



**HAL**  
open science

## Degradation and deactivation of a plasmid-encoded extracellular antibiotic resistance gene during separate and combined exposures to UV254 and radicals

Maolida Nihemaiti, Younggun Yoon, Huan He, Michael C. Dodd,  
Jean-Philippe Croué, Yunho Lee

### ► To cite this version:

Maolida Nihemaiti, Younggun Yoon, Huan He, Michael C. Dodd, Jean-Philippe Croué, et al.. Degradation and deactivation of a plasmid-encoded extracellular antibiotic resistance gene during separate and combined exposures to UV254 and radicals. *Water Research*, 2020, 182, pp.115921 -. 10.1016/j.watres.2020.115921 . hal-03492495

**HAL Id: hal-03492495**

**<https://hal.science/hal-03492495>**

Submitted on 15 Jul 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1     **Degradation and deactivation of a plasmid-encoded extracellular**  
2     **antibiotic resistance gene during separate and combined exposures**  
3                     **to UV<sub>254</sub> and radicals**

4     Maolida Nihemaiti <sup>a, 1</sup>, Younggun Yoon <sup>b, 1</sup>, Huan He <sup>c</sup>, Michael C. Dodd <sup>c</sup>, Jean-Philippe Croué  
5                                     <sup>a, d, \*</sup>, and Yunho Lee <sup>b, \*</sup>

6     <sup>a</sup> Curtin Water Quality Research Centre, Department of Chemistry, Curtin University, GPO Box  
7     U1987, Perth 6845, Australia

8     <sup>b</sup> School of Earth Sciences and Environmental Engineering, Gwangju Institute of Science and  
9     Technology (GIST), Gwangju 61005, Republic of Korea

10    <sup>c</sup> Department of Civil and Environmental Engineering, University of Washington, Seattle,  
11    Washington 98195-2700, United States

12    <sup>d</sup> Institut de Chimie des Milieux et des Matériaux IC2MP UMR 7285 CNRS, Université de  
13    Poitiers, France

14    <sup>1</sup> M. Nihemaiti and Y. Yoon contributed equally to this work

15    \*Corresponding authors: Yunho Lee, Email: [yhlee42@gist.ac.kr](mailto:yhlee42@gist.ac.kr)

16    Jean-Philippe Croué, Email: [jean.philippe.croue@univ-poitiers.fr](mailto:jean.philippe.croue@univ-poitiers.fr)

17                                     **Submitted to Water Research**

## 18 **Abstract**

19 This study investigated the degradation and deactivation of an extracellular ampicillin resistance  
20 gene ( $amp^R$ ) encoded in plasmid pUC19 during exposure to UV<sub>254</sub>, •OH (generated by  
21 UV<sub>>290</sub>/H<sub>2</sub>O<sub>2</sub>), and combined exposure to UV<sub>254</sub> and •OH (SO<sub>4</sub><sup>•-</sup>) using UV<sub>254</sub>/H<sub>2</sub>O<sub>2</sub> and  
22 UV<sub>254</sub>/S<sub>2</sub>O<sub>8</sub><sup>2-</sup>. The degradation rates of  $amp^R$  measured by quantitative polymerase chain  
23 reaction increased with increasing target amplicon length (192–851 bps). The rate constants for  
24 the degradation of pUC19 (2686 bps) were calculated as 0.26 cm<sup>2</sup>/mJ for UV<sub>254</sub> and 1.5×10<sup>11</sup> M<sup>-1</sup>  
25 s<sup>-1</sup> for •OH, based on the degradation rates of  $amp^R$  amplicons and assuming an equal  
26 sensitivity of DNA damage across the entire plasmid. DNA repair-proficient *Escherichia coli* (*E.*  
27 *coli*) AB1157 strain (wild-type) and its repair-deficient mutants including AB1886 (*uvrA*<sup>-</sup>),  
28 AB2463 (*recA*<sup>-</sup>), AB2480 (*uvrA*<sup>-</sup>, *recA*<sup>-</sup>), and DH5α (*recA*<sup>-</sup>, *endA*<sup>-</sup>) were applied as recipient  
29 cells in gene transformation assays. Results suggested that the elimination efficiency of  
30 transforming activity during UV<sub>254</sub> and •OH exposure was dependent on the type of DNA repair  
31 genes in recipient *E. coli* strains. Losses of transforming activity were slower than the  
32 degradation of pUC19 by a factor of up to ~5 (for *E. coli* DH5α), highlighting the importance of  
33 DNA repair in recipient cell. The degradation rates of  $amp^R$  amplicons were much larger (by a  
34 factor of ~4) in UV<sub>254</sub>/H<sub>2</sub>O<sub>2</sub> and UV<sub>254</sub>/S<sub>2</sub>O<sub>8</sub><sup>2-</sup> than UV<sub>254</sub> direct photolysis, indicating the  
35 significant contribution of •OH and SO<sub>4</sub><sup>•-</sup> to the gene degradation. Not only UV<sub>254</sub> and SO<sub>4</sub><sup>•-</sup>,  
36 but also •OH contributed to the degradation of  $amp^R$  during UV<sub>254</sub>/S<sub>2</sub>O<sub>8</sub><sup>2-</sup>, which was attributed  
37 to the conversion of SO<sub>4</sub><sup>•-</sup> to •OH and a 10-fold larger reactivity of •OH towards  $amp^R$  as  
38 compared to SO<sub>4</sub><sup>•-</sup>. However, the enhanced gene degradation by radicals did not lead to a faster  
39 elimination of gene transforming activity during UV<sub>254</sub>/H<sub>2</sub>O<sub>2</sub> and UV<sub>254</sub>/S<sub>2</sub>O<sub>8</sub><sup>2-</sup>, suggesting that  
40 UV<sub>254</sub>- and radical-induced DNA damage were not additive in their contributions to losses of  
41 gene transforming activity. Wastewater effluent organic matter (EfOM) accelerated the  
42 degradation of  $amp^R$  during UV<sub>254</sub> irradiation by means of reactive species production through

43 indirect photolysis reactions, whereas EfOM mainly acted as a radical scavenger during  
44  $UV_{254}/H_2O_2$  and  $UV_{254}/S_2O_8^{2-}$  treatments.

45 **Keywords**

46 Antibiotic resistant bacteria, antibiotic resistance genes, gene transformation, UV, hydroxyl  
47 radical, sulfate radical

48 **Abbreviations**

49	<i>amp<sup>R</sup></i>	Ampicillin Resistance Gene
50	AOPs	Advanced Oxidation Processes
51	Amp	Amplicon
52	ARB	Antibiotic Resistant Bacteria
53	ARG(s)	Antibiotic Resistance Gene(s)
54	bp(s)	Nucleotide Base Pair(s)
55	DOM	Dissolved Organic Matter
56	e-ARG	Extracellular Antibiotic Resistance Genes
57	<i>E. coli</i>	<i>Escherichia coli</i>
58	EfOM	Effluent Organic Matter
59	<i>p</i> CBA	<i>para</i> -Chlorobenzoic Acid
60	qPCR	Quantitative Polymerase Chain Reaction

## 61 **1. Introduction**

62 Antibiotic resistance is a natural phenomenon. However, anthropogenic activities (e.g.,  
63 overuse and disposal of antibiotics) are providing constant selection pressure on antibiotic  
64 resistant bacteria (ARB) (Vikesland, Pruden et al. 2017). Antibiotic resistance can be developed  
65 and disseminated within the bacterial population by genetic mutation, cell division, and the  
66 transfer of antibiotic resistance genes (ARGs) (Davies and Davies 2010). ARGs are  
67 contaminants of concern in natural and engineered aquatic systems, as ARG transfer can be  
68 linked with the spread of antibiotic resistance to human pathogens (Dodd 2012, Pruden 2014).  
69 Mobile genetic elements carrying ARGs (e.g., plasmids, integrons, and transposons) can be  
70 disseminated to and expressed by recipient cells through horizontal gene transfer (HGT),  
71 including conjugation (mediated by cell to cell contact), transduction (bacteriophage mediated),  
72 and transformation (mediated by extracellular DNA) (Lorenz and Wackernagel 1994) Of these,  
73 transformation can be understood to represent the most basic mode of HGT, as it does not  
74 require the presence of live donor cells or bacteriophages (Lorenz and Wackernagel 1994).  
75 Consequently, quantification of the frequency with which an ARG in extracellular DNA can be  
76 transported into and expressed by a recipient cell through transformation (hereafter referred to as  
77 *transforming activity*) can provide a conservative measure of the ability of an ARG to undergo  
78 HGT. Transforming activity encompasses (a) efficiency of extracellular DNA transport into a  
79 recipient cell, (b) any repair of that DNA by the recipient cell in the event the DNA is damaged,  
80 and (c) heritable incorporation and expression of the DNA's genetic information by recipient  
81 cells, and is therefore a function of both the recipient cell's ability to import, repair, and express  
82 a given ARG and the quality and quantity of the extracellular DNA containing the ARG.  
83 Extracellular DNA can be released into the environment though secretion by live cells and lysis  
84 of dead cells. While unstable to nucleases likely to be present in many aquatic matrixes, it can

85 persist in the environment through adsorption onto soil and sediments (Lorenz and Wackernagel  
86 1994, Mao, Luo et al. 2014, Nagler, Insam et al. 2018).

87 Although conventional disinfection processes during water treatment (e.g., chlorine and UV)  
88 efficiently inactivate ARB, intracellular ARGs are more difficult to degrade (McKinney and  
89 Pruden 2012, Yoon, Chung et al. 2017, He, Zhou et al. 2019) and can be released into water  
90 following the death of ARB cells (Zheng, Su et al. 2017). Sub-inhibitory concentrations of  
91 disinfectants (e.g., chlorine and monochloramine) were even reported to promote the horizontal  
92 transfer of ARGs by increasing the permeability of cell membrane and altering the expression of  
93 conjugation-related genes (Guo, Yuan et al. 2015, Zhang, Gu et al. 2017). The proportion of  
94 extracellular DNA carrying ARGs was found to increase after various biological and chemical  
95 processes in wastewater treatment, revealing the potential risk of antibiotic resistance  
96 dissemination in discharged effluent and receiving environments (Liu, Qu et al. 2018, Zhang, Li  
97 et al. 2018). Previous studies have reported the enrichment of ARGs in surface water due to the  
98 discharged effluents from upstream wastewater plants (Cacace, Fatta-Kassinos et al. 2019, Wu,  
99 Su et al. 2019, Osińska, Korzeniewska et al. 2020). High diversity of ARGs in reclaimed  
100 wastewater (e.g., agriculture irrigation, potable reuse) may pose risks of antibiotic resistance  
101 dissemination to environmental bacteria and even human pathogens (Christou, Agüera et al.  
102 2017, Hong, Julian et al. 2018).

103 UV-based advanced oxidation processes (UV-AOPs) are increasingly applied for the removal  
104 of refractory contaminants during water treatment. The elimination of contaminants in UV-AOP  
105 treatment is achieved by dual pathways, namely direct UV photolysis (depending on the UV  
106 absorbances and quantum yields of target compounds) and oxidation by powerful radical species  
107 (Stefan 2018). The activation of hydrogen peroxide (UV/H<sub>2</sub>O<sub>2</sub>) and peroxydisulfate (UV/S<sub>2</sub>O<sub>8</sub><sup>2-</sup>)  
108 by UV light produces hydroxyl radical (•OH) and sulfate radical (SO<sub>4</sub>•<sup>-</sup>), respectively, which  
109 can degrade a wide range of contaminants, as these two radicals react with many compounds at

110 or near diffusion-controlled reaction rates (Buxton, Greenstock et al. 1988, Neta, Huie et al.  
111 1988).

112 UV/H<sub>2</sub>O<sub>2</sub> and UV/S<sub>2</sub>O<sub>8</sub><sup>2-</sup> have also been reported to efficiently inactivate ARB cells  
113 (Michael-Kordatou, Iacovou et al. 2015, Ferro, Guarino et al. 2016, Giannakis, Le et al. 2018).  
114 However, detailed investigation into the reactivity of •OH with intracellular ARGs in these  
115 studies was limited due to the significant consumption of •OH by constituents of the water  
116 matrix and cell membranes before reaching the cell interiors (Ferro, Guarino et al. 2017, Yoon,  
117 Chung et al. 2017, Zhang, Hu et al. 2019). Recent studies have reported that the reactivity of  
118 •OH with extracellular chromosomal ARGs is highly dependent on the length of the monitored  
119 ARG targets (He, Zhou et al. 2019). More studies are needed to assess whether the observed  
120 reactivities are generally applicable to other types of DNA such as plasmid-encoded ARGs. To  
121 the best of the authors' knowledge, little is known on the fate of ARGs during SO<sub>4</sub><sup>•-</sup>-based  
122 treatment processes.

123 Quantitative polymerase chain reaction (qPCR) has been widely applied to quantify  
124 structurally-intact ARGs or to measure their degradation rates in water and wastewater treatment  
125 (McKinney and Pruden 2012, Luby, Ibekwe et al. 2016, Hiller, Hübner et al. 2019). It should be  
126 noted that qPCR methods usually record the degradation of a small fraction of a target ARG's  
127 sequence length (with amplicon sizes typically <1000 bps), and not the entirety of a gene  
128 (McKinney and Pruden 2012, Chang, Juhrend et al. 2017, Yoon, Dodd et al. 2018). In addition,  
129 the efficiency of DNA replication (particularly of damaged DNA) in qPCR methods can be  
130 different from that observed in bacterial cells subjected to transformation with the same DNA.  
131 Furthermore, the uptake and repair of DNA by recipient cells can play important roles in  
132 modulating gene transforming activity (Chang, Juhrend et al. 2017, Yoon, Dodd et al. 2018).  
133 While the correlation between the degradation of target ARG amplicons (measured by qPCR)



134 and the elimination of ARG transforming activity was reasonably well explained for  
135 chromosomal ARGs (He, Zhou et al. 2019), it is still less clear for plasmid-borne ARGs.

136 Exposure of DNA to UV and •OH is known to induce different types of DNA damage (von  
137 Sonntag 2006). UV-C generates cyclobutane-pyrimidine dimers (CPDs) as the predominant  
138 DNA lesions, while •OH can induce DNA damage ranging from base oxidation to phosphate-  
139 sugar backbone breakages (Görner 1994, Sinha and Häder 2002, von Sonntag 2006). It is  
140 currently not completely clear how the various type(s) of DNA damage resulting from UV, •OH,  
141 and  $\text{SO}_4^{\bullet-}$  are related to the deactivation of ARGs; for example, whether DNA lesions such as  
142 CPDs (formed by direct UV irradiation) have the same effect on DNA transforming activity as  
143 phosphate-sugar backbone breakage by •OH (generated by  $\text{H}_2\text{O}_2$  or  $\text{S}_2\text{O}_8^{2-}$  photolysis).  
144 Furthermore, in UV-AOPs, DNA lesions can be generated by both direct UV irradiation and  
145 radicals simultaneously.

146 The aim of this study was to investigate the fate of plasmid-encoded e-ARGs during the  
147 common UV at 254 nm water disinfection process ( $\text{UV}_{254}$ ) and its related AOPs. Plasmid  
148 (pUC19) encoding an ampicillin resistance gene ( $\text{amp}^R$ ) was purified from *Escherichia coli* (*E.*  
149 *coli*) host cells and exposed to  $\text{UV}_{254}$ , to  $\text{UV}_{254}$  in combination with •OH or  $\text{SO}_4^{\bullet-}$  (by using  
150  $\text{UV}_{254}/\text{H}_2\text{O}_2$  and  $\text{UV}_{254}/\text{S}_2\text{O}_8^{2-}$ ), or to •OH only (by combining UV at  $>290$  nm with  $\text{H}_2\text{O}_2$ ) (i.e.,  
151  $\text{UV}_{>290}/\text{H}_2\text{O}_2$ ).  $\text{UV}_{>290}/\text{H}_2\text{O}_2$  treatment was chosen to study the effect of •OH only exposure  
152 because irradiation of  $\text{H}_2\text{O}_2$  by  $\text{UV}_{>290}$  can generate •OH, while neither  $\text{UV}_{>290}$  irradiation nor  
153  $\text{H}_2\text{O}_2$  directly affect  $\text{amp}^R$  under these experimental conditions (detailed in Section 3.1). The  
154 treated plasmid samples were analysed by qPCR, gel electrophoresis, and ARG transformation  
155 assay. DNA repair-proficient *E. coli* AB1157 (wild-type), as well as repair-deficient *E. coli*  
156 mutants including AB1886 ( $\text{uvrA}^-$ ), AB2463 ( $\text{recA}^-$ ), AB2480 ( $\text{uvrA}^-$ ,  $\text{recA}^-$ ), and DH5 $\alpha$   
157 ( $\text{recA}^-$ ,  $\text{endA}^-$ ) were employed as recipient cells in the ARG transformation assay. Competition  
158 kinetics methods using radical probe compounds were applied to measure the second-order rate

159 constants of  $\bullet\text{OH}$  and  $\text{SO}_4^{\bullet-}$  with  $\text{amp}^R$  segments. Experiments were also conducted in the  
160 presence of wastewater effluent organic matter (EfOM) to study the effect of the dissolved  
161 organic matter (DOM) on the removal of e-ARGs.

## 162 **2. Materials and Methods**

### 163 **2.1 Chemical Reagents**

164 All chemicals were in analytical grade or higher and used as received without further  
165 purification. Ampicillin sodium salt (#A0166), agar powder (#A1296), sodium chloride  
166 ( $\geq 99.5\%$ ), *para*-chlorobenzoic acid (99%), and nitrobenzene ( $\geq 99\%$ ) were purchased from  
167 Sigma-Aldrich. Tryptone (#1612) and yeast extract (#1702) were supplied from Laboratorios  
168 CONDA. Hydrogen peroxide 30% (Thermo Fisher Scientific) and sodium peroxydisulfate  
169 ( $\geq 98\%$ , Sigma-Aldrich) were used to prepare the stock solutions of  $\text{H}_2\text{O}_2$  and  $\text{S}_2\text{O}_8^{2-}$ ,  
170 respectively. All solutions were prepared in Milli-Q water (18  $\text{M}\Omega\cdot\text{cm}$ , Millipore). EfOM was  
171 previously extracted from the discharged effluent of a wastewater treatment plant in Jeddah,  
172 Saudi-Arabia, with XAD resins (Zheng, Khan et al. 2014). Solutions of EfOM were prepared by  
173 dissolving the hydrophobic and transphilic fractions (2:1 by mass) of EfOM in phosphate buffer.

### 174 **2.2 Plasmid Preparation**

175 Plasmid pUC19 was extracted from *E. coli* DH5 $\alpha$ . pUC19 (2686 bp) is a commercially  
176 available *E. coli* vector carrying an ampicillin resistance gene ( $\text{amp}^R$ , 861 bp). One hundred  $\mu\text{L}$   
177 of *E. coli* DH5 $\alpha$  mid-exponential growth phase culture in LB broth medium with 50 mg/L of  
178 ampicillin was transferred into 150 mL of LB broth medium containing 50 mg/L of ampicillin  
179 and incubated overnight (200 rpm, 37°C). Plasmids were extracted from this overnight stock  
180 solution using an AccuPrep Plasmid Mini Extraction kit (Bioneer 2016). The concentration of  
181 recovered plasmid DNA was measured on a NanoDrop ND-2000 Spectrophotometer  
182 (NanoDrop Products, Wilmington, USA).

### 183 2.3 UV Experiments

184 UV<sub>254</sub>-based experiments were carried out with a UV quasi-collimated beam device equipped  
185 with a low-pressure mercury lamp emitting UV light primarily at 254 nm (Sankyo Denki Ltd.,  
186 Tokyo, Japan). The UV light was collimated onto the experimental solution, which was  
187 contained in a petri dish placed on a magnetic stirrer. The average UV intensity (0.3 mW/cm<sup>2</sup>)  
188 was determined with atrazine chemical actinometry (Lee, Gerrity et al. 2016). Experimental  
189 solutions were prepared in autoclaved 2 mM phosphate buffer at pH 7. The initial concentration  
190 of plasmid DNA used in experiments was 0.3 or 1 µg/mL. The applied UV fluences ranged from  
191 0–180 mJ/cm<sup>2</sup>. The initial concentrations of H<sub>2</sub>O<sub>2</sub> and S<sub>2</sub>O<sub>8</sub><sup>2-</sup> were 0.5 mM for UV<sub>254</sub>/H<sub>2</sub>O<sub>2</sub> and  
192 UV<sub>254</sub>/S<sub>2</sub>O<sub>8</sub><sup>2-</sup>, respectively. UV<sub>>290</sub>/H<sub>2</sub>O<sub>2</sub> experiments were conducted using a Model 66924 arc  
193 lamp source fitted with a 450-W O<sub>3</sub>-free Xe arc lamp and focusing collimator, and equipped  
194 with an atmospheric attenuation filter and dichroic mirror to restrict lamp output to near-UV  
195 wavelengths (290 nm < λ < 400 nm) (Newport-Oriel Model 66924; Stratford, CT). Relatively  
196 high H<sub>2</sub>O<sub>2</sub> concentrations (i.e., 10 mM) were applied for UV<sub>>290</sub>/H<sub>2</sub>O<sub>2</sub>, in order to increase the  
197 yield of •OH due to the low absorbance of UV light by H<sub>2</sub>O<sub>2</sub> in this wavelength region. Samples  
198 were withdrawn from the irradiated solutions in each experimental setup at predetermined time  
199 intervals for DNA and probe compound analyses. Residual H<sub>2</sub>O<sub>2</sub> and S<sub>2</sub>O<sub>8</sub><sup>2-</sup> were quenched  
200 with excess bovine catalase and sodium thiosulfate, respectively. Samples for DNA analysis  
201 (500 µL) were stored at -20°C and analysed within 24 h. Control experiments indicated that  
202 H<sub>2</sub>O<sub>2</sub> and S<sub>2</sub>O<sub>8</sub><sup>2-</sup> did not affect qPCR analyses within these experimental conditions (Figure S1).

### 203 2.4 Radical probe compounds

204 *para*-chlorobenzoic acid (*p*CBA) was used as •OH probe during UV<sub>254</sub>/H<sub>2</sub>O<sub>2</sub> and  
205 UV<sub>>290</sub>/H<sub>2</sub>O<sub>2</sub> experiments. Both *p*CBA and nitrobenzene were applied during UV<sub>254</sub>/S<sub>2</sub>O<sub>8</sub><sup>2-</sup>.  
206 Nitrobenzene can be used as a selective •OH probe compound ( $k_{\bullet\text{OH}}=3.9\times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ ) (Buxton,

207 Greenstock et al. 1988) due to its low reactivity to  $\text{SO}_4^{\bullet-}$  ( $k_{\text{SO}_4^{\bullet-}} < 10^6 \text{ M}^{-1} \text{ s}^{-1}$ ) (Neta, Madhavan et  
208 al. 1977), whereas *p*CBA reacts with both radicals (i.e.,  $k_{\text{SO}_4^{\bullet-}} = 3.6 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$  and  $k_{\bullet\text{OH}} = 5 \times 10^9$   
209  $\text{M}^{-1} \text{ s}^{-1}$ ) (Neta, Madhavan et al. 1977, Buxton, Greenstock et al. 1988). *p*CBA and nitrobenzene  
210 were analysed on a high-performance liquid chromatograph (HPLC, Dionex Ultimate 3000,  
211 Thermo Scientific) equipped with an XDB-C18 column (5  $\mu\text{m}$ , 4.6  $\times$  150 mm, Agilent). The  
212 mobile phase comprised acetonitrile and 10 mM phosphoric acid (40:60, v/v). The two probe  
213 compounds were analysed at their maximum absorbance wavelengths (i.e., 240 and 270 nm for  
214 *p*CBA and nitrobenzene, respectively).

## 215 **2.5 qPCR Analysis**

216 Gene degradation was measured by qPCR using a CFX96 real-time PCR detection system  
217 (Bio-Rad, Hercules, CA, USA). Four different amplicons (i.e., 192, 400, 603, and 851 bp) were  
218 monitored, which covered varying fractions of *amp<sup>R</sup>* as shown in Figure S2. The longest size of  
219 *amp<sup>R</sup>* segment targeted in qPCR was 851 bp instead of the whole *amp<sup>R</sup>* gene (861 bp), due to the  
220 low amplification efficiency of the 861 bp amplicon in the qPCR method. The reaction mixture  
221 (20  $\mu\text{L}$ ) consisted of 1  $\mu\text{L}$  of each primer, 1  $\mu\text{L}$  of sample, 10  $\mu\text{L}$  of EvaGreen® supermix, and 7  
222  $\mu\text{L}$  of autoclaved DNase free water. The qPCR protocol included one cycle at 95°C for 2 min,  
223 30 cycles at 95°C for 5 s, an annealing step at 55°C for 60 s, and an elongation at 72°C for 20 s,  
224 followed by a melt curve analysis from 65°C to 95°C. Each sample was analysed in triplicate.  
225 More information on qPCR analysis, the DNA sequence of *amp<sup>R</sup>*, and the monitored qPCR  
226 target amplicons is described elsewhere (Yoon, Dodd et al. 2018).

## 227 **2.6 Gel Electrophoresis Analysis**

228 pUC19 plasmid (1  $\mu\text{g}/\text{mL}$ ) treated by  $\text{UV}_{254}$  and  $\bullet\text{OH}$  ( $\text{UV}_{>290}/\text{H}_2\text{O}_2$ ) was analysed by gel  
229 electrophoresis to investigate the structural change of plasmid DNA. Linearized pUC19 was also  
230 prepared for a reference by incubating the plasmid with type II restriction enzyme *EcoRI* (NEB,

231 USA) at 37 °C for 1 h, followed by enzyme inactivation at 65°C for 20 min. Plasmid samples  
232 from UV<sub>254</sub>, •OH (UV<sub>>290</sub>/H<sub>2</sub>O<sub>2</sub>), and enzyme treatments, as well as a 1 kb DNA ladder  
233 (Enzynomics, Korea) were loaded on 0.8% agarose gels at 4 V cm<sup>-1</sup> for 35 min. The bands were  
234 visualized by ethidium bromide staining. Gel images were captured on a UV transilluminator  
235 (Universal mutation detection system, UVP, LLC, USA).

## 236 2.7 Gene Transformation Assays

237 Non-ampicillin resistant *E.coli* K12 bacteria strains DH5α (*recA*<sup>-</sup>, *endA*<sup>-</sup>), AB1157 (wild-  
238 type), AB1886 (*uvrA*<sup>-</sup>), AB2463 (*recA*<sup>-</sup>), and AB2480 (*uvrA*<sup>-</sup>, *recA*<sup>-</sup>) were used as recipient  
239 cells for gene transformation assays. DH5α strain was commercially available from ATCC;  
240 others were provided by CGSC (Coli Genetic Stock Center at Yale University). Details on  
241 transformation assays were described by Yoon, Dodd et al. (2018). In brief, competent cells  
242 were prepared by chemical treatment of non-resistant *E. coli* K12 strains using calcium chloride  
243 and glycerol (Shanebandi, Saei et al. 2013) and preserved at -80°C until use. Fifty μL of  
244 treated plasmid sample was mixed with 100 μL of thawed competent cells. After incubating in  
245 ice for 30 min, the mixture was quickly transferred onto a digital test tube heater (45°C) for 45 s  
246 and then placed back in ice for 2 min. After the heat shock, the samples were mixed with 900  
247 μL of LB broth and cultured for 45 min (200 rpm, 37°C). Finally, the incubated samples were  
248 serially diluted with LB broth and plated onto two types of LB agar plates (i.e., with and without  
249 inclusion of 50 mg/L of ampicillin). After 24 h of incubation at 37°C, the number of ARB  
250 colonies (transformants) detected on selective agar plates (with ampicillin) were compared with  
251 the total recipient cells growing on nonselective agar plates (without ampicillin). The gene  
252 transformation efficiency was calculated as follows:

$$253 \quad \text{Transformation efficiency} = \frac{\text{Transformant cells}_{\text{selective plate}} \text{ (CFU/mL)}}{\text{Total recipient cells}_{\text{nonselective plate}} \text{ (CFU/mL)}}$$

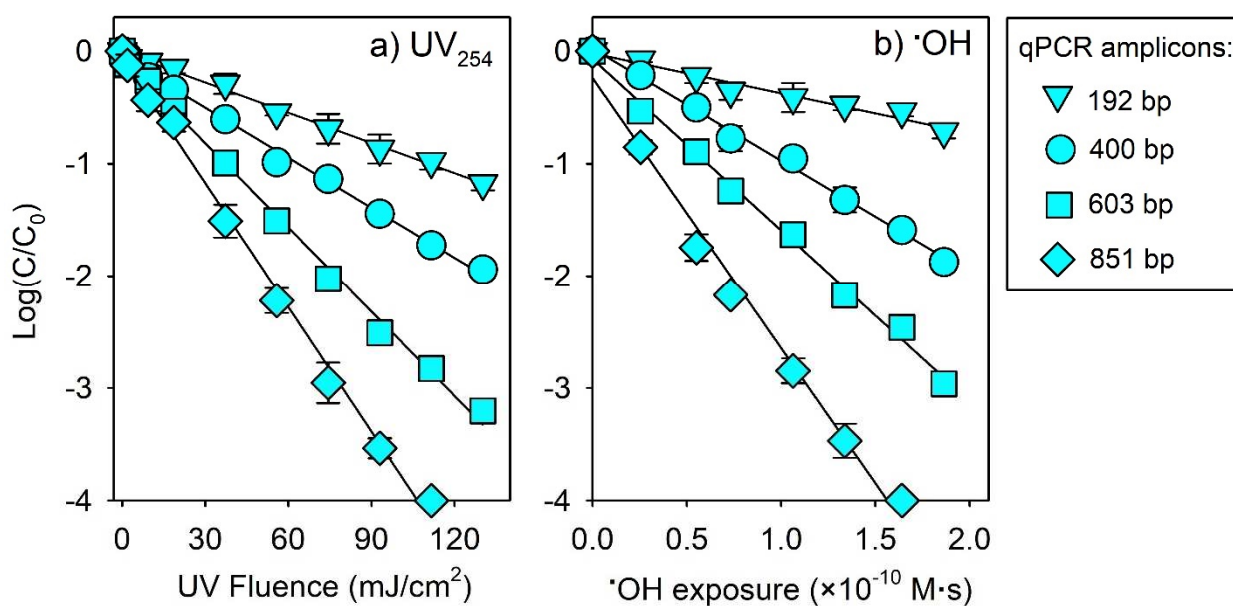
254 The typical concentrations of total recipient cells, as measured by culturing on nonselective agar  
255 plates (without ampicillin), were approximately  $3 \times 10^8$  and  $2 \times 10^8$  CFU/mL for *E. coli* DH5 $\alpha$  and  
256 *E. coli* AB strains, respectively. Control experiments conducted by directly plating the recipient  
257 cells (without heat shock) on nonselective plates showed <10% variation in the final colony  
258 counts of recipient cells as compared to the conditions incorporating heat shock (Figure S3), and  
259 <50% variation in colony counts between strains. These results indicated that the type of  
260 bacterial strain used, or stresses to which the strains were subjected during transformation assays  
261 (e.g., heat shock), did not significantly impact the fitness or growth rates of recipient cells on  
262 nonselective plates. Calibration curves prepared with known concentrations of plasmid DNA  
263 indicated that the number of transformants linearly increased with increasing plasmid DNA  
264 concentration from  $10^{-5}$ – $10^{-1}$   $\mu$ g/mL (Figure S4). The transformation efficiency of *amp*<sup>R</sup>  
265 calculated for *E. coli* K12 strains was in the range of  $10^{-4}$  –  $10^{-8}$ , which was comparable with  
266 previous studies (Chang, Juhrend et al. 2017, Yoon, Dodd et al. 2018).

## 267 **2.8 Statistical analysis**

268 Statistical analysis was conducted using SigmaPlot 12.0 and GraphPad Prism 7.0. The  
269 fluence-based rate constants for gene degradation and transformation efficiency loss under  
270 various experimental conditions were compared by multiple linear regression analysis. The null  
271 hypothesis was that the first-order rate constants were identical, with  $p=0.05$  as the threshold  
272 significance level.

## 273 **3. Results and Discussions**

### 274 **3.1 e-ARG degradation during separate exposure to UV<sub>254</sub> and •OH**



275  
 276 **Figure 1.** Degradation of *amp<sup>R</sup>* segments during the treatment of pUC19 (1 µg/mL) with (a)  
 277 UV<sub>254</sub> and (b) •OH (UV<sub>>290</sub>/H<sub>2</sub>O<sub>2</sub>) at pH 7 (2 mM phosphate buffer). 10 mM of H<sub>2</sub>O<sub>2</sub> and 1 µM  
 278 of *pCBA* were added in UV<sub>>290</sub>/H<sub>2</sub>O<sub>2</sub> experiments. Error bars represent the standard deviations  
 279 of triplicate experiments. Lines are the linear regressions of the experimental data.

280 *UV<sub>254</sub> exposure (Figure 1a).* The solution containing pUC19 was irradiated with UV<sub>254</sub> light  
 281 (0–130 mJ/cm<sup>2</sup>). The degradation of *amp<sup>R</sup>* segments (192, 400, 603, and 851 bp) measured by  
 282 qPCR followed first-order kinetics with respect to UV fluence ( $r^2 > 0.99$ ) (Figure 1a). The 192 bp  
 283 *amp<sup>R</sup>* segment was degraded by about 1-log at 110 mJ/cm<sup>2</sup>, whereas 4-log reduction was already  
 284 achieved for the 851 bp segment, which was close to the overall size of *amp<sup>R</sup>* (861 bp). The  
 285 resulting fluence-based rate constants derived from the slopes of linear curves,  $k_{UV, Amp} = 2.303$   
 286  $\times$  slope, are summarized in Table 1. The  $k_{UV, Amp}$  of *amp<sup>R</sup>* segments increased with amplicon  
 287 size, which was consistent with the increasing number of potential reaction sites with increasing  
 288 number of nucleotide bps. The  $k_{UV, Amp}$  values obtained from this study were comparable to  
 289 previously-reported values for the same *amp<sup>R</sup>* segments (Yoon, Dodd et al. 2018). The  
 290  $k_{UV, plasmid}$  of pUC19 plasmid could be estimated by extrapolating the  $k_{UV, Amp}$  of each

291 segment to the entire plasmid (e.g.,  $k_{UV,plasmid}=k_{UV,192bp}\times\frac{2686\text{ bp}}{192\text{ bp}}$ ), based on an assumption  
 292 that the UV reactivity per base pair is the same across the entire plasmid. The  $k_{UV,plasmid}$  values  
 293 estimated from the 192, 400, 603, and 851 bp amplicons were  $2.9(\pm 0.06)\times 10^{-1}$ ,  $2.3(\pm 0.04)\times 10^{-1}$ ,  
 294  $2.6(\pm 0.05)\times 10^{-1}$ , and  $2.7(\pm 0.06)\times 10^{-1}$  cm<sup>2</sup>/mJ, respectively, yielding an average  
 295  $k_{UV,plasmid}$  value of  $2.6(\pm 0.05)\times 10^{-1}$  cm<sup>2</sup>/mJ (Table 1).

296 A theoretical approach was recently employed to estimate the photoreactivity of DNA (i.e.,  
 297 formation rate of CPDs and 6,4-photoproducts) based on the molar absorption coefficients,  
 298 quantum yields, and the number of 5'-bipyrimidine-3' doublets of a given DNA (Yoon, Dodd et  
 299 al. 2018, He, Zhou et al. 2019). By applying this approach, the overall DNA lesion formation  
 300 rate on pUC19 upon UV<sub>254</sub> exposure,  $k_{all\ lesions}$ , was calculated as  $2.1\times 10^{-1}$  cm<sup>2</sup>/mJ (Text S1),  
 301 which was close to the average  $k_{UV,plasmid}$  ( $2.6\times 10^{-1}$  cm<sup>2</sup>/mJ) predicted from qPCR results as  
 302 mentioned above.

303 **Table 1.** Apparent rate constants for the degradation of  $amp^R$  segments and pUC19 plasmid, and  
 304 the elimination of gene transforming activity during UV<sub>254</sub> and •OH only treatments <sup>a</sup>

$amp^R$ segments		pUC19 plasmid <sup>b</sup>		Loss of gene transformation	
#base pairs (bps)	$k_{Amp}$	$k_{plasmid}$	average	Recipient cells	$k_{transformation}$
UV <sub>254</sub> (cm <sup>2</sup> /mJ)					
192	$2.0(\pm 0.05)\times 10^{-2}$	$2.9(\pm 0.06)\times 10^{-1}$	$2.6(\pm 0.05)\times 10^{-1}$	DH5α	$6.5(\pm 0.21)\times 10^{-2}$
400	$3.4(\pm 0.07)\times 10^{-2}$	$2.3(\pm 0.04)\times 10^{-1}$		AB1157	$1.0(\pm 0.03)\times 10^{-1}$
603	$5.8(\pm 0.11)\times 10^{-2}$	$2.6(\pm 0.05)\times 10^{-1}$		AB1886	$2.4(\pm 0.06)\times 10^{-1}$
851	$8.9(\pm 0.16)\times 10^{-2}$	$2.7(\pm 0.06)\times 10^{-1}$		AB2463	$1.6(\pm 0.06)\times 10^{-1}$
•OH (UV <sub>&gt;290</sub> /H <sub>2</sub> O <sub>2</sub> ) (M <sup>-1</sup> s <sup>-1</sup> )					
192	$8.1(\pm 0.69)\times 10^9$	$1.2(\pm 0.10)\times 10^{11}$	$1.5(\pm 0.07)\times 10^{11}$	DH5α	$2.7(\pm 0.05)\times 10^{10}$
400	$2.2(\pm 0.07)\times 10^{10}$	$1.5(\pm 0.05)\times 10^{11}$		AB1157	$4.1(\pm 0.14)\times 10^{10}$
603	$3.5(\pm 0.10)\times 10^{10}$	$1.6(\pm 0.04)\times 10^{11}$		AB1886	$6.5(\pm 0.18)\times 10^{10}$
851	$5.6(\pm 0.27)\times 10^{10}$	$1.8(\pm 0.08)\times 10^{11}$		AB2463	$8.2(\pm 0.23)\times 10^{10}$

<sup>a</sup> Experimental conditions: pUC19: 1 μg/mL, 2 mM phosphate buffer at pH 7, fluence range for UV<sub>254</sub>: 0–130 mJ/cm<sup>2</sup>, 10 mM of H<sub>2</sub>O<sub>2</sub> and 1 μM of pCBA were added in UV<sub>>290</sub>/H<sub>2</sub>O<sub>2</sub>. The apparent rate constant values were reported in the form of Mean ± Standard Error.

<sup>b</sup> The  $k_{plasmid}$  values of pUC19 plasmid were estimated based on the  $k_{Amp}$  of  $amp^R$



---

segments obtained from qPCR analyses (see Section 3.1 for details).

---

305  
306  $\bullet$ OH exposure (Figure 1b). Control experiments have confirmed that  $amp^R$  segments were  
307 stable under  $UV_{>290}$  irradiation alone (Figure S5), which was attributed to the low UV  
308 absorbance of plasmid DNA above 290 nm. Moreover, 10 mM (340 mg/L) of  $H_2O_2$  had  
309 negligible effect on  $amp^R$  segment (Figure S1). Thus,  $UV_{>290}/H_2O_2$  was applied to investigate  
310 the influence of  $\bullet$ OH on the degradation of e-ARG. The  $\bullet$ OH exposure,  $\int[\bullet OH]dt$ , in the  
311  $UV_{>290}/H_2O_2$  system was obtained by following the degradation of  $pCBA$ , which was  
312 additionally added in the experimental solution. No  $UV_{>290}$  direct photolysis of  $pCBA$  was  
313 observed in the absence of  $H_2O_2$  (Figure S6). Thus, the  $\bullet$ OH exposure was calculated from  
314 
$$\int[\bullet OH] dt = -\frac{\ln\frac{[pCBA]}{[pCBA]_0}}{k_{\bullet OH,pCBA}}$$
 (Elovitz and von Gunten 1999), where the  $k_{\bullet OH,pCBA}$  is the second-  
315 order rate constant of  $\bullet$ OH with  $pCBA$  ( $5\times 10^9 M^{-1} s^{-1}$ ) (Buxton, Greenstock et al. 1988). The  
316 logarithmic-scale degradation of  $amp^R$  segments exhibited linear correlations with  $\bullet$ OH exposure  
317 during  $UV_{>290}/H_2O_2$  (Figure 1b). The resulting bimolecular rate constants derived from the  
318 slopes of linear curves,  $k_{\bullet OH,Amp} = 2.303 \times \text{slope}$ , are summarized in Table 1.

319 The  $k_{\bullet OH,Amp}$  of  $amp^R$  segments increased with amplicon size, ranging from  $8.1\times 10^9 M^{-1} s^{-1}$   
320 for the 192 bp amplicon to  $5.6\times 10^{10} M^{-1} s^{-1}$  for the 851 bp amplicon (Table 1). A good linear  
321 relationship ( $r^2=0.996$ ) was observed between the  $k_{\bullet OH,Amp}$  and the base pair number of  $amp^R$   
322 segments (Figure S7), which was consistent with previous findings that  $\bullet$ OH non-selectively  
323 reacts with all nucleotides in a DNA sequence (both AT and GC contents) (He, Zhou et al.  
324 2019). The  $k_{\bullet OH,plasmid}$  of the entire plasmid was predicted to be  $1.8\times 10^{11} M^{-1} s^{-1}$  by applying  
325 the base pair number of pUC19 (2686 bps) into the regression equation of this linear curve  
326 (Figure S7). Alternatively, similar to the  $k_{UV,plasmid}$  as mentioned above, the  $k_{\bullet OH,plasmid}$  of

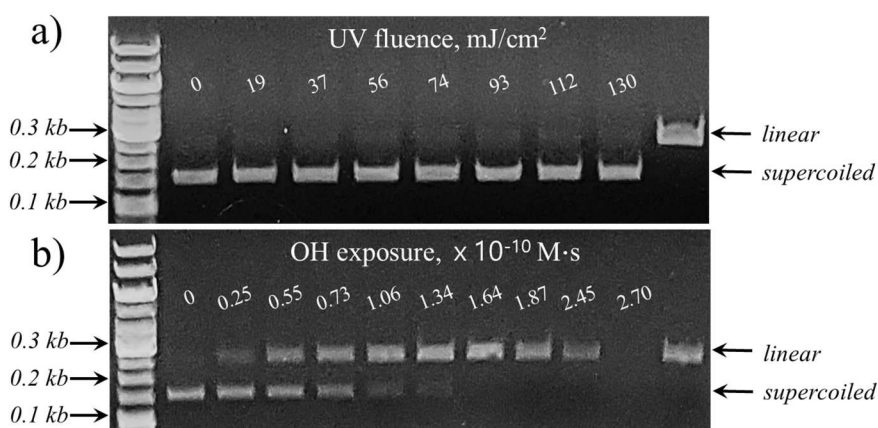
327 pUC19 was also estimated by normalizing the  $k_{\bullet OH, Amp}$  of each segment (qPCR analyses) to the  
328 entire plasmid and calculating the average value as  $1.5(\pm 0.07) \times 10^{11} \text{ M}^{-1} \text{ s}^{-1}$  (Table 1).

329 The  $k_{\bullet OH, Amp}$  values for the plasmid-encoded  $amp^R$  segments in this study were lower by a  
330 factor of  $\sim 3$  than those reported for the chromosome-encoded *blt* genes with similar amplicon  
331 length (He, Zhou et al. 2019). To see whether the reactivity difference is due to the  
332 conformational difference between the supercoiled-form plasmid and linear-form genomic DNA,  
333 the theoretical diffusion-controlled rate constant for the reaction of  $\bullet OH$  with supercoiled  
334 pUC19 was calculated based on a method proposed by He, Zhou et al. (2019). The theoretical  
335 maximum  $k_{\bullet OH}$  value was predicted as  $3.84 \times 10^{11} \text{ M}^{-1} \text{ s}^{-1}$  for pUC19, which yields a bp-specific  
336  $k_{\bullet OH}$  value of  $1.43 \times 10^8 \text{ M}_{AT+GC}^{-1} \text{ s}^{-1}$  when normalized to the length (in AT+GC bps) of pUC19  
337 (2686 bps) (Text S2). For comparison, the theoretical maximum  $k_{\bullet OH}$  value was  $7.97 \times 10^{12} \text{ M}^{-1}$   
338  $\text{s}^{-1}$  for a hypothetical 50-kbp linear-form segment of genomic DNA, yielding a bp-specific  $k_{\bullet OH}$   
339 value of  $1.59 \times 10^8 \text{ M}_{AT+GC}^{-1} \text{ s}^{-1}$  (He, Zhou et al. 2019). This indicates that the conformational  
340 difference between the plasmid (supercoiled) and genomic (linear) DNA (the bp-specific  $k_{\bullet OH}$   
341 differed only by a factor of 1.1) does not explain the observed  $\bullet OH$  reactivity difference. The  
342 maximum  $k_{\bullet OH}$  values for the  $amp^R$  amplicons can also be calculated by multiplying the bp-  
343 specific  $k_{\bullet OH}$  ( $1.43 \times 10^8 \text{ M}_{AT+GC}^{-1} \text{ s}^{-1}$ ) with the length of each amplicon, and ranged from  
344  $2.57 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$  for 192 bp to  $1.14 \times 10^{11} \text{ M}^{-1} \text{ s}^{-1}$  for 851 bp (Text S2). Thus, the experimental  
345  $k_{\bullet OH, Amp}$  for pUC19 were lower by a factor of 2.5 than the theoretical maximum  
346  $k_{\bullet OH, Amp}$  values. This difference is acceptable considering all possible experimental errors as  
347 well as the hypothesis applied in the theoretical calculation.

348 *Structural degradation of plasmid (Figure 2)*. Figure 2a shows the electrophoresis gel of  
349 pUC19 exposed to  $UV_{254}$ . The intact pUC19 before the  $UV_{254}$  exposure showed a band located  
350 lower than the linearized pUC19 by *EcoRI*. Plasmid DNA generally exists in a supercoiled form,

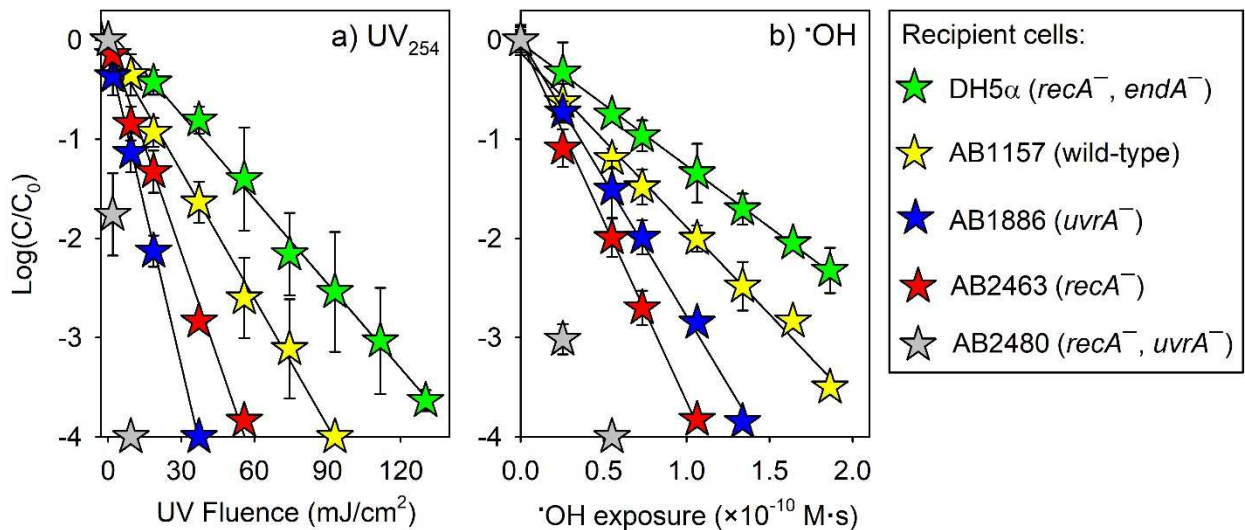
351 which is smaller than its linear form and migrates faster in electrophoresis gel. The UV<sub>254</sub>  
 352 irradiation (0–130 mJ/cm<sup>2</sup>) did not change the position of the band, suggesting that the pUC19  
 353 kept its supercoiled form without significant conformational change (e.g., strand breakage). It is  
 354 well documented that the gene damage by UV<sub>254</sub> is mainly caused by the formation of DNA  
 355 lesions such as CPDs and 6,4-photoproducts (pyrimidine adducts) (Siřha and Häder 2002, Cadet  
 356 and Douki 2018), which are generally not detectable by gel electrophoresis analysis (Yoo,  
 357 Dodd et al. 2018).

358 The reaction pathways of •OH with DNA include addition to nucleobases – producing adduct  
 359 radicals – and hydrogen-abstraction from 2-deoxyribose (sugar moiety) (von Sonntag 2006,  
 360 Dizdaroglu and Jaruga 2012), resulting in not only oxidized bases and abasic sites, but also  
 361 strand breaks (Cadet, Delatour et al. 1999, von Sonntag 2006). The double-strand breaks are  
 362 expected to be mainly responsible for the conformational change from supercoiled to linear  
 363 plasmid form. Gel electrophoresis analysis showed that the pUC19 plasmid band gradually  
 364 moved from the supercoiled to linear form position with increasing •OH exposure (Figure 2b),  
 365 revealing the formation of double-strand breaks in pUC19.



366  
 367 **Figure 2.** DNA electrophoresis gel of pUC19 treated by (a) UV<sub>254</sub> and (b) •OH (UV<sub>>290</sub>/H<sub>2</sub>O<sub>2</sub>).  
 368 First column shows the standard DNA ladders. Last column shows the linearized pUC19 after  
 369 treating with *EcoRI* restriction digestion.

370 **3.2 e-ARG deactivation (loss of transforming activity) during separate exposure to UV<sub>254</sub>**  
 371 **and •OH**



372  
 373 **Figure 3.** Elimination of gene transforming activity during the treatment of pUC19 (1 µg/mL)  
 374 with (a) UV<sub>254</sub> and (b) •OH (UV<sub>>290</sub>/H<sub>2</sub>O<sub>2</sub>) at pH 7 (2 mM phosphate buffer). 10 mM of H<sub>2</sub>O<sub>2</sub>  
 375 and 1 µM of *pCBA* were added in UV<sub>>290</sub>/H<sub>2</sub>O<sub>2</sub> experiments. The parentheses in legend show  
 376 the deficient DNA repair genes in recipient cells. Error bars represent the standard deviations of  
 377 triplicate experiments. Lines are the linear regressions of the experimental data.

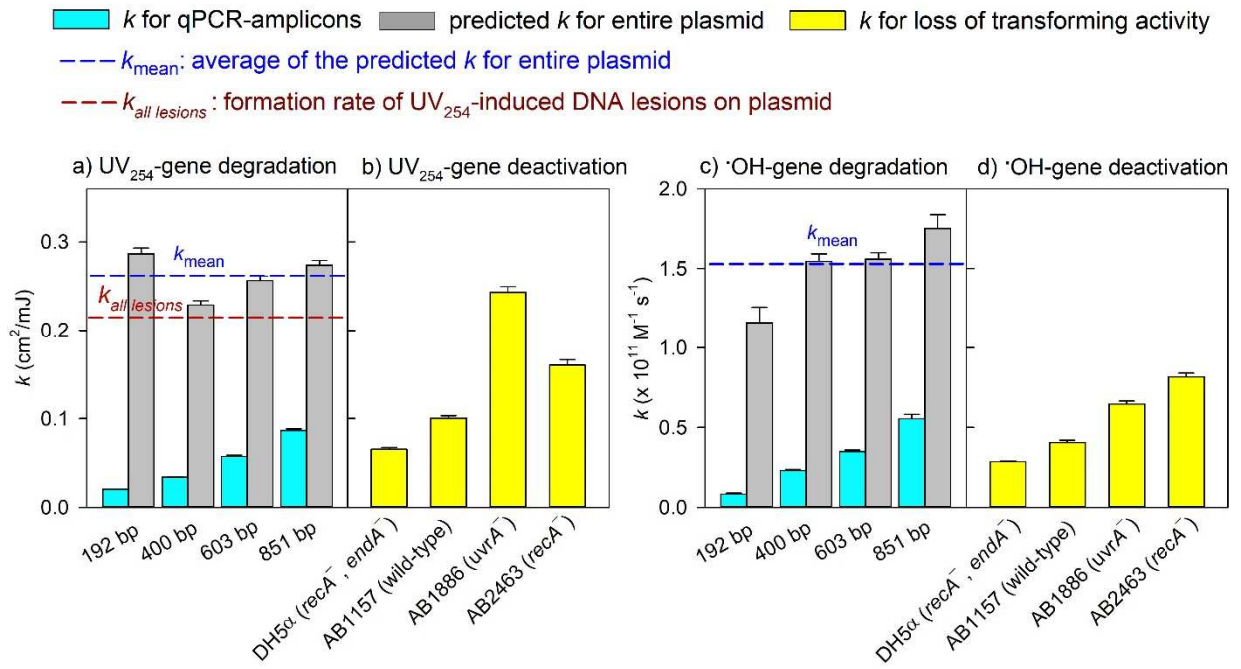
378 Bacterial cells have developed the ability of repairing DNA lesions induced by UV  
 379 irradiation and oxidant stress (e.g., radicals) using several enzyme systems (Teebor, Boorstein et  
 380 al. 1988, Sinha and Häder 2002). The biological deactivation of pUC19 upon separate treatment  
 381 with UV<sub>254</sub> and •OH was studied by analysing its transformation of non-resistant recipient cells  
 382 having different DNA repair abilities. The decrease of transforming activity of pUC19 followed  
 383 first-order kinetics with respect to UV fluence or •OH exposure, and was variable depending on  
 384 the recipient cells used (Figure 3). The most rapid decrease was observed for AB2480 (*uvrA*<sup>-</sup>,  
 385 *recA*<sup>-</sup>) during each separate treatment with UV<sub>254</sub> and •OH. No transformant was detectable  
 386 from AB2480 (more than 4-log decrease) when the UV fluence was above 5 mJ/cm<sup>2</sup>, or when

387 the  $\bullet\text{OH}$  exposure was higher than  $5.5 \times 10^{-11}$  M·s. Thus, the fluence-based rate constant for UV  
388 ( $k_{UV,transformation}$ ) and second-order rate constant for  $\bullet\text{OH}$  ( $k_{\bullet\text{OH},transformation}$ ) for the  
389 transforming activity loss for AB2480 were not calculated in this case due to the limited data  
390 points.

391 During UV<sub>254</sub> exposure (Figure 3a), the loss of transforming activity was slower in AB1886  
392 (*uvrA*<sup>-</sup>, 0.24 cm<sup>2</sup>/mJ) than AB2480, followed by AB2463 (*recA*<sup>-</sup>, 0.16 cm<sup>2</sup>/mJ), AB1157 (wild-  
393 type, 0.10 cm<sup>2</sup>/mJ), and DH5 $\alpha$  (*recA*<sup>-</sup>, *endA*<sup>-</sup>, 0.07 cm<sup>2</sup>/mJ), in which the values in parentheses  
394 show  $k_{UV,transformation}$  (Table 1). The variation in the transformation efficiency of plasmids  
395 carrying the same DNA damage indicated that the UV-induced damage can be repaired by  
396 recipient cells to different extents, depending on their repair gene proficiencies (or deficiencies).  
397 The *uvrABC* proteins involve the nucleotide excision repair of a variety of DNA lesions  
398 including the UV-induced CPDs and 6,4-photoproducts. This process is initiated by the  
399 interaction of *uvrA* protein with DNA to recognize the damaged sites (Kisker, Kuper et al.  
400 2013). The *recA* protein is essential for the post-replication repair of double-strand breaks and  
401 single-strand gaps (e.g., T-T dimers) by homologous recombination (Smith and Wang 1989,  
402 Shinohara and Ogawa 1995). These roles of *uvrA* and *recA* proteins well explained the trend  
403 observed from the transformation rates of *E. coli* strains. AB2480 was most sensitive to UV-  
404 induced DNA damage on pUC19 due to lack of both *uvrA* and *recA* genes. The *uvrA* deficient  
405 AB1886 was the second most sensitive, followed by the *recA* deficient AB2463, indicating that  
406 *uvrA* played a more important role than *recA* for the repair of UV-induced DNA damage. The  
407 wild-type AB1157 was less sensitive than the other three *E. coli* mutant strains owing to its  
408 DNA repair capability by *uvrA* and *recA*. Interestingly, despite its deficiency in *recA*, the DH5 $\alpha$   
409 strain exhibited even lower sensitivity to UV-induced DNA damage compared to wild-type  
410 AB1157. This was possibly attributable to the lack of a functional *endA* gene in DH5 $\alpha$ . The  
411 *endA* gene is associated with the formation of extracellular DNase I, which can hydrolyze

412 exogenous plasmid DNA, consequently reducing its uptake and transformation (Shou, Kang et  
413 al. 2019). In line with this, the DH5 $\alpha$  showed ~10-fold higher transformation efficiency than the  
414 other *E. coli* strains proficient in *endA* when all were transformed with equivalent concentrations  
415 of undamaged pUC19 (Figure S4). This *endA* deficiency might therefore render *E. coli* DH5 $\alpha$   
416 inherently more active for DNA damage repair than the other strains, despite its lack of *recA*.

417 During  $\bullet$ OH exposure (Figure 3b), the rate of transforming activity loss was the highest for  
418 AB2480 followed by AB2463 (*recA*<sup>-</sup>,  $8.2 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ ), AB1886 (*uvrA*<sup>-</sup>,  $6.5 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ ),  
419 AB1157 (wild-type,  $4.1 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ ), and DH5 $\alpha$  (*recA*<sup>-</sup>, *endA*<sup>-</sup>,  $2.9 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ ) in which the  
420 values in parentheses show  $k_{\bullet\text{OH},\text{transformation}}$  (Table 1). The *recA* deficient AB2463 showed  
421 higher sensitivity to  $\bullet$ OH-induced DNA damage than the *uvrA* deficient AB1886, which was  
422 opposite compared to the case for UV<sub>254</sub> (Figure 3a). This suggests that *recA* is more efficient  
423 for repairing  $\bullet$ OH-induced DNA damage, as homologous recombination is based on exchange of  
424 (damaged) nucleotide sequences (Alberts, Johnson et al. 2002) and capable of repairing not only  
425 base oxidation but also double-strand breaks. In contrast, *uvrA* is based on nucleotide excision  
426 repair and more specific to the repair of UV-induced DNA lesions (base modification such as  
427 CPDs), but not efficient for double-strand breaks (Alberts, Johnson et al. 2002).



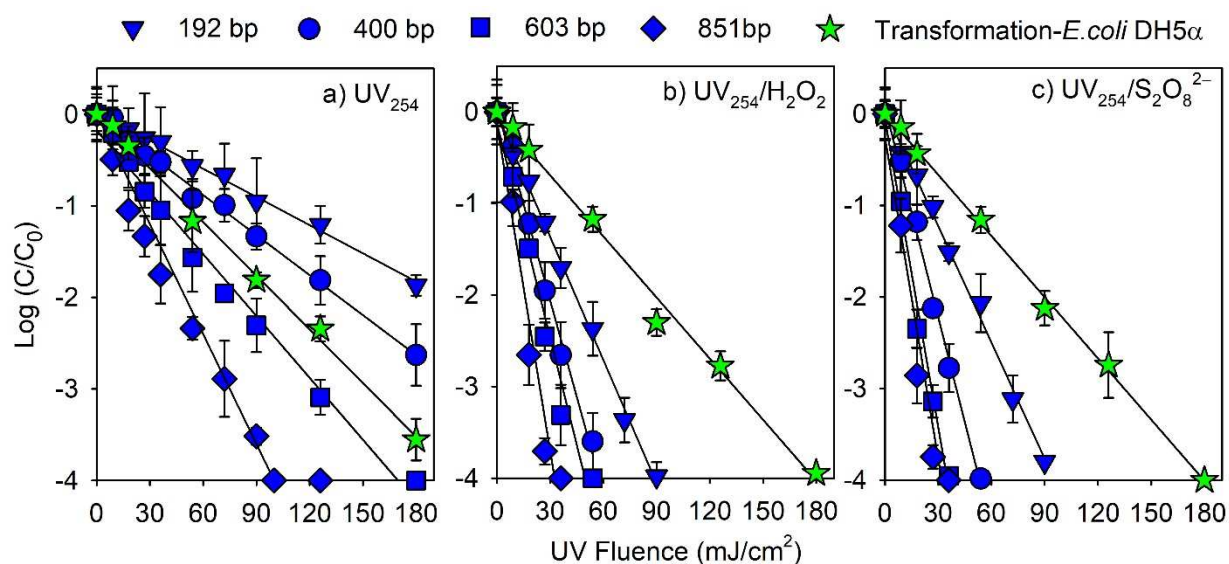
428

429 **Figure 4.** Apparent rate constants for the degradation of  $\text{amp}^R$  segments, pUC19 plasmid, and  
 430 the elimination of gene transforming activity during exposure to (a, b)  $\text{UV}_{254}$  and (c, d)  $\bullet\text{OH}$   
 431 ( $\text{UV}_{>290}/\text{H}_2\text{O}_2$ ). Specific kinetic parameters are summarized in Table 1 and error bars represent  
 432 the standard errors of apparent rate constants. Rate constants for the loss of transforming activity  
 433 in AB2480 were not calculated due to the limited data points.

434 The apparent rate constants for the degradation of  $\text{amp}^R$  segments, pUC19 plasmid, and the  
 435 loss of gene transforming activity are compared in Figure 4. For both  $\text{UV}_{254}$  direct photolysis  
 436 and  $\bullet\text{OH}$  oxidation, the estimated degradation rates of the entire plasmid (based on qPCR  
 437 analyses) overestimated the rates of pUC19 deactivation (loss of transforming activity) observed  
 438 for all repair-proficient *E. coli* recipient strains, which can be explained by the repair of  $\text{UV}_{254}$ -  
 439 and  $\bullet\text{OH}$ -induced damage by repair-proficient recipient cells. Our results were in agreement  
 440 with previous findings on the  $\text{UV}_{254}$  treated pWH1266 plasmid and its transformation of  
 441 *Acinetobacter baylyi* (Chang, Juhrend et al. 2017). However, the rate of gene deactivation  
 442 during  $\text{UV}_{254}$  direct photolysis measured using *uvrA* deficient AB1886 as the recipient strain  
 443 was close to the estimated degradation rate of pUC19 and the formation rate of DNA lesions

444 (Figure 4a, b). The minimum rates of gene deactivation measured by double mutant AB2480  
 445 (*uvrA*<sup>-</sup>, *recA*<sup>-</sup>) were estimated based on the limited data points shown in Figure 3, which gave  
 446 0.91 cm<sup>2</sup>/mJ and 1.7×10<sup>11</sup> M<sup>-1</sup> s<sup>-1</sup> for UV<sub>254</sub> and •OH exposure, respectively. These values were  
 447 significantly larger than the gene deactivation rates measured by any of the repair proficient  
 448 strains. Furthermore, the degradation rate of the entire plasmid underestimated the loss of  
 449 pUC19 transforming activity measured by AB2480 (*uvrA*<sup>-</sup>, *recA*<sup>-</sup>) during UV<sub>254</sub> direct  
 450 photolysis and •OH oxidation.

### 451 3.3 e-ARG degradation and deactivation by combined exposure to UV<sub>254</sub> and radicals

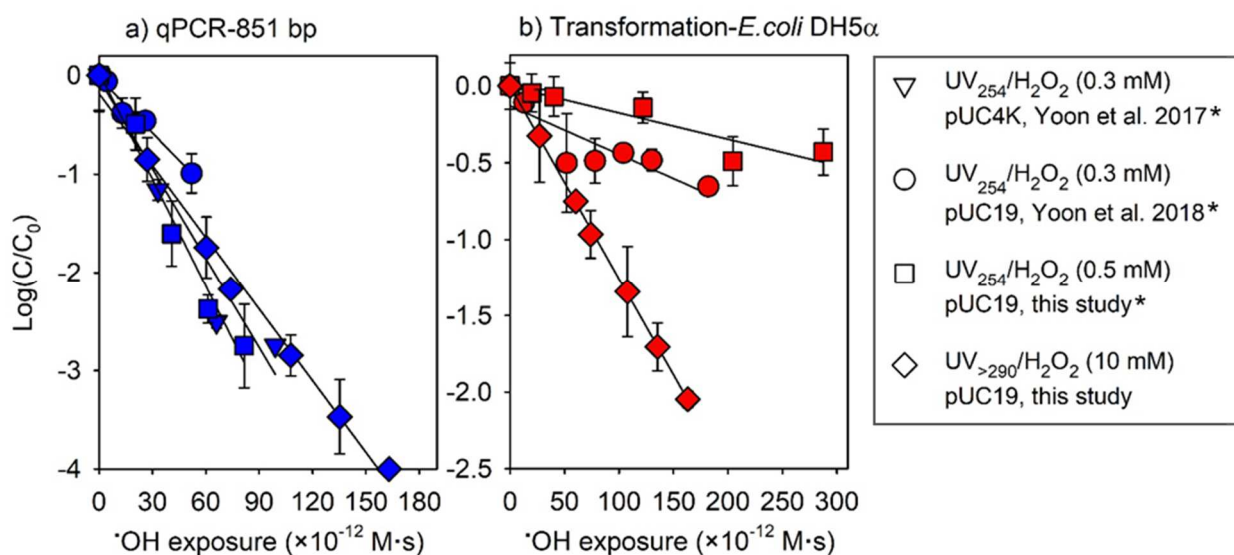


452 **Figure 5.** Degradation of *amp*<sup>R</sup> segments and elimination of gene transforming activity (*E. coli*  
 453 DH5α as recipient cells) with increasing UV fluence (0–180 mJ/cm<sup>2</sup>) during treatment of  
 454 pUC19 (0.3 μg/mL) with (a) UV<sub>254</sub>, (b) UV<sub>254</sub>/H<sub>2</sub>O<sub>2</sub> (0.5 mM), and (c) UV<sub>254</sub>/S<sub>2</sub>O<sub>8</sub><sup>2-</sup> (0.5 mM)  
 455 at pH 7 (2 mM phosphate buffer). *pCBA* (1 μM) was added in UV<sub>254</sub>/H<sub>2</sub>O<sub>2</sub> experiments for the  
 456 quantification of •OH, and both *pCBA* and nitrobenzene (each 1 μM) were added in UV/S<sub>2</sub>O<sub>8</sub><sup>2-</sup>  
 457 experiments for the quantification of •OH and SO<sub>4</sub><sup>•-</sup>. Error bars represent the standard  
 458 deviations of triplicate experiments. Lines represent the linear regressions of experimental data.



460 The degradation of *amp<sup>R</sup>* and the elimination of gene transforming activity (*E. coli* DH5 $\alpha$  as  
461 recipient cells) were tested during combined exposure to UV<sub>254</sub> and radicals ( $\bullet$ OH and SO<sub>4</sub> $\bullet^-$ ), in  
462 comparison to UV<sub>254</sub> direct photolysis. The degradation of *amp<sup>R</sup>* amplicons followed first-order  
463 kinetics with respect to UV fluence ( $r^2 > 0.99$ ) during UV<sub>254</sub>, UV<sub>254</sub>/H<sub>2</sub>O<sub>2</sub>, and UV<sub>254</sub>/S<sub>2</sub>O<sub>8</sub><sup>2-</sup>  
464 treatments (Figure 5). A lower initial concentration of pUC19 (0.3  $\mu$ g/mL) and a higher fluence  
465 range (0–180 mJ/cm<sup>2</sup>) were applied in these tests than in the experiments described in the  
466 preceding sections. However, the degradation rate constants of *amp<sup>R</sup>* amplicons upon UV<sub>254</sub>  
467 direct photolysis (i.e.,  $2.4 \times 10^{-2}$ – $8.2 \times 10^{-2}$  cm<sup>2</sup>/mJ) (Table S1) were in good agreement with  
468 those obtained during the treatment of 1  $\mu$ g/mL of pUC19 by 0–130 mJ/cm<sup>2</sup> of UV<sub>254</sub> (i.e.,  
469  $2.0 \times 10^{-2}$ – $8.9 \times 10^{-2}$  cm<sup>2</sup>/mJ, Table 1). The  $k_{UV_{254}/H_2O_2}$  of *amp<sup>R</sup>* amplicons during UV<sub>254</sub>/H<sub>2</sub>O<sub>2</sub>  
470 were in the range of  $1.0 \times 10^{-1}$ – $3.0 \times 10^{-1}$  cm<sup>2</sup>/mJ, which were larger than the  $k_{UV_{254}}$  by a factor of  
471  $\sim 4$  (Table S1). Therefore, the contribution of UV<sub>254</sub> direct photolysis to the overall gene  
472 degradation during UV<sub>254</sub>/H<sub>2</sub>O<sub>2</sub> was only about 25% ( $k_{UV_{254}}/k_{UV_{254}/H_2O_2} \times 100\%$ ), revealing  
473 the significant role of  $\bullet$ OH exposure (75%).

474 The  $k_{UV_{254}/H_2O_2}$  for the elimination of gene transforming activity was  $(5.1 \pm 0.2) \times 10^{-2}$  cm<sup>2</sup>/mJ,  
475 which was larger than the  $k_{UV_{254}}$  by a factor of only 1.1 ( $k_{UV_{254}/H_2O_2}/k_{UV_{254}} = 1.1$ , Table S1).  
476 Thus, even though the  $\bullet$ OH exposure significantly accelerated gene degradation (by a factor of  
477 4), its contribution to the elimination of gene transforming activity was not as efficient as  
478 expected.



479  
 480 **Figure 6.** (a) Degradation of *amp<sup>R</sup>* segment (851 bp) and (b) elimination of gene tra<sup>s</sup>formi<sup>g</sup>  
 481 activity (*E. coli* DH5 $\alpha$  as recipient cells) as a function of  $\bullet$ OH exposure during UV/H<sub>2</sub>O<sub>2</sub>  
 482 treatments at different UV (254 nm and >290nm) and H<sub>2</sub>O<sub>2</sub> (0.3 mM, 0.5 mM, and 10 mM)  
 483 conditions. The gene transforming activity was not measured in the study of Yoon et al. 2017  
 484 for treating pUC4k with UV<sub>254</sub>/H<sub>2</sub>O<sub>2</sub> (0.3 mM). The values for the data sets designated by (\*)  
 485 represent contributions from  $\bullet$ OH exposure only (estimated by subtracting UV<sub>254</sub> contributions  
 486 from overall measurements of qPCR signal or transforming activity loss). Error bars represent  
 487 the standard deviations of triplicate experiments. Lines represent the linear regressions of  
 488 experimental data.

489 Figure 6 summarizes the results from this study and the literature on the effect of  $\bullet$ OH  
 490 exposure on degradation of the 851 bp *amp<sup>R</sup>* segment and loss of gene transforming activity  
 491 (measured using *E. coli* DH5 $\alpha$  as recipient strain). Plasmid pUC4k or pUC19 carrying *amp<sup>R</sup>*  
 492 was treated with UV/H<sub>2</sub>O<sub>2</sub> at different conditions of UV wavelength and initial H<sub>2</sub>O<sub>2</sub>  
 493 concentrations, i.e., UV<sub>254</sub>/H<sub>2</sub>O<sub>2</sub> (0.3 mM or 0.5 mM) and UV<sub>>290</sub>/H<sub>2</sub>O<sub>2</sub> (10 mM). The  $\bullet$ OH-  
 494 induced degradation or deactivation were calculated by subtracting the effect of UV<sub>254</sub> direct  
 495 photolysis from the overall results of corresponding UV<sub>254</sub>/H<sub>2</sub>O<sub>2</sub>. Although the statistical  
 496 analysis showed a significant variation ( $p=0.0149$ ) in the slopes of linear curves in Figure 6a,

497 the  $k_{\bullet\text{OH},851\text{ bp}}$  values during UV<sub>254</sub>/H<sub>2</sub>O<sub>2</sub> treatment differed only by factors of 0.8–1.5 from that  
498 during  $\bullet\text{OH}$  only process (UV<sub>>290</sub>/H<sub>2</sub>O<sub>2</sub>). This suggested that UV<sub>254</sub>-induced gene damage on the  
499 plasmid (e.g., CPDs and pyrimidine adducts) did not cause significant impact on the reactivity  
500 of  $\bullet\text{OH}$  with nucleobase and sugar moieties during UV<sub>254</sub>/H<sub>2</sub>O<sub>2</sub>. However, the elimination of  
501 gene transforming activity (measured using *E. coli* DH5 $\alpha$  as recipient strain) varied significantly  
502 in the different treatments. The loss of gene transforming activity was ~5 times slower in  
503 UV<sub>254</sub>/H<sub>2</sub>O<sub>2</sub> processes (UV<sub>254</sub> and  $\bullet\text{OH}$  co-exist) as compared to that obtained from  
504 UV<sub>>290</sub>/H<sub>2</sub>O<sub>2</sub> ( $\bullet\text{OH}$  only). This indicates that the elimination of gene transforming activity  
505 caused by UV<sub>254</sub> direct photolysis and  $\bullet\text{OH}$  oxidation were not additive during UV<sub>254</sub>/H<sub>2</sub>O<sub>2</sub>. One  
506 possible explanation could be that some DNA damage repair pathways such as nucleotide  
507 excision repair by *uvrABC* also repaired  $\bullet\text{OH}$ -induced lesions (e.g., base oxidation) located near  
508 the UV-induced lesions, leading to much less extensive loss of transforming activity than would  
509 be expected from simple summation of the losses in transforming activity observed for UV<sub>254</sub>  
510 and  $\bullet\text{OH}$  exposure separately.

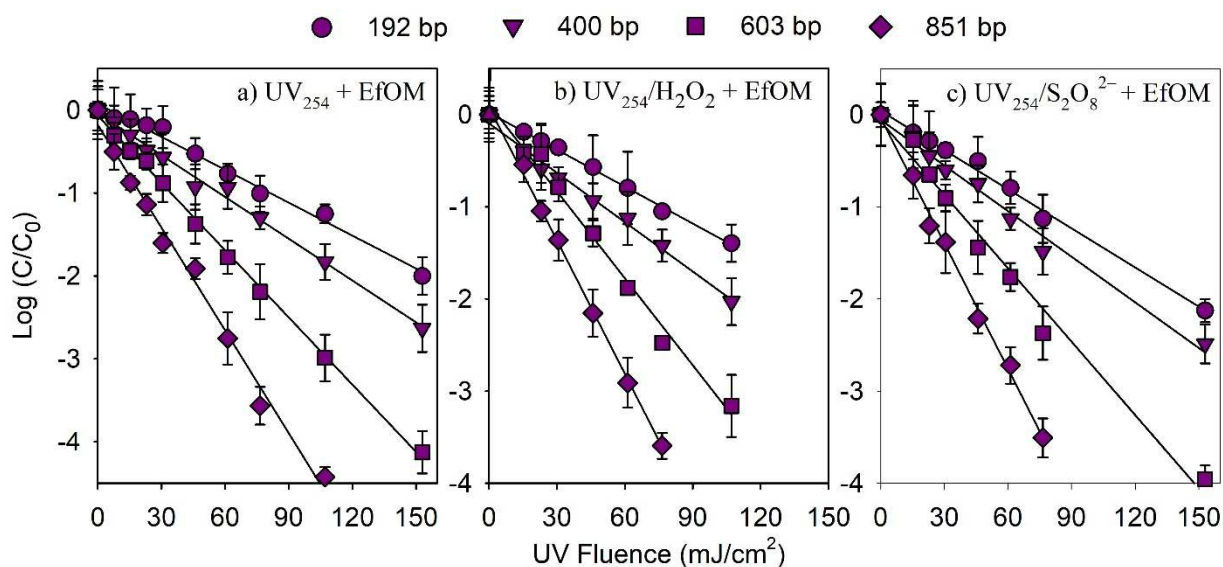
511 The degradation rates of *amp*<sup>R</sup> amplicons ( $1.0\times 10^{-1}$ – $3.2\times 10^{-1}$  cm<sup>2</sup>/mJ) during UV<sub>254</sub>/S<sub>2</sub>O<sub>8</sub><sup>2-</sup>  
512 were also ~4-fold larger than that obtained during UV<sub>254</sub> (Table S1). SO<sub>4</sub><sup>•-</sup> is the primary radical  
513 generated during UV<sub>254</sub> photolysis of S<sub>2</sub>O<sub>8</sub><sup>2-</sup>. However, SO<sub>4</sub><sup>•-</sup> can further react with H<sub>2</sub>O, OH<sup>-</sup>,  
514 and Cl<sup>-</sup> (sometimes present as an impurity) in aqueous solution to produce  $\bullet\text{OH}$  (Lutze, Kerlin  
515 et al. 2015). Using the degradation kinetics of radical probe compounds (nitrobenzene and  
516 *p*CBA) (Figures S7 and S8), the steady-state concentration of  $\bullet\text{OH}$  during UV<sub>254</sub>/S<sub>2</sub>O<sub>8</sub><sup>2-</sup> was  
517 determined to be  $6.4\times 10^{-13}$  M, which was approximately two orders of magnitude smaller than  
518 that of SO<sub>4</sub><sup>•-</sup> ( $1.8\times 10^{-11}$  M) under experimental conditions used here (Text S3). This was  
519 consistent with the theoretically estimated values considering all the possible reactions related to  
520  $\bullet\text{OH}$  formation in UV<sub>254</sub>/S<sub>2</sub>O<sub>8</sub><sup>2-</sup> system (Text S4). Despite the much lower concentration, the  
521 contribution of  $\bullet\text{OH}$  to the overall gene damage cannot be ignored, as the *amp*<sup>R</sup> segments

522 appeared to be much more reactive to •OH than SO<sub>4</sub>•<sup>-</sup> (see discussions below). Thus, the  
523 percentages of gene degradation attributable to UV<sub>254</sub> direct photolysis, SO<sub>4</sub>•<sup>-</sup>, and •OH during  
524 UV<sub>254</sub>/S<sub>2</sub>O<sub>8</sub><sup>2-</sup> treatment could be estimated as ~23%, ~33%, and ~44%, respectively, in which  
525 the relative contributions of each pathway to the overall gene damage were calculated based on  
526 the observed rate constants for degradation of *amp*<sup>R</sup> amplicons during UV<sub>254</sub> and  
527 UV<sub>254</sub>/S<sub>2</sub>O<sub>8</sub><sup>2-</sup> treatments, the steady-state concentrations of •OH and SO<sub>4</sub>•<sup>-</sup> in UV<sub>254</sub>/S<sub>2</sub>O<sub>8</sub><sup>2-</sup>  
528 experiments, and the second-order rate constants for reactions of •OH and SO<sub>4</sub>•<sup>-</sup> with the *amp*<sup>R</sup>  
529 amplicons (Text S5).

530 The second-order rate constants of SO<sub>4</sub>•<sup>-</sup> with *amp*<sup>R</sup> segments were determined to be in the  
531 range of (1.07–1.96)×10<sup>9</sup> M<sup>-1</sup> s<sup>-1</sup> (Text S5 and Table S2), which were about an order of  
532 magnitude lower than the •OH rate constants (Table 1). SO<sub>4</sub>•<sup>-</sup> preferentially adds to the electron-  
533 rich sites of the nucleobases (e.g., C<sub>5</sub> of pyrimidines) to generate radical adducts, which decay to  
534 nucleobase radicals as intermediates, followed by hydroxylation and oxidation in aqueous  
535 solution (von Sonntag 2006). However, SO<sub>4</sub>•<sup>-</sup> is larger than •OH and negatively charged.  
536 Therefore, steric hindrance and electrostatic repulsion might decrease the reactivity of SO<sub>4</sub>•<sup>-</sup>  
537 toward negatively charged DNA molecules in comparison to •OH.

538 Similar to UV<sub>254</sub>/H<sub>2</sub>O<sub>2</sub> processes, the elimination rate of pUC19 transforming activity during  
539 UV<sub>254</sub>/S<sub>2</sub>O<sub>8</sub><sup>2-</sup> treatment was also only slightly larger than that in UV<sub>254</sub> direct photolysis  
540 ( $k_{UV_{254}/S_2O_8^{2-}}/k_{UV_{254}} = 1.1$ , Table S1), even while the observed amplicon *degradation* rates  
541 were ~4-fold faster for UV<sub>254</sub>/S<sub>2</sub>O<sub>8</sub><sup>2-</sup> compared to UV<sub>254</sub> direct photolysis. This further supports  
542 the above findings that co-exposure to UV<sub>254</sub> and radicals during UV<sub>254</sub>-AOPs did not lead to  
543 faster transforming activity loss than the separate exposures to UV<sub>254</sub> and radicals, due to the  
544 facile repair of UV<sub>254</sub> and/or radical-induced DNA damage in host cells.

545 **3.5 Effect of wastewater effluent organic matter on the degradation of *amp<sup>R</sup>* during UV<sub>254</sub>-**  
 546 **AOPs**



547  
 548 **Figure 7.** Degradation of *amp<sup>R</sup>* segments in the presence of wastewater effluent organic matter  
 549 (5.4 mg C/L) during the treatment of pUC19 (0.31 µg/mL) with (a) UV<sub>254</sub>, (b) UV<sub>254</sub>/H<sub>2</sub>O<sub>2</sub> (0.5  
 550 mM), and (c) UV<sub>254</sub>/S<sub>2</sub>O<sub>8</sub><sup>2-</sup> (0.5 mM) at pH 7 (2 mM phosphate buffer). *p*CBA (1 µM) was  
 551 added in UV<sub>254</sub>/H<sub>2</sub>O<sub>2</sub> experiments for the quantification of •OH, and both *p*CBA and  
 552 nitrobenzene (each 1 µM) were added in UV/S<sub>2</sub>O<sub>8</sub><sup>2-</sup> experiments for the quantification of •OH  
 553 and SO<sub>4</sub><sup>•-</sup>. Error bars represent the standard deviations of triplicate experiments. Lines represent  
 554 the linear regressions of experimental data.

555 The efficiencies of *amp<sup>R</sup>* degradation during UV<sub>254</sub>, UV<sub>254</sub>/H<sub>2</sub>O<sub>2</sub>, and UV<sub>254</sub>/S<sub>2</sub>O<sub>8</sub><sup>2-</sup>  
 556 experiments were investigated in the presence of EfOM (5.4 mg C/L). The UV 254 nm  
 557 absorbance of EfOM solution (*a*<sub>254</sub>) was 0.1 cm<sup>-1</sup>. The path length in the petri dish (*L*) was 1.6  
 558 cm. Thus, the water factor,  $WF = (1 - 10^{-a_{254} \cdot L}) / (a_{254} \cdot L \cdot \ln 10)$  (Bolton and Linden 2003), was  
 559 calculated to be 0.84. The average UV intensity in the solution containing EfOM was then  
 560 corrected from 0.30 mW/cm<sup>2</sup> to 0.25 mW/cm<sup>2</sup> using the calculated water factor (Lee, Gerrity et  
 561 al. 2016). The *amp<sup>R</sup>* segments were gradually degraded with increasing UV fluence following

562 first-order kinetics (Figure 7). Interestingly, the  $k_{UV,Amp}$  of each segment during UV<sub>254</sub>  
563 irradiation in the presence of EfOM was approximately 1.2 times higher than the corresponding  
564 conditions without EfOM ( $p < 0.0001$ ) (Table S1). Chromophoric dissolved organic matter  
565 (DOM) is known to generate reactive species upon UV irradiation, such as triplet excited states  
566 of DOM (<sup>3</sup>DOM\*), singlet oxygen, and •OH, which have been proven to contribute to the  
567 oxidation of organic pollutants (Chin, Miller et al. 2004, Lester, Sharpless et al. 2013, Batista,  
568 Teixeira et al. 2016, Rosario-Ortiz and Canonica 2016). Bacteriophage MS2 was found to be  
569 inactivated by <sup>3</sup>DOM\* and singlet oxygen generated from sunlight irradiation of wastewater and  
570 river water DOM isolates (Kohn, Grandbois et al. 2007, Kohn and Nelson 2007, Rosado-Lausell,  
571 Wang et al. 2013). The reactive oxygen species (e.g., singlet oxygen, superoxide, H<sub>2</sub>O<sub>2</sub>)  
572 generated during photooxidations involving <sup>3</sup>DOM\* were proposed to inactivate bacteria by  
573 damaging DNA, proteins, and cell membranes (Song, Mohseni et al. 2016, Nelson, Boehm et al.  
574 2018). Our findings suggest that through its photosensitizing properties, DOM can indirectly  
575 contribute to the photodegradation of e-ARGs. Similar results were reported recently on the  
576 enhanced photo-degradation of pBR322 plasmid-encoded *tet A* and *bla<sub>TEM-1</sub>* genes in DOM  
577 solution (Zhang, Li et al. 2019). This degradation pathway might play an especially important  
578 role during solar disinfection processes, due to the low UV absorbance characteristics of DNA at  
579 high wavelengths (and consequent minimal direct UV photolysis under such conditions).

580 In contrast, EfOM mainly acted as radical scavenger during UV<sub>254</sub>/H<sub>2</sub>O<sub>2</sub> and UV<sub>254</sub>/S<sub>2</sub>O<sub>8</sub><sup>2-</sup>  
581 experiments. The  $k_{UV_{254}/H_2O_2}$  and  $k_{UV_{254}/S_2O_8^{2-}}$  of *amp<sup>R</sup>* segments in the presence of EfOM were  
582 each decreased by a factor of ~3 compared to the conditions without EfOM (Table S1). The  
583 degradation kinetics of *pCBA* and nitrobenzene (Figure S10) also confirmed the much lower  
584 levels of radicals in the presence of EfOM (e.g., [SO<sub>4</sub>•<sup>-</sup>]<sub>ss</sub>: 9.5×10<sup>-13</sup> M with EfOM vs. 1.8×10<sup>-11</sup>  
585 M without EfOM). Overall, the degradation efficiency of *amp<sup>R</sup>* in the presence of EfOM

586 increased only marginally (within a factor of 1.4) during UV<sub>254</sub>/H<sub>2</sub>O<sub>2</sub> and UV<sub>254</sub>/S<sub>2</sub>O<sub>8</sub><sup>2-</sup>,  
587 compared to UV<sub>254</sub> alone, due to the significant scavenging of radicals by EfOM.

## 588 4. Conclusions

- 589 • The degradation of *amp<sup>R</sup>* segments and the loss of gene transforming activity followed first-  
590 order kinetics with respect to UV fluence and •OH exposure during UV<sub>254</sub> direct photolysis  
591 and •OH oxidation (UV<sub>>290</sub>/H<sub>2</sub>O<sub>2</sub>). The degradation rate constants of *amp<sup>R</sup>* segments  
592 increased with amplicon size, which corresponded to the increase in nucleotide bps (and  
593 hence, reaction sites) with increased amplicon length. Gel electrophoresis results indicated  
594 that UV<sub>254</sub> (0–130 mJ/cm<sup>2</sup>) did not change the supercoiled conformation of pUC19 plasmid,  
595 whereas •OH exposure (0–2.7×10<sup>-10</sup> M·s) led to strand-breaks.
- 596 • The loss of gene transforming activity varied depending on the type of recipient cells.  
597 Double mutant AB2480, which was deficient in repair genes *uvrA* and *recA*, was the most  
598 sensitive to DNA damage induced by UV<sub>254</sub> direct photolysis and •OH oxidation. The  
599 predicted degradation rates for the entire pUC19 plasmid overestimated the loss in  
600 transforming activity of pUC19 for all recipient cells other than AB2480, apparently due to  
601 the repair of DNA damage by repair-proficient strains.
- 602 • The second-order rate constants of •OH with *amp<sup>R</sup>* segments were determined to be in the  
603 range of 8.1×10<sup>9</sup> – 5.6×10<sup>10</sup> (M<sup>-1</sup> s<sup>-1</sup>) and showed a strong linear relationship with the base  
604 pair number of *amp<sup>R</sup>* segments, suggesting that •OH reacts with all nucleobases of plasmid  
605 DNA non-selectively. The rate constants of SO<sub>4</sub>•<sup>-</sup> with *amp<sup>R</sup>* segments were an order of  
606 magnitude lower than the corresponding •OH rate constants; thus, the contribution of the  
607 trace amount of •OH to the overall gene damage cannot be ignored during UV<sub>254</sub>/S<sub>2</sub>O<sub>8</sub><sup>2-</sup>  
608 treatment.
- 609 • Although •OH and SO<sub>4</sub>•<sup>-</sup> significantly accelerated (~75%) the degradation of *amp<sup>R</sup>*  
610 amplicons measured by qPCR during UV<sub>254</sub>/H<sub>2</sub>O<sub>2</sub> and UV<sub>254</sub>/S<sub>2</sub>O<sub>8</sub><sup>2-</sup> experiments, this only  
611 marginally increased the gene deactivation rate (~11%), as gene damage caused by UV<sub>254</sub>  
612 and radical co-exposure appeared to be repaired more efficiently by recipient cells than the



613 gene damage from the separate UV<sub>254</sub> and radical exposures. Thus, the elimination  
614 efficiency of the ARGs' transforming activities was similar for UV<sub>254</sub> vs UV<sub>254</sub>-AOPs at the  
615 same UV fluence, and the extent of elimination was mainly determined by the level of UV  
616 fluence. Because the transforming activity of the *amp*<sup>R</sup> gene could be lowered by only ~1-  
617 log at a typical UV disinfection fluence for water treatment (e.g., 40 mJ/cm<sup>2</sup>) but increased  
618 to more than 4-logs at an elevated UV fluence for UV-AOPs (e.g., 500 mJ/cm<sup>2</sup>), the use of  
619 UV-AOPs may still prove beneficial for ARG degradation and deactivation, with the  
620 accompanying benefit of improved degradation of trace organic contaminants.

- 621 • Reactive species formed from the excitation of chromophoric organic matter appear to  
622 contribute to accelerated degradation of *amp*<sup>R</sup> during UV<sub>254</sub> irradiation in the presence of  
623 EfOM (compared to direct photolysis by UV<sub>254</sub> alone), whereas EfOM mainly acted as a  
624 radical scavenger in UV<sub>254</sub>/H<sub>2</sub>O<sub>2</sub> and UV<sub>254</sub>/S<sub>2</sub>O<sub>8</sub><sup>2-</sup> experiments.

## 625 **Acknowledgements**

626 This study was supported by the Korea Institute of Marine Science & Technology Promotion  
627 funded by the National Research Foundation funded by the Ministry of Science, ICT and Future  
628 Planning (NRF-2020R1A2C2011951). Curtin University (Curtin International Postgraduate  
629 Research Scholarship) and Water Research Australia (WaterRA Postgraduate Scholarship) are  
630 gratefully acknowledged for providing financial support for M. Nihemaiti. Additional support  
631 for H. He and M. C. Dodd from U.S. National Science Foundation Grant Number CBET-  
632 1254929 is gratefully acknowledged.

633

634 **Reference**

635 Alberts, B., et al. (2002). Molecular Biology of the Cell, 4th edition. New York, Garland  
636 Science.

637  
638 Batista, A. P. S., et al. (2016). "Correlating the chemical and spectroscopic characteristics of  
639 natural organic matter with the photodegradation of sulfamerazine." Water Research **93**: 20-29.

640  
641 Bioneer (2016). "AccuPrep® Nano-Plus Plasmid Mini/Midi/Maxi Extraction Kit." User's Guide  
642 **Available at [http://us.bioneer.com/Protocol/AccuPrep%20Nano-](http://us.bioneer.com/Protocol/AccuPrep%20Nano-Plus%20Plasmid%20Extraction%20Kit.pdf)**  
643 **Plus%20Plasmid%20Extraction%20Kit.pdf**.

644  
645 Bolton, J. R. and K. G. Linden (2003). "Standardization of methods for fluence (UV Dose)  
646 determination in bench-scale UV experiments." Journal of Environmental Engineering **129**(3):  
647 209-215.

648  
649 Buxton, G. V., et al. (1988). "Critical Review of rate constants for reactions of hydrated  
650 electrons, hydrogen atoms and hydroxyl radicals ( $\cdot\text{OH}/\cdot\text{O}-$ ) in Aqueous Solution." Journal of  
651 Physical and Chemical Reference Data **17**(2): 513-886.

652  
653 Cacace, D., et al. (2019). "Antibiotic resistance genes in treated wastewater and in the receiving  
654 water bodies: A pan-European survey of urban settings." Water Research **162**: 320-330.

655  
656 Cadet, J., et al. (1999). "Hydroxyl radicals and DNA base damage." Mutation  
657 Research/Fundamental and Molecular Mechanisms of Mutagenesis **424**(1): 9-21.

658  
659 Cadet, J. and T. Douki (2018). "Formation of UV-induced DNA damage contributing to skin  
660 cancer development." Photochemical & Photobiological Sciences **17**(12): 1816-1841.

661  
662 Chang, P. H., et al. (2017). "Degradation of Extracellular Antibiotic Resistance Genes with  
663 UV254 Treatment." Environmental Science & Technology **51**(11): 6185-6192.

664  
665 Chin, Y.-P., et al. (2004). "Photosensitized Degradation of Bisphenol A by Dissolved Organic  
666 Matter." Environmental Science & Technology **38**(22): 5888-5894.

667  
668 Christou, A., et al. (2017). "The potential implications of reclaimed wastewater reuse for  
669 irrigation on the agricultural environment: The knowns and unknowns of the fate of antibiotics  
670 and antibiotic resistant bacteria and resistance genes – A review." Water Research **123**: 448-467.

671  
672 Davies, J. and D. Davies (2010). "Origins and Evolution of Antibiotic Resistance."  
673 Microbiology and Molecular Biology Reviews : MMBR **74**(3): 417-433.

674

675 Dizdaroglu, M. and P. Jaruga (2012). "Mechanisms of free radical-induced damage to DNA."  
676 Free Radical Research **46**(4): 382-419.

677

678 Dodd, M. C. (2012). "Potential impacts of disinfection processes on elimination and  
679 deactivation of antibiotic resistance genes during water and wastewater treatment." Journal of  
680 Environmental Monitoring **14**(7): 1754-1771.

681

682 Elovitz, M. S. and U. von Gunten (1999). "Hydroxyl Radical/Ozone Ratios During Ozonation  
683 Processes. I. The Rct Concept." Ozone: Science & Engineering **21**(3): 239-260.

684

685 Ferro, G., et al. (2016). "Antibiotic resistance spread potential in urban wastewater effluents  
686 disinfected by UV/H<sub>2</sub>O<sub>2</sub> process." Science of the Total Environment **560-561**: 29-35.

687

688 Ferro, G., et al. (2017). "β-lactams resistance gene quantification in an antibiotic resistant  
689 Escherichia coli water suspension treated by advanced oxidation with UV/H<sub>2</sub>O<sub>2</sub>." Journal of  
690 Hazardous Materials **323**: 426-433.

691

692 Giannakis, S., et al. (2018). "Solar photo-Fenton disinfection of 11 antibiotic-resistant bacteria  
693 (ARB) and elimination of representative AR genes. Evidence that antibiotic resistance does not  
694 imply resistance to oxidative treatment." Water Research **143**: 334-345.

695

696 Görner, H. (1994). "New trends in photobiology: Photochemistry of DNA and related  
697 biomolecules: Quantum yields and consequences of photoionization." Journal of Photochemistry  
698 and Photobiology B: Biology **26**(2): 117-139.

699

700 Guo, M.-T., et al. (2015). "Distinguishing Effects of Ultraviolet Exposure and Chlorination on  
701 the Horizontal Transfer of Antibiotic Resistance Genes in Municipal Wastewater."  
702 Environmental Science & Technology **49**(9): 5771-5778.

703

704 He, H., et al. (2019). "Degradation and Deactivation of Bacterial Antibiotic Resistance Genes  
705 during Exposure to Free Chlorine, Monochloramine, Chlorine Dioxide, Ozone, Ultraviolet Light,  
706 and Hydroxyl Radical." Environmental Science & Technology **53**(4): 2013-2026.

707

708 Hiller, C. X., et al. (2019). "Antibiotic microbial resistance (AMR) removal efficiencies by  
709 conventional and advanced wastewater treatment processes: A review." Science of the Total  
710 Environment **685**: 596-608.

711

712 Hong, P.-Y., et al. (2018). "Reusing Treated Wastewater: Consideration of the Safety Aspects  
713 Associated with Antibiotic-Resistant Bacteria and Antibiotic Resistance Genes." Water **10**(3).

714

715 Kisker, C., et al. (2013). "Prokaryotic Nucleotide Excision Repair." **5**(3): a012591-a012591.

716

717 Kohn, T., et al. (2007). "Association with Natural Organic Matter Enhances the Sunlight-  
718 Mediated Inactivation of MS2 Coliphage by Singlet Oxygen." Environmental Science &  
719 Technology **41**(13): 4626-4632.

720  
721 Kohn, T. and K. L. Nelson (2007). "Sunlight-Mediated Inactivation of MS2 Coliphage via  
722 Exogenous Singlet Oxygen Produced by Sensitizers in Natural Waters." Environmental Science  
723 & Technology **41**(1): 192-197.

724  
725 Lee, Y., et al. (2016). "Organic Contaminant Abatement in Reclaimed Water by UV/H<sub>2</sub>O<sub>2</sub> and  
726 a Combined Process Consisting of O<sub>3</sub>/H<sub>2</sub>O<sub>2</sub> Followed by UV/H<sub>2</sub>O<sub>2</sub>: Prediction of Abatement  
727 Efficiency, Energy Consumption, and Byproduct Formation." Environmental Science &  
728 Technology **50**(7): 3809-3819.

729  
730 Lester, Y., et al. (2013). "Production of Photo-oxidants by Dissolved Organic Matter During UV  
731 Water Treatment." Environmental Science & Technology **47**(20): 11726-11733.

732  
733 Liu, S.-S., et al. (2018). "Chlorine disinfection increases both intracellular and extracellular  
734 antibiotic resistance genes in a full-scale wastewater treatment plant." Water Research **136**: 131-  
735 136.

736  
737 Lorenz, M. G. and W. Wackernagel (1994). "Bacterial gene transfer by natural genetic  
738 transformation in the environment." Microbiological Reviews **58**(3): 563-602.

739  
740 Lorenz, M. G. and W. Wackernagel (1994). "Bacterial gene transfer by natural genetic  
741 transformation in the environment." Microbiol. Mol. Biol. Rev. **58**(3): 563-602.

742  
743 Luby, E., et al. (2016). "Molecular Methods for Assessment of Antibiotic Resistance in  
744 Agricultural Ecosystems: Prospects and Challenges." Journal of Environment Quality **45**(2): 441.

745  
746 Lutze, H. V., et al. (2015). "Sulfate radical-based water treatment in presence of chloride:  
747 Formation of chlorate, inter-conversion of sulfate radicals into hydroxyl radicals and influence  
748 of bicarbonate." Water Research **72**: 349-360.

749  
750 Mao, D., et al. (2014). "Persistence of Extracellular DNA in River Sediment Facilitates  
751 Antibiotic Resistance Gene Propagation." Environmental Science & Technology **48**(1): 71-78.

752  
753 McKinney, C. W. and A. Pruden (2012). "Ultraviolet disinfection of antibiotic resistant bacteria  
754 and their antibiotic resistance genes in water and wastewater." Environmental Science and  
755 Technology **46**(24): 13393-13400.

756  
757 Michael-Kordatou, I., et al. (2015). "Erythromycin oxidation and ERY-resistant Escherichia coli  
758 inactivation in urban wastewater by sulfate radical-based oxidation process under UV-C  
759 irradiation." Water Research **85**: 346-358.

760  
761 Nagler, M., et al. (2018). "Extracellular DNA in natural environments: features, relevance and  
762 applications." Applied Microbiology and Biotechnology **102**(15): 6343-6356.

763  
764 Nelson, K. L., et al. (2018). "Sunlight-mediated inactivation of health-relevant microorganisms  
765 in water: a review of mechanisms and modeling approaches." Environmental Science: Processes  
766 & Impacts **20**(8): 1089-1122.

767  
768 Neta, P., et al. (1988). "Rate Constants for Reactions of Inorganic Radicals in Aqueous  
769 Solution." Journal of Physical and Chemical Reference Data **17**(3): 1027-1284.

770  
771 Neta, P., et al. (1977). "Rate constants and mechanism of reaction of SO<sub>4</sub><sup>·-</sup> with aromatic  
772 compounds." Journal of the American Chemical Society **99**(1): 163-164.

773  
774 Osińska, A., et al. (2020). "Small-scale wastewater treatment plants as a source of the  
775 dissemination of antibiotic resistance genes in the aquatic environment." Journal of Hazardous  
776 Materials **381**: 121221.

777  
778 Pruden, A. (2014). "Balancing Water Sustainability and Public Health Goals in the Face of  
779 Growing Concerns about Antibiotic Resistance." Environmental Science & Technology **48**(1):  
780 5-14.

781  
782 Rosado-Lausell, S. L., et al. (2013). "Roles of singlet oxygen and triplet excited state of  
783 dissolved organic matter formed by different organic matters in bacteriophage MS2  
784 inactivation." Water Research **47**(14): 4869-4879.

785  
786 Rosario-Ortiz, F. L. and S. Canonica (2016). "Probe Compounds to Assess the Photochemical  
787 Activity of Dissolved Organic Matter." Environmental Science & Technology **50**(23): 12532-  
788 12547.

789  
790 Shanehbandi, D., et al. (2013). "Vibration and glycerol-mediated plasmid DNA transformation  
791 for Escherichia coli." FEMS Microbiology Letters **348**(1): 74-78.

792  
793 Shinohara, A. and T. Ogawa (1995). "Homologous recombination and the roles of double-strand  
794 breaks." Trends in Biochemical Sciences **20**(10): 387-391.

795  
796 Shou, W., et al. (2019). "Substituted Aromatic-Facilitated Dissemination of Mobile Antibiotic  
797 Resistance Genes via an Antihydrolysis Mechanism Across an Extracellular Polymeric  
798 Substance Permeable Barrier." Environmental Science & Technology **53**(2): 604-613.

799  
800 Sinha, R. P. and D.-P. Häder (2002). "UV-induced DNA damage and repair: a review."  
801 Photochemical & Photobiological Sciences **1**(4): 225-236.

802

803 Smith, K. C. and T.-C. V. Wang (1989). "recA-dependent DNA repair processes." BioEssays  
804 **10**(1): 12-16.

805

806 Song, K., et al. (2016). "Application of ultraviolet light-emitting diodes (UV-LEDs) for water  
807 disinfection: A review." Water Research **94**: 341-349.

808

809 Stefan, M. I. (2018). Advanced Oxidation Processes for Water Treatment. Fundamentals and  
810 Applications, IWA Publishing.

811

812 Teebor, G. W., et al. (1988). "The Repairability of Oxidative Free Radical Mediated Damage to  
813 DNA: A Review." International Journal of Radiation Biology **54**(2): 131-150.

814

815 Vikesland, P. J., et al. (2017). "Toward a Comprehensive Strategy to Mitigate Dissemination of  
816 Environmental Sources of Antibiotic Resistance." Environmental Science & Technology **51**(22):  
817 13061-13069.

818

819 von Sonntag, C. (2006). Free-radical-induced DNA damage and its repair: A chemical  
820 perspective. Heidelberg, Berlin, Springer.

821

822 Wu, D., et al. (2019). "Urban and agriculturally influenced water contribute differently to the  
823 spread of antibiotic resistance genes in a mega-city river network." Water Research **158**: 11-21.

824

825 Yoon, Y., et al. (2017). "Inactivation efficiency of plasmid-encoded antibiotic resistance genes  
826 during water treatment with chlorine, UV, and UV/H<sub>2</sub>O<sub>2</sub>." Water Research **123**: 783-793.

827

828 Yoon, Y., et al. (2018). "Elimination of transforming activity and gene degradation during UV  
829 and UV/H<sub>2</sub>O<sub>2</sub> treatment of plasmid-encoded antibiotic resistance genes." Environmental  
830 Science: Water Research & Technology.

831

832 Zhang, T., et al. (2019). "Removal of antibiotic resistance genes and control of horizontal  
833 transfer risk by UV, chlorination and UV/chlorination treatments of drinking water." Chemical  
834 Engineering Journal **358**: 589-597.

835

836 Zhang, X., et al. (2019). "Enhanced Photodegradation of Extracellular Antibiotic Resistance  
837 Genes by Dissolved Organic Matter Photosensitization." Environmental Science & Technology  
838 **53**(18): 10732-10740.

839

840 Zhang, Y., et al. (2017). "Subinhibitory Concentrations of Disinfectants Promote the Horizontal  
841 Transfer of Multidrug Resistance Genes within and across Genera." Environmental Science &  
842 Technology **51**(1): 570-580.

843

844 Zhang, Y., et al. (2018). "Cell-free DNA: A Neglected Source for Antibiotic Resistance Genes  
845 Spreading from WWTPs." Environmental Science & Technology **52**(1): 248-257.

846  
847 Zheng, J., et al. (2017). "Effects and mechanisms of ultraviolet, chlorination, and ozone  
848 disinfection on antibiotic resistance genes in secondary effluents of municipal wastewater  
849 treatment plants." Chemical Engineering Journal **317**: 309-316.

850  
851 Zheng, X., et al. (2014). "Contribution of effluent organic matter (EfOM) to ultrafiltration (UF)  
852 membrane fouling: Isolation, characterization, and fouling effect of EfOM fractions." Water  
853 Research **65**: 414-424.

854

855

## Graphical Abstract

