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Review

Effect of ozonation-based disinfection methods on the removal of antibiotic resistant bacteria and resistance genes (ARB/ARGs) in water and wastewater treatment: a systematic review

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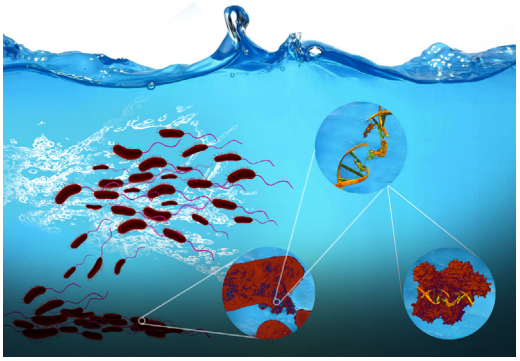
HIGHLIGHTS

- The ozone-based strategies for removing ARB & ARGs are classified and discussed.
- Mechanism of action and repairment phenomenon are informatively presented.
- The operational parameters of ozonation are characterized in detail.
- The main perspectives for future plans are provided.

GRAPHICAL ABSTRACT



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ABSTRACT

Antibiotic resistance is considered a universal health threat of the 21st century which its distribution and even development are mainly mediated by water-based media. Disinfection processes with the conventional methods are still the most promising options to combat such crises in aqueous matrices especially wastewater. Knowing that the extent of effectiveness and quality of disinfection is of great importance, this paper aimed to systematically review and discuss ozonation (as one of the main disinfectants with large scale application) effect on removing antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) from aqueous solutions, for which no study has been reported. For this, a comprehensive literature survey was performed within the international databases using appropriate keywords which yielded several studies involving different aspects and the effectiveness extent of ozonation on ARB & ARGs. The results showed that no definite conclusion could be drawn about the superiority of ozone alone or in a hybrid form. Mechanism of action was carefully evaluated and discussed although it is still poorly understood. Evaluation of the studies from denaturation and repairment perspectives showed that regrowth cannot be avoided after ozonation, especially for some ARB & ARGs variants. In addition, the comparison of the effectiveness on ARB & ARGs showed that ozonation is more effective for

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Oxidation
Antibiotic resistance

resistant bacteria than their respective genes. The degradation efficiency was found to be mainly influenced by operational parameters of *CT* (i.e. ozone dose & contact time), solids, alkalinity, pH, and type of pathogens and genes. Moreover, the correlation between ARB & ARGs removal and stressors (such as antibiotic residuals, heavy metals, aromatic matters, microcystins, opportunistic pathogens, etc.) has been reviewed to give the optimal references for further in-depth studies. The future perspectives have also been reported.

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1. Introduction

Antibiotics as a group of powerful medicines in the therapy of infectious diseases have been widely used worldwide for both humans and animals. However, most of these compounds (between 30 and 90% for different types) are excreted as pristine forms or metabolites in urine and feces, which in turn, leads to their continuous and intensive release into wastewater treatment plants (WWTPs) (Foroughi et al., 2017; Liu et al., 2019). WWTPs, which are not designed for the effective elimination of antibiotics, provide the ideal conditions for MOs to develop and/or acquire antibiotic resistance, owning to three pivotal items i.e. a substantial number of microorganisms (MOs); antibiotic residuals; and enough contact time (Karkman et al., 2018; Makowska et al., 2016). Therefore, these facilities not only are not able to remove antibiotics and the resistance species, but they also act as hotspots and sink for the propagation of ARB and ARGs in the environment (Kampouris et al., 2021; Xia et al., 2019). On the other hand, the released antibiotic residuals can further promote the development and dissemination of antibiotic resistance by various mechanisms. The developed ARB & ARGs are doomed to release into the different environmental matrices including natural and drinking water bodies (Voigt et al., 2020; Xu et al., 2016). The development of antibiotic resistance has turned into a serious and growing threat at all ecological aspects; influencing human, animal, and environmental health as it now has been recognized as a global public health crisis of the 21st century by The World Health Organization (WHO) (Ávila et al., 2021; Zieliński et al., 2021). It is estimated that if countermeasures are not adopted, antibiotic resistance will kill 10 million people/year globally by 2050, which is higher than those caused by cancer (Azuma and Hayashi, 2021).

Unfortunately, ARB and ARGs are not presently included in water or wastewater treatment control parameters mainly because no admitted regulation or standard for either antibiotic residuals or antibiotic resistance (Raza et al., 2021; Zhang et al., 2020). Nonetheless, the requirements of the European directive 2000/60/EG focus on the improvement of the aquatic matrices through the reduction/loss of discharge of hazardous/priority substances like pesticides, heavy metals, or nonylphenol. Since June 2018, specific antibiotics like azithromycin, erythromycin, clarithromycin, amoxicillin, and ciprofloxacin have been added to the "watch list of substances for union-wide monitoring in the field of water policy" in order to improve tracking the occurrence and spread of antimicrobial substances in water systems. Whereas water and wastewater treatment plants (W&WWTPs) should be redesigned so that efficient control strategies can be adopted to relieve the propagation of antibiotic resistance in the environment from these *the calm before the storm* sources (Ahmed et al., 2020; Voigt et al., 2020). In the light of the mentioned facts, it seems that there is an imminent need for a move-in approach from conventional pathogenic inactivation to destroying ARB and ARGs (Umar et al., 2019). Nevertheless, in a multi-barrier approach implemented in W&WWTPs flowchart, tertiary treatment plays a critical role in minimizing the release of antibiotic residuals and the risk of antibiotic resistance spread into the environment, although many aspects of them have not been fully understood (Rizzo et al., 2019). Disinfection may still offer an excellent opportunity to restrict the discharge of ARB and ARGs into the

environmental matrices and therefore reduce the risk of their spreading to/and via other pathogens (McKinney and Pruden, 2012).

Ultraviolet (UV), chlorine, and ozone are considered as the routine disinfectants in W&WWTPs as well as the most promising ones in the removal of ARB and ARGs (Gelete et al., 2020; Manaia et al., 2018). At present, chlorination is still one of the most common disinfection techniques mainly because simplicity as well as its economical and systematic consumption. Besides, ozone is promisingly applied for disinfection owing to its high biocidal efficacy over a wide antimicrobial spectrum, in addition to decolorization, taste & odour control, and reduction of trace organics in the treated effluent or water. UV disinfection is an important physical technology known as an effective alternative for many waterborne pathogens with no production of detrimental disinfection by-products (DBPs) or other chemical residuals (Shi et al., 2021). Although the mentioned disinfectants have their own disadvantages, in the face of ARB & ARGs they still considered as the best practical option. However, their efficiency not fully understood neither quantitatively (how much they can affect different types of ARB & ARGs) nor qualitatively (which mechanism and combination, or other operational parameters). In addition, their effectiveness on ARB and ARGs removal sometimes can be controversial. Therefore, there is a significant research and knowledge gap in this area (Ni et al., 2020). While in the last few years a growing number of studies toward different disinfection approaches on ARB & ARGs removal has been published or reviewed (Hiller et al., 2019; Liu et al., 2018; Umar et al., 2019; Yang et al., 2019b), to the best of the authors' knowledge no review article is available on the effect of ozone-based strategies for ARB and ARGs removal. For this reason, the current study aimed to systematically review the effects of ozonation on ARB & ARGs removal from aqueous solutions.

2. Material & methods

The literature collection was conducted according to the PRISMA statement in four consecutive stages of searching, selecting, data extraction, and data synthesis and analysis.

2.1. Information sources

The main international databases including Scopus, PubMed, and Web of Science (WoS) were comprehensively searched by using both terminologies and MeSH terms to identify all articles regarding the elimination of ARB and/or ARGs from water and/or wastewater media using ozonation (alone or in combination with other techniques) between Jan 2000 and May 24, 2021. The search was conducted with no limitation in the geographical area, the scale of experiments (i.e. batch, bench, pilot, and real scales), and the method for analysis of bacteria and/or genes. Besides, only studies published in English and available in the full text were reviewed.

2.2. Search strategy

The search MESH terms were “wastewater” OR “water” OR “effluent” OR “sewage” OR “wastewater treatment plant” OR “water treatment plant” AND “ozone” OR “ozonation” OR “advanced oxidation process” OR “O_3” OR “oxidation” OR “disinfection” OR “disinfectant” AND “antibiotic resistance” along with all possible combinations.

2.3. Inclusion/exclusion criteria and data extraction

After removing duplicates, the obtained studies were reviewed and the eligible ones were selected according to the following criteria: (1) being an original study that examined ozonation effect on removal, destruction, or deactivation of ARB and/or ARGs from (waste) water matrices or their related products such as sludge or biological solids; (2) expressing the removal efficiency (as either % or log) or designing in a way that the removal efficiency can be obtained or calculated from data; and (3) published in English. The following search results were excluded: book chapters, review articles, conference papers, letters to the editor, short communications, studies written in languages other than English, and studies that their data were not represented quantitively or cannot be calculated. The obtained results were independently screened and assessed for eligibility by three authors (M.F., V.Kh., and M.K.), first by reviewing their titles and abstracts and then full texts regarding the mentioned inclusion and exclusion criteria. Any disagreement was resolved by consulting the corresponding authors (S.K. and Kh. N.)

2.4. Data extraction

The required information was extracted from the eligible articles with regard to the essential items that had already been provided as the study goals. The items were the type of medium or media (i.e., water, wastewater, sludge, etc.); type of ARB and/or ARGs; type of antibiotic studied (if any); the operational conditions such as pH, temperature, ozone dose, etc.; the performance of applied process as units of either removal efficiency (%) or log value; and the differences between ozone and its modifications for the elimination of ARB and/or ARGs. The latter was considered for the studies which applied ozonation with other techniques (such as UV, ultrasound (US), etc.). The data was either directly sourced from the selected articles if it was available or extracted from graphs and calculated manually.

3. Results and discussion

A total of 40 studies were distinguished to be in the defined framework from 2742 firstly obtained records through database searching. Fig. 1 shows the summary profile of the search strategy used to identify eligible studies and the number of studies identified and selected during each phase of the search. All 40 studies were fully reviewed and categorized separately as presented detail in Tables 1 and 2 and discussed in the following sections.

3.1. Application of ozone for ARB & ARGs removal

From the studied bacteria for degradation using ozone-based processes (Fig. 2) special attention have been paid to *E. coli*, *Enterococcus*, and *Staphylococcus* with 25%, 25%, and 15% relative fraction, respectively. Since the main MOs in the activated sludge-based processes are *Zooglea*, *Pseudomonas*, *Flavobacterium*, *Alcaligenes*, *Achromobacter*, *Corynebacterium*, *Comomonas*, *Brevibacterium*, *Acinetobacter*, and *Bacillus* (Bitton, 2011), more investigations are needed to assess the influence of the other resistant bacteria. Moreover, the data shows that sulfonamide- and tetracycline- resistance genes (22% and 21%, respectively), have been studied more frequently than other most common genes in wastewater (as reviewed by Pazda et al., 2019). This is welcomed because these two classes of antibiotics are the two first produced and consumed ones in the world (García-Delgado et al., 2018).

The ozonation process for the elimination of ARB & ARGs from water and wastewater as well as their related media (such as sludge) have been reported in many studies and found to be significantly effective. The effect of ozonation on the removal of ARB & ARGs, when used as the unique disinfectant agent, is summarized in Table 1. As can be seen from this table, ozone can remove at least 2 log of both ARB & ARGs in the mentioned media. The removal efficiency, however, is different and depends on the type of bacteria, genes, and antibiotics. For example, for *E-coli*, a removal efficiency as high as 3 log, 4 log, and 5 log have been reported by Hembach et al. (2019), Pak et al. (2016), and Stange et al. (2019), respectively. However, it was found a 100% removal efficiency for Erythromycin and ethylparaben resistance *E-coli*. Moreover, *Enterobacteria* and *Enterococci* have shown the highest removal efficiencies of >5 log and 5 log as reported by Sousa et al. (2017) and Stange et al. (2019), respectively. The removal efficiency

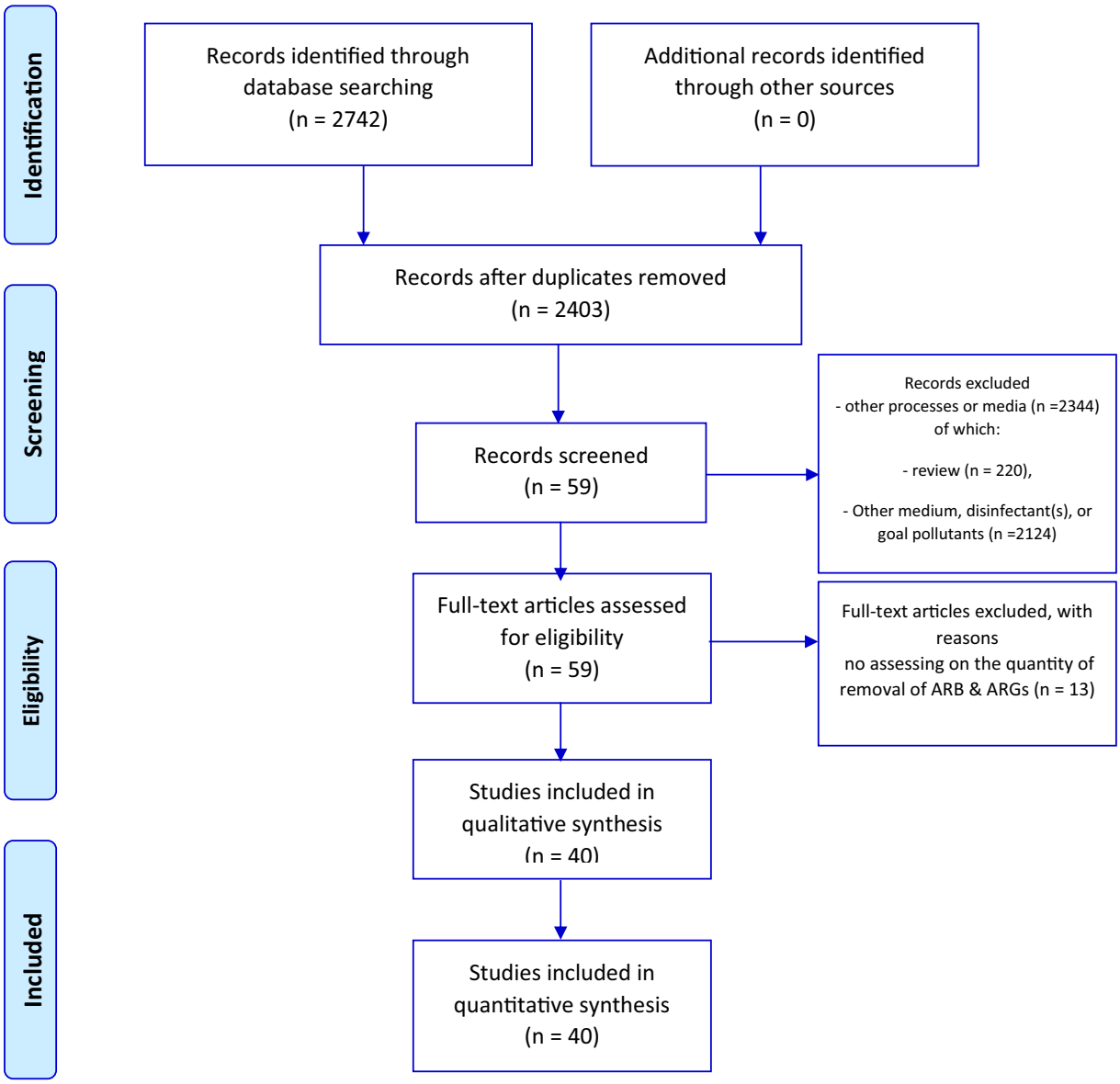

Fig. 1. Flow diagram of the studies selection procedure based on the PRISMA.

for the latter is however different for its various species (from 1.5 log for *E. casseliflavus* to 3.8 log for *E. gallinarum* (Hess and Gallert, 2015)). A range of 1.17 log to 3.16 log has also been stated by Tong et al. (2017) and Wei et al. (2020), respectively. Nonetheless, a wider range of removal was found for *Staphylococcus*; from −78.1% (Alexander et al., 2016) to 35% (Chu et al., 2020) and 69% (Kanaan, 2018). From the MOs, *P. aeruginosa* showed the highest tolerance (Alexander et al., 2016). Therefore, it appears that the susceptibility of ARB followed the order of *Enterobacteria* > *Enterococci* > *E-coli* > *Staphylococcus* > *P. aeruginosa*. According to Table 1, most of ARGs studied are also significantly influenced by ozone treatment within the range of 0.02 - >5 log. Nevertheless, there is almost no certainty about the extent of removal even for a given gene. For example, while Sousa et al. (2017) reported 4 log removal for *sul1*, Su et al. (2018) reported 0.03 log removal. This dramatic difference can be due to ozone concentration, or other operational parameters, such as pH, temperature, etc. (Hiller et al., 2019). However, it also depends on the measuring method (Rizzo et al., 2013). Besides, for some ARGs the concentrations increased after processing through the unit (Su et al., 2018). This can be due to regrowth or repairment ability, the increase of detectable fraction, etc., which are discussed in Sections 3.4 and 3.7, respectively. Even in high removal efficiencies of ARGs, ozone may need to be injected at a very high dose as reported by Zhuang et al. (2015) (over 177.6 mg/L), which impose a high cost in comparison with conventional disinfection methods. Therefore, it seems that ozone could not dramatically remove the antibiotic resistance in wastewater effluents in already used dosages for disinfection, especially for some ARGs. The main reason is that the majority of O_3 is primarily utilized by the dissolved organic matter of effluents (Ben et al., 2017). Another important conclusion from the articles listed in Table 1 is that although the optimum conditions are considered in maintaining efficiencies, whenever two concentration of ozone are compared, the removal efficiencies increased with increasing ozone initial concentrations (Zheng et al., 2017). It has been already recognized that the suspended solids, the dissolved organic matter, and the complexity of the microbiota in an aqueous medium may influence the efficiency of the ozonation process mainly due to the effect on the reaction mechanism of ozone. All the effective factors are discussed in Section 3.6.

Table 1
Ozonation process for removal of ARB & ARGs (obtained directly or calculated by the average values in the references, positive values represent removal, negative values represent increase).

Medium	The declared operational condition(s)	Name of bacterium or bacteria	Removal of ARB (% or log)	Name of gene(s)	Removal of ARG (% or log)	Ref.
MWWTP	Real scale Dose: 1 g ozone/g DOC Flow rate = 7 m^3/h, (→ T = 5 min) pH = 8.1 ± 0.7 Temperature = 16.0 °C ± 3.2 °C DOC = 4.3 g ± 1.2 g Conductivity = 828.1 μS/cm ± 193.1 μS/cm SAC (spectral adsorption coefficient) = 9.4 ± 1.7	- *E-coli* - *Acinetobacter baumannii* - intestinal *enterococci* - *eubacteria*	- 3 log - 3 log - 3 log - 2 log	- *sul1* - *blaTEM* - *tetM* - *CTX-M30*, - *CTX-M-3228*, - *blaOXA-48* - *blaVIM* - *CMY-2* - *vanA* - *mcr-1* - *blaNDM*	- *sul1*: 2 log - *blaTEM*: <2 log (97%) - *tetM*: 2 log - *CTX-M30*, 4 log - *CTX-M-3228*, <2 log (81.77%) - *blaOXA-48*: 100% - *blaVIM*: ND - *CMY-2*: ND - *vanA*: ND - *mcr-1*: ND - *blaNDM*: ND	(Hembach et al., 2019)
Synthetic and urban treated wastewater (after secondary treatment)	Batch experiments Dose: 50 g/Nm^3 Flow rate: 150 cm^3/min Temperature: 25 ± 2 °C Times: 15, 30 or 60 min	- *Heterotrophs* - *Enterobacteria* - *Enterococci* - Fungi (*Rhodotorula rubra* and *Aspergillus niger*)	Removal (as % and log, respectively) Synthetic wastewater: Time 30 min: - >99.99 (6.5 ± 0.1) - >99.99 (>5.0) - >99.99 - 99.99 ± 0.01 (3.3 ± 0.2) Urban treated wastewater: Time 30 min: - 99.96 ± 0.06 - 99.93 ± 0.11 - 99.78 ± 0.33 - 99.97 ± 0.03	- *intI1* - *sul1* - *blaTEM* - *qnrS* - *vanA*	Synthetic wastewater: Time 30 min: - 99.99 ± 0.00% - ND - 99.99 ± 0.00% - ND - >99.99% Urban treated wastewater: - 98.03 ± 2.16% - >99.99 % - >99.99% - >99.99% - ND	(Sousa et al., 2017)
Drinking water	Batch experiments Dose: 1 mg O_3/L Time: 5 min	*E-coli K12 DH1 RU2945* *Enterococcus faecium*	- 5.0 log reduction of bacterial cells (99.999%) - 5.0 log (99.999%)	*tet(A)* *ampC* *ermB* *vanA*	*tet(A)*: 99.9968% *ampC*: 99.995% *ermB*:99.999% *vanA*: 99.995% 4.3–4.6 log reduction of ARGs.	(Stange et al., 2019)
WTP	Full scale	No	No	-*sul1*, *sul2*, *ermB* *tetA*, *tetM*, *tetO* *tetQ*, *tetS*, *tetW* *tetX*, *cfr*, *cmlA*, *fexA*, *fexB*, *floR* *oqxB*, *qepA*, *qnrA* *qnrB*, *qnrD*, *qnrS*, ∑ARGs	Removal (as log): sul1: 0.03, sul2: 0.46, *ermB*: 0.38, *tetA*: −3.39, *tetM*: −0.31, *tetO*: 0.09, *tetQ*: 0.02, *tetS*: 0, *tetW*: −0.44, *tetX*: 0.97, *cfr*: −0.44, *cmlA*: −0.55, *fexA*: 4.03, *fexB*: −0.03, *floR*: 0.34, *oqxB*: 0.31, *qepA*: 0, *qnrA*: 0, *qnrB* −0.17, *qnrD*: 0, *qnrS* −3.75, ∑ARGs: −0.07	(Su et al., 2018)
Pharmaceutical Waste Sludge	Batch experiments Dose: 0.11 mg O_3/L Temperature: 38 °C	- Total heterotrophic bacteria - *Enterococci* - azithromycin (15 μg), - erythromycin (15 μg) - spiramycin (100 μg) - clarithromycin	Total heterotrophic bacteria: 1.39 log CFU/g *Enterococci* ~ 1.17 log CFU/g	-*ereA* - *ermB* - *ermF* -*wrmX* -*mphB* - *mefA*/E) - *tetM* - *tetO* -*intI1* - *ISCR1*	-*ereA*: 2.3 log - *ermB* ~ 1.6 log - *ermF* ~ 0.1 log -*wrmX* ~ 0.6 log -*mphB* ~ 0.7 log - *mefA*/E) ~ 0.5 log - *tetM* ~ 0.3 log - *tetO* ~ 1 log -*intI1* ~ 1.5 log - *ISCR1* ~ 1.5 log	(Tong et al., 2017)

(continued on next page)

Table 1 (*continued*)

Medium	The declared operational condition(s)	Name of bacterium or bacteria	Removal of ARB (% or log)	Name of gene(s)	Removal of ARG (% or log)	Ref.
		(15 μg) - tetracycline (30 μg), - vancomycin (30 μg)		-*Tn916*/1545	-*Tn916/1545* ~ −1.2 log	
Wastewater from high-speed railway trains	Pilot scale Continuous mood (1 m^3/day real wastewater) Dose: 91 mg O_3/L Flow rate: 25 L/h	*Enterococci*	3.16 logs CFU/mL	- *tetA* - *tetG* - *qnrA* - *qnrs* - *blaNDM-1* - *ermF* - *intI1* - *Tn916*/615	- *tetA*: 2.95 ± 0.21 log - *tetG*: 1.79 ± 0.40 log - *qnrA*: 1.97 ± 0.35 log - *qnrs*: 1.43 ± 0.45 log - *blaNDM-1*: 2.01 ± 0.29 log - *ermF*: 0.47 ± 0.23 log - *intI1*: 2.80 ± 0.35 log - *Tn916/615*: 4.59 ± 0.13 log	(Wei et al., 2020)
MWWTP (receiving domestic sewage and approximately 30% of pharmaceutical and other industrial wastewaters.)	Batch experiments Dose = 20 mg O_3/L Time: 20 min On the secondary effluent Coal-based granular activated carbon, upward, at a flow rate of 0.6 L/h.	–	–	- *tetA* - *tetO* - *tetW* - *sulI* - *sulII* - *blaCTX-M*	- *tetA* ~ 0.5 log (70%) - *tetO* ~ 0.3 log (52%) - *tetW* ~ 0.3 (53%) - *sulI* ~ 0.4 (60%) - *sulII* ~ 0.38 (58) - *blaCTX-M* ~ <0.2 log (30%)	(Yang et al., 2019a)
Secondary effluents from a MWWTP[a]	Dose: 2 mg O_3/L Time: 10 min	Heterotrophic plate count (HPC) method	10 mg/L O_3: - No culturable bacteria detected 1 mg/L O_3: TC resistant bacteria: 2.46 Sulfamethoxazole-resistant bacteria: 2.10 orders of magnitude	- *tetA* - *tetM* - *tetO* - *tetQ* - *tetW* - *sulI* - *sulII*	Dose: 2 mg O_3/L: - *tetA* ~ 52% - *tetM* ~ 50% - *tetO* ~ 50% - *tetQ* ~ 59% - *tetW* ~ 47 - *sulI* ~ 25% - *sulII* ~ 43%	(Zheng et al., 2017)
MWWTP	Bench scale Dose: 2.66 mg O_3/L Flow rate: 8.3 L/min.			- *sul1* - *tetG*, - *intI1*	- *sul1*: 1.75 log - *tetG*: 2.25 log - *intI1*: 1.5 log	(Zhuang et al., 2015)
WTP	Full scale	–	–	The resistance genes to: - Aminoghycoside - Beta Lactamase - FAC - MLSB - Other/efflus - Tetracycline - Sulfonamide - Vancomycin	Removal (as %) 99.09 93.87 95.45 94.33 98.67 97.67 99.50 99.90	(Xu et al., 2020)
A synthetic wastewater with p-aminophenol (PAP) concentration of 100 mg/L	Dose: 25 mg/L pH = 7.0 Time: 15–140 min The initial sludge concentration in each reactor was maintained at 3 g/L	–	–	*novA*, *kdpE*, *aadA2*, *APH(6)-Id*, *uppP*, *bcrA*, *BacA*, *pbp1a*, *pbp2b*, *K678_12262*, *CCC_03326*, *mrdA*, *AIM-1*, *NmcR*, *ampC*, *murA*, *VanRC*, *vanRO*, vanRI, *VanRE*, *KsgA*, macB, *AMS22_10315*, *msrB*, *tlrC*, *oleB*, *carA*, *srmB*, *Rahaq2_0060*, *msrE*, *MuxB*, *mexK*, *MexD*, *acrB*, *ceoB*, *mdtc*, *adeb*, *adeG*, *mexF*, *mdtb*, *MuxC*, *floR*, *mexW*, *mexB*, *bepE*, *MexC*, *msbA*, *AmrB*, *mexN*, *smeE*, *adeL*, *mtrD*, *smeR*, *sav1866*, *oprJ*, *adeJ*, *emrB*, *mexi*, *oprM*, *OprA*, *baeS*, *adeF*, *mexQ*, *golS*, mepB, *cpxr*, *opcm*, *mdtF*, *CeoA*, *smeF*, *MuxA*, *smeC*, *mdsb*, *AcrA*, *mdtD*, *qepA*, *mexA*, *mexE*, *mexL*, *adeH*, *mexJ*, *baeR*, *adeS*, *smeB*, *smeD*, *arlR*, *farA*, *cfr*, *mdta*, *oprN*, *adeR*, *abcA*, *adeK*, *ileS1*, *ileS*, *TaeA*, *RosB*, *basR*, *PmrE*, *rosA*, *MCR-1*, *mfd*, *oqxB*, *patA*, *oqxA*, rphB, sul1, *sul2*, *tetG*, *tetC*, *tet41*, *tetA(48)*, *tetB(46)*, *TriC*, *dfrE*, *dfrA3*	The relative abundance (as %): *novA*: 26.21, *kdpE*: 80.78, *aadA2*: 0.00, *APH(6)-Id*: 75.00, *uppP*: 18.95, *bcrA*: 3.37, *BacA*: 38.98, *pbp1a*: 29.18, *pbp2b*: 69.84, *K678_12262*: −24.05, CCC_03326: −93.55, *mrdA*: 25.81, AIM-1: 61.90, *NmcR*: 81.91, ampC: −462.50, *murA*: −73.58, *VanRC*: 58.68, *vanRO*: −42.50, *vanRI*: −11.11, *VanRE*: 31.58, *KsgA*: 58.19, *macB*: 16.45, *AMS22_10315*: −12.88, *msrB*: −25.76, *tlrC*: 16.95, *oleB*: −39.05 *carA*: 9.84, *srmB*: 13.07, *Rahaq2_0060*: −9.21, *msrE*: 82.71, *MuxB*: 76.16, *mexK*: 59.31, *MexD*: 47.40, acrB: 38.08, *ceoB*: 57.47, *mdtc*: 79.15, *adeB*: 90.14, *adeG*: 78.14, *mexF*: 37.83, *mdtb*: 77.59, *MuxC*: 78.37, *floR*: −55.84, *mexW*:	(Xia et al., 2020)

					−27.72, *mexB*: 14.17, *bepE*: 60.46, *MexC*: 79.89, *msbA*: 6.91, *AmrB*: 50.00, mexN: 24.31, smeE: 6.55, *adeL*: 85.39, *mtrD*: 53.44, *smeR*: 25.81, sav1866: 22.61, *oprJ*: 69.60, *adeJ*: 42.40, *emrB*: 70.95, *mexi*: −1.83, *oprM*: 62.16, *OprA*: 64.36, *baeS*: 79.26, *adeF*: 88.19, *mexQ*: 37.14, golS: −25.83, *mepB*: 0.00, *cpxr*: 26.49, *opcm*: 87.84, *mdtF*: 14.38, *CeoA*: 64.18, *smeF*: 80.00, *MuxA*: 78.15, *smeC*: 79.73, mdsb: 29.17, *AcrA*: 37.18, *mdtD*: 82.30, *qepA*: 71.88, *mexA*: 75.5, *mexE*: 28.57, *mexL*: −100.00, *adeH*: 86.57, *mexJ*: −37.50, *baeR*: 59.17, *adeS*: 97.22, smeB: 71.68, *smeD*: 23.28, *arlR*: 31.30, *farA*: 73.58, *cfr*: 3.09, *mdta*: 61.00, *oprN*: 87.10, *adeR*: 96.43, *abcA*: −16.67, adeK: 82.76, *ileS1*: 36.77, *ileS*: −54.17, *TaeA*: 4.06, *RosB*: 62.75, *basR*: 45.48, *PmrE*: −201.52, *rosA*: −45.16, MCR-1: 70.18, *mfd*: 12.89, *oqxB*: 71.21, *patA*: 8.64, *oqxA*: 77.42, *rphB*: 41.94, *sul1*: 34.67, *sul2*: −1163.64, *tetG*: 0.00, *tetC*: 89.19, *tet41*: 65.71, *tetA*(48): −177.42, *tetB*(46): 4.23, *TriC*: −6.25, *dfrE*: 21.88, *dfrA3*: 37.68	
Clinical and wastewater samples (secondary treated effluent)	Dose: 4 mg O_3/L pH = 7.0 Time: 20 min, Autoclave time: 15 min with 5.5 mg DOC/ L	- *Enterococcus* - *Staphylococcus* - *E.coli*	**- *Enterococcus*** - *E. casseliflavus*: 1.5 log - *E. gallinarum* 3.8 log - *E. durans* 3.6 log - *E. hirae* 2.5 log - *E. faecalis* 3.0 log - *E. faecium* 3.2 log **- *Staphylococcus:*** *S. aureus* 2.1 log units *S. saprophyticus* 3.2 log *S. sciuri* 3.1 log *S. xylosus* 2.7 log **- *E-coli*** median 2.8 log Resistant strains against: - ampicillin (AM-r) ~ 0.7–4 log - cotrimoxazol (SXT-r) ~ 1.5–3.3 - ciprofloxacin (CIP-r) ~ 1.4–3.7 -both CIP-r & SXT-r ~ 3.6–4.1 -extended-spectrum-ß-lactamase producers ESBL ~1.6–3.3	–	–	(Hess and Gallert, 2015)
Synthetic water	Batch scale Dose: 1 mg O_3/L Flow rate: 24–50 mL/min pH = 7 Temperature: 20 ± 1 °C Time: 0.047 to 1.3 s.	*B. subtilis* strains (2 mg/L): - 1A1 (nonresistant) - 1A189 (multidrug-resistant)	–	Extra- and intracellular antibiotic resistance genes (eARGs and iARGs)	≥90% (CT required for 2-log10 iARG degradation or deactivation: >0.12) ≥90% (CT required for 2-log10 eARG degradation or deactivation:0.1-NA)	(He et al., 2019)
UWWTP	Scale: laboratory Dose: 90 ppm Time: 20 min	Coliform and antibiotic resistant bacteria	100%	- *blaCTX* - *blaOXA* - *blaTEM* - *blaNDM-1*	*CTX* ~ 77.69%, *OXA* ~ 60.08%, *TEM* ~ 64.91%, *NDM-1*– 77.69%.	(Lamba and Ahammad, 2017)
WWTP	Pilot scale Dose: 0.5 to 11 mg O_3/L pH: 6.8	- *E. coli* - *Enterococcus spp*	- Up to 50% - Up to 15%		–	(Luczkiewicz et al., 2011)
Urban wastewater[b]	Batch scale	*E-coli*	100%	–	–	(Michael-Kordatou

(continued on next page)

Table 1 (*continued*)

Medium	The declared operational condition(s)	Name of bacterium or bacteria	Removal of ARB (% or log)	Name of gene(s)	Removal of ARG (% or log)	Ref.
	Dose: 0.3 mg O_3/L pH:8 Temperature: 25 °C Time:15 min					et al., 2017)
Synthetic wastewater	Flow rate: 4–5 L/min Dose: 3, 5, 7, and 10 mg /L Time: 1, 3, 5, and 10 min Scale: laboratory	*E. coli DH5α (pB10)*	CT = 101.4 mg.min/L Dose: 7 mg/L: 4 log	PB10 plasmid	CT = 127.15 mg.min/L Dose: 7 mg/L: 4 log	(Pak et al., 2016)
Conventional clarified wastewater.	Pilot scale A concentration of 0.9 ± 0.1 g O_3/1 g DOC Real wastewater	- *Enterococci* - *Staphylococci* - *P. aeruginosa* - *Enterobacteria*	The relative & absolute abundance, respectively: - *Enterococci*: (−98%), −98.9%, - *Staphylococci*: reduction (−83%), −78.1% - *P. aeruginosa*: increase (+43%), − 60.2% - Enterobacteria: reduction (−9%), −69. 8%	*ermB* *ampC* *vanA* *blaVIM*	The relative & absolute abundance, respectively: Reduced: *ermB* ~ −99% (−99.3%) *ampC* ~ −9% (−69.8%) Increased: *vanA* ~ +405% (−49.9%) *blaVIM* ~ +764% (−18.7%)	(Alexander et al., 2016)
Hospital effluent, sewage treatment plant (STP) and river water	Full scale Dose: 8.6 mg O_3/L Time: 100 min	Carbapenem-resistant *entero-bacteriaceae* - Vancomycin-resistant *enterococci* - Methicillin-resistant *Staphylococcus aureus* - Multi-drug-resistant *Acinetobacter* - Multi-drug-resistant *Pseudomonas aeruginosa*	ND	–	–	(Azuma et al., 2019)
WTP	Full-scale Continues mood	–	–	*tetA*, *sul1*, *sul2*, *ampc*, *tetM*, *intI1*,	Relative abundances: *tetA*: −496.33, *sul1*: 99, *sul2*: 0.00, *ampc*: −392, *tetM*: 0.00, *intI1*: −376.19,	(Chen et al., 2020a)
Cephalosporin C fermentation residues	Batch scale Dose: 5.2 g O_3/L Flow rate: 0.6 L/min Continuous mood Time: 2 h	*Staphylococcus aureus*	35%	*tolC*	64.6% by ozonation Relative abundance increased to 1.6% (from around 1.4% in raw residues) after ozonation	(Chu et al., 2020)
WWTP	Batch & full scales pH = 7.8	*E. coli*	Batch scale: 4 log With kb [L/mg s] = 32 (±2) Full scale: 0.55 gO_3/g DOC 1.4–1.6-log	- *sul1*	Batch scale: 4 log CT_{lag} [mgs/ L], k [L/mg s], and ozone exposure [mg s/ L], respectively for: - *sul1*: 0.76 (±0.49), 2, 5.15. Full scale: 0.55 gO_3/g DOC - *sul1* ~ 0.0 log	(Czekalski et al., 2016)
Real pharmaceutical wastewater	Batch scale Dose: 50 mg/L (normal dose) pH = 7.0 ± 0.3 Temperature: 25 ± 2 °C	–	–	*sul*, *sul2*, *tetW*, *tetQ*, *tetM*, *tetO*, *qnrD*, *ermB*, *blaOXA-1*, *blaOXA-10*	Absolute abundance *sul1*: 99.90, *sul2*: 99.90, *tetW*: 99.99, *tetQ*: 100.00, *tetM*: 100.00, *tetO*: 100.00, *qnrD*: 100.00, *ermB*: 100.00, *blaOXA-1*: 100.00, *blaOXA*-10: 100.00	(Hou et al., 2019)
WTP	Dose: 1.0–2.0 mg/L O_3	–	–	*intI*, *sul1*, *sul2*, *sul3*, *qepA*, *qnrA*, *qnrB*, *tetA*, *tetB*, *tetC*, *tetM*, *tetW*, *strA*, *strB*, *blaOXA-1*, *blaTEM-1*,	Removal (as log) *intI*: 0.4, *sul1*: 0.3, *sul2*: 0.8, *sul3*: −0.2, *qepA*:	(Hu et al., 2019)

				ermB, *ermC*	0.2, *qnrA*: −0.3, qnrB: 0.6, *tetA*: 0.8, *tetB*: 0.7, *tetC* 0.6, *tetM*: 0.4, *tetW*: 0.3, *strA*: −0.1, *strB*: −0.2, *blaOXA-1*: −0.5, *blaTEM-1*: −0.3, *ermB*: 0.5, *ermC*: −1.8,		
Urban wastewater[c]	Continuous mood Dose: 0.125 gO_3/g DOC pH 7.3–7.8. Temperature: 22–25 °C HRT: 40 min Real scale	*E. coli*	HRT: 40 min Dose: 0.75 gO_3/DOC <<LOD = 0.01 CFU/mL	*intI1* *aadA1* *dfrA1* *qacED1* *sul1*	HRT: 40 min Dose: 0.75 gO_3/DOC Removal (as log): *intI1*: 2.3 *aadA1* ~ 2.2 log *dfrA1*: <LOD *qacED1*: 4 log *sul1*: 2 log	(Iakovides et al., 2019)
Synthetic wastewater	Batch scale Cons: 1 mM Time: 0–45 s Temperature 20 °C	*Staphylococcus aureus*	5 log	*mecA*	3 log	(Choi et al., 2021)
Hospital wastewater	Real scale No more data	–	–	*Aph3a*, *blaKPC*, *blaOXA*, *blaSHV*, *ermB*, *ermF*, *Int1*, *MecA*, *qnrs*, *Sul1*, *TetB*, *TetM*, *VanA*, *VanB*	Removal (as %) *Aph3a*: 98.52, *blaKPC*: 0.00, *blaOXA*: 49.50, *blaSHV*: 0.00, *ermB*: 62.35, *ermF*: 65.90, *Int1*: −15,742.58, *MecA*: 0.00, *qnrs*: 0.00, *Sul1*: −4912.96, *TetB*: 0.00, *TetM*: 0.00, *VanA*: 0.00, *VanB*: 0.00	(Paulus et al., 2019)

Abbreviations: ND: not determined, CT: concentration × time, WTP: water treatment plant, WWTP: wastewater treatment plant, MWTP: Municipal wastewater treatment plant, MOs: microorganisms, HWW: Hospital wastewater, US: ultrasound, AD: anaerobic digestion, PWS: pharmaceutical waste sludge, and MWS: Municipal waste sludge, GAC: Granulated Activated Charcoal.
Scales: are as batch, bench, pilot, and full scales. From the place of doing are as laboratory or full (in the place) scales.
Name and concentration of antibiotic(s).
[a] TC (16.0 mg/L), sulfamethoxazole (SMX, 50.4 mg/L), separately and with each other.
[b] Erythromycin (100 g/L) & ethylparaben (100 g/L and 1 mg/L).
[c] AMP, AZM, CLA, ERY, OFL, SMX, TMP and TC with an initial concentration of 100 mg/L.

3.2. Ozonation in combination with other techniques

From the above-mentioned hybrid techniques, catalytic ozonation (including O_3/persulfate (PS) and O_3/monopersulfate), O_3/UV, and O_3/sand filtration or granular activated carbon (GAC) adsorption have been used for removal of ARB & ARGs until now (Table 2). Moreover, application of O_3/anaerobic digestion (AD), O_3/US, O_3/US+AD, O_3/autoclave, O_3/chlorine, and O_3/biological activated carbon filtration (O_3-BAC) has also been reported in this field (Table 2). As can be observed from Table 2, the elimination efficiency of ARB and/or ARGs increases in hybrid processes in comparison with ozonation alone either by influence on removal efficiency (Pei et al., 2016) or its related parameters such as *CT* values in (Oh et al., 2014) study, who reported that adding the PS-based catalysts significantly reduces the required contact time to attain a 2-log removal, which in turn, enhances the process from kinetics standpoint, or denaturation in Somensi et al. (2015) study which was significantly reduced by using ozone/US relative to ozonation alone. Nevertheless, the hybrid process has not always been reported effective than ozone alone. For example, Jia et al. (2020) showed that there is no significant difference between the performance of O_3/chlorine relative to O_3 alone ($p = 0.93$). The effect of O_3/UV seems controversial; while some studies reported no significant effect relative to O_3 alone (Jager et al., 2018). Others discussed a significant effect of this hybrid process (Lee et al., 2011). This is also applicable to O_3/US for which some literature reported a significant performance (X. Chen et al., 2020; Somensi et al., 2015) and others declared an insignificant performance versus O_3 alone (Lan et al., 2021). Despite O_3/US, Lan et al. (2021) also reported a significant improvement in removing ARGs in O_3/autoclave process (Table 2). Moreover, the addition of certain agents has been reported effective. For instance, Zheng et al. (2017) reported that the mean values of the removal efficiency increased to 91.4% when Deoxyribonuclease (DNase) was added to the ozonation process. These controversial results in addition to disrupting the comparison of them, indicate that the findings should assess according to the effective parameters as discusses in Section 3.9.

3.3. Mechanism of action

In an aqueous solution, the dissolved ozone is rapidly decomposed into the radicals of superoxide ($\bullet O_2^-$), hydroperoxyl ($HO_2\bullet$), and OH• according to the following mechanism:

$$O_3 \rightarrow {}^{\circ}OH, {}^{\circ}O_2, H_2O_2$$

The above-mentioned radicals besides organic radicals, which generated due to the reaction of hydroxide ions with ozone as well as OH radicals, attack non-selectively the bacterial cell wall and oxidize mainly two groups of polyunsaturated fatty acids and amino acids from peptides, enzymes, or proteins, which in turn can result in leakage of cellular constituents. The penetrated radicals into the cytoplasm also may induce multiple oxidation reactions with intracellular substances. For example, they can destroy the enzymes that contributed to the decomposition of glucose inside the bacterium cells; protein; fatty acids; and even purines and pyrimidines of DNA and RNA, which impacts the reparation and transcription and then may result in cell lysis or death (Pei et al., 2019; Stange et al., 2019; Zhang et al., 2018; Zheng et al., 2017) (Fig. 3). Although no explicitly have been distinguished between the role of direct attack to the cellular components by ozone or indirect reactions with OH•, which is produced continuously simultaneous with auto-decomposition of ozone, and is recognized to be fairly reactive toward free DNA (Dodd, 2012), the stated consecutive reactions are highly-dose dependence (Pei et al., 2016).

Microbial community type and aqueous solution metrics condition are also effective on ozone mechanism for degradation of bacteria and/or genes. From a bacteria types point of view, ozone effectiveness strongly depends on several parameters, including but not limited to

Table 2
The hybrid ozonation processes for removal of ARB & ARGs.

Medium, disinfection technique	Operational condition(s)	Name of bacterium or bacteria Name of antibiotic (s) + concentration	Removal of ARB (% or log)	Name of gene(s)	Removal of ARG (% or log)	Status[a]	Ref.
Synthetic wastewater **- O_3 alone** **- O_3+ persulfate** **- O_3+ monopersulfate**	Scale[b]: laboratory - Dose: 3 mg O_3/L - Catalyst concentration = 1 mg/L - Time = 10 min	*E. coli DH5α* TC (2 mg/L)	- CT [(mg.min)/L] for removal of 90% or 2 log ARB: **- O_3 alone:** 31 **- O_3+ persulfate:** 15.9 **- O_3+ monopersulfate:** 2	MRG (*pB10*)	- CT [(mg.min)/L] for removal of 90% or 2 log ARG **- O_3 alone:** 33 **- O_3+ persulfate:** 18.5 **- O_3+ monopersulfate:** 14.5	Significant reduction of contact time to achieve 2-log removal	(Oh et al., 2014)
MWTP **- O_3 alone** **- O_3 + UV**	Real scale - Dose: 1.0 g ozone/DOC - Time ~5 min (flow rate ca. 7 m^3/h) - UV dose: 400 J/m^2, low pressure lamp	- *enterococci* (*23S rRNA*), - *Pseudomonas aeruginosa* (*ecfX*) - *E- coli* (*yccT*)	**O_3 alone and ozone + UV, respectively:** - *enterococci* = 98.4% - *ecfx*: <LOD - *E coli*: - *yccT*: −99.2%	- *mecA* - *ctx-M32* - *ermB* - *blaTEM* - *sul1* - *vanA* - *intI1*	**O_3 alone and ozone + UV, respectively:** - *23S*: −99.7%, −98.4% - *mecA*: <LOD, <LOD - *ctxM32*: −85.5%, −84.1% - *ermB*: −98.1%, −98.0% - *blaTEM*: −91.0%, −90.8% - *sul1*: −94.9%, −95.6% - *intI1*: 94.7, 99.	*p*-Value for: 1 vs. 0 <0.05 2 vs 1 Insignificant 2 vs. 0 <0.05	(Jager et al., 2018)
Piggery livestock wastewater **- O_3 alone** **- O_3 + UV**	Scale: laboratory **- O_3** Dose: 0.6 mg/L Flow rate = 4.9 mg/min.L **- O_3 + UV** Dose: 0.6 mg/L UV: 15 W low-pressure lamp **- O_3 + H2O2+ UV** Dose: 0.6 mg/L H_2O_2: 0–200 mg/L UV: 15 W mercury low-pressure lamp	Chlortetracycline resistant bacteria 20 mg/mL Chlortetracycline	**- O_3** 10 min: <2 log **- O_3 + UV** 20 min: >5 log	–		1 vs. 0: significant 2 vs. 1: significant	(Lee et al., 2011)
HWW **- O_3 alone** **- O_3 + US** (100w/L)	Batch scale For raw and treated HWW Dose: 0.5 mg/L pH =7.0 Time = 40 min, Sonolysis amplitudes = 50 and 100 W/L (=amplitudes of 50 and 70%) Temperature: 57 °C **For 99% reduction of MOs:** O_3 CT = 7.5 mg/L min O_3 + US (100 W) = 1.9 mg L min **For 99.99% reduction of MOs:** O_3 CT = 75.7 mg L min O_3 + US (100 W) = 12.9 mg L min	Raw: different types of MOs (450 CFU/mL) - Doxorubicin - Methotrexate - Chloramphenicol - Amoxicillin - Gentamicin - Tetracycline	–	–	Ozone: 99% Denaturation: Negligible Ozone +US: 99% Denaturation: 81%	2 vs. 1: Significant for both R (%) and Denaturation	(Somensi et al., 2015)
Dairy wastewater **- O_3 alone**	Batch scale Flow rate of 600 mL/min	–	–	*SulI*, *sulII*, *tetW*, *tetO*, *tetG*, *intI1*,	Relative abundance of ARGs (as %) **- O_3 alone**	2 vs 1 significant	(Chen et al., 2020a, 2020b)

- Ozone + US	US power 200 W Dose: 4.2 mg O_3/L, Pretreatment time 20 min.				*SulI*: 87.60, *sulII*: 92.00, *tetW*: 45.31, *tetO*: 46.81, *tetG*: 48.28, *intI1*: 48.28, **- O_3 + US** *SulI*: 87.60, *sulII*:33.33, *tetW*: 45.31, *tetO*: 41.49, *tetG*:39.66, *intI1*: 39.66,		
Biological laboratory wastewater **- O_3 alone** **- O_3 + US** **- O_3 + Autoclave**	Bench-scale Dose: 6 mg O_3 /L.min **- O_3 alone** Dose: 30 mg/L **- O_3 + US** Dose: 30 mg/L Flow rate: 0.16 L/min US power: 150 W **- O_3 + Autoclave** Autoclave: 121 °C at 0.1 MPa, 15 min Dose: 30 mg/L Flow rate: 0.16 L/min	*E. coli DH5α* (~2 × 10^9 CFU/mL)	Time: 30 min 5 log Time: 60 min No culturable bacteria	Log 10 copy number: *sul1* (14) *sul2* (14) *tetW* (11) *tetM* (12) *amp* (14) *intI1* (9)	Time: 60 min **- O_3 alone** *sul1* ~ 3.5 log *sul2* ~ 3.5 log *tetW* ~ 5.5 log *tetM* ~ 3.5 log *amp* ~ 3.5 log *intI1* ~ 4 log **- O_3 + US** *sul1* ~ 2.5 log *sul2* ~ 3 log *tetW* ~ 3.5 log *tetM* ~ 3.5 log *amp* ~ 2.5 log *intI1* ~ 4 log **- O_3 + Autoclave** *sul1* ~ 7.5 *sul2* ~ 7.5 *tetW* ~ 7 *tetM* ~ 7.5 *amp* ~ 7.5 *intI1* ~ 7	In the optimum time (60 min) 1 vs. 0: significant 2 vs. 1: Insignificant 3 vs. 1: significant	(Lan et al., 2021)
Pretreatment of anaerobic digestion Biosludge **US/O3 + AD**	Bench-scale US: 20 kHz, 100 W Dose: 200 mg O_3/L pH = 8.5 ± 0.3 Time: 20 min The specific energy applied to sludge was around 8000 kJ/kgTS The feed sludge was centrifuged at 6000 rpm and 25 °C	*Enterococcus*, *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*. *Thermotogae* Levofloxacin (LEVO) concentration 1 ± 0.7 ng/L	>96%	- *qnrA* - *qnrS*	- Both *qnrA* and *qnrS* declined by 1–1.5 log,	1 vs. 0: significant	(Zhao et al., 2020)
Drinking water treatment plant **- O_3 alone** **- O_3 → Cl**	Dose: 2–3 mg/L Bacteria (CFU/mL) **O_3 alone**: 420 ± 58 **O_3 → Cl**: 41 ± 11	*Pseudomonas* *Fusobacteriales* *Aeromonadaceae* *Mycobacterium* *Methylosinus* *Acinetobacter* Planctomyces	**O_3 alone** Not mentioned **- O_3 → Cl** *Mycobacterium*, *Methylosinus*, *Acinetobacter*, *Planctomyces*, and *Pseudomonas* were dramatically enriched after the O_3/Cl_2 disinfection, averagely accounting for 42.91% of the total total relative abundance ranged	A total of 297 ARGs within 17 types: *aac(2′)-I*, *aac(3)-II*, *aac(6′)-31*, *aac(6′)-I*, *aac(6′)-II*, *aad(6)*, *aadA*, *aadB*, *aadE*, *ant(2″)-I*, *ant(3″)-Ih-aac(6′)-IId*, *ant(9)-I*, *aph(2″)-Ie*, *aph(2″)-IV*, *aph(3′)-I*, *aph(3″)-I*, *aph(3‴)-III*, *aph(6)-I*, bifunctional aminoglycoside *N*-acetyltransferase and aminoglycoside phosphotransferase encoding gene, *bacA*, *bcrA*, *blaACT-1*, *blaACT-2*, *blaAER-1*, *ampC*, *blaCARB-12*, *blaCARB-6*, *cfxA2*, class A beta-lactamase--encoding gene, class B beta-lactamase-encoding gene, class C beta-lactamase-encoding gene, *blaCMY-1*, *blaCMY-11*, *blaCMY-19*, *blaCMY-51*, *blaCMY-74*, *blaCMY-78*, *blaCMY-9*, *blaCTX-M*, *blaCTX-M-1*, *blaCTX-M-15*, *blaCTX-M-53*,	**O_3 alone** (as %) Relative abundance of all ARGs (copy/cell) *aac(2′)-I*: 0.00, *aac(3)-II*: 0.00, *aac(6′)-31*: 0.00, *aac(6′)-I*: 77.86, *aac(6′)-II*: 54.76, *aad(6)*: 0.00, *aadA*: 62.16, *aadB*: 0.00, *aadE*: 47.06, *ant(2″)-I*: 57.14, *ant(3″)-Ih-aac(6′)-IId*: −50.00, *ant(9)-I*: 100.00, *aph(2″)-Ie*: 100.00, *aph(2″)-IV*: 100.00, *aph(3′)-I*: −114.29, *aph(3″)-I*: −97.53, *aph(3‴)-III*: −28.57, *aph(6)-I*: −157.63, bifunctional aminoglycoside *N*-acetyltransferase and aminoglycoside phosphotransferase encoding gene: 0.00, *bacA*: 55.19, *bcrA*: −300.00, *blaACT-1*: 0.00, *blaACT-2*: 0.00, *blaAER-1*: 0.00, *ampC*: 46.43, *blaCARB-12*: 100.00, *blaCARB-6*: 0.00, *cfxA2*: 66.67, class A beta-lactamase-encoding gene: 95.62, class B beta-lactamase-encoding gene:	*p*-Value for 1vs. 0: 0.00 2vs. 1: 0.93 2 vs. 0: $p < 0.05$	(Jia et al., 2020)

(continued on next page)

Table 2 (*continued*)

Medium, disinfection technique	Operational condition(s)	Name of bacterium or bacteria Name of antibiotic (s) + concentration	Removal of ARB (% or log)	Name of gene(s)	Removal of ARG (% or log)	Status[a]	Ref.
			from 195.49 ± 24.85 to 626.31 ± 38.61 copies of ARGs per cell.	*blaCTX-M-55*, *blaCTX-M-62*, *blaFEZ-1*, *blaFOX-1*, *blaFOX-2*, *blaFOX-3*, *blaFOX-4*, *blaFOX-5*, *blaFOX-7*, *blaFOX-8*, *blaFOX-9*, *blaGES-12*, *blaGES-13*, *blaGES-14*, *blaGES-15*, *blaGES-19*, *blaGES-21*, *blaGES-4*, *blaIMP*, *blaIMP-13*, *blaIMP-14*, *blaIMP-18*, *blaIMP-19*, *blaIMP-20*, *blaIMP-24*, *blaIMP-32*, *blaIMP-33*, *blaIMP-37*, *blaIMP-8*, *blaJOHN-1*, *blaLRA-9*, metallo-beta--lactamase-encoding gene, *blaMIR-1*, *blaMIR-6*, *blaMOX-1*, *blaMOX-2*, *blaMOX-3*, *blaMOX-4*, *blaMOX-5*, *blaMOX-6*, *blaMOX-7*, *blaNPS-1*, *blaOXA-1*, *blaOXA-10*, *blaOXA-118*, *blaOXA-119*, *blaOXA-12*, *blaOXA-129*, *blaOXA-13*, *blaOXA-141*, *blaOXA-142*, *blaOXA-147*, *blaOXA-15*, *blaOXA-162*, *blaOXA-163*, *blaOXA-181*, *blaOXA-19*, *blaOXA-2*, *blaOXA-20*, *blaOXA-205*, *blaOXA-209*, *blaOXA-21*, *blaOXA-211*, *blaOXA-212*, *blaOXA-247*, *blaOXA-251*, *blaOXA-28*, *blaOXA-29*, *blaOXA-3*, *blaOXA-309*, *blaOXA-333*, *blaOXA-334*, *blaOXA-4*, *blaOXA-47*, *blaOXA-48*, *blaOXA-5*, *blaOXA-53*, *blaOXA-54*, *blaOXA-56*, *blaPER-1*, *blaPER-2*, *blaPER-3*, *blaPER-4*, *blaPER-6*, *blaPER-7*, *blaPSE-1*, *blaSMB-1*, *blaTEM-1*, *blaTEM-117*, *blaTEM-118*, *blaTEM-178*, *blaTEM-187*, *blaTEM-209*, *blaTEM-6*, *blaTEM-63*, *blaTEM-75*, *blaTEM-86*, *blaTEM-89*, *blaTEM-91*, *blaTHIN-B*, *blaVEB-1*, *blaVEB-2*, *blaVEB-3*, *blaVEB-5*, *blaVEB-7*, *blaVEB-8*, cat chloramphenicol acetyltransferase-encoding gene, *catA*, *catB*, *catQ*, chloramphenicol and florfenicol exporter--encoding gene, chloramphenicol and florfenicol resistance gene, chloramphenicol exporter--encoding gene, *cmlA*, *floR*, *fosA*, *fosB*, *rosA*, *rosB*, *ksgA*, *ereA*, *ereB*, *erm(38)*, *erm(TR)*, *ermA*, *ermB*, *ermF*, *ermG*, *ermX*, *lnuB*, *macA*, *macB*, *mefA*, *mphA*, *mphB*, *vatB*, *vatG*, *acrA*, *acrB*, *acrF*, *adeB*, *adeC*, adeJ, *adeK*, *amrB*, *bcr*, *bpeF*, *ceoB*, *cmeB*, *emrA*, *emrB*, *EmrB-QacA* family major facilitator transporter-encoding gene, *emrD*, *emrE*, MFS transporter-encoding gene, *marR*, *mdfA*, *mdtA*, *mdtB*, *mdtC*, *mdtD*, *mdtE*, *mdtF*, *mdtG*, *mdtH*, *mdtK*, *mdtL*, *mdtM*, *mdtN*, *mdtO*, *mexA*, *mexB*, *mexC*, *mexD*, *mexE*, *mexF*, *mexI*, *mexT*, *mexW*, *mexX*, *mexY*, ABC transporter-encoding gene, multidrug transporter-encoding gene, *omp36*, *ompF*, *ompR*, *opmD*, *oprA*, *oprC*, *oprJ*, *oprM*, *oprN*, *pmrA*, *sdeY*, *smeB*, *smeE*, *tolC*, *arnA*, *mfpA*, *qepA*, *qnrA*, *qnrS*, ADP-ribosylating transferase--encoding gene, rifampin monooxygenase--encoding gene, sulI, *sulII*, *sulIII*, *tet32*, *tet35*, *tet36*, *tet39*, *tet40*, *tet41*, *tet44*, *tetA*, *tetC*, *tetE*, *tetG*, *tetH*, *tetJ*, *tetL*, *tetM*, *tetO*, *tetP*, *tetQ*, tetracycline resistance protein-encoding gene, *tetW*, *tetX*, *tetZ*, *dfrA1*, *dfrA12*, *dfrA14*, *dfrA15*, *dfrA16*, *dfrA17*,	−7.18, class C beta-lactamase-encoding gene: −41.22, blaCMY-1: 0.00, *blaCMY-11*: 0.00, *blaCMY-19*: 100.00, *blaCMY-51*: 100.00, *blaCMY-74*: 100.00, *blaCMY-78*: 100.00, *blaCMY-9*: 100.00, *blaCTX-M*: 100.00, *blaCTX--M-1*: 0.00, *blaCTX-M-15*: 42.86, *blaCTX-M-53*: 0.00, *blaCTX-M-55*: 0.00, *blaCTX-M-62*: 0.00, *blaFEZ-1*: 0.00, *blaFOX-1*: 0.00, *blaFOX-2*: −40.00, *blaFOX-3*: −9.09, *blaFOX-4*: −80.00, *blaFOX-5*: −20.41, *blaFOX-7*: 60.00, *blaFOX-8*: 100.00, *blaFOX-9*: −92.31, *blaGES-12*: 0.00, *blaGES-13*: 0.00, *blaGES-14*: 100.00, *blaGES-15*: 100.00, *blaGES-19*: 0.00, *blaGES-21*: 100.00, *blaGES-4*: 100.00, *blaIMP*: 0.00, *blaIMP-13*: 0.00, *blaIMP-14*: 0.00, *blaIMP-18*: 100.00, *blaIMP-19*: 0.00, *blaIMP-20*: 0.00, *blaIMP-24*: 0.00, *blaIMP-32*: 0.00, *blaIMP-33*: 0.00, *blaIMP-37*: 0.00, *blaIMP-8*: 0.00, *blaJOHN-1*: 100.00, *blaLRA-9*: 0.00, metallo-beta-lactamase-encoding gene: −41.67, *blaMIR-1*: 0.00, *blaMIR-6*: 0.00, *blaMOX-1*: 81.25, *blaMOX-2*: 0.00, *blaMOX-3*: 100.00, *blaMOX-4*: 0.00, *blaMOX-5*: 0.00, *blaMOX-6*: 100.00, *blaMOX-7*: 100.00, *blaNPS-1*: 100.00, *blaOXA-1*: 94.38, *blaOXA-10*: 84.85, *blaOXA-118*: 71.43, *blaOXA-119*: 0.00, *blaOXA-12*: 13.57, *blaOXA-129*: 100.00, *blaOXA-13*: 44.44, *blaOXA-141*: 0.00, *blaOXA-142*: 0.00, *blaOXA-147*: 100.00, *blaOXA-15*: 100.00, *blaOXA-162*: 0.00, *blaOXA-163*: −323.08, *blaOXA-181*: −71.43, *blaOXA-19*: 0.00, *blaOXA-2*: 100.00, *blaOXA-20*: 0.00, *blaOXA-205*: 0.00, *blaOXA-209*: −638.89, *blaOXA-21*: 97.01, *blaOXA-211*: 0.00, *blaOXA-212*: 0.00, *blaOXA-247*: −347.14, *blaOXA-251*: 0.00, *blaOXA-28*: 100.00, *blaOXA-29*: 0.00, *blaOXA-3*: 100.00, *blaOXA-309*: 0.00, *blaOXA-333*: −25.00, *blaOXA-334*: −100.00, *blaOXA-4*: 0.00, *blaOXA-47*: 71.43, *blaOXA-48*: 0.00, *blaOXA-5*: 100.00, *blaOXA-53*: 100.00, *blaOXA-54*: −137.84, *blaOXA-56*: 0.00, *blaPER-1*: 0.00, *blaPER-2*: 0.00, b*laPER-3*: 0.00, *blaPER-4*: 0.00, *blaPER-6*: 0.00, *blaPER-7*: 0.00, *blaPSE-1*: 100.00, *blaSMB-1*: 0.00, *blaTEM-1*: 100.00, *blaTEM-117*: 50.00, *blaTEM-118*: 100.00, *blaTEM-178*: 100.00, *blaTEM-187*: 0.00, *blaTEM-209*: 100.00, *blaTEM-6*: 0.00, *blaTEM-63*: 0.00, *blaTEM-75*: 100.00, *blaTEM-86*: 0.00, *blaTEM-89*: 100.00, *blaTEM-91*: 100.00, *blaTHIN-B*: 0.00, *blaVEB-1*: 96.74, *blaVEB-2*: 100.00, *blaVEB-3*: 100.00, *blaVEB-5*: 100.00, *blaVEB-7*: 100.00, *blaVEB-8*: 100.00, cat chloramphenicol acetyltransferase--encoding gene: −1140.00, *catA*: 100.00, *catB*: 39.78, *catQ*: 100.00, chloramphenicol and florfenicol exporter-encoding gene: 0.00, chloramphenicol and florfenicol resistance gene: 0.00,		

dfrA5, *dfrA7*, *dfrB1*, *dfrB2*, *dfrB3*, *dfrB6*, bacterial regulatory protein LuxR encoding gene, cAMP--regulatory protein-encoding gene, cob(*I*)alamin adenolsyltransferase-encoding gene, DNA--binding_protein_H-NS-encoding gene, rpsD, sdiA, thiostrepton resistance methylase tsnR encoding gene, cpxR, truncated putative response regulator *ArlR* encoding gene, *vanB*, *vanR*, *vanS*, *vanX*, *vanZ*

chloramphenicol exporter-encoding gene: 57.14, *cmlA*: 72.97, *floR*: −29.41, *fosA*: 0.00, *fosB*: 0.00, *rosA*: 55.56, *rosB*: −105.56, *ksgA*: 0.00, *ereA*: 91.36, *ereB*: 0.00, *erm*(38): 0.00, *erm(TR)*: 100.00, *ermA*: 100.00, *ermB*: 0.00, *ermF*: 90.63, *ermG*: 0.00, *ermX*: −17.39, *lnuB*: 100.00, *macA*: 0.00, *macB*: −103.53, *mefA*: 96.70, *mphA*: 94.59, *mphB*: 0.00, *vatB*: −120.00, *vatG*: 0.00, *acrA*: 0.00, *acrB*: −94.96, *acrF*: 0.00, *adeB*: −1100.00, *adeC*: 0.00, *adeJ*: −1750.00, *adeK*: 0.00, *amrB*: 33.33, *bcr*: 0.00, *bpeF*: −40.00, *ceoB*: 0.00, *cmeB*: 0.00, *emrA*: 0.00, *emrB*: −100.00, *EmrB-QacA* family major facilitator transporter-encoding gene: 0.00, *emrD*: 57.89, *emrE*: 25.49, MFS transporter-encoding gene: −850.00, *marR*: −1133.33, *mdfA*: −180.00, *mdtA*: 0.00, *mdtB*: −56.33, *mdtC*: −94.74, *mdtD*: 0.00, *mdtE*: 0.00, *mdtF*: −33.33, *mdtG*: 100.00, *mdtH*: 0.00, *mdtK*: −300.00, *mdtL*: −50.00, *mdtM*: 0.00, *mdtN*: 100.00, mdtO: 100.00, *mexA*: −90.91, *mexB*: −140.00, *mexC*: −613.33, *mexD*: −44.00, *mexE*: −61.11, *mexF*: −348.44, *mexI*: −1833.33, *mexT*: −572.86, *mexW*: −262.07, *mexX*: 0.00, *mexY*: 0.00, ABC transporter-encoding gene: −1223.33, multidrug transporter-encoding gene: −370.13, omp36: 0.00, *ompF*: 0.00, *ompR*: −38.26, *opmD*: 0.00, *oprA*: 0.00, *oprC*: 0.00, *oprJ*: 0.00, *oprM*: 0.00, *oprN*: 0.00, *pmrA*: 0.00, *sdeY*: 0.00, *smeB*: 100.00, *smeE*: 83.33, *tolC*: 3.13, *arnA*: −32.65, *mfpA*: 0.00, *qepA*: 0.00, *qnrA*: 0.00, *qnrS*: 42.11, ADP-ribosylating transferase-encoding gene: 82.50, rifampin monooxygenase-encoding gene: 0.00, *sulI*: 69.09, *sulII*: 73.12, *sulIII*: 0.00, *tet32*: 33.33, *tet35*: 100.00, *tet36*: 100.00, *tet39*: −380.00, tet40: 0.00, tet41: 100.00, tet44: 100.00, tetA: 68.62, *tetC*: 35.24, *tetE*: 52.57, *tetG*: −98.04, *tetH*: 60.00, *tetJ*: 100.00, *tetL*: 100.00, *tetM*: 60.00, *tetO*: 66.67, *tetP*: 26.83, *tetQ*: −100.00, tetracycline resistance protein--encoding gene: 26.09, *tetW*: 0.00, *tetX*: 90.97, tetZ: 0.00, *dfrA1*: 100.00, *dfrA12*: 100.00, *dfrA14*: 100.00, *dfrA15*: 0.00, *dfrA16*: 0.00, *dfrA17*: 100.00, *dfrA5*: −500.00, *dfrA7*: 0.00, *dfrB1*: 100.00, *dfrB2*: 0.00, *dfrB3*: −508.33, *dfrB6*: 0.00, bacterial regulatory protein LuxR encoding gene: 0.00, cAMP-regulatory protein-encoding gene: −22.62, cob(*I*)alamin adenolsyltransferase--encoding gene: 0.00, DNA-binding_protein_H--NS-encoding gene: 0.00, rpsD: 0.00, sdiA: 0.00, thiostrepton resistance methylase tsnR encoding gene: 0.00, *cpxR*: −245.45, truncated putative response regulator *ArlR* encoding gene: 0.00, *vanB*: 0.00, *vanR*: 0.00, *vanS*: 0.00, *vanX*: 0.00, *vanZ*: 0.00,

- $O_3 \rightarrow Cl$
Relative abundance of all ARGs (copy/cell)
aac(2′)-I: 100, *aac(3)-II*: 0.00, *aac(6′)-31*: 68.97, *aac(6′)-I*: 85.00, *aac(6′)-II*: 5.26, *aad(6)*: 0.00,

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Table 2 (*continued*)

Medium, disinfection technique	Operational condition(s)	Name of bacterium or bacteria Name of antibiotic (s) + concentration	Removal of ARB (% or log)	Name of gene(s)	Removal of ARG (% or log)	Status[a]	Ref.
					aadA: 64.71, *aadB*: 0.00, *aadE*: −811.11, *ant (2″)-I*: 100.00, *ant(3″)-Ih-aac(6′)-IId*: 100.00, *ant (9)-I*: 0.00, *aph(2″)-Ie*: 0.00 *aph(2″)-IV*: 0.00, *aph (3′)-I*: 100.00, *aph(3″)-I*: 100.00, *aph(3‴)-III*: 100.00, *aph(6)-I*: 96.71, bifunctional aminoglycoside *N*-acetyltransferase and amino-glycoside phosphotransferase encoding gene: 0.00, *bacA*: 71.73, *bcrA*: 100.00, *blaACT-1*: 0.00, *blaACT-2*: 0.00, *blaAER-1*: 100.00, *ampC*: 100.00, *blaCARB-12*: 0.00, *blaCARB-6*: 0.00, *cfxA2*: 100.00, class A beta-lactamase-encoding gene: 9.68, class B beta-lactamase-encoding gene: 100.00, class C beta-lactamase-encoding gene: 100.00, *blaCMY-1*: 100.00, *blaCMY-11*: 0.00, *blaCMY-19*: 0.00, *blaCMY-51*: 0.00, *blaCMY-74*: 0.00, *blaCMY-78*: 0.00, *blaCMY-9*: 0.00, *blaCTX-M*: 0.00, *blaCTX-M-1*: 100.00, *blaCTX-M-15*: 100.00, *blaCTX-M-53*: 0.00, *blaCTX-M-55*: 0.00, *blaCTX--M-62*: 0.00, *blaFEZ-1*: 0.00, *blaFOX-1*: 100.00, *blaFOX-2*: 100.00, *blaFOX-3*: 100.00, *blaFOX-4*: 100.00, *blaFOX-5*: 100.00, *blaFOX-7*: 100.00, *blaFOX-8*: 0.00, *blaFOX-9*: 100.00, *blaGES-12*: 0.00, *blaGES-13*: 0.00, *blaGES-14*: 0.00, *blaGES-15*: 0.00, *blaGES-19*: 0.00, *blaGES-21*: 0.00, *blaGES-4*: 0.00, *blaIMP*: 0.00, *blaIMP-13*: 0.00, *blaIMP-14*: 0.00, *blaIMP-18*: 0.00, *blaIMP-19*: 0.00, *blaIMP-20*: 0.00, *blaIMP-24*: 0.00, *blaIMP-32*: 0.00, *blaIMP-33*: 0.00, *blaIMP-37*: 0.00, *blaIMP-8*: 0.00, *blaJOHN-1*: 0.00, *blaLRA-9*: 0.00, metallo-beta-lactamase--encoding gene: 94.57, *blaMIR-1*: 0.00, *blaMIR-6*: 0.00, *blaMOX-1*: 100.00, *blaMOX-2*: 0.00, *blaMOX-3*: 0.00, *blaMOX-4*: 100.00, *blaMOX-5*: 0.00, *blaMOX-6*: 0.00, *blaMOX-7*: 0.00, *blaNPS-1*: 0.00, *blaOXA-1*: −720.00, *blaOXA-10*: 100.00, *blaOXA-118*: 100.00, *blaOXA-119*: 100.00, *blaOXA-12*: 95.49, *blaOXA-129*: 0.00, *blaOXA-13*: 100.00, *blaOXA-141*: 0.00, blaOXA-142: 0.00, *blaOXA-147*: 0.00, *blaOXA-15*: 0.00, *blaOXA-162*: 100.00, *blaOXA-163*: 100.00, *blaOXA-181*: 100.00, *blaOXA-19*: 0.00, *blaOXA-2*: 0.00, *blaOXA-20*: 0.00, *blaOXA-205*: 0.00, *blaOXA-209*: 88.72, *blaOXA-21*: 100.00, *blaOXA-211*: 100.00, *blaOXA-212*: 100.00, *blaOXA-247*: 98.08, *blaOXA-251*: 0.00, *blaOXA-28*: 0.00, *blaOXA-29*: −50.00, *blaOXA-3*: 0.00, *blaOXA-309*: 100.00, *blaOXA-333*: 100.00, *blaOXA-334*: 100.00, *blaOXA-4*: 0.00, *blaOXA-47*: 100.00, *blaOXA-48*: 100.00, *blaOXA-5*: 0.00, *blaOXA-53*: 0.00, *blaOXA-54*: 100.00, *blaOXA-56*: 0.00, *blaPER-1*: 0.00, *blaPER-2*: 100.00, *blaPER-3*: 0.00, *blaPER-4*: 0.00, *blaPER-6*: −175.00, *blaPER-7*: 0.00, *blaPSE-1*: 0.00, *blaSMB-1*: 100.00, *blaTEM-1*: 0.00, *blaTEM-117*: −75.00, *blaTEM-118*: 0.00,		

blaTEM-178: 0.00, *blaTEM-187*: −20.00, *blaTEM-209*: 0.00, *blaTEM-6*: 100.00, *blaTEM-63*: 0.00, *blaTEM-75*: 0.00, *blaTEM-86*: 100.00, *blaTEM-89*: 0.00, *blaTEM-91*: 0.00, *blaTHIN-B*: 100.00, *blaVEB-1*: 47.62, *blaVEB-2*: 0.00, *blaVEB-3*: 0.00, *blaVEB-5*: 0.00, *blaVEB-7*: 0.00, *blaVEB-8*: 0.00, cat chloramphenicol acetyltransferase-encoding gene: 48.39, *catA*: 0.00, *catB*: 85.80, *catQ*: 0.00, chloramphenicol and florfenicol exporter-encoding gene: 100.00, chloramphenicol and florfenicol resistance gene: 0.00, chloramphenicol exporter-encoding gene: 33.33, *cmlA*: 100.00, *floR*: 100.00, *fosA*: 0.00, *fosB*: 0.00, *rosA*: 100.00, *rosB*: 83.78, *ksgA*: −50.00, *ereA*: 69.23, *ereB*: 0.00, *erm(38)*: 100.00, *erm (TR)*: 0.00, *ermA*: 0.00, *ermB*: 0.00, *ermF*: −177.78, *ermG*: 100.00, *ermX*: 55.56, *lnuB*: 0.00, *macA*: −400.00, *macB*: 32.95, *mefA*: −3100.00, *mphA*: 100.00, *mphB*: 0.00, *vatB*: 100.00, *vatG*: 100.0, *acrA*: 0.00, *acrB*: 77.16, *acrF*: 50.00, *adeB*: 75.00, *adeC*: −1180.00, *adeJ*: −2102.70, *adeK*: −14,100.00, *amrB*: 25.00, *bcr*: 0.00, *bpeF*: 100.00, *ceoB*: 100.00, *cmeB*: −100.00, *emrA*: 33.33, *emrB*: −6712.50, *EmrB-QacA* family major facilitator transporter-encoding gene: 0.00, *emrD*: 100.00, *emrE*: −39.47, *MFS* transporter-encoding gene: −521.05, *marR*: −2532.43, *mdfA*: −3857.14, *mdtA*: 0.00, *mdtB*: 53.85, *mdtC*: 18.92, *mdtD*: 100.00, *mdtE*: 100.00, *mdtF*: 100.00, *mdtG*: 0.00, *mdtH*: 0.00, *mdtK*: −6950.00, *mdtL*: 100.00, *mdtM*: 0.00, *mdtN*: 0.00, *mdtO*: 0.00, *mexA*: 76.19, *mexB*: 62.50, *mexC*: 71.96, *mexD*: 100.00, *mexE*: 58.62, *mexF*: −10.28, *mexI*: 100.00, *mexT*: −415.29, *mexW*: −41.43, *mexX*: 0.00, *mexY*: 100.00, ABC transporter-encoding gene: 86.90, multidrug transporter-encoding gene: −28.52, *omp36*: 100.00, *ompF*: 0.00, *ompR*: −1060.38, *opmD*: 100.00, *oprA*: 100.00, *oprC*: 70.00, *oprJ*: 0.00, *oprM*: −1921.43, *oprN*: −96.55, *pmrA*: 100.00, *sdeY*: 0.00, *smeB*: 0.00, *smeE*: 100.00, *tolC*: 41.94, *arnA*: 9.23, *mfpA*: 0.00, *qepA*: 0.00, *qnrA*: 100.00, *qnrS*: 100.00, ADP-ribosylating transferase-encoding gene: 100.00, rifampin monooxygenase-encoding gene: 0.00, *sulI*: 67.78, *sulII*: 76.52, *sulIII*: 0.00, tet32: −700.00, *tet35*: 0.00, *tet36*: 0.00, *tet39*: 66.67, *tet40*: 0.00, *tet41*: 0.00, *tet44*: 0.00, tetA: 67.03, tetC: 100.00, *tetE*: 100.00, *tetG*: 100.00, *tetH*: 100.00, *tetJ*: 0.00, *tetL*: 0.00, *tetM*: −250.00, *tetO*: −1900.00, *tetP*: −203.33, *tetQ*: −150.00, tetracycline resistance protein-encoding gene: 70.59, *tetW*: −700.00, *tetX*: 51.22, *tetZ*: 0.00, *dfrA1*: 0.00, *dfrA12*: 0.00, *dfrA14*: 0.00, *dfrA15*: 100.00, *dfrA16*: 100.00, *dfrA17*: 0.00, *dfrA5*: 100.00, *dfrA7*: 0.00, *dfrB1*: 0.00, *dfrB2*: 100.00, *dfrB3*: 100.00, *dfrB6*: 100.00, bacterial regulatory protein *LuxR* encoding gene: 100.00, *cAMP*-regulatory protein-encoding gene: 100.00, *cob(I)*alamin adenolsyltransferase--encoding gene: 0.00,

(continued on next page)

Table 2 (*continued*)

Medium, disinfection technique	Operational condition(s)	Name of bacterium or bacteria Name of antibiotic (s) + concentration	Removal of ARB (% or log)	Name of gene(s)	Removal of ARG (% or log)	Status[a]	Ref.
					DNA-binding_protein_H-NS-encoding gene: 100.00, *rpsD*: 100.00, *sdiA*: 100.00, thiostrepton resistance methylase *tsnR* encoding gene: 100.00, *cpxR*: −97.37, truncated putative response regulator *ArlR* encoding gene: 0.00, *vanB*: 0.00, *vanR*: −92.00, *vanS*: 100.00, *vanX*: 0.00, *vanZ*: 100.00,		
WWTP **- O_3 alone** **- UV → O_3** **- UV → O_3 → Cl**	Large scale Secondary effluent Dose = 3.5 mg/L Time = 25 min. the effect of ozonation was investigated for two gene types: Cell-associated and Cell-free fractions	–	–	- *sulII* - *tetC* - *blaPSE-1* - *ermB*	**Mean** ozonation effect on cell-associated, Cell-free, and total fractions, respectively: (log) **- O_3 alone** - *tetC*: 0.01, 0.57, 0.04 - *sulII*: −0.13, 0.19, −0.13 - *ermB*: −0.63, 0.03, −0.07 -*blaPSE*-1: 0.47, 0.65, 0.49 **UV → O_3** - *tetC*: 2.75, 1.76, 2.74 - *sulII*: 2.55, 1.87, 2.81 - *ermB*: 3.38, 0.44, 2.81 -*blaPSE-1*: 2.82, 2.13, 2.80 **UV → O_3 → Cl** - *tetC*: 3.54, 2.06, 3.51 - *sulII*: 3.68, 2.68, 3.67 - *ermB*: 4.14, 0.36, 2.84 -*blaPSE*-1: 3.21, 2.04, 3.12	1 vs. 0: significant 2 vs. 1: significant 3 vs. 1: significant	(Zhang et al., 2018)
PWS and WMS Pre-treatment of sludge with: **- O_3 alone** **- O_3 + AD**	Batch scale Dose: 0.1 g O_3/g TS Fow rate was 2 L/min with an ozone concentration of ~9 mg/L. The ozone transfer efficiency was over 90%.	No Bactria Oxytetracycline: 40.7–170.2 mg/kg in PWS sludge	–	- tet(A) - tet(G) - tet(Q) - tet(W) - tet(X) - intI1	Log (Concentration, Copies/g) reduction for PWS and MWS, respectively: **- O_3 alone** - 0.07, 0.55 - 0,17, 0.57 −0.04, 1.03 - -0.13, 0.66 −0.05, 0.81 −0.01, all **O_3 + AD** - 0.67, 0.78 - 1.19, 1.35 - -1.27, 0.95 - -0.28, 0.77 - 1.64, 1.48 - 0.7, all	*p*-Value for: 2 vs. 1: <0.05 except for *tet (Q)* (both sludges) *tet(W)* (PWS sludge)	(Pei et al., 2016)
WWTP - **O_3 alone** - **O_3+ Sand Filtration** - **O_{3+} (GAC)** - **O_3+ Sand Filtration + GAC**	Pilot scale **- O_3 alone** Dose: 0.73 mg O_3/mg DOC Time: 20 min **- O_3+ Sand Filtration** sand filter (back-flush interval 43 h) contained 0.3 m gravel support layer, a 0.6 m sand and a 0.8 m hydroanthracite layer **- O_{3+} GAC** GAC filter (back-flush	*E. coli*, *enterococci* and *staphylococci* Panel of antibiotic	**- O_3 alone** *E. coli*: ~3.5 log *Enterococci*: ~4.2 log *Staphylococci*: ~3.7 log **- O_3+ Sand Filtration** *E. coli*: ~3.8 log *Enterococci*: ~3.4 log *Staphylococci*: ~4.4 log **- O_{3+} (GAC)** *E. coli*: ~3.8 log *Enterococci*: ~3.6 log	–	–	1 vs. 0: significant 2 vs. 1: Insignificant 3 vs. 1: Insignificant 4 vs. 1: Insignificant	(Lüddeke et al., 2015)

	interval 5 h) contained 0.3 m gravel support layer and a 2.2 m charcoal layer				*Staphylococci*: ~4.5 log		
	- O_3+ Sand Filtration + GAC The combined sand/GAC filter contained 0.6 m sand and 2.2 m GAC				**- O_3+ Sand Filtration + GAC** *E. coli*: ~3.5 log *Enterococci*: ~3.6 log *Staphylococci*: ~4.7 log		
Urban wastewater and surface water	Scale: laboratory continuous mode Dose: 50 g/Nm^3 Flow rate: 15 Ncm^3/min	*Enterococci* & *Enterobacteria* ciprofloxacin (4 mg/L), gentamicin (16 mg/L) meropenem (4 mg/L)	>99%	multi-resistance gene		3 log to limit of quantification	1 vs. 0: significant	(Moreira et al., 2016)
-Photocatalytic ozonation (O_3 + UV + TiO_2)							

Abbreviations: - CT: concentration × time, WWTP: wastewater treatment plant, MWTP: Municipal wastewater treatment plant, MOs: microorganisms, HWW: Hospital wastewater, US: ultrasound, AD: anaerobic digestion, PWS: pharmaceutical waste sludge, and MWS: Municipal waste sludge, GAC: Granulated Activated Charcoal.
LOD: limit of detection; LOQ: limit of quantification; N.A.: not applicable.
[a] The stated differences between the methods applied. For comparison 0 implies on no disinfection and 1, 2, 3, and 4 imply on the first, second, third, and fourth applied methods at the first columns, respectively.
[b] Scales are as batch, bench, pilot, and full scales. From the place of performing are as laboratory or full (in the place) scales.

growth stage, type of cell envelope, repairing ability, and the type of viability indicator employed. Interfering agents present in the aqueous medium, such as dissolved organic matters and flocs or particles can diminish the stability of O_3 or protect MOs from exposure to ozone, thus disrupt the disinfection performance (Czekalski et al., 2016). The effects of these agents are discussed in the next sections. Nevertheless, no exact mechanism is known for ozone disinfection yet. And although damage of cell membranes is assumed even before nucleoids damage, it has been acclaimed that cell lysis might not be the foremost inactivation mechanism. Besides, the damages induced by O_3 can be repaired because of the high activity of the related cellular enzymes. Therefore, it appears more studies are required to understand ozone inactivation mechanisms especially for ARB and ARGs (Hiller et al., 2019).

3.4. Denaturation or repairment?

Disinfection efficiency is not identical to destroying the integrity of DNA molecules (known as denaturation) and any inactivation of the genes in a disinfection process can be reversible, and the genome can be repaired by several mechanisms when the disinfection stress is relieved (Pei et al., 2019). Therefore, it is of great importance to carry regrow ability and its extent in this pivotal process (Moreira et al., 2016). Unfortunately, repairment cannot also be ignored after disinfection with O_3, as it has been proved that living cells may still present in the treated media even after an extended time of reactions (Iakovides et al., 2019). About ozonation, some studies showed repairment for some bacteria/genes and not for others. For example, (Zheng et al., 2017) declared that though after storage total heterotrophic and bacteria resistant to ciprofloxacin, gentamicin, and meropenem as well as the genes of 16S rRNA and *intI1* returned to values close to the preliminary levels, this didn't occur for ARGs of *blaTEM*, *qnrS*, and *sul* even after three days storing of the treated aqueous solution. Moreover, DNase I processing trials showed that it produced a large number of free DNA containing ARGs after the inactivation of bacteria. (Sousa et al., 2017) reported a removal efficiency of 2 log for cultivable fungi, 16S rRNA, and *intI1* genes; ~3–4 log for total *heterotrophs*, *enterobacteria*, and *enterococci*; and close or below the detection limits for ARGs, over a time of 30 min. However, most of the mentioned populations, 16S rRNA, and *intI1* genes, along with ARGs (except *qnrS*) returned to the preliminary levels after three days, indicating a transitory inactivation. However, owning higher microbial diversity and more complicated interaction, real wastewater might experience more intense conditions from a regrowth point of view. Indeed, in the stored real medium, the abundance of total *heterotrophs*, *enterobacteria*, and fungi was found to be as same or above as the pre-ozonation levels. In agreement with the culture-dependent data, the genes of 16S rRNA and *blaTEM* almost returned to their initial values and *intI1* and *sul1* to about 1 log-unit below pre-ozonation levels after storage, suggesting that the process left viable cells which can regrow after relieving the stress (Sousa et al., 2017). Nevertheless, some literatures reported no repairment for the studied bacteria/genes. For example, Moreira et al. (2016) observed no evidence of regrowth of the host, as inferred from the absence of an increase in the relative abundance of the respective ARGs after the 3-days storage of treated aqueous medium at room temperature. Nevertheless, both culture-based techniques (dependent & independent) proved that part of the MOs or genes are viable and able to repair or regrow, whose potential risk is still needed to be assessed.

3.5. Antibiotic resistant bacteria or resistance genes; this is a problem!

It is well-identified that the multiple obstacles employed in W & WWTPs, especially with the bitter end of disinfection, result in mortality of 90–99% of the MOs, which in turn can cause releasing a huge amount of dead and broken MOs as well as extracellular DNA (eDNA) into the environment receiving the discharged effluents (Sivalingam et al.,

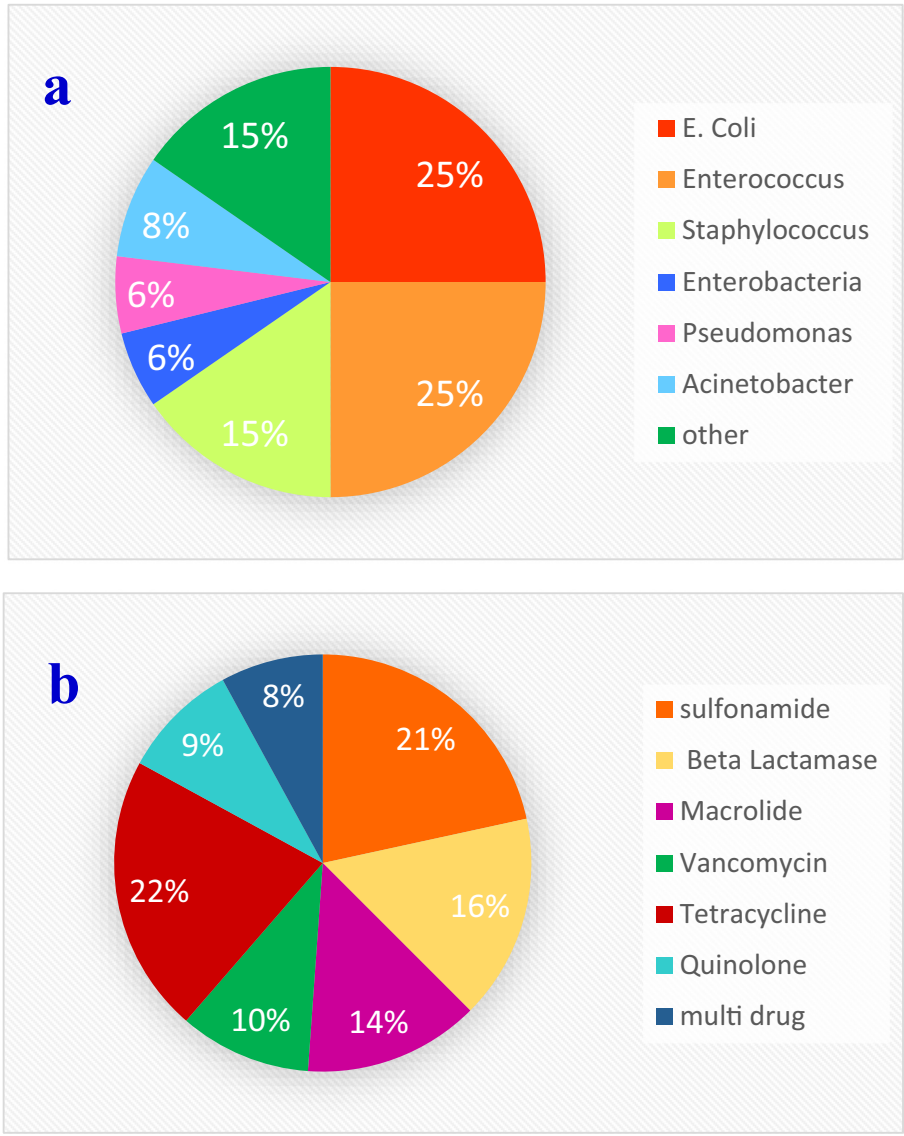

Fig. 2. The frequency of studied a) ARB and b) ARGs classes removal using ozonation-based processes.

2020). In fact, inactivation of MOs during disinfection may not assurance removal of intracellular ARGs even where 4 log removal of ARB was achieved (Sharma et al., 2016). Unfortunately, cell-free ARGs in WWTPs' effluents is known as an important contamination type of ARGs (Zhang et al., 2018). Therefore, removing these orphaned genes is of great importance and a turning point in disinfection.

As explained in the ozone disinfection mechanism (Section 3.6), O_3 likely inactivates ARB cells by damaging their own envelope well before reaching to ARGs. Hence, it can be expected that ARGs deactivation face delays behind ARB inactivation, though even after which ARGs may remain active and able to repair themselves as discussed before (Dodd, 2012). In fact, even when significant removal of bacteria is achieved, the results cannot be applicable for their respective genes. For example, Zheng et al. (2017) reported that a great number of free DNA after the appropriate inactivation of MOs by O_3 disinfection. In addition, Alexander et al. (2016), Czekalski et al. (2016), and Xu et al. (2016) showed a limited ARGs removal in ozonated wastewater and water, respectively.

3.6. Operational parameters

Several parameters are suspected to be effective in a process and the actual performance in the reduction of ARB and/or ARGs depends on the operational situations, such as (waste)water characteristics, O_3 dose, and reaction time (Ben et al., 2017; Luczkiewicz et al., 2011). The former include pH, alkalinity, organic carbon content as either total or dissolved, bicarbonate and carbonate concentrations, and particles (as total suspended solids (TSS) and turbidity) (Association, 2011). Besides, effects of humic acids (Pak et al., 2016) and the presence of flocs in an aqueous medium (Czekalski et al., 2016) especially in low concentrations are also important (Yang et al., 2019a). Ozone dose and contact time can be adapted to increase elimination effects on bacteria carrying ARGs (Jager et al., 2018). When dealing with microbes, the importance of the type of bacteria and type of genes are added in the mentioned list (Czekalski et al., 2016; Wang and Zhuan, 2020).

3.6.1. Ozone dose & contact time: the concept of CT

For an effective deactivation of ARB and/or ARGs a minimum dose is needed. In fact, above a given level, ozone can destroy the proteins and intensify the permeability of cell walls, resulting in its deep penetration into the cytoplasm and destroying ARGs. It is well known that 90% of the ARB and ARGs can be eliminated by a 3 mg/L ozone concentration (Hu et al., 2019). However, the ozone-dose effect is controversial, while some researchers declared that increasing ozone dose improve the

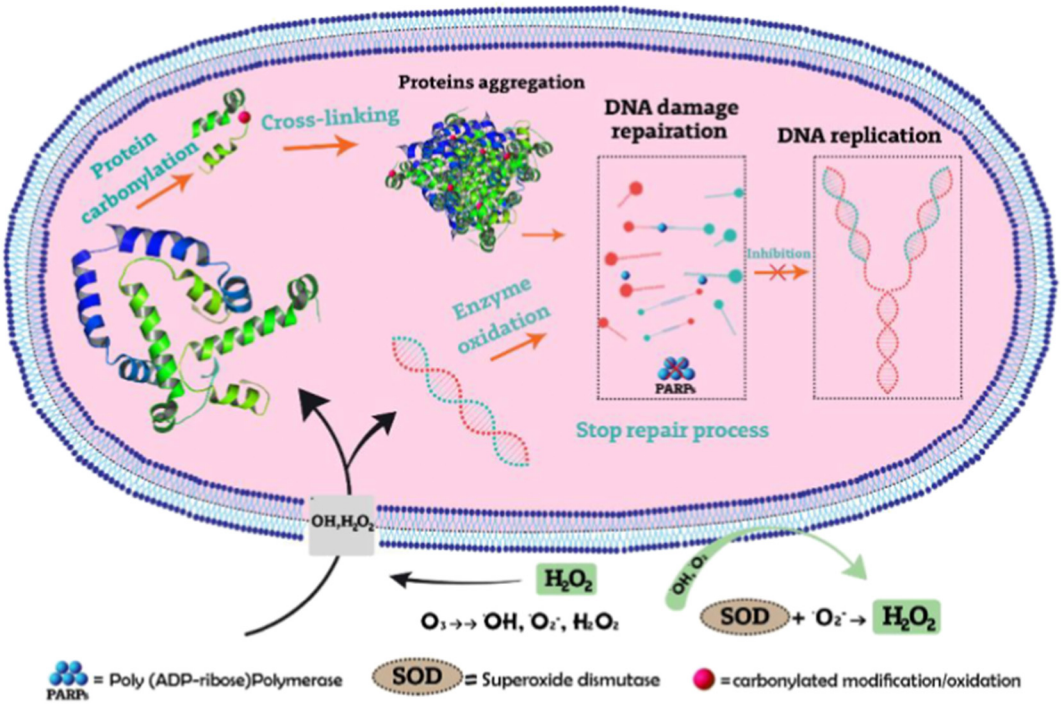

Fig. 3. The general ozone mechanism of action for removal of MOs.

removal efficiency (Zhuang et al., 2015), others argue that the ability to eliminate ARGs was not dramatically improved by increasing the ozone dose (Zheng et al., 2017). This is attributed to ozone's strong oxidizing ability which results in its reaction with a variety of cell material and reduces the opportunity to target DNA or ARGs (Zheng et al., 2017). This can also be due to accelerated self-decomposition of ozone at a high concentration (Macauley et al., 2006). The first group, however, acclaimed from the appreciable reactivity of double-stranded DNA (dsDNA) toward O_3, that the deactivation of ARGs could be enhanced by an order of 1–2 at ozone dose and contact time (*CT*) ranges employed in water disinfection (i.e. 0.1–1 mg/L.min at 25 °C) compared to very low O_3 *CTs* needed to achieve MOs inactivation (Dodd, 2012). Nonetheless, since the existence of orphaned genes in aquatic matrices might intensify the public health risk as they can transform via horizontal gene transfer (HGT) mechanism special attention need to be paid to the irreversible removal of ARGs (Tan et al., 2019). As such, there are a few reports on the effect of O_3 on ARGs and their recovery after treatment.

For addressing these controversial results, more attention needs to be paid to both ozone dose and contact time. Some studies reported that from two main parameters of ozone dose and contact time, it is operationally possible and even better to maneuver more on the latter instead of increasing ozone dose which imposes lateral side effects such as the generation of the carcinogenic by-product as bromate or nitrosamines (Hiller et al., 2019). For example, (Iakovides et al., 2019) showed that with increasing the retention time to 40 min at a low ozone concentration (i.e. 0.25 gO_3/g dissolved organic carbon, DOC) the ARGs reduction was the same as that of applying the higher concentration (i.e. 0.75 gO_3/gDOC). These findings suggested that a proper reaction time would result in the desired disintegration values of the examined ARGs, even with low O_3 concentrations. These can be attributed to the opportunity for interaction between O_3 and OH• with the naked DNA after the destruction of the bacteria cell surface (or the opportunity for O_3 and OH• to interact with the naked DNA after the destruction of the bacteria cell surface) (Iakovides et al., 2019). It should be noted that the concept of *CT* is in turn highly affected by the specific aqueous solution ingredients such as DOC, suspended solids (SS), residual nitrite concentrations, and particulate matter as they can cause a rapid consumption of O_3 and failed performance, which will be discussed in the following sections (Hiller et al., 2019). Another important consideration is that the practical application of *CT* concept is still challenging because it is currently used for disinfection of routine MOs and not for antibiotic resistant species in the (waste)water treatment procedures (Pak et al., 2016).

3.6.2. Solids in forms of SS and natural organic matter (NOM)

Apart from the shielding of MOs, solids can scavenge various types of radicals generated by ozonation. In fact, it seems that SS is first disintegrated into at first high- and then low-molecular-weight compounds when exposed with O_3 which results in decreasing its disinfection efficiency (Pak et al., 2016). The presence of NOM in water bodies is always problematic for every operational or process units in water and wastewater treatment, especially in disinfection. Once ozone is used as a disinfectant, its main part is preferentially consumed by NOM present in lagoon samples especially at low ozone doses (Macauley et al., 2006). However, when it is applied as an oxidant (e.g. pre-oxidation with ozone unit) it can positively reduce NOM as reflected by the values of COD and DOC (Hu et al., 2019). Nevertheless, some studies have reported a positive effect of humic acid (HA) in the removal of ARB and/or ARGs. For example, Pak et al. (2016) observed a higher elimination in antibiotic resistance at higher HA concentrations. In fact, O_3 disintegration can be started by substances that are able to induce the formation of a superoxide anion (O_2^-), hydroxide ions, peroxides, ferrous iron, or humic substances. The latter is also identified as promoters, by which O_2^- can be regenerated from OH•. Therefore, humic substances can integrate with O_3 and initiate the formation of OH•, and therefore, improve the oxidative effect of the process. The presence of flocs and biofilms are also reported that can decrease the stability of O_3 or shield MOs from its exposure (Czekalski et al., 2016).

3.6.3. Alkalinity

Apart from NOM, the presence of alkalinity may inhibit chain reactions responsible for the generation of OH• radical from ozone decomposition (Deng, 2020). Carbonate and bicarbonate (HCO_3 and CO_3^-, respectively), are the main alkalinity agents which react with OH• and

then increase the ozone lifetime, thereby they slow down the chain reaction mechanism. Besides, the radical-scavenging activity of the CO_3^- species enhances at higher pH values, as carbonate has more scavenging ability in comparison with bicarbonate. Therefore, the more rapid velocity of the hydroxide-originated initiation reaction at higher pH values would be decreased in parallel when alkalinity is high. Since hydroxyl radicals which are known as one of the strongest chemical oxidants ($E^0_{red} = 1.78$), its production is operationally pivotal and its quenching agents should be regarded before generalization of the results from experiments to real scale application (Association, 2011). Nevertheless, in alkaline aqueous solutions, this phenomenon leads to retaining an ozone residual for longer periods which is important from a disinfection point of view.

3.6.4. pH

pH is regarded as a main effective parameter on ozone efficiency for removing ARB & ARGs by several studies. The effect of pH on the disinfection performance of O_3 is multifaceted. It is recognized that O_3 decomposition rate rises when the concentration of OH• increases (Eq. (6)) which then react with O_3 molecules and results in producing OH• (Eq. (7)).

$$O_3 + OH^- \rightarrow HO_2^- + O_2 \quad (6)$$

$$O_3 + HO_2^- \rightarrow {}^\circ OH + O^\circ{}_2^- + O_2 \quad (7)$$

Since in the above-mentioned reactions, OH• produces which have a stronger oxidation potential (2.86 V) than O_3 (2.07 V), the influence of pH on MOs inactivation is a trade-off status; raised pH reduces the O_3 concentration but generates more radical species. Therefore, the net influence of pH is not fully understood or accepted (Pak et al., 2016). Besides, as mentioned before, the effect of other confounders (such as alkalinity agents) on ozone performance can be influenced by pH. In the United States Environmental Protection Agency (USEPA) *CT* tables, the efficacy of O_3 residuals is viewed independently from pH. Because the influence of pH on O_3 capability for MOs inactivation seems to be predominantly related to changing the stability of its residual, though further investigations are needed (Association, 2011). Therefore, it seems that more studies are required to explain pH effectiveness on the removal of ARB & ARGs by ozonation.

3.6.5. Microbial types or their respective genes

Depending on the ARB species or these related ARGs, different extents of resistance is imagined in confronting oxidative stress such as ozone (Alexander et al., 2016). It is reported that antibiotic resistant bacteria (such as *E. coli* and *staphylococci*) survived better than the sensitive strains after ozone treatment. Moreover, the reduced abundance of antibiotic resistant *enterococci* after treatment may be explained by a different O_3 sensitivity for the species: *Enterococcus faecium* and *Enterococcus faecalis* reported to be more sensitive than other *Enterococcus*-species (Lüddeke et al., 2015). The extent of resistance strongly depends on several parameters, including but not limited to growth stage, type of cell envelope, repairing ability, and the type of viability-indicator method employed (e.g., culture-based against others), abatement, and intracellular ARGs disruption (Czekalski et al., 2016). The latter can also be attained by considering higher ozone doses (Czekalski et al., 2016). Investigation of the cell membrane permeability with 1-*N*-phenylnaphthylamine (NPN) showed increased uptake of NPN after ozonation in *E. coli* strains, indicating that the structure of the outer membrane is important for permeability relative to ozone (Patil et al., 2011). Moreover, it is supposed that the microbial cells experience the lowest and highest inactivation rates at the stationary and exponential growth phases, respectively. The results, however, can not be generalized but indicates different inactivation rates during various stages of growth (Cherchi and Gu, 2011). The diversity in resistance is more highlighted for genes (Paulus et al., 2019). For example, it becomes obvious that ozone affected the abundances of *vanA* and *blaVIM* quite differently relative to the *ermB* resistance gene (Alexander et al., 2016). This can be attributed to potential transfers of these highly abundant genes induced by oxidative stress. Besides, the species-specific quantification discovered different levels of susceptibilities from high (*enterococci*) to low (*P. aeruginosa*), representing different mechanisms for coping with the resistance.

3.7. Other important considerations

3.7.1. Other confounders in performance evaluation

Apart from the above-discussed effective parameters on ozone performance for removing ARB & ARGs, the reported removal efficiencies for ozone may not be realistic even for the controlled, optimized conditions of experiments. This is mainly because of the culture-based ARB detection method as well as ARGs transformation. During ozonation, a number of reactive oxygen species including H_2O_2 can be generated which causes bacteria to be in the status of "viable but non-culturable", therefore, they may not be distinguished by culture-based procedures despite their existence and the ability for transferring their respective ARGs (Pak et al., 2016). The application of culture-independent procedures is essential for the fact that most of MOs in aqueous solutions are not cultivable, and also the advanced treatment alternatives could increase the fraction of non-cultivable populations, (Moreira et al., 2016).

Apart from the mentioned considerations, the outcomes obtained by the reviewed studies (Tables 1 and 2) may be still hard to be judged and compared. Because in addition to the lack and/or heterogeneity of the scientific information (for instance different target micro contaminants, experimental situations, etc.), the results have not been homogenized for performance unit expression; while some studies have been reported removal as the only absolute abundance, others normalized the data to the quantity of 16S rRNA gene (i.e. relative abundance) and remain as both of them. These make it difficult to accurately evaluate the ozonation efficiency.

3.7.2. Side effects

Another consideration in using ozone as a disinfectant agent for the removal of ARB & ARGs is its adverse side effects. For instance, the potential mutation of nucleoids after ozone contact and toxic transformation products (such as bromate and nitrosamines) are of great importance. Although addressing some strategies such as biological filtration with a medium of sand or activated carbon after disinfection with ozone is found to be beneficial, they bear the risk of microbial regrowth of ARGs (Jager et al., 2018). Increasing the formation potential (FP) of trihalomethanes (THMs) and haloacetic acids (HAAs) especially in low concentrations of ozone (0.5 mg/L) has also been reported by Li et al. (2018) who attributed this to the increased content of reactive organic compounds. At the higher ozone dose (i.e. 2.5 mg/L) THMsFP was smaller mainly due to its effective performance in oxidizing the reactive organic compounds. However, when ozonation is integrated with biological activated carbon filtration, the FP of the mentioned by-products is reduced even more than when BAC is used alone. The authors acclaimed that although ozone enhances the contents of THMsFP and HAAsFP, it also increases the biodegradability of the maternal organic compounds; thus, contributing to their removal in the subsequent treatment by BAC filtration.

4. Conclusions and perspectives

The current study comprehensively reviewed the literature published on the removal of ARB & ARGs from aqueous environments using ozone-based disinfection processes. Although the results of the assessed studies were relatively scarce and not fully consistent, it seems that ozonation can be considered as a potential option in removing ARB & ARGs. However, there is still a significant knowledge

deficiency so that the results cannot be generalized and used in real scale treatment systems. In fact, a comprehensive investigation of the process is required for being able to have confident results for scaling up the results. As discussed, the application of ozone for ARB & ARGs suffers from several major operation and process obstacles for upscaling which are summarized as follow:

- First of all, the best process needs to be recognized (ozone alone or in combination with other techniques, for the latter option the question that may arise is which technique?). Afterwards, for the results that the scale-up is allowed, the following considerations should be regarded: the role of operation/process parameters including ozone dose and reaction time (*CT* value), pH, alkalinity, etc., and secondary parameters such as the presence of SS & NOM, antibiotic residual (not only related but also unrelated antibiotics), and other ARB, ARGs and stressors should be fully understood and optimized for different types of water or wastewater media. Moreover, the type of MOs containing the resistance and their detection methods should be considered.
- Repairment after treatment is a crucial challenge that should be kept in mind for scaling up the process.
- If determined that higher doses of ozone than normal values are needed to achieve enough deactivation or for preventing repairment, the challenges of by-products, in particular, bromate must carefully be considered.
- After optimizing for the critical factors, biological safety and toxicity assessment should be passed and the process must give acceptable mineralization.
- All the findings of optimization experiments should be tested and passed for real water or wastewater matrices in which numerous agents exist and can influence the ozone-based process and their roles are neither fully understood nor even investigated. For instance, the suggested *CT* values in the reviewed studies might not be practically attainable to avoid ARB to spread and plasmid which still is able to transfer to microorganisms.
- The results reported until now are mainly on the given or a narrow collection of ARB and/or ARGs. Evaluation of a method for the performance on removing ARB and/or ARGs as well as multidrug resistance genes (MRGs) must be included a collective different bacteria and genes.
- The results of any new study should be based on culture-independent methods because ozonation could increase the fraction of non-cultivable populations, in addition to that the most of the MOs in aqueous solutions are not cultivable.
- The ozonation-based processes should be defined somehow to give appropriate removal of both ARB and ARGs along with MRGs. Because the orphaned genes will be problematic.
- Techno-economic analyses of the process are of great importance.
- A deeper understanding of the ozonation mechanism would help to explain the findings and to improve the process performance.

All in all, this systematic review presents a comprehensive study of ARB & ARGs removal using ozonation-based strategies in W&WWTPs, which include a large body of information to be used in any future study.

CRediT authorship contribution statement

Maryam Foroughi: Conceived and designed the analysis; Contributed data or analysis tools; Wrote the paper.

Mehdi Khiadani: Wrote the paper; Other contribution, revised the paper from operation & process point of views.

Samaneh Kakhki: Conceived and designed the analysis; Collected the data; Wrote the paper.

Vahid Kholghi: Conceived and designed the analysis; Collected the data.

Khosro Naderi: Conceived and designed the analysis; Collected the data; Contributed data or analysis tools.

Sama Yektay: Collected the data; Contributed data or analysis tools.

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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