGO- $Bi_2O_2CO_3$ *i-*bla_{NDM-1} = 5 log *e-*bla_{NDM-1} < LOD Vis/RGO-$Bi_2O_2CO_3$ *i-*bla_{NDM-1} = 3.5 log *e-*bla_{NDM-1} -increase ~0.5 log Vis/$Bi_2O_2CO_3$ $ibla_{NDM-1}$ = 0.6 log $ebla_{NDM-1}$ -increase ~3.5 log Vis/TiO_2 i-bla_{NDM-1} = 0.5 log e-bla_{NDM-1}-increase ~3.5 log	Simulated sunlight Xe lamp (340 < λ < 700 nm), 300 W Photon fluence rate: 8.68 × 10^{-6} E/dm^3·s	Up to 180 min	Water matrix: phosphate buffer solution (pH 7.0), membrane-filtered secondary effluents from WWTP [PC] = 200 mg/dm^3	Reactor: quartz reactor in a black acrylic box, Temp:25 °C (controlled) Volume: 150 cm^3	(D. Li et al., 2020; G. Li et al., 2020)
Vis/TiO_2 Vis/$Mn^{0.1\%}$-TiO_2 Vis/$Co^{0.1\%}$-TiO_2 Vis/$(Mn/Co)^{0.04\%}$-TiO_2	Suspended system Precursor: P25, Evonik Degussa	*tetA*, *tetM*, *sul1*, *blaTEM*, *ampC* *Klebsiella pneumoniae*	Vis/TiO_2 effective: *tetM*, *blaTEM*, not effective: *ampC*, *tetA*, *sul1*, *blaTEM*, Vis/$Mn^{0.1\%}$-TiO_2 Not effective for all ARGs Vis/$Co^{0.1\%}$-TiO_2 effective: *tetM*, *tetA*, *blaTEM*, *sul1*,	Simulated solar irradiation XBO lamp 150 W Simulated: Photon fluence rate: 5.8 × 10^{-7} E/dm^3 s;	n.a.	Matrix: effluent after conventional activated sludge from WWTP spiked with *K. pneumonia* 1.5 × 10^8 CFU/cm^3 [PC] = 250 mg/dm^3	Reactor: -Newport, model 96,000, double-walled Pyrex glass batch reactor stirred, aerated Volume: 300 cm^3 (working vol.), Temp:25 °C (controlled)	(Venieri et al., 2017)

			not effective: *ampC* Vis/(Mn/Co)$^{0.04\%}$-TiO_2 effective: *tetM*, *tetA*, *blaTEM*, not effective: *ampC*, *sul1*	Irradiance: 1.31 × 10^{-3} mW/cm^2				
Vis/Ag-AgBr/$_gC_3N_4$ Vis/$_gC_3N_4$	Suspended System	*tetA*, *tetM*, *tetQ*, intI1 *Escherichia coli K12*	Vis/Ag-AgBr/$_gC_3N_4$//Vis/$_gC_3N_4$ Removal: -*tetA* ~ 49%//~12%, -*tetM* ~ 86%//~0% -*tetQ* ~ 69%//~8% -int1 ~ 86%//~15%	Simulated sunlight xenon arc lamp with a cutoff filter (<420 nm) 500 W Irradiance: 6.14–123.86 mW/cm^2 For ARGs 96.0 mW/cm^2	Up to 120 min	Matrix: secondary effluents from WWTP [PC] = 211.0 mg/dm^3, [PC] = 31.82–368.18 mg/dm^3 (for *E. coli*)	Reactor: CEL-WLAX500, with cold water jacket to temp. Control 23.7 °C,	(Yu et al., 2020),
UVA/TiO_2/O_3	Immobilized system TiO_2-coated glass Raschig rings Precursor: n.a.	*sul1*, *qnrS*, bla_{TEM}, intI1, 16S rRNA *Enterococci*, *Enterobacteria*	Wastewater: Reduction: -All ARGs were below limit of quantitation, 16S rRNA ~4log - intI1 ~ 3log surface water ARGs reduction: bla_{TEM} was below LOQ (*sul1*, *qnrS*, were below the LOD in surface water) 16S rRNA ~5log - intI1 ~ 3.5log	UVA radiation (370 nm) 10 W LEDs UV fluence/ UV intensity n.a.	26 min	Matrix: Effluents from UWWTP and surface water [PC] = NA, [O_3] = 50 g/Nm^3	Reactor: A bubble column reactor equipped with a loop column; operate in continuous mode, with continuous recirculation (60 cm^3/min) between the columns. Loop column holding the photocatalyst.	(Moreira et al., 2016)
Sunlight/TiO_2 Sunlight/TiO_2/H_2O_2 Sunlight/GO-TiO_2 Sunlight/H_2O_2	Suspended system Precursor: P25, Evonik Aeroxide 4.0 wt% of graphene oxide	*qnrS*, bla_{CTX-M}, *sul1*, bla_{TEM} 16S rRNA, intI1,	Sunlight/TiO_2/H_2O_2 and sunlight/H_2O_2 reduced *sul1*, *qnrS*, 16S rRNA, intI1 by 1 log bla_{CTX-M} by 3 log rest treatments led slightly removal	Natural sunlight Plataforma Solar de Almeria (PSA), Spain Fluence/ Irradiance n.a.	180 min	Matrix: Effluents from UWWTP [PC] = 200 mg/dm^3, [H_2O_2] = 20 mg/dm^3	Reactor: The CPC photoreactor tube module (37°) connected to a recirculation tank and a centrifugal pump, the irradiated collector equal 1m^2 Flow rate: 10 dm^3/min, Volume: 20 dm^3 (illuminated volume (15 dm^3))	(Moreira et al., 2018)

Escherichia coli inactivation to make the bacteria more sensitive and weak to H_2O_2 (Rincón and Pulgarin, 2006). The mechanism of inactivation of bac teria during photo Fenton process occurs by two pathways: (i) via light ox idation and (ii) via ROS (mainly H_2O_2 and HO• radicals) (Fig. 5) (Polo López et al., 2019). ROS lead to membrane damage (1). UVC and UVB light, as well as ROS, cause the DNA lesions directly (2). While UVA and vis ible light inactivate CAT and ROS enzymes (3) and indirectly lead to DNA damage (2) (Schauen et al., 2007). Fe^{2+} and H_2O_2 diffuse inside bacteria cells promoting an extra internal photoreaction, especially with naturally occurring iron, resulting in intracellular photo Fenton reaction (4) (Aguas et al., 2017). Additional H_2O_2 disrupts the intracellular steady state concen tration of ROS (3). Intracellular production of ROS and the increase of water temperature is also responsible for bacteria inactivation (Moreira et al., 2018).

Although pH is an important operational parameter, the application of the Fenton based process can be used also in neutral conditions (Ahmed et al., 2020; Giannakis et al., 2018a; O'Dowd and Pillai, 2020; Vilela et al., 2021). The most relevant parameter is the ratio between iron and hy drogen peroxide. It has a crucial influence on efficiency (Cengiz et al., 2010; Fiorentino et al., 2015; Polo López et al., 2019; Rubio et al., 2013; Serna Galvis et al., 2019; Zhang et al., 2016). For the Fenton process, the ARG re moval increased considerably with increasing the $[Fe^{2+}/H_2O_2]$ molar ratio (Zhang et al., 2016). The effectivity of removal was comparable for neutral and acidic environment (pH 3, range from 2.58 to 3.79 log removal, while at pH 7 from 2.26 to 3.35 log removal for ARGs and intI1, 16SrRNA) (Zhang et al., 2016). Furthermore, in manure (pH 8.5), the Fenton process was ef fective in *tetM* removal and dependent on the reagent ratio (Cengiz et al., 2010). The Fenton process gave the best sul1, *tetG*, and intI1 removal (2.63 3.48 log) in comparison to UVC/H_2O_2, chlorination, UVC and UVC/Chlorination (1.55 2.32 log, 1.65 2.28 log, 0.80 1.21 log, 1.12 1.91 log, respectively) (Zhang et al., 2016; Zhuang et al., 2015). How ever, when the chemical requirements are taken into account, Fenton pro cess is not economically viable (0.312 \$/m^3 wastewater) (Zhang et al., 2016; Zhuang et al., 2015), while H_2O_2/UVC despite a worse ARGs removal is not only cheaper (0.296 \$/m^3 wastewater), but also less time consuming (Zhang et al., 2016). To reduce operating costs, the photo Fenton as a disin fection process is applied at under natural and artificial (mostly visible) light. Although the sunlight photo Fenton was not recommended by Moreira and co workers for ARGs removal, due to its low efficiency in the degradation of organic micropollutants, a few papers showed that despite that it can be a promising method (Table 5).

In contrast to TiO_2/H_2O_2/sunlight process, sunlight photoFenton pro cess was efficient in multidrug resistant *Escherichia coli* inactivation (Fiorentino et al., 2015). The complete inactivation was obtained when Fe^{2+}/H_2O_2 ratio was equal to 5:10 under fluence equals 33,300 mJ/cm^2. Much higher TiO_2/H_2O_2 ratios were required to achieve comparable inac tivation (TiO_2/H_2O_2 ratio 50:100 under 13,320 mJ/cm^2) (Fiorentino et al., 2015). Despite the higher energy consumption of the photoFenton process, the operational costs are lower than for sunlight/TiO_2/H_2O_2. It should be noted that at in the studied conditions, none of the processes did affect the antibiotic resistance of survived colonies (Fiorentino et al., 2015). Interesting results were obtained in synthetic wastewater. The sim ulated Vis photo Fenton has the highest efficiency in resistance conferring plasmids (RCPs) removal, while simulated Vis/H_2O_2 and Fenton required twice as much time to achieve the same efficiency (Vilela et al., 2021). That fast inactivation in comparison to the other methods was the result of the higher H_2O_2 consumption in this system (60 70%), which enhanced ROS formation capable of damaging and inactivating cell free (Vilela et al., 2021). The differences in plasmid removal were observed when a real mu nicipal wastewater treatment plant effluent was used. The Fenton process did not reach any removal of RCPs, while H_2O_2 and simulated sunlight/ H_2O_2 showed a relatively low percentage of reduction (Vilela et al., 2021). Again, the simulated Vis photo Fenton process was the most effec tive, but, still after 60 min removal reached only 80% of cell free RCPs. The efficiency was nearly 30% lower in real municipal wastewater than under the same conditions in synthetic wastewater (Vilela et al., 2021). The H_2O_2 consumption in real municipal wastewater was lower (~40%) than in wastewater (~30%), but the differences were not proportional to the removal decrease, justifying that effluent organic matter scavenged through the HO• (Vilela et al., 2021).

Taxon specific markers Enc and *ecfX*, and *ampC*, *sul1*, and *ermB* from membrane bioreactor (MBR) effluent were treated by sunlight photo Fenton (Karaolia et al., 2017). The total DNA concentration was reduced by 97%, however, Enc, *ecfX*, *sul1* and *ermB* were still present after treat ment. Karaolia et al. concluded that the treatment of MBR effluent by the sunlight photo Fenton process may impair total DNA inherence (both eDNA and iDNA) as well as ARGs, therefore another removal approach is recommended (Karaolia et al., 2017).

After the simulated solar photo Fenton process any amplification was observed after 15 min of exposure, proving this method as a sufficient in $bla_{CTX\ M\ 9}$ removing (Giannakis et al., 2018a). Unfortunately, photo Fenton initiated by UVA light was not effective in removing bla_{KPC} gene. Although *K. pneumoniae* was completely inactivated after 120 min of treat ment, the gene was not degraded even after 240 min of photo Fenton action (Serna Galvis et al., 2019). Therefore, to avoid resistance spread, the treat ment longer than 240 min under working conditions (UVA light, $[Fe^{2+}/H_2O_2]_{ratio}$ = 5/50) is required to guarantee the complete bla_{KPC} degradation.

The reagent dosage ratio for ARG (*tetA* and $bla_{TEM\ 1}$) removal was inves tigated as well. It was found, that 10 min of treatment by a simulated Vis photoFenton process initiated by LED lamp at the $[Fe^{2+}/H_2O_2]_{ratio}$ equal ing 1:20 can obtain very satisfactory removal of *tetA* and $bla_{TEM\ 1}$ genes (7.62 log and 8.56 log, respectively) (Ahmed et al., 2020). However, with a lower $[Fe^{2+}/H_2O_2]_{ratio}$ of 1:3, lower removal of *tetA* and $bla_{TEM\ 1}$ was ob tained (4.2 log and 6.5 log, respectively). For both ratios, after 20 min of treatment, the detection limit of eARGs was reached. The atomic force mi croscopy images confirmed that the simulated Vis photoFenton process can damage extracellular DNA, causing direct exposure of the plasmid DNA to the ROS, and finally bacterial cell wall was damaged. Only, 10 min of treat ment at the $[Fe^{2+}/H_2O_2]_{ratio}$ equal to 1:20, was required to damage the

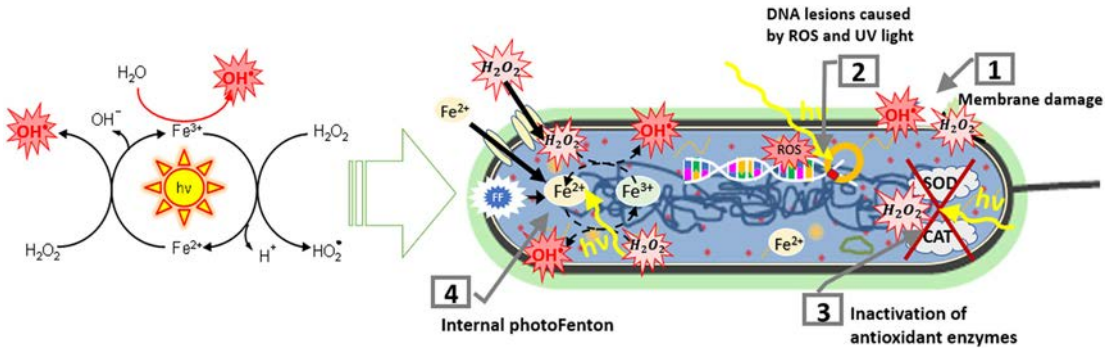

Fig. 5. Bacteria inactivation mechanism during PhotoFenton disinfection (based on Polo-López et al., 2019).

Table 5
Operational condition for photo-Fenton ARGs removal. Removal efficiency for a proper time treatment is provided as log unit removal.

Methods	Molecular target	ARGs removal efficiency	Light source	Operational parameters			Ref.
				Reaction time	Operational conditions	Reactor parameters	
Vis-Photo-Fenton	Plasmids pSB1A2 (resistance to ampicillin) and pSB1K3 (resistance to kanamycin)	Synthetic secondary wastewater t = 30 min sufficient for total inactivation of cell-free RCPs real municipal wastewater *t* = 60 min 80% of cell-free RCPs was removed	Simulated solar light Fluence: 96,480 mJ/cm^2 Irradiance: 26.8 mW/cm^2 (accumulated radiation after 1 h equal to 5.57 kJ/dm^3)	60 min	Matrix: synthetic secondary wastewater (SWW) and municipal wastewater treatment plant effluent (MWWTPE), neutral pH $[Fe^{2+}/H_2O_2]_{ratio}$ = 30:50 $[H_2O_2]$ = 50 mg/dm^3, Note: intermittent iron addition strategy was used as follows: Fe^{2+} additions at times 0, 5, 10 and 15 min was 15 mg/dm^3, 5 mg/dm^3, 5 mg/dm^3 and 5 mg/dm^3 respectively.	Reactor: glass reactor placed inside a bench-scale solar simulator chamber (SUNTEST CPS +, ATLAS). Volume: 400 cm^3	(Vilela et al., 2021)
Sunlight Photo-Fenton	*ermB*, *sul1*, *mecA*, *ampC*, Enc, ecfX	t = 120 min All ARGs $\log_{10}$ abundance increase Enc and ecfx achieved twice highest abundance *t* = 180 min abundance CE/100 ng DNA ermB = 1.53 log sul1 = 1.56 log	Natural solar irradiation Fluence/ Irradiance n.a.	Up to 180 min	Matrix: MBR effluent pH = 2.8 (adjusted with 1 M H_2SO_4) $[Fe^{2+}/H_2O_2]_{ratiomass}$ = 1:10 $[H_2O_2]$ = 50 mg/dm^3	Reactor: compound parabolic collector (CPC) pilot plant fixed platform (angle (35°)), consists of 6 mounted glass tubes (55 mm × 1.5 m), Flow rate: 150 dm^3/h, Volume: 60 dm^3. (irradiated tube volume 21.4 dm^3),	(Karaolia et al., 2017)
Vis-Photo-Fenton	$bla_{CTX\ M\ 9}$ present in *E. coli* ESBL 8543	Vis- sunlight PhotoFenton any amplification was observed after 15 min vis light after 300 min $bla_{CTX\ M\ 9}$ still was observed	Xe 150 W lamp Fluence: Up to 405,000 mJ/cm^2 Irradiance: 75 mW/cm^2.	Up to 90 min	Matrix: simulated wastewater; -ultrapure water spiked by bacterial concentration 10^6 CFU/cm^3, pH = 6.5 (without adjustment, after processes 6.1–6.2) $[Fe^{2+}/H_2O_2]_{ratio}$ = 1:10 $[H_2O_2]$ = 10 mg/dm^3	Reactor: Suntest apparatus (Heraeus, Hanau, Germany) occupied with XBO lamp. Pyrex Erlenmeyer flasks with screw cap were used as reactors Volume: 40 cm^3	(Giannakis et al., 2018a)
UVA-Photo-Fenton	*bla-KPC* *K. pneumoniae*	Not effective after 240 min, *bla-KPC* was still present after the treatment despite decreasing trend	UVA lamp main emission at 365 nm 5 lamps (20 W each) UV Fluence: up to 5616 mJ/cm^2 UV Irradiance: 0.39 mW/cm^2	Up to 240 min	Matrix: distilled water spiked with *K. pneumoniae* 10^6 CFU/cm^3, hospital wastewater pH = 6.5 $[Fe^{2+}/H_2O_2]_{ratio}$ = 5:50 $[H_2O_2]$ = 50 mg/dm^3,	Reactor: homemade aluminum reflective reactor occupied in magnetic stiller in which beakers were used as a reactor. Volume:150 cm^3	(Serna-Galvis et al., 2019)
Vis-Photo-Fenton	*tetA*, $bla_{TEM\ 1}$	*t* = 10 min $[H_2O_2]$ = 340.2 mg/dm^3 Reduction of ARGs for Long/short amplification qPCR -*tetA* 7.62 log/6.75 log -$bla_{TEM\ 1}$ 8.56 log/7.49 log $[H_2O_2]$ = 18.7 mg/dm^3 Reduction of ARGs for Long amplification qPCR -*tetA* 4.2 log -$bla_{TEM\ 1}$ 6.5 log *T* = 20 min All ARGs below LOD	96 visible LED lamps with a peak emission at 425–525 nm Fluence: 240 mJ/cm^2 Irradiance: 0.2 mW/cm^2	20 min	Matrix: PBS solution containing ~10^6 CFU/cm^3. *E. coli* DH5a (pH 6.85–6.96) $[Fe^{2+}/H_2O_2]_{ratio}$ = 1: 18.7; 2.8: 340.2 $[H_2O_2]$ = 18.7 mg/dm^3; 340.2 mg/dm^3	Reactor: cell culture flash, lamps were employed from the bottom and up the side of the reactor. Half of the lamps were fixed 5 cm away from the reactor and the rest lamps were fixed over the magnetic stirrer speed (250 rpm). Temp. = 22 ± 2 °C) Volume: 250 cm^3	(Ahmed et al., 2020)

naked DNA compared to the contact time for ARB inactivation (30 min) (Ahmed et al., 2020). Despite good results for eARGs Vis photo Fenton pro cess showed limited potential to destruct iARGs (Ahmed et al., 2020).

As it was mentioned above, based on the worst results obtained by sunlight photo Fenton at circumneutral pH for antibiotic degradation (20 mg H_2O_2/dm^3, 10 mg Fe^{2+}/dm^3), Moreira and co workers did not en dorse this method for ARG removal (Moreira et al., 2018). However, de spite any improvement for antibiotics photodegradation compared to direct photolysis, sunlight photo Fenton process was the most efficient treatment for reduction of resistant and non resistant faecal coliforms and enterococci (Moreira et al., 2018). The low oxidizing efficiency could be re lated due to the circumneutral pH (5.5), while it is known to be maximized at pH values around 3. Besides, sunlight photo Fenton showed similar dis infection profiles to sunlight/H_2O_2 for faecal coliforms. It is worth to notice that almost for all sunlight driven AOPs in combination with H_2O_2 (photo Fenton, sunlight/H_2O_2, sunlight/GO TiO_2/H_2O_2), the inactivation of bacte ria was achieved for an accumulated energy of 12 kJ/dm^3 (Moreira et al., 2018). Only TiO_2/H_2O_2 required 28 kJ/dm^3 (Moreira et al., 2018). That finding suggests that H_2O_2 plays a crucial role in disinfection, regardless of low effectiveness for the removal of organic micropollutants.

The application of sunlight photo Fenton as a SODIS method seems to be promising since bacteria can be eliminated and at the same time natural organic matter loads can be reduced (Ozores Diez et al., 2020). However, its action is mostly related with the action of H_2O_2, rather than hydroxyl radicals.

In the final analysis, the ARGs inactivation is a result of photochemical reactions initiated by the absorption of UV light by components of cells, that ultimately lead to DNA damage. UV disinfection results in damage to nucleic acids, because nucleotides absorb UV light in the range from 200 to 300 nm with a peak absorption at 265 nm (Bolton and Cotton, 2008; Hockberger, 2002; Jungfer et al., 2007). The photons absorbed by DNA cause several types of damage resulting in cell inactivation by interfering with transcription and replication (Goosen and Moolenaar, 2008; Mullenders, 2018; Pullerits et al., 2020). The UVC and UVB radiation trig ger the formation of mutagenic DNA lesions like photodimers at a di pyrimidine site (cyclobutane type pyrimidine dimers) and pyrimidine 6,4 pyrimidone photoproducts (Mullenders, 2018; Pullerits et al., 2020). Under disinfection, where ROS is produced, the balance of ROS is dis turbed. In the equilibrium state, the enzymes superoxide dismutase (SOD) and catalase (CAT) prevent the accumulation of $O_2^{\bullet -}$ and H_2O_2. Photocat alytic and photo Fenton processes, where UVC/UVA light, as well as solar light, were applied, have a detrimental effect on these enzymes, causing its loss of enzymatic activity, and finally $O_2^{\bullet -}$ and H_2O_2 will be accumu lated (Castro Alférez et al., 2016; Marugán et al., 2020; Mullenders, 2018). ROS are major causes for activating lipid peroxidation that alters the fluidity and permeability but affects the integrity of biological mem branes (by reacting with proteins and lipids) (Castro Alférez et al., 2016; Polo López et al., 2019). ROS attack DNA generating a variety of DNA le sions, such as strand breaks, oxidized bases, base release mutations, and other genetic alterations (Castro Alférez et al., 2016; Cooke et al., 2003; Helena et al., 2018; Polo López et al., 2019). To conclude, there are several light based AOPs applied for ARG removal. Nevertheless, it is still difficult to predict the inactivation levels of various kinds of ARGs during disinfec tion. Mostly all from the light driven AOPs have been reported as an effi cient disinfection method concerning spread of antibiotic resistance in the natural environment. Bearing in mind that UV light and simulated sunlight promote the spread of ARGs mediated by conjugative transfer, while visible light does not influence on it, photocatalytic as well as PhotoFenton pro cesses seems to be the most promising technologies.

4. Regrowth after photo-based disinfection

The impact of all light based disinfection technologies has to be consid ered not only for its direct application but also from its influence on micro bial dynamics within the water distribution system (Pullerits et al., 2020). Bacterial regrowth may be a problem when treated water is consumed or when treated wastewater is reused (Fiorentino et al., 2015). Moreover, the bacterial regrowth leads to the spread of antibiotic resistance genes (Sousa et al., 2017). The regrowth may occur only when bacteria can repro duce (Wang et al., 2021). To avoid regrowth, the disinfection process has to cause the loss of reproducibility by photo and/or ROS induced DNA dam age or entry into a viable but non culturable (VBNC) state (Wang et al., 2021).

Bacteria exhibit a maximum UVC absorption at approx. 260 nm, which matches the absorbance of the nucleic acid, therefore the highest lethal ef fect toward microorganisms at wavelengths near to 260 nm is observed (Bolton and Cotton, 2008; Gallagher, 2001; Hockberger, 2002; Jungfer et al., 2007). The effect of solar light (long UVA and short VIS (350 490 nm)) on *E. coli* indicates that radiation of 265 nm directly dam ages nucleic acids, while short VIS causes the production of toxic com pounds that destroy other cell components (Hollaender, 1943). It can be conclude that UVC and UVB light cause the DNA lesions directly, while UVA and visible light indirectly via CAT and ROS enzymes inactivation and indirectly lead to DNA damage (Schauen et al., 2007). When the cell is damaged by UV or sunlight, several different mechanisms can be in volved in the repairment. The first important repair mechanism is photore activation, often called 'light repair'. It takes place during the exposure to near UV and visible light (300 500 nm), which consists of direct reversal of the damage by photolyase (Goosen and Moolenaar, 2008; Mullenders, 2018). The second mechanism of excision repair pathways is referred as "dark repair" and includes: (i) removal of the damaged base by a DNA glycosylase (base excision repair, BER); (ii) incision of the DNA adjacent to the damage by an endonuclease (UV damage endonuclease, UVDE) and (iii) removal of a complete oligonucleotide containing the damage (nu cleotide excision repair, NER) (Goosen and Moolenaar, 2008; Jungfer et al., 2007; Sinha and Häder, 2002). Base excision repair (BER) recognizes and repairs base modifications, as well as abasic sites and DNA single strand breaks (Helena et al., 2018; Goosen and Moolenaar, 2008; Jungfer et al., 2007; Sinha and Häder, 2002). The repair mechanism depends on the types of DNA lesions caused by a variety of DNA damaging agents (UV light, visible light, or ROS) (Fig. S4). VBNC is one of the nongrowth states (like sporulation, persistence, and dormancy) but with a particular survival strategy that permits endurance to unfavorable environmental conditions (Wang et al., 2021; Yang et al., 2020). Unfortunately, if the disinfection pro cess is not enough efficient, the reactivation from the VBNC state occurs. It results in the recovering of bacteria ability to reproduce and enlarge their population (Wang et al., 2021). To mimic the condition in drinking water distribution systems, post disinfection bacterial regrowth tests are mostly conducted in darkness by storing treated water samples for 1 3 days. How ever, the ability to regrowth is also relevant when wastewater reclamation is taken into consideration.

4.1. Regrowth after UVC based treatment

Interestingly, tetracycline resistant *Bacillus* bacteria were able to repair within 24 h after UVC_{LED} irradiation, mainly via photoreactivation (Shen et al., 2020). When two different wavelengths were used for inactivation, the lower regrowth was observed for 268 nm than for 275 nm (Shen et al., 2020). This was surprising because in case of non resistant bacteria reverse tendency was observed (Nyangaresi et al., 2019).

Although the UVC_{254nm} and ozonation treatment lead to a reduction in ARG abundances, after 3 days of storage 16S rRNA and bla_{TEM}, reached levels close to the pre treatment values (Sousa et al., 2017). The results in dicated that neither UVC nor O_3 can prevent ARGs spread because both pro cesses left viable cells capable to recover and grow after the stress relief (Sousa et al., 2017). The integrase genes of a class of integron (*intI1*) and *sul1* gene were also detected after storage, but slightly lower reactivation was observed after ozonation. The gene *qnrS* was fully inactivated after ozonation, while after UVC treatment and storage, it was able to reactivate. It was noticed that UV → chlorination was quite effective to control the re growth of opportunistic pathogens. However, at the same time, it was noted that the microorganisms creating biofilms, inhabiting corrosion products

and loose deposits, were more tolerant to treatment with UV → chlorination (Liu et al., 2019). When UV disinfection (8 mJ/cm^2) was enhanced by 2 mg Cl_2/dm^3 the recovery of bacteria was not observed. Potentially, as suggested before (Dodd, 2012; Ersoy et al., 2019), chlorine together with formed free radicals cause perforation of bacterial cell walls that cannot be mitigated by the typical repairment processes. An increase of bacterial inactivation of 1.4 log unit was observed with UV/chlorination compared to UV alone (Wang et al., 2020).

When *Klebsiella pneumoniae* was exposed to 15 s of UVC, and UVC/H_2O_2 process, the survivor bacteria exhibited higher susceptibility for antibiotic treatment, compared to the situation when they were treated with UVC/H_2O_2 (Serna Galvis et al., 2020). Potentially, an attack of radical species on cells may increase antibiotic permeation. The complete inactivation of bacteria, with no subsequent regrowth after 24 h was achieved only after 60 s of water treatment using UVC, UVC/H_2O_2 (1 mM of H_2O_2) and UVC/PS (Serna Galvis et al., 2020). UVC/H_2O_2 (50 mg H_2O_2/dm^3, 730,000 mJ/cm^2) treatment was more efficient than a photo Fenton like process (UVC/H_2O_2/Cu IDS, additional 6.2 mg Cu IDS/dm^3) ARGs (*tetA*, *qnrS*, *sul2*) reduction, but only to limited types of putative pathogenic bacteria (*Aeromonas* spp., *Escherichia/Shigella* spp.) (Di Cesare et al., 2020). Compared to untreated wastewater, a significant abundance decrease was noted after UVC/H_2O_2 process concerning *Bacteroides* spp., *Morganella* spp., *Proteus* spp. and *Streptococcus* spp. The measurement of intact and dead cells clearly showed that these AOPs are not invasive enough to achieve a permanent disinfection effect, and regrowth of bacteria was observed (Di Cesare et al., 2020). However, after UVC/H_2O_2 process *Escherichia coli* and *Pseudomonas aeruginosa* were inactivated after 8 min (1248 mJ/cm^2) and after 3 min (480 mJ/cm^2), in wastewater and surface water, respectively, and no regrowth was observed after 48 h (Michael et al., 2020).

4.2. Regrowth after UVA or visible light based treatment

No bacterial regrowth after 3 days storage was observed after treatment of surface water and wastewater with photocatalytic ozonation (initiated by UVA_{LED}) (Moreira et al., 2016). Thus, the two kinds of possible DNA repair mechanisms were investigated (photoreactivation as well as "dark repair"). For all investigated ARGs no regrowth after 72 h of light or dark storage was proved (Moreira et al., 2016). Nevertheless, both culture dependent and culture independent methods showed that a part of the surface water and wastewater microbiota was viable with the ability to regrow (Moreira et al., 2016).

It should be noted, the combined effect of UVC, UVB, UVA and visible light is expected under natural sunlight condition. Even if just visible light (VIS) is used for disinfection (mostly XBO lamps in solar simulators), UVC and UVB are cut off but UVA and visible light are responsible for bacteria inactivation. It was found, that in the case of 16S rRNA, *intI1* as well as ARGs, some of the solar treatments were insufficient to prevent regrowth. After 3 days of storage, the 16S rRNA abundance was close or even higher (up to 1 log for sunlight/TiO_2/H_2O_2) than before treatment (Moreira et al., 2018). *intI1* and *sul1* were also detected after the storage suggesting the ability of some bacteria to recover after the sunlight/TiO_2/H_2O_2 treatment. The reactivation of bla_{TEM}, *sul1* and *qnrS* was detected after sunlight/TiO_2 (abundances above the pre treatment levels) (Moreira et al., 2018). However, sunlight/GO TiO_2 was able to prevent the reactivation of $bla_{CTX\ M}$, bla_{TEM} and *qnrS* genes above the pre treatment levels, while for the *qnrS* gene also sunlight/TiO_2/H_2O_2 prevented its reactivation (Moreira et al., 2018). The reactivation and regrowth experiments suggest that at least part of the ARG removal was more connected to cell inactivation than to ARG loss itself. That implies that at least some of the bacterial cells survived these several sunlight treatments and saved their ability to reproduce during storage (Moreira et al., 2018).

After 24 h of dark storage of effluents treated by 180 min of photocatalytic disinfection, no regrow of *Escherichia coli* was observed (Karaolia et al., 2018). However, when the process was conducted for a shorter time the regrowth was observed (despite physical cell damage and reduction in colony counts) (Karaolia et al., 2018). The higher *Escherichia coli* reactivation was found for Vis/TiO_2 and Vis/TiO_2 rGO PH, indicating the growth on solid media (Karaolia et al., 2018). While after Vis/TiO_2 rGO HD treatment, no regrowth during the first 60 min of dark treatment compared to the other two photocatalysts was achieved, suggesting that the physical contact and bacterial surface damage incurred by TiO_2 rGO HD may produce a more severe and permanent inactivation effect on bacteria (Karaolia et al., 2018).

The photo Fenton process favors ARG removal. But considering operational condition, the high light intensity with broad spectrum but with lower H_2O_2 dose (75 mW/cm^2, 10 mg H_2O_2/dm^3, 1 mg Fe^{2+}/dm^3) required at least 90 min to completely inactivate ARB (Giannakis et al., 2018a). The treatment based on VIS photo Fenton (425 525 nm) with much lower light intensity but with higher H_2O_2 concentration (0.2 mW/cm^2, 170 mg H_2O_2/dm^3, 1 mg Fe^{2+}/dm^3) required 30 min for complete inactivation of ARB (Ahmed et al., 2020). Both photo Fenton experiments did not cause any bacterial regrowth. Ahmed et al. (2020) demonstrated that after 30 min of photo Fenton treatment, no regrowth was observed neither for 24 h, 48 h, nor 7 days, indicating a complete inactivation of ARB. The study of Giannakis et al. showed no regrow, even for the disinfection time of 15 min. Interestingly, at the same time, after such short disinfection, still not all ARBs were inactivated. Furthermore, the effect of residual H_2O_2 was confirmed, leading to decrease in bacteria abounded even in dark condition (Giannakis et al., 2018a).

The light exposure time, is important when only DNA damages caused by light are considered. Not enough time result in the inactivation process induced limited cell damage. Longer exposure times cause the irreversible DNA damages ultimately preventing the bacteria multiplication (Valero et al., 2017). To investigate the influence of ROS on DNA damage repair, the simulated sunlight was compared with simulated solar photoFenton (10 mg H_2O_2/dm^3, 1 mg Fe^{2+}/dm^3) at the same light condition (260 700 nm, 75 mW/cm^2). When just sunlight was applied for disinfection, the ability to regrow after 48 h of dark storage was observed for light exposure time below 120 min, revealing the photo Fenton advantage (Giannakis et al., 2018a).

When visible light disinfection is considered, DNA damage is mostly indirect via ROS, less by UVA or visible light itself. During photocatalytic disinfection or photo Fenton, the fundamental question is: Are the ROS generated at sufficient level? It is necessary to overcome the defense systems of the microorganisms for both achieving complete inactivation and avoiding bacterial recovery or regrowth. Due to "residual disinfecting effect" which is a remarkable advantage of photocatalytic disinfection or photo Fenton process, those light based AOPs seem to give many possibilities (Rincón and Pulgarin, 2004; Xiong and Hu, 2013). As it was mentioned above, H_2O_2 has a questionable privilege and prevalence in disinfection, therefore its extra generation during photocatalytic processes causes an additional residual effect. Moreover, further ROS generation from H_2O_2 inhibits bacteria reproduction, acting as an "after illumination residual disinfectant" (Xiong and Hu, 2013). When PhotoFenton is performed, the light intensity as well as H_2O_2 concentration are a key factor (at low concentrations it acts by causing DNA damage and at high concentrations, by oxidizing other molecules in the cell).

4.3. Prevention of bacterial regrowth after light based treatment

In general, the topic of bacterial regrowth and possible increase of ARG abundance after treatment is rarely investigated than removal of ARB and ARGs. However, its importance is undeniable. An increase of ARG abundance in water without bacterial regrowth after a certain period following the treatment clearly indicates presence of other bacteria (not covered by this study). These bacteria might be either cultivable or non cultivable, and be the consequence of incomplete bacterial inactivation by disinfection, or activation of the repair systems in the bacterial cells damaged during disinfection. However, as it is discussed above, the light based tertiary treatment could prevent the spread of antibiotic resistance genes when a proper treatment is applied. The best regrowth prevention seems to be photocatalytic ozonation, sunlight/TiO_2/H_2O_2 or photo Fenton. As it was

shown, the light source does not have much influence on the regrowth. Nevertheless, the lack of a residual effect is a major disadvantage of UV or sunlight/solar disinfection compared to other light based disinfection processes where additional oxidants such as O_3, H_2O_2 are supplemented. It is well known, that during ozonation in an acidic environment direct oxidation with molecular ozone is favored, whereas in alkaline environments, the HO• pathway is promoted (Gmurek et al., 2019). An improvement of the disinfection process in the presence of additional oxidants can be explained by the nucleobases' reactivity with molecular ozone and hydroxyl radicals. The bimolecular rate constants with ozone in neutral solution show that the most reactive site in the DNA molecule are thymine and guanine bases (Table S2) (Nompex et al., 1991; Theruvathu et al., 2001). Cytosine is one order of magnitude less reactive. Considering second order rate constants and purine/pyrimidine base pair, the higher reactivity toward O_3 has thymine/adenine pair (6.8×10^3 dm^3/mol s and 2.2×10^3 dm^3/mol s, respectively) than guanine/cytosine pair (3.5×10^3 dm^3/mol s and 7.6×10^2 dm^3/mol s, respectively) (Nompex et al., 1991). Alexander and coworkers hypothesized that higher stability of DNA with a high GC content toward ozone is related to higher number of hydrogen bonds between the nucleobases (two and three hydrogen bonds per pair for AT and GC, respectively) (Alexander et al., 2016). What is more the bimolecular rate constants with HO• demonstrated that these DNA degradation pathways strongly depend on cytosine and adenine (Table S2) because these two nucleotide bases are most susceptible to HO• oxidation (Buxton et al., 1988; Joseph and Aravindakumar, 2000). The values of second order rate constants concerning the reaction between the nucleobases and sunlight generated HO• radicals suggest their lower reactivity toward guanosine and thymine (2.3×10^9 dm^3/mol s and 3.2×10^9 dm^3/mol s) compared to HO• generated during pulse radiolysis (7.8×10^9 dm^3/mol s and 4.6×10^9 dm^3/mol s, respectively) (Joseph and Aravindakumar, 2000).

In presence of compounds serving as an additional electron acceptor, such as H_2O_2 or O_3 the observed enhanced efficiency is due to accelerated ROS generation ($^1O_2$, $O_2^{\bullet -}$, and •OH). Additionally, H_2O_2 retards the electron hole recombination. It is well known that the differences between photocatalytic ozonation and photocatalysis (in oxygen environment) are related to the different electron affinity of ozone compared to oxygen. Lower reactivity can be observed due slower electron transfer from photocatalyst to oxygen than to ozone. To generate HO• one electron must be trapped by ozone while three electrons are necessary when oxygen acts as the electron acceptor. It is also estimated that 80% of the detected lesion following UVA mediated disinfection of cellular DNA is affected by $^1O_2$ (Ravanat et al., 2004). Due to the lowest reduction potential among the four DNA bases, guanine nucleoside is the most susceptible target attacked by $^1O_2$ at neutral pH (Lu et al., 2018; Ravanat et al., 2004). The bimolecular rate constant of $^1O_2$ chemical reaction with guanine is four order higher than with adenine (1.7×10^7 dm^3/mol s and 8×10^3 dm^3/mol s, respectively) (Petroselli et al., 2008). Therefore, predominant pathway of the $^1O_2$ deactivation by DNA is via reaction with guanine.

As can be seen ozone rate constants are much lower, than those for HO• and even $^1O_2$. However, during the applied treatment the ozone dose was in excess. It can be suspected that during treatment with ozone not only a higher HO• concentration but also $^1O_2$ is generated, which are responsible for higher degradation of GC rich DNA. Despite differences in magnitude between the second order rate constants (Table S2), ozone plays a significant role in DNA degeneration causing a strong action of ozone on AT rich DNA. Therefore, the combined action of ROS prevents bacterial regrowth and leads to inhibit ARG spread.

5. DBPs and DOM influence on ARGs removal

When conventional UV treatment is considered, the DBPs are formed below 400 mJ/cm^2 does (Malley et al., 1996). While, when subsequent chlorination processes are applied, the UV disinfection plays a significant role in DBPs formation. UV is capable of transforming regulated DBP precursors but at doses significantly higher than those required for primary disinfection. Unfortunately, these conditions are necessary for complete removal of ARGs. Hence, despite limiting the risk coming from the presence of ARGs, we create a new thread that is formation of DBPs. Especially when recently AMR spread have been linked to DBPs and residual disinfectants (Amarasiri et al., 2020; Li and Gu, 2019; Mantilla Calderon et al., 2019).

It was confirmed that the increase of individual and multiple antibiotic resistance was caused by mutagenic DBPs activity at above minimal inhibitory concentration (MIC) levels (antibiotic concentration that inhibits 90% of growth) (Lv et al., 2014, 2015). Antibiotic resistance is also influenced by oxidative stress, stronger stress response promotes HGT of ARGs (Chen et al., 2019; Rodríguez Rosado et al., 2018). DBPs have been reported to activate the oxidative stress response system at both sub MIC and high near MIC concentrations, that favored ARGs spread (D. Li et al., 2016). The conjugation rate increased among diverse lineages of bacteria inducted by DBPs and residual disinfectants (Mantilla Calderon et al., 2019). It is known that, HGT of ARGs can be decreased above MIC by repressing the conjugative transfer through inactivating donor and receipt bacteria (Guo et al., 2015; Li and Gu, 2019; Zhang et al., 2017). Moreover, strong activation of DNA repair mechanisms (BER, NER that are responsible for repair DNA lesions caused by ROS and UV light) were also widely observed for various DBPs at multiple concentrations (Lan et al., 2018). The acceleration of DNA repair by the presence of DBPs may contribute in the spread of ARGs. Dissolved organic matter (DOM) acts as an important precursor of DBPs and enables biological regrowth in water distribution systems. While DBPs have a negative influence on ARG removal, DOM was found to be an important driving factor in ARG removal (Chen et al., 2015; Feng et al., 2021; Riquelme Breazeal et al., 2013; X. Zhang et al., 2019; Y. Zhang et al., 2020). Breazeal et al., found that the protein, polysaccharide, and total organic carbon colloidal fraction originated from wastewater effluent resulted in enhanced removal of *vanA* and bla_{TEM} removal (Riquelme Breazeal et al., 2013). Chen et al. also reported that complexation of tetracycline and DOM and inhibition of tetracycline diffusion by adsorption of DOM on bacterial cells caused diminishment of tetracycline bioavailability in *E. coli* and reduction of *tetM* and *tetR* expression (Chen et al., 2015). The Suwannee River DOM photoactivation by medium pressure UV lamp (290 400 nm) caused acceleration in eARG removal (X. Zhang et al., 2019). DOM was photosensitized to an excited triplet form (^{3}DOM*) that leads to ROS ($^1O_2$ and HO•) generation. It was found that $^1O_2$ enhances guanine oxidation, HO• promotes plasmid strand breaks, while ^{3}DOM* did not play a role in *tetA* and $bla_{TEM\ 1}$ removal (X. Zhang et al., 2019). However, Suwannee River DOM under simulated sunlight did not cause a reduction in iARG as well as eARG (maintained at the same level under dark conditions) even though heavy membrane damage and inactivation of *E. coli* was observed (Y. Zhang et al., 2020). It was concluded that, the severe damage of the *E. coli* cell membrane inducted by the action of DOM and simulated sunlight inhibited the expression of *tetA* by damaging the efflux system (Y. Zhang et al., 2020). Feng applied fluorescence excitation emission matrix (EEM) spectroscopy to determine the DOM dynamics, investigating the relation between ARG elimination and DOM removal (Feng et al., 2021). DOM removal showed an obvious correlation with the elimination of ARGs (*tetA*, *tetO*, *tetM*), only *tetW* was not influenced by humic like components (Feng et al., 2021). However, it was demonstrated that DOM with high molecular weight was not beneficial for ARG elimination (Feng et al., 2021).

DOM plays crucial role in deciding the fate of contaminants and ARGs. However, effluent organic matter (EfOM) is responsible for the majority of the coagulant and disinfectant demand, membrane fouling, contributes to corrosion, acts as a substrate for bacterial growth in distribution systems and can interfere with the removal of other contaminants. Furthermore, the toxicity of newly formed trace organic transformation products (TrTPs) during an AOP is critical to its implementation. In addition, the efficient removal of EfOM by methods based on photocatalysis and photo Fenton can result in secondary benefits. It is well known that during the oxidation processes, EfOM is modified and broken down into smaller compounds, which affect the characteristics of the treated effluent. Still, we need to remember, that formed DBPs induce oxidative stress, DNA

damage, and activate DNA repair system at environmental concentration. Therefore, when secondary effluent needs to be disinfected, the above discussed issues need to be considered.

6. Remarks for the future

The WHO named the antimicrobial resistance development one of biggest challenge for the global public health due to the increasing numbers of critical infection rates together with limited therapeutic possibilities (WHO, 2020). In that concern, worldwide programs and strategies aiming on the resistance evolution are existing as well as the political declaration of the United Nation according to antimicrobial resistance is enacted. Especially this EU action plan emphasizes the role of the environment and a gap of knowledge regarding the entry and dissemination of resistances to the aquatic environment via municipal wastewaters and the subsequent consequences. Hence, the identification of potentials risks with a subsequent risk assessment via detection of relevant bacterial species, resistance genes, and chemical analytical estimation of antibiotic residues is a fundamental requirement.

Unfortunately, despite the assumption that disinfection methods should potentially help in limiting the spread of AMR, numerous studies showed that those currently in use are not effective. Furthermore, even when disinfection is effective toward the inactivation of living ARB, the possibility of transferring intact DNA containing ARGs via natural transformation or transduction remains. The removal of ARGs in municipal wastewater effluents requires a new approach, new methods. Therefore, the efficiency of disinfection should be expressed via the destruction of bacterial DNA rather than via the vitality of bacteria.

Regarding application of sulfate radicals based photo AOPs for ARG removal, it can be concluded that this method is promising with respect to regrowth. Nevertheless, extension of the research on new ARG targets is recommended. Second option seems to be photocatalytic disinfection. However, its mechanism of ARG removal is tricky. If immobilized photocatalysts are used, the photocatalyst penetration inside the cell does not occur, which leads to lower DNA damage. It is also likely that this lack of photocatalysts penetration hinders the removal of more challenging ARGs.

Considering that upgrading wastewater treatment plants with advanced oxidation technologies has already been recommended, its application for removal of ARB and ARGs seems to be the next logical step. To gain these goals some crucial actions have to be considered:

- The research should focus on challenges related to controlling the effectivity of ARG removal, instead of simultaneous removal with ARB and micropollutants. Establishing the best operational condition concerning ARG removal could overcome the insufficient dosage problem and may lead to higher safety of effluent treatment.
- The photocatalytic processes led to the highest ARG removal. But as can be concluded based on the presented results, neither UVC, UVA nor visible light photocatalysis is able to completely remove ARGs. Therefore, it is reasonable to enhance the efficiency by using electron acceptors such as H_2O_2 or O_3. That increases its photocatalytic activity by accelerating reactive oxygen species generation ($^1O_2$, $O_2{\bullet}^-$, and •OH) and in the case of the H_2O_2 retards the electron hole recombination. The use of an additional oxidants in combination with an immobilized photocatalyst may lead to increase the reaction rate and prevent regrowth. Effective ARG removal could be achieved after application of visible light induced recyclable heterogeneous photocatalysis (reusable catalyst elimination of catalyst separation problem) combined with O_3 or/and H_2O_2, solar photocatalytic ozonation and photo Fenton, which allow to propose the most efficient and cost effective tertiary appropriate treatment for the treatment of secondary effluents.
- An efficient removal of particulate matter together with associated ARB with ARGs from wastewater effluent by separation techniques seems to be promising in decreasing the spread of ARGs. The association of DNA with particles can gain an advantage over the molecular properties of DNA leading to much higher DNA removal via the membrane is expected (Riquelme Breazeal et al., 2013).
- It should be considered, that additional purification should be applied. As it was shown, after secondary treatment (activated sludge systems/membrane biological reactors) tertiary treatment has to be employed. Due to high effectivity of membrane technology toward ARGs (Hembach et al., 2019; Krzeminski et al., 2020; Lan et al., 2019; Liang et al., 2021; Lu et al., 2020; Niestroj Pahl et al., 2020; Riquelme Breazeal et al., 2013; Schwermer et al., 2018; Slipko et al., 2019; Sun et al., 2019), the combined action should be considered. From ARB and ARGs point of view, the hybrid technology based on AOPs and membrane could be an interesting option. Coupling AOPs allows to reduce the time required for micropollutants complete elimination or post treatment repairing oxidative damage and subsequent recovery of the bacterial species. Furthermore, investigation of the combination of an AOP with membrane pretreatment increased the efficiency in resistance removal.

CRediT authorship contribution statement

M. Gmurek: Conceptualization, Visualization, Writing original draft, Writing review & editing. **E. Borowska:** Writing original draft. **T. Schwartz:** Supervision, Writing review & editing. **H. Horn:** Supervision, Writing review & editing.

Declaration of competing interest

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

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