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Review

Regrowth of bacteria after light-based disinfection — What we know and where we go from here

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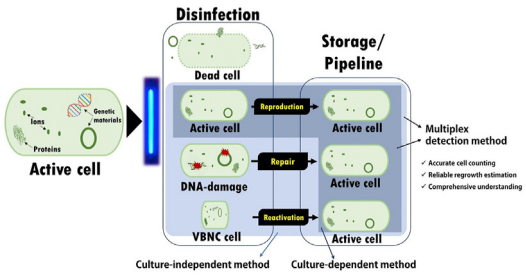
HIGHLIGHTS

- Regrowth mechanisms after disinfection are reactivation, repair and reproduction.
- More comprehensive regrowth tests at environmentally relevant conditions are needed.
- Both culture-base and culture-independent approaches should be adopted.

GRAPHICAL ABSTRACT



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ABSTRACT

Regrowth of bacteria after water/wastewater disinfection is a serious risk to public health, particularly when such pathogens carry antibiotic resistance genes. Despite increasing interest in light-based disinfection using ultraviolet or solar radiation, the mechanism of bacterial regrowth and their concentration upon light exposure (i.e., during storage, or after discharge into rivers or lakes) remain poorly understood. Therefore, we present a focused critical review to 1) elucidate regrowth mechanisms, 2) summarize the pros and cons of available experimental designs and detection techniques for regrowth evaluation, and 3) provide an outlook of key research directions for further investigations of post-disinfection bacterial regrowth. Bacterial regrowth can occur through reactivation from a viable but non-culturable state, repair of photo-induced DNA damage, and reproduction of bacteria surviving disinfection. Many studies have underestimated the degree of actual regrowth because of the use of simple experimental designs and plate count methods, which cannot quantify actual abundance of viable bacteria. Further research should investigate the effects of various factors on bacterial regrowth in realistic conditions in regrowth tests and adopt multiplex detection methods that combine culture-based and culture-independent approaches. An accurate understanding of the mechanisms involved in bacterial regrowth following disinfection is critical for safeguarding public health and aquatic environments.

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Contents

Abbreviations

SODIS	Solar disinfection
LP UV	Low-pressure ultraviolet
LEDs	Light-emitting diodes
AOPs	Advanced oxidation processes
ROS	Reactive oxygen species
ARGs	Antibiotic resistance gene
VBNC	Viable but nonculturable
CDM	Culture-dependent method
CIMs	Culture-independent methods
EMA	Ethidium monoazide
PMA	Propidium monoazide
qPCR	Quantitative polymerase chain reaction
RT-qPCR	Reverse transcription qPCR
FM	Fluorescence microscopy
FCM	Flow cytometry

1. Introduction

Continuous discharge of pathogenic microorganisms (i.e., bacteria, virus, protozoa, and fungi) into aquatic environments threatens human health and leads to many fatal diseases, such as diarrhea and hepatitis (Bennett, 2008; Wilson et al., 2019). Thus, development of effective disinfection technology is of great importance. Despite the germicidal effects of disinfection techniques used during water and wastewater treatment, recent studies have reported an undesirable increase in microbial populations after disinfection in both discharged effluents and water distribution systems (Thayanukul et al., 2013; Lin et al., 2016). The ability of bacteria to reactivate and repair damage allows their continued survival, rendering disinfection a temporary state and increasing the risk to public health. The spread of pathogenic bacteria can lead to fatal diseases such as diarrhea and pneumonia (Bennett, 2008; Wilson et al., 2019). For these reasons, the World Health Organization (WHO) has identified infections by antimicrobial resistant pathogens, including antibiotic resistant bacteria (ARB), as one of the world's greatest health risks (WHO, 2017a). This issue becomes particularly serious when pathogenic bacteria carrying antibiotic resistance genes (ARGs) regrow after disinfection in treated effluents for drinking water or reclaimed water (Li et al., 2014; Deng et al., 2019).

For instance, conventional chemical disinfection methods such as chlorine and chloramine inactivate bacteria primarily by damaging the outer membrane and subsequently cytoplasmic components. However, these disinfectants are not effective against all bacterial species (e.g., chlorine-resistant species) and hence allow their regrowth (Huang et al., 2011; Sharma et al., 2016), thereby weakening the desired residual disinfection effect of chlorine in water supply practices (WHO, 2017b). Meanwhile, conventional physical disinfection techniques such as ultraviolet (UV) irradiation, particularly UV-C, directly target intracellular DNA molecules. Yet, DNA damage induced by UV-C or UV-B irradiation can be repaired depending on the post-disinfection conditions (e.g., favorable light conditions), which may substantially enhance cell viability and lead to bacterial regrowth (Liltved and Landfald, 1996; Guo et al., 2015; Sousa et al., 2017; Hu et al., 2019). Solar disinfection (SODIS) can inactivate bacteria through the effect of UV irradiation, intracellular oxidative damage, and thermal heating and has great potential for application in developing countries around the equator that receive abundant solar energy (Pichel et al., 2019). Similar to UV disinfection, the SODIS process lacks residual disinfection activity and allows for some DNA repair afterwards (McGuigan et al., 2012; Pichel et al., 2019). Bacterial regrowth after disinfection is of particular concern because the surviving microorganisms may become more tolerant to stress factors (i.e., they may become superbugs that are harder to disinfect) (Michael et al., 2020).

The emergence of new technologies such as advanced oxidation processes (AOPs) (e.g., Sánchez-Montes et al., 2020), and photothermal and high electrical field induced-inactivation using nanomaterials (e.g., Wang et al., 2018a; Shimizu et al., 2019), provides

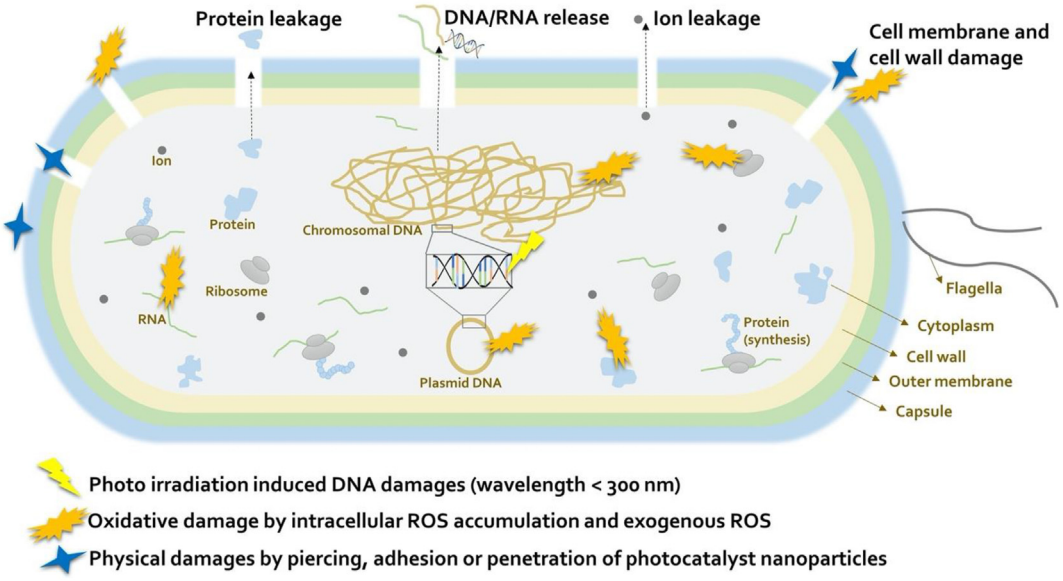

Fig. 1. The mechanisms of light-based disinfection technologies. The disinfection processes are light irradiation, photo/Fenton, photo/H_2O_2, photocatalysis, photo/chlorination and photo/ozonation. Exogenous ROS attacks cell membrane/wall and intracellular components when cell membrane/wall is damaged. Cell membrane damage through physical disruption by nanoparticles is also included because many types of nanoparticles work as photocatalyst. Note that the illustrated cell structure represents gram-negative bacteria, and gram-positive bacteria do not have outer membrane, but the disinfection mechanisms are similar.

alternative options for water and wastewater disinfection. Most AOPs involve solar or UV light as an assistive energy source, to produce reactive oxygen species (ROS), which are so-called light-based AOPs. The resulting ROS (e.g., hydroxyl radicals) can cause strong oxidative damage in bacteria through such mechanisms as lipid peroxidation and protein oxidation (Michael-Kordatou et al., 2018). Most previous studies of light-based AOPs reported limited or no bacterial regrowth, especially when the treatment was enhanced, for example by increasing photocatalyst loading, extending treatment time, and adjusting light wavelength (e.g., Moreira et al., 2018; Das et al., 2019; Ugwuja et al., 2019). Such light-based AOPs, UV disinfection, and SODIS are collectively known as light-based disinfection. The group of light-based disinfection inactivate bacteria through three major mechanisms (Fig. 1): (1) photo-damage of DNA by light irradiation (wavelength < 300 nm); (2) oxidation of cell membrane, cell wall and intracellular components by photo/Fenton, photo/H_2O_2, photocatalysis, photo/chlorination and photo/ozonation; (3) physical damages by piercing, adhesion or penetration of photocatalyst nanoparticles (Huo et al., 2020). Though the lack of a residual disinfection effect is a drawback of using most of light-based disinfection techniques in the water supply, these processes do not result in harmful byproducts and have no detrimental effects on organisms such as fish inhabiting natural waters.

Several reviews have discussed the disinfection performance and mechanisms of specific light-based disinfection techniques, including SODIS (Giannakis et al., 2016a, 2016b; Pichel et al., 2019), UV-C or UV light-emitting diodes (UV-LEDs) (Song et al., 2016; Li et al., 2019; Umar et al., 2019), and photocatalysis (Uyguner Demirel et al., 2018; Gong et al., 2019; You et al., 2019; Zhang et al., 2019) as well as general disinfection processes (Dodd, 2012; Giannakis et al., 2017; Miklos et al., 2018; Hiller et al., 2019). However, the topic of bacterial regrowth after light-based disinfection has received little attention in comprehensive reviews. Regrowth of ARB after advanced oxidation processes was discussed partially in one review paper, which highlighted the need for investigations of how AOPs operating conditions affect bacterial regrowth (Michael-Kordatou et al., 2018). An earlier review discussed the regrowth of bacteria and phytoplankton in a ship's ballast water tank and urged stakeholders to consider this issue while selecting ballast water treatment systems (Grob and Pollet, 2016). To our knowledge, the present review is the first to focus on bacterial regrowth after light-based disinfection (solar/UV irradiation and light-based AOPs) in water supplies and wastewater treatment. We present a critical review of the available literature, covering aspects of the underlying mechanisms, experimental designs, and detection methods with the aim to 1) elucidate the underlying bacterial regrowth mechanisms, 2) summarize the pros and cons of experimental designs and detection techniques for regrowth evaluation, and 3) provide an outlook of key research directions for further investigations of post-disinfection bacterial regrowth.

2. Regrowth: *reactivation, repair, and reproduction*

The phenomenon of post-disinfection increases in population of bacteria has been variously termed regrowth, recovery, and reactivation in the literature. In this article, we refer to the phenomenon as "regrowth" to avoid confusion, and only discuss the possible mechanisms happening during and after disinfection. Regrowth can happen as long as bacteria has the ability to reproduce. Upon disinfection treatments, bacteria may lose reproducibility due to the entry into viable but non-culturable (VBNC) state or the occurrence of photo-induced DNA damages. The reactivation from VBNC state, and the repair of DNA damages would allow bacteria to recover their ability to reproduce and enlarge their population. We consider these as two mechanisms of regrowth, namely reactivation and repair. In addition, the reproduction of intact bacterial cells is separately noted as the third regrowth mechanism, namely, reproduction. In the following subsections, we discuss the three underlying mechanisms of bacterial regrowth, namely, reactivation, repair, and reproduction (Fig. 2).

2.1. Reactivation from viable but non-culturable state

Currently, over 100 species of bacteria, including *Escherichia coli*

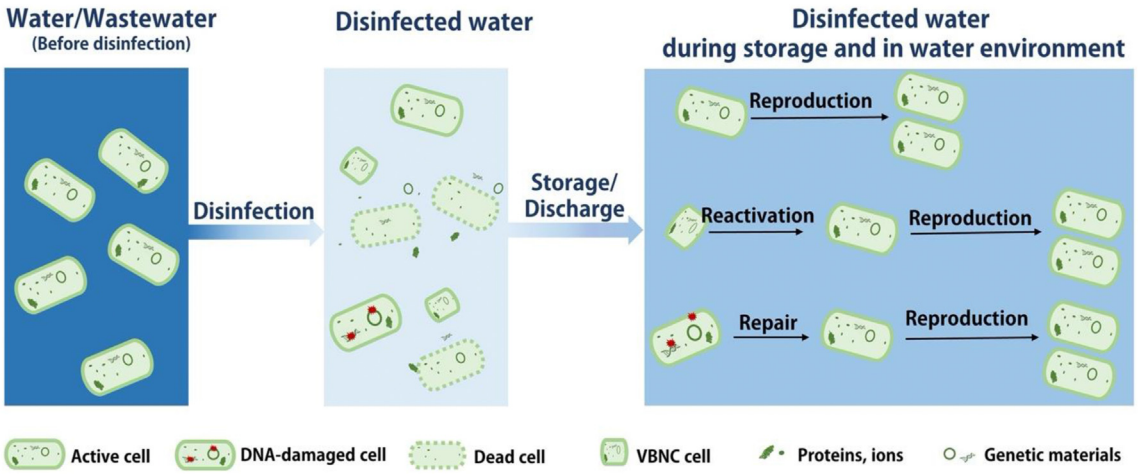

Fig. 2. Bacterial regrowth mechanisms: reactivation, repair, and reproduction. VBNC: viable but nonculturable; genetic materials include chromosome DNA and plasmid DNA.

(*E. coli*) and *Enterococcus* and *Salmonella* species, have been documented to have the ability to enter VBNC state (Ayrapetyan et al., 2018; Xu et al., 1982) under harsh environmental stresses (e.g., starvation, UV radiation exposure, oxidative stress, extreme temperatures) (Xu et al., 1982; Nowakowska and Oliver, 2013; Lin et al., 2016). Unlike transient stress responses, the VBNC state is an adaptive survival strategy that can continue for years (Stokell and Steck, 2012), while maintaining certain features of viable cells, such as membrane integrity, undamaged genetic information, and metabolic activity (Li et al., 2014; Robben et al., 2018). However, bacteria in the VBNC state are physiologically and molecularly different from viable cells (e.g., cellular morphology, cell wall and membrane composition, gene expression) (Stokell and Steck, 2012). VBNC bacteria are not able to divide and form colonies, so they do not appear on normal cultivation media as other viable cells, but they are alive and retain the ability to reactivate and regrow when the external stressors disappear (e.g., during water storage and distribution) (Ayrapetyan et al., 2018; Karaolia et al., 2018). Note that we consider this mechanism as reactivation from the VBNC state.

Several pathogenic bacteria (e.g., *E. coli* O8:H14, *Listeria monocytogenes*) have been confirmed to enter a VBNC state after being exposed to non-ionic surfactants (a common ingredient in household cleaners), but remained intact and continued their metabolic activity (i.e., ATP production, fermentation of sugars) (Robben et al., 2018). Low doses of disinfectants, such as 0.7 mg/L monochloramine and 1.5 mg/L total chloramine, could induce a VBNC state in pathogenic bacteria *E. coli* O157:H7 within 15 min in tap water or within 14 weeks in river water (Liu et al., 2009). The study also observed subsequent reactivation of *E. coli* O157:H7 in Eagle's minimal essential medium (Liu et al., 2009). When applying higher doses of chlorine (up to 4 mg/L), *E. coli* entered into a VBNC state, then reactivated in Luria-Bertani (LB) broth (Chen et al., 2018). Two indicators of pathogenic bacteria (*E. coli* and *Pseudomonas aeruginosa*) entered a VBNC state upon UVC (254 nm) irradiation up to 100 mJ cm^{-2}, during which expression of their virulence genes (*gad*A and *opr*L, respectively) remained at high levels (Zhang et al., 2015). These reports demonstrated the potential health risk of VBNC bacteria and the importance of this survival strategy in water environments.

2.2. Repair of photo-induced DNA damages

UV disinfection inactivates bacteria through direct damage to DNA and/or indirect damage by inducing internal oxidative stress through endogenous photosensitizers (Kielbassa et al., 1997). In the former, short-wave UV (wavelength $\lambda < 300$ nm, UVB and UVC) irradiation causes various mutagenic and cytotoxic DNA lesions such as cross-linkage between DNA bases that produce cyclobutene pyrimidine dimers and 6-4 photoproducts, thereby interrupting DNA replication and transcription (Lyons et al., 1998; Sinha and Häder, 2002). However, such DNA damage can be repaired in many microorganisms via photoreactivation and dark-repair mechanisms (Stephanie et al., 2011). In the photoreactivation mechanism, exposure to light at wavelengths of around 300–500 nm, DNA photolyases alter the DNA conformation and break apart the dimers formed under UV exposure (Sinha and Häder, 2002). Without requiring energy from light, dark-repair mechanisms work with enzymes like N-glycosylase to cleave DNA crosslinks, hence the name "excision repair" (Kielbassa et al., 1997; Sinha and Häder, 2002). It should be noted that bacterial regrowth due to the recovery of reproducibility through either photoreactivation or dark-repair mechanisms after UV disinfection is considered repair of photo-damaged DNA.

In a study of UV-LED disinfection, inactivation of *E. coli* at an irradiation wavelength of 280 nm led to more severe damage and less photoreactivation and dark repair compared with samples irradiated at a wavelength of 265 nm, which was probably attributed to the effect of 280-nm UV irradiation on proteins such as photolayse (Li et al., 2017a). Following irradiation of up to 10 mJ cm^{-2} using a conventional low-pressure UV lamp (254 nm), antibiotic-resistant *E. coli* and *P. aeruginosa* showed regrowth through photo repair and dark repair at 3, 15 and 24 h after disinfection (Destiani and Templeton, 2019).

2.3. Reproduction of viable and culturable bacteria

Unless complete inactivation is achieved (i.e., the detection limit of culture-dependent method is reached), we can always expect an increase in bacterial populations in water with a sufficient nutrient supply due to the ability of surviving bacteria to multiply. This mechanism is different from the reactivation of bacteria from the VBNC state because this fraction of bacteria remains viable and culturable throughout the entire process.

Bacterial regrowth essentially results from bacterial cell reproduction. The mechanisms of reactivation from VBNC state and repair of photo-induced damages are means by which bacterial cells recover their reproducibility, while the third mechanism is

Table 1
Studies of disinfection and post-disinfection regrowth of bacteria (2015–2020).

Treatment	Target bacterium	Light source and intensity	Water matrices	Measurement	Experiments details of regrowth test						Regrowth or not	Ref.
					Tested sample	Light source and distance to sample	Temperature	Receiving water	Storage time	Other conditions		
Photolysis disinfection (solar/UV irradiation)												
UVC-LED	Tetracycline resistant *Bacillus* sp.	•UVC-LED (265 nm, 275 nm) •0.038 W m^{-2}	0.9% sterile saline solution	Plate count (Tetracycline agar)	Treated water (varying fluences)	•Natural light •Near a transparent window	25 ± 1 °C	Original sample	24 h	–	Yes (Increase with regrowth time; decrease with UV fluence)	Shen et al., (2020)
						Dark	25 ± 1 °C	Original sample	24 h			
LP-UV	*E. coli*	•Collimated beam apparatus with LP UV lamp •120 W, 30% UVC	0.9% sterile saline water	Standard plate count (LB agar containing 50 mg/L kanamycin and 160 mg/L rifampicin)	Treated water	Similar to sunlight (1000 lux)	25 °C	Original sample	8 h	–	Yes	Guo and Kong, (2019)
						Dark	37 °C	1 mL sample to 50 mL LB broth	20 h	Shaker 150 rpm	Yes	
LP-UV	*E. coli*	•Collimated beam apparatus with LP UV lamp •0.02 W m^{-2}	PBS (pH 7.4)	Membrane filtration	Treated water	•Daylight bulb (270 lumen) •Distance 18.5 cm	Room temperature	Original sample	24 h	Magnetic stir	Yes	Destiani and Templeton, (2019)
						Dark	Room temperature	Original sample	24 h		Yes	
UVC-LED	*E. coli*	•Pulsed and continuous UVC-LED (268 nm, 275 nm) •0. 028 W m^{-2}	Sterile saline solution	Standard plate count (LB agar)	Treated water	•Fluorescent lamp (15 W, 395 nm) •Distance 30 cm	25.2 °C	Original sample	3 h	–	Yes (depending on wavelength)	Nyangaresi et al. (2019b)
						Dark					No	
UV-LED	*E. coli*	•UV-LED (267, 275, 267/275 and 275/310 nm) •0.0384 W m^{-2}	Sterile saline solution	Standard plate count (LB agar)	Treated water (3-and 4-log inactivation)	•Fluorescent lamp (15 W, 395 nm) •Distance 30 cm	Room temperature	Original sample	9 h	–	Yes (least for 275 nm irradiation)	Nyangaresi et al., (2018)
						Dark					Yes (less than photoreactivation)	
UV-LED LP-UV	*E. coli*	•UV-LED 265 nm, 280 nm, combination of 265 nm and 280 nm, and LP UV lamp •0.005, 0.01, 0.0065 and 0.009 W m^{-2}, respectively	0.9% sterile saline water	Standard plate count (Nutrient agar)	Treated water (3.0 or 4.5 log removal)	•Five fluorescent lamps (11 W, 300–500 nm, 0.012 W m^{-2}) •Distance 38 cm	25 °C	Original sample	8 h	Glass tube	Yes	Li et al. (2017a)
						Dark	25 °C	Original sample	8 h	Glass tube	Yes	
LP-UVC	Heterotrophs, Enterobacteria, Enterococci	LP UV lamp	Synthetic wastewater; Secondary treated wastewater	Membrane filtration (PCA agar for heterotrophs, m-Enterococcus agar for enterobacteria)	Before, immediately after treatment, and after 3-day storage	Room light	20–22 °C	Original sample	12 h	Liquid: air = 1:2	Yes	Sousa et al., (2017)
						Dark	20–22 °C	Original sample	12 h		Yes	
Solar (CPCs)	*E. coli*	•Simulated sunlight (0.5% UVB and 5% UVA) •500, 600, 700, 800, 900, 1000, 1200, 1400 and 1600 W m^{-2}	Synthetic secondary effluent	Plate count (PCA agar)	Treated water (varying time)	Dark	–	Original sample	48 h	–	Yes for 0-40-min irradiation at all intensities (500–1600 W m^{-2})	Giannakis et al. (2015a)
Solar (double-wall reactor) (varying temperature)	*E. coli*	•Simulated sunlight (0.5% UVB, 7% in 300–400 nm) •800 and 1200 W m^{-2}	Synthetic secondary effluent	Plate count (PCA agar)	Treated water (varying time)	Dark	25 °C	Original sample	48 h	–	Yes for low dose-low intensity; No for fully inactivated samples.	Giannakis et al. (2015b)

(*continued on next page*)

Table 1 (*continued*)

Treatment	Target bacterium	Light source and intensity	Water matrices	Measurement	Experiments details of regrowth test						Regrowth or not	Ref.
					Tested sample	Light source and distance to sample	Temperature	Receiving water	Storage time	Other conditions		
Solar (with dark exposure)	*E. coli*	•Simulated sunlight (0.5% UVB and 5% UVA) •500, 600, 1000 and 1200 W m^{-2}	Synthetic secondary treated wastewater	Plate count (PCA agar)	Treated water (Varying time & light intensity)	Dark	Room temperature	Sample mixed with lake water (10% or 1% dilutions)	48 h	–	Yes (Outright regrowth; decay then regrowth) No (highest intensities and/or exposure times)	Giannakis et al. (2015c)
Solar	*E. coli*	•Simulated sunlight (0.5% UVB and 5–7% UVA) •1000 W m^{-2}	Synthetic secondary effluent	Plate count (PCA agar)	Treated water (Varying time)	Dark •Monochromatic lamps (18 W; blacklight blue, actinic blacklight, blue, green and yellow) •Less than 80 W m^{-2} •Distance 15 cm	–	Original sample	48 h 2, 4, 8 h under light then 48 h in dark	–	Yes/No (depends on wavelengths of reactivation light and time)	Giannakis et al. (2015d)
Peroxide-based disinfection												
UVC/persulfate **UVC/H_2O_2**	Carbapenem resistant *Klebsiella pneumoniae*	•UV-C lamp (8 W) •2.3 × 10^{-6} W m^{-2}	Secondary effluent; Deionized water	Plate count (PCA agar, ChromIDRCARBA agar)	Treated water (after 60-second treatment)	Dark	37 °C	Water sample to Brain Heart Infusion broth	24 h	Incubator	No	Serna-Galvis et al., (2020)
UV **UV/H_2O_2** **UV/PMS**	Chlorine-resistant bacteria & their spores	•Collimated beam apparatus with LP UV lamp (40 W, 30% UVC) •2 × 10^{-4} W m^{-2}	Treated drinking water	DNA & protein quantification by spectrometer	–	–	–	–	–	Judge the regrowth potential by DNA/protein damages	Yes for UV; No for UV/H_2O_2 and UV/PMS	Zeng et al., (2020)
UV/H_2O_2	*E. coli* *Pseudomonas aeruginosa*	•UVC lamps (230 W) •26 W m^{-2}	0.9% sterile saline water;	Plate count (McConkey agar, tryptone agar)	Treated sample	Dark	25 °C	Original sample	48 h	–	No	Michael et al., (2020)
Solar/H_2O_2		Natural sunlight on sunny days	Secondary treated wastewater								Yes	
UV/H_2O_2	Total coliforms, *E. coli*	•LP UVC lamp •Flux of 9.5 × 10^{-7} E s^{-1}	Secondary effluent	Enzymatic substrate method	Treated water	Dark	Room temperature	Original sample	72 h	Unmixed closed glass bottle	Yes	Malvestiti and Dantas, (2019)
Sunlight/H_2O_2	*E. coli*, multidrug resistant *E. coli*	•-Natural sunlight •37.34 ± 4.30 W m^{-2}	Effluent of biological process in wastewater treatment plant	Plate count (Endo agar; coliform agar)	Untreated, treated (in the middle & end) samples	–	–	–	6, 12, 24, 48 h	–	Yes (less than chlorination	Fiorentino et al., (2015)
Fenton-based disinfection												
Solar in PET bottle Solar/Fenton in iron-deposed PET bottle)	*E. coli*	•Simulated sunlight •Global irradiance of 900 W m^{-2}	Milli-Q; Lake water	Plate count (PCA agar)	Treated water (varying time)	Dark	20–25 °C	Original sample	24 h, 48 h and 1 week	In PET bottles	Yes No	Shekoohiyan et al., (2019)
Solar Solar/Fenton	*E. coli*	•Simulated solar light •300, 600, 900 and 1200 W m^{-2}	Wastewater (before & after activated sludge)	Standard plate count (TSA agar)	Treated water (varying time)	Dark	22 ± 2 °C	Original sample	72 h	–	Yes No	Giannakis et al. (2018b)
UVA Fenton	Total coliform (TC) *E. coli* *Enterococcus* sp.	•Natural sunlight 13 ± 1 W m^{-2} (winter condition)	Secondary treated wastewater	Plate count (Chromocult agar)	Treated water	Dark	Room temperature	Original sample	Overnight	–	No	de la Obra Jimenez et al., (2019)
Solar Fenton	*E. coli*	•Simulated solar light 63 W m^{-2}	Secondary treated wastewater	Membrane filtration	Treated water	–	37 °C	Original sample	48 h	Incubator	No	Ioannou-Ttofa et al., (2019)

				(Glucuronide medium agar)								
UVA-Fenton/H_2O_2	*Klebsiella pneumoniae*	•UVA lamps (365 nm) 3.9 W m^{-2}	Bio-treated hospital wastewater	Plate count (PCA agar, ChromIDRCARBA agar)	Treated water	Dark	Room temperature	Original sample	48 h	–	No	Serna-Galvis et al., (2019)
Photocatalysis disinfection												
Solar/H_2O_2 Solar/TiO_2 Solar/ Fenton Solar/GO-TiO_2	Enterococci, fecal coliforms (FC)	•Natural sunlight on sunny days •40 W m^{-2}	Secondary treated wastewater	Membrane filtration (m-FC agar, S&B agar)	Treated water	Dark	Room temperature	Original sample	72 h	–	*Enterococci*-No FC-yes	Moreira et al., (2018)
UVA/TiO_2	*E. coli*, *Enterococcus* sp.	•Simulated sunlight •500 W m^{-2}	0.9% sterile saline solution; simulated municipal wastewater treatment plant effluent	Membrane filtration (MacConkey or Slanetz for *E. coli* and Bartley agar for *Enterococcus* sp.)	Treated water	Dark	–	Original sample	48 h	–	No. (But for UVA without TiO_2, *E. coli* and *Enterococcus* sp. showed little after short disinfection in saline solution wastewater, respectively.	Moles et al., (2020)
Solar light-Ag@SnO_2@ZnO core-shell nanocomposite	*Bacillus* sp.	•Natural sunlight •100, 000 ± 5000 lx (0.0079 W m^{-2} per lux)	0.9% sterile saline water;	Standard plate count (Nutrient agar)	Treated water	Dark	–	Original sample	96 h	Magnetic stir	No	Das et al., (2019)
UVA-LEDs/TiO_2	heterotrophs, *E. coli*, enterococci	•UVA-LED (381 nm) •Maximum 515 W m^{-2} (center of the irradiation area)	Secondary treated wastewater	Membrane filtration (PCA agar, TBX agar, m-Ent agar)	Treated water	Dark	24 °C	Original sample	72 h	–	Yes	Biancullo et al., (2019)
UVA-LED/TiO_2	*E. coli*	•365 nm UV-LED •0.049 W m^{-2}	0.85% saline solution	Standard plate count (LB agar)	Before and after treatment	•White light fluorescent lamp (15 W) •Distance 30 cm	Room temperature	Original sample	8 h	Magnetic stir; petri dish	No (residual disinfection)	Nyangaresi et al. (2019a)
						Dark	Room temperature	Original sample	8 h		No (residual disinfection)	
Visible light/Cu–Zn clay	*E. coli*	•Visible light from fluorescence lamps in laboratory •Natural solar light	Sterile water	Optical method	Treated water (varying time)	Laboratory light (407 nm)	–	Original sample	–	Laboratory bench	No	Ugwuja et al., (2019)
						Dark	–	Original sample	–	Incubator	No	
Solar/TiO_2 Solar/TiO_2-rGO	*E. coli*	•Simulated solar light •63 W m^{-2}	MBR treated wastewater	Membrane filtration (TSA agar)	Treated water (varying time)	Dark	44 °C	Original sample	24 h	Incubator	No (Except for TiO_2 30/ 60 min)	Karaolia et al., (2018)
Other light-based disinfection												
UV UV/persulfate	*E. faecalis* *E. coli*	•LP UVC lamp (10 W) •0.02 W m^{-2}	Distilled water Seawater	Plate count (S&B agar, Chromogenic Collinstant agar)	Treated water	Dark	Ambient temperature	Original sample	48 h	Residual persulfate was not neutralized	Yes No	Moreno-Andrés et al., (2019)
Photocatalytic ozonation	*E. coli* *Salmonella* sp. *Shigella* sp. *Vibrio cholerae*	•UV lamp (366 nm; 7 W m^{-2}) •Sunlight (3.76 W m^{-2})	Synthetic water Secondary municipal wastewater	Pour plate method (TSA agar, XLD agar, TCBS agar)	Treated water where no bacteria were detected	Dark	25–30 °C	Original sample	24, 48 h	–	No	Mecha et al., (2017)
Photocatalytic ozonation	Heterotrophs; Enterobacteria; Enterococci	UV LED (382 nm)	Secondary treated wastewater	Membrane filtration (PCA agar, m-FC agar, m-Ent agar)	Treated water	Light Dark	Room temperature	Original sample	72 h	–	No	Moreira et al., (2016)

CPCs, compound parabolic collector reactors. LB agar: Luria-Bertani agar; PCA agar, plate count agar; TSA agar: Tryptic Soy agar; ChromIDRCARBA agar contains 0.3 g L^{-1} of selective carbapenem antibiotics mixture; m-FC agar: membrane Faecal Coliforms agar; S&B agar: Slanetz & Bartley agar; TBX agar: Tryptone Bile X-Glucuronide agar; m-Ent agar: m Enterococcus agar; XLD agar: xylose lysine deoxycholate agar; TCBS agar: thiosulfate-citrate-bile salts-sucrose agar.

through the inherent reproducibility of intact bacterial cells. Nevertheless, the presence of VBNC cells, DNA-damaged cells and intact cells in disinfected water always signals the potential of observing regrowth after disinfection. Although the three mechanisms can be clarified theoretically, doing so practically is more challenging because of issues concerning experimental design and selection of bacterial detection methods. Therefore, in the following sections we review the available studies related to regrowth tests, discuss the essential factors to be considered in regrowth tests, and explain the use of various detection methods.

3. Experimental design for regrowth test

Research on regrowth tests has considered a large range of disinfection techniques, including conventional UV (Li et al., 2017a) and advanced processes such as photocatalysis (Biancullo et al., 2019) and UV/solar H_2O_2 (Michael et al., 2020). Microbial regrowth is influenced by various factors such as temperature (Sanders et al., 2005; Salcedo et al., 2007; Li et al., 2017a), availability of nutrients (Giannakis et al., 2014), dark delay (i.e., the period of dark storage before exposure to light) (Wen et al., 2019), storage/residence time (Giannakis et al., 2014; Moreno-Andrés et al., 2019), water matrix (Giannakis et al., 2014; Shafaei et al., 2017), and residual oxidants (Mao et al., 2018). These factors create plenty of possible conditions for regrowth tests, as seen in recent studies (Table 1). Generally, *E. coli* was chosen in most studies in Table 1. Though a single bacterial species may not be representative of all bacteria, *E. coli* is a main species of fecal coliform group that are the most common microbials in wastewater (Crittenden et al., 1991; Lin et al., 2016; Li et al., 2017b, 2017c), so the findings and conclusions from those studies can still be informative. The typical bacterial regrowth test consists of five steps (Fig. 3): 1) water disinfection, 2) sampling during disinfection, 3) storage of treated samples, 4) sampling during storage, and 5) measurements of samples (e.g., bacterial population). In this section, we summarize and critically review more than 20 recent studies to elucidate the role of disinfection processes and storage conditions (i.e., temperature, light, and water matrix) in the regrowth of bacteria after disinfection (Table 2).

3.1. Different disinfection processes primarily determine the regrowth potential

Insufficient disinfection leads to incomplete inactivation of microorganisms, leaving only a subpopulation injured and capable of reactivating and repairing. Thus, ensuring complete inactivation and residual disinfection is of great importance. UV disinfection is prone to bacterial regrowth because the primary disinfection mechanism is through the damage to nucleic acid by UV light adsorption that results in the loss of culturability. This has been observed in studies using low-pressure (LP) UV (Destiani and Templeton, 2019; Guo and Kong, 2019), UV-LED (Li et al., 2017a), and solar irradiation (Giannakis et al., 2014). However, bacterial cellular membranes undergo oxidative damage (e.g., lipid peroxidation) at considerably high UV doses, and such damage is thought to be irreversible and lethal (McKinney and Pruden, 2012; Xu et al., 2018). The lack of a residual effect is another critical disadvantage of light-based disinfection using UV or solar radiation compared with chemical disinfectants such as chlorine. For example, UV-LED (265 nm) irradiation (13.03 mJ cm^{-2}) of an *E. coli* suspension in buffer solution resulted in 4.5 log removal, and subsequent exposure to florescent light recovered the population to 35.23% of that before UV irradiation (Li et al., 2017a).

Therefore, it would seem that replacing UV disinfection with chemical disinfectants should prevent regrowth. Unfortunately, it is not that simple. We must keep in mind that DNA repair is not the only regrowth mechanism. Chlorination is known to act as a selection pressure on virus (Rachmadi et al., 2018a, 2018b). Similarly, chlorination also induces bacteria to enter a VBNC state, acting as a selective pressure for resistant species, including ARB- and chlorine-resistant species, and eventually contributes to the reproduction of such superbugs in treated waters (Huang et al., 2011; Lin et al., 2017; Liu et al., 2018; Hou et al., 2019; Zeng et al., 2020). Interestingly, despite the faster inactivation rate of chlorination than sunlight/H_2O_2, more regrowth of multi-drug resistant (MDR) *E. coli* was observed in chlorinated water due to the resistance of MDR *E. coli* to chlorine and the less lethal effect of chlorine at the investigated dose compared with highly reactive hydroxyl radicals produced by sunlight/H_2O_2 (Fiorentino et al., 2015). Photocatalysis and other AOPs generate ROS (e.g., hydroxyl radicals) that causes oxidative damage to bacterial membranes and subsequently intracellular components. Ideally, complete inactivation by those processes ensures prevention of post-disinfection regrowth, and many studies of photocatalytic disinfection indeed reported consistent findings of no regrowth post-treatment (e.g., Moreira et al., 2018; Das et al., 2019; Ugwuja et al., 2019) (Table 1). However, disinfectants (i.e., chlorine species, ROS) in real water matrices also react with organic matter and pollutants and produce assimilable organic matter, which can provide a carbon source for heterotrophic bacteria to utilize for reproduction (Thayanukul et al., 2013; Sousa et al., 2017; Pei et al., 2019). Thus, it was not surprising to observe higher regrowth after photocatalysis (UVA-LEDs/TiO_2) compared with photolysis (UVA-LEDs) of urban wastewater contaminated with antibiotics (Biancullo et al., 2019).

3.2. Storage conditions

Treated water is not immediately used or discharged because the water remains for a period of hours or days in reservoirs and/or distribution pipes. Therefore, it is essential that regrowth tests simulate realistic storage conditions, including time, temperature, light, and the water matrix.

3.2.1. Storage time

Reactivation from a VBNC state and repair of DNA damage takes minutes or hours, so there is normally a time lag until regrowth is first observed. A common practice is to store and observe disinfected water for a period ranging from a few hours to days (Table 1). Despite various doses of LP-UV irradiation (3, 5, and 10 mJ cm^{-2}) for disinfection, light/dark conditions during storage,

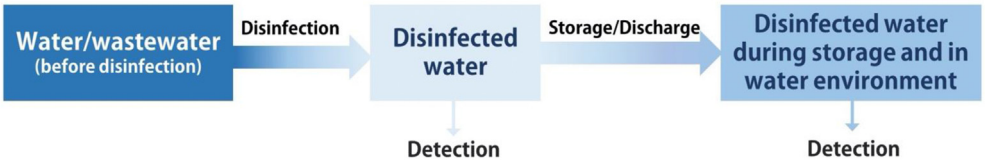

Fig. 3. Typical experimental flow of regrowth test.

and bacterial species (*E. coli* and *P. aeruginosa*), the percentages of regrowth all increased with time (3, 15, and 24 h) (Destiani and Templeton, 2019). Similar findings were also reported in studies with a storage time of 0–48 h (Fiorentino et al., 2015; Moreno-Andrés et al., 2019). Many studies have chosen instead to report only the regrowth result at the end of the storage period (e.g., Mecha et al., 2017; Malvestiti and Dantas, 2018), using indicators such as the percentage of regrowth (Linden and Darby, 1997) or the regrowth ratio (Karaolia et al., 2018), calculated by Equations (1) and (2), respectively.

$$Percentageofregrowth(\%) = \frac{N_r - N}{N_0 - N} \times 100 \quad (1)$$

$$\mathrm{Re}growthratio = \frac{N_r}{N} \quad (2)$$

Here, N_0 is the cell number before treatment, N is the cell number immediately after treatment, N_r is the cell number after repair, $(N_r - N)$ is the number of recovered or reactivated cells, and $(N_0 - N)$ is the total number of inactivated cells.

Kinetic models were developed to simulate the photoreactivation and/or dark-repair process after UV disinfection, assuming a saturation-type first-order reaction (Equation (3)) (Kashimada et al., 1996) or second-order kinetics (Equation (4) and (5)) (Sanz et al., 2007; Li et al., 2017a; Nyangaresi et al., 2018). Based on the model assumptions, bacterial populations increase logistically (first-order reaction, Equation (3)) or exponentially (second-order reaction, Equation (4)) with time, finally stabilizing when regrowth stops. Additionally, growth occurs less frequently in darkness than in light exposure conditions, and the growth curve in darkness may fall after reaching the maximum (Equation (4) and (5)) (Sanz et al., 2007). The proposed models could accurately fit the experimental data in those studies and provide a method for predicting the regrowth process after UV irradiation. However, these models are limited to UV disinfection and simplified regrowth conditions such as simple background water. More effort is required to develop or modify existing models that consider more realistic disinfection and regrowth conditions to perform reliable assessments and make accurate predictions.

$$\frac{dS}{dt} = k_1(S_m - S) \quad (3)$$

$$\frac{dS}{dt} = k_2(S_m - S)S \quad (4)$$

$$Decayphaseindarkness: \frac{dS}{dt} = -Mt \quad (5)$$

Here, S is survival ratio (N_r/N_0), S_m is the maximum survival ratio, k is reactivation rate constant, M is the decay coefficient at decay phase in darkness, and t is the time of regrowth.

Whether conducting a modeling analysis or not, it is always recommended to extend the timespan of regrowth tests and conduct frequent observations, especially in the first few hours.

3.2.2. Storage temperature

Tests conducted at low (4 °C), ambient (20 °C), and high (37 °C) temperatures have revealed that bacterial regrowth under dark storage conditions is dependent on temperature. Low temperature (4 °C) hindered bacterial regrowth, whereas high temperature promoted it (Giannakis et al., 2014). This supports an early investigation of temperature effects on photoreactivation and dark repair in UV-treated microorganisms, which observed an increased survival ratio and decreased reactivation rates (i.e., the reaction rate to reach the maximum survival ratio) with a temperature increase (5°C–30 °C) (Salcedo et al., 2007). In one study's regrowth test, a relatively high temperature of 44 °C was set as the storage temperature to simulate the wastewater storage conditions in Mediterranean countries and the optimum temperature for *E. coli* growth (Karaolia et al., 2018). Otherwise, we noticed that a temperature of 20°C-25 °C (i.e., room temperature) was commonly used for regrowth storage (Table 1); however, few studies considered the regrowth temperature for simulating actual conditions and/or for maximizing bacterial growth.

The temperature effect on bacterial regrowth can be attributed to two factors. First, metabolic activities involved in bacterial growth are influenced by temperature (Price and Sowers, 2004). Second, reactivation from a VBNC state and DNA repair processes also can be temperature-dependent. For example, temperature is an influential factor in the photoreactivation process because it affects the complex between DNA photolyases and dimers (Lindenauer and Darby, 1994). Given the considerable temperature effect on bacterial regrowth, it is critical to select an appropriate temperature when designing regrowth tests.

3.2.3. Light conditions

Post-disinfection regrowth tests are mostly conducted in darkness to mimic the conditions of drinking water distribution systems. Meanwhile, treated wastewater spends an average of 3 h in the outfall tunnel (Hallmich and Gehr, 2010) before being either discharged to a natural water body or delivered for water reuse (e.g., irrigation), thereby exposing it to natural light. Yet, the majority of previous studies only evaluated regrowth by storing treated water samples under dark conditions for 1–3 days, despite targeting wastewater treatment facilities (e.g., Sousa et al., 2017). Bacterial regrowth behavior is influenced by light/dark conditions (i.e., the duration of light exposure, and light characteristics) during post-disinfection storage, especially when UV-induced damage is the mechanism of the disinfection process. After reaching 1–4.5 log inactivation by LP-UV or UV-LED irradiation, the regrowth of *E. coli* occurred at a considerably lower degree under dark conditions compared with light exposure under fluorescent lamps (Emission wavelength: 300–400 nm, 0.012 W m^{-2} (Li et al., 2017); Emission peak wavelength = 395 nm (Nyangaresi et al, 2018, 2019a, 2019b)), in line with the reports on UV disinfection of fecal coliforms and fungal spores (Hallmich and Gehr, 2010; Wen et al., 2019). These consistent findings highlight the importance of light/dark conditions in determining bacterial regrowth. Interestingly, *E. coli* regrowth after solar disinfection showed dependence on the wavelengths of fluorescent light prior to dark storage; only blue (peak wavelength around 380 nm; irradiation less than 80 W m^{-2}) and green (peak wavelength around 550 nm; irradiation less than 80 W m^{-2}) light caused noticeable photoreactivation of bacteria that were partially and heavily damaged by solar irradiation, respectively, though the regrowth extent after fluorescent light exposure and dark storage was more correlated with the dose of solar irradiation ($R^2 > 0.7$, p value < 0.001) (Giannakis et al., 2015d). Furthermore, prolonged dark conditions prior to light exposure, which hindered photoreactivation of microorganisms such as fecal coliforms (Hallmich and Gehr, 2010; Wen et al., 2019), is another crucial factor to consider in regrowth tests. However, this factor was often overlooked (Table 1). Considering the increasing interest in water and wastewater disinfection by AOPs, especially photocatalysis and photo-Fenton, it is important to carefully design the light/dark conditions of regrowth tests.

3.2.4. Water matrix

Water characteristics (e.g., nutrient concentration, osmotic pressure) substantially influence the severity of the germicidal

effect and have the potential to alter post-disinfection regrowth patterns. Spiking filtered river water into wastewater effluent decreased the concentration of dissolved organic carbon (17.7–15.6 mg/L), orthophosphate (1.5–1.0 mg/L), ammonia (0.11–0.03 mg/L), and alkalinity (294.5–248 mg $CaCO_3$ L^{-1}), thereby hindering the regrowth of UV-irradiated bacteria under sunlight (Shafaei et al., 2017); this indicates the important role of nutrients in DNA repair activities. Solar-disinfected *E. coli* was introduced to synthetic wastewater and synthetic seawater (salinity = 3.5%), to reach 50%, 10% and 1% dilution rates, then stored under dark condition for regrowth tests (Giannakis et al., 2014). Regardless of the dilution rate, initial populations of 10^4–10^6 CFU mL^{-1} increased to the same eventual population of 10^8 CFU mL^{-1} after five-day storage in wastewater. This study also confirmed that 50% dilution could prolong *E. coli* survival (10^6–10^7 CFU mL^{-1} across the five days of storage), while higher dilution rates (i.e., 10% and 1%) lead to a decay of the *E. coli* population (10^2–10^5 CFU mL^{-1} to 1 CFU mL^{-1} for 10% dilution; 10^1–10^4 CFU mL^{-1} to 10^1–10^2 CFU mL^{-1} for 1% dilution) during five-day storage in synthetic seawater, indicating the significant effect of salinity on bacterial regrowth process (Giannakis et al., 2014). In another study, lower regrowth of *E. faecalis* was observed in artificial seawater (conductivity = 34.82 mS cm^{-2}; $[Cl^-] = 16.66$ g L^{-1}; $[SO_4^{-2}] = 0.44$ g L^{-1}) than in distilled water after UVC and UV/persulfate disinfection (UV dose = 40 mJ cm^{-2}; [persulfate] = 1 mM), despite the lower disinfection effectiveness of seawater (Moreno-Andrés et al., 2019). In addition to the nutrients and salts in water, other factors such as turbidity may also play important roles in the fate of bacteria after light-based disinfection, but are not discussed here due to scarcity in the literature. In wastewater disinfection, the effluent is often discharged into natural water bodies (e.g., rivers and oceans), resulting in a change to the environment where bacteria live. Nevertheless, most previous studies have overlooked such changes in water characteristics while conducting regrowth tests (Table 1); therefore, their evaluations of the degree of regrowth and the effectiveness of disinfection techniques may be somewhat misleading.

4. Detection of bacterial regrowth

4.1. Culture-dependent method: plate count

Previous studies confirmed regrowth by standard plate count, which is a culture-dependent method (CDM). With this method, regrowth is confirmed if more coliforms are observed during storage after disinfection. However, such an approach counts only the culturable fraction of total viable cells, leaving bacteria in a VBNC state unrecognized, and thus underestimating their biological risks. Apart from the potential to reactivate and regrow, VBNC bacteria carrying ARGs retain a certain level of plasmid gene transfer efficiency (Salcedo and Kim, 2017) as well as their resistance to antibiotics (Amarasiri et al., 2019). Therefore, it is important to distinguish and quantify both culturable and VBNC bacteria in samples. Moreover, photo-induced DNA damage interrupts DNA replication and transcription; therefore, cells with such damage lose their culturability on nutrient medium. However, photodamage does not necessarily lead to death. Therefore, it is also important to quantify cells with DNA damage because, like VBNC cells, they can potentially recover. The use of nonselective and low-nutrient media for bacteria cultivation (e.g., plate count agar and Reasoner's 2 A agar) would allow for the growth of some injured cells and result in a higher count than high-nutrient media such as Luria-Bertani agar (Valero et al., 2017), but would fail to accurately ascertain viability. In a nutshell, plate count does not satisfy the requirement for carefully quantifying total viable cells (including VBNC cells) and bacteria with repairable DNA damages, and so more advanced detection methods are urgently needed.

4.2. Culture-independent method: molecular-based techniques

Culture-independent methods (CIMs) that equate cell membrane integrity with viability have made possible the detection of total viable cells. The two most common approaches are a quantitative real-time polymerase chain reaction (qPCR)-based method using either ethidium monoazide bromine (EMA)- or propidium monoazide (PMA)-qPCR with reverse transcription (RT)-qPCR, and a viability assay using LIVE/DEAD BacLight Bacterial Viability Kit (Fig. 4).

Table 2
Summary of commonly used culture-independent methods for quantification of viable bacterial cells.

Culture-independent method	Principle	Advantages	Disadvantages	References
LIVE/DEAD BacLight Bacterial Viability test	SYTO 9 labels all bacterial cells; propidium iodide stains dead bacterial cells with damaged membrane.	•Direct cell observation and quantification •Able to further analyze by fluorescence microscope or flow cytometry •Relatively cheap	•Only applicable when membrane damage is the primary inactivation mechanism	(Ding et al., 2017; Song et al., 2020; Stokell and Steck, 2012; Xu et al., 2018)
EMA- or PMA-qPCR	EMA and PMA combine with intracellular DNA of membrane-damaged cells, so that PCR reaction is hindered.	•Easy to perform •Acute detection •Able to monitor certain genes	•Only applicable when membrane damage is the primary inactivation mechanism •High cost	(Ding et al., 2017; Emerson et al., 2017; Lin et al., 2016; Polo-López et al., 2017; Zhang et al., 2015)
RT-qPCR	mRNA has a short half-life time in dead bacterial cells, so mRNA only exists in living cells and can be quantified by qPCR after reverse transcription.	•Accurate differentiation between viable and dead cells •Able to detect the damages of certain genes	•Difficulty in selection of indicator gen •Complex procedures and high cost	(Ding et al., 2017; Emerson et al., 2017; Lin et al., 2016; Stokell and Steck, 2012; Xu et al., 2018; Zhang et al., 2015)

EMA, ethidium monoazide; PMA, propidium monoazide; qPCR, quantitative polymerase chain reaction; RT-qPCR, reverse transcription qPCR.

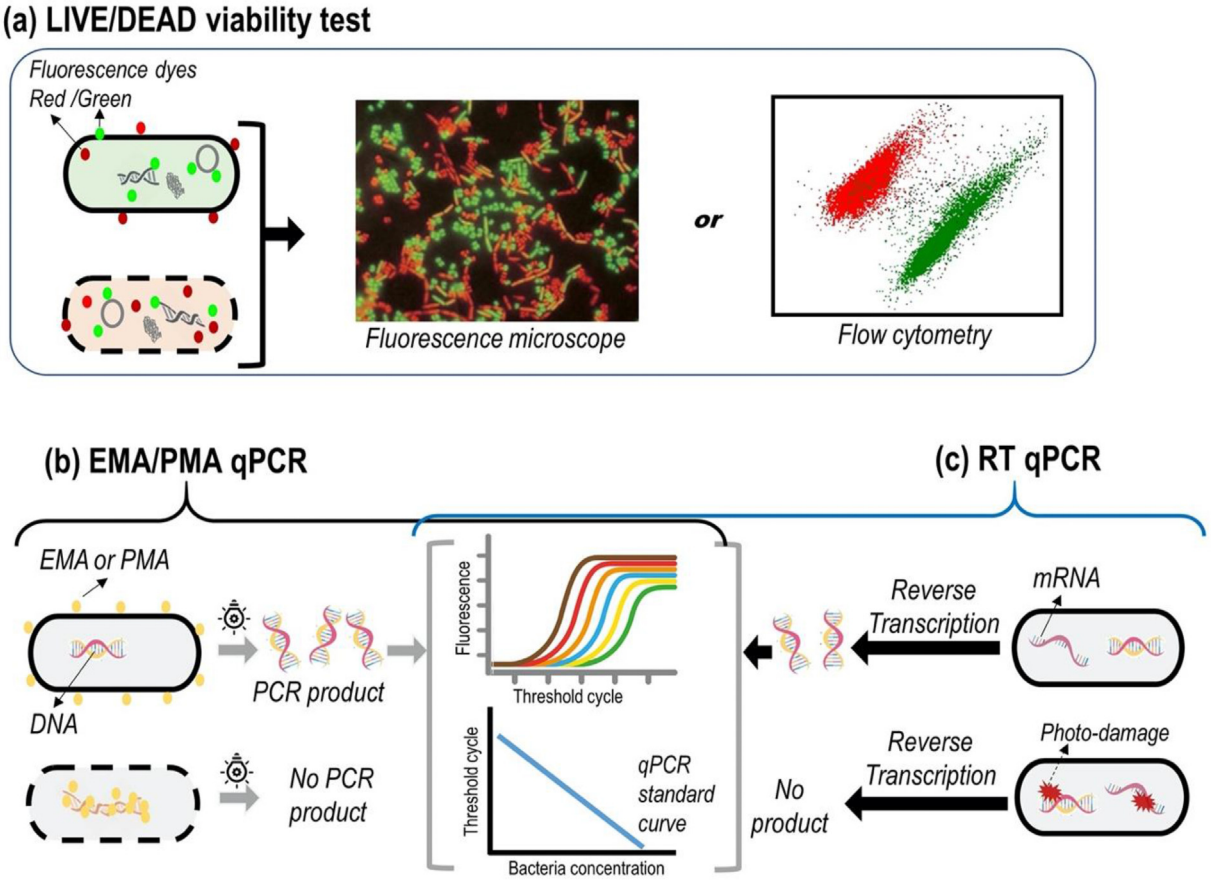

Fig. 4. Principle and analysis workflow of three culture-independent approaches. EMA, ethidium monoazide; PMA, propidium monoazide; qPCR, quantitative polymerase chain reaction; RT-qPCR, reverse transcription qPCR.

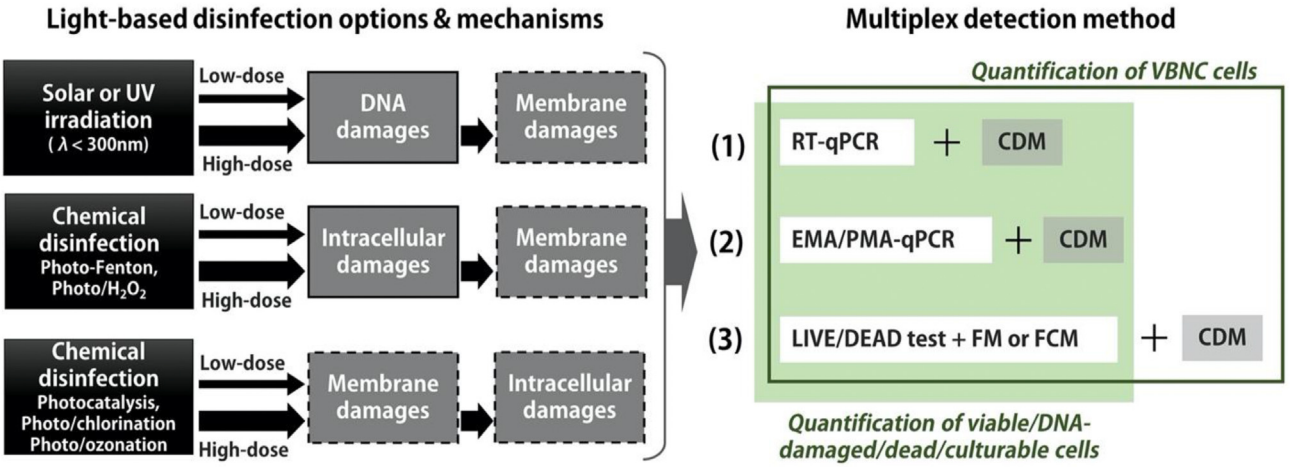

Fig. 5. Proposed multiplex detection method for the measurement of bacterial regrowth after various light-based disinfection treatments. qPCR, quantitative polymerase chain reaction; RT-qPCR, reverse transcription qPCR; EMA, ethidium monoazide; PMA, propidium monoazide; FM, fluorescence microscopy; FCM: flow cytometry; CDM, culture-dependent method (i.e., plate count method).

Both EMA- and PMA-qPCR methods establish a relationship between the number of viable cells and the cycle threshold in qPCR on the basis that EMA/PMA binds to intracellular DNA in dead cells with damaged membranes and stops DNA amplification in the qPCR process. A comparison between EMA-qPCR and plate count was made for the quantification of *Legionella* spp. during solar-driven disinfection treatments, including solar, solar/H_2O_2 and solar/TiO_2/H_2O_2 (Polo-López et al., 2017). The results demonstrated that EMA-qPCR could detect dead cells when treatment damaged the cell membrane, but it could not distinguish live and dead cells in treatments where the membrane was undamaged. Similarly, the LIVE/DEAD BacLight Bacterial Viability Kit is also based on membrane integrity and uses a green dye to label total cells and a red dye to label only dead cells (Stokell and Steck, 2012), making it possible

to quantify viable and dead cells via fluorescent microscopy (FM) or flow cytometry (FCM). However, FCM with fluorescing dyes was also found unsuitable for UV disinfection, where the primary inactivation mechanism is damage to nucleic acid by irradiation (Xu et al., 2018). Generally, mRNA has a very short half-life (range: 0.5−50 min; typical average: 3 min) and exists only in viable cells (Takayama and Kjelleberg, 2000), So, the detection of mRNA by RT-qPCR can be used to reflect bacterial viability, to elucidate the potential for regrowth, or to identify DNA damage if an appropriate indicator gene is selected (Li et al., 2014; Ding et al., 2017; Zhang et al., 2015; Lin et al., 2016). It should be noted that for cell quantification purposes, qPCR methods need the help of conventional plate count to prepare the standard curve for cycle threshold and the number of bacterial subpopulations (Polo-López et al., 2017).

4.3. Multiplex detection method

Multiplex detection method (Fig. 5) is defined as the combination of culture-dependent and -independent approaches (Fig. 4) to realize quantitative testing and deeper understanding of the dynamic changes of bacterial population in different physiological states (Fig. 2). Among the studies on post-disinfection regrowth of bacteria as summarized in Table 1, we found no application of multiplex detection method for the assessment of bacterial regrowth after light-based disinfection.

There is no consensus on which multiplex detection method is the most precise and accurate. However, one simple validation method is proposed to ensure and improve the precision and accuracy of multiplex detection methods. Before applying those culture-independent methods, validation on standard bacterial suspensions with known concentrations (CFU mL^{-1}) is recommended. By comparing the results from plate count method and culture-independent methods of standard bacterial suspensions, judgement on the precision and accuracy of the detection methods can be made. Following are the procedures to prepare the standard bacterial suspensions to be examined. Cultures of 100% living (culturable) and 100% dead cells are needed for preparing the standard bacterial suspensions. No harmful chemicals and treatments should be involved to obtain the pure culture of living cells (also culturable in this case), while germicidal chemicals (e.g., 70% isopropyl alcohol) or physical treatments (e.g., heating and UVC irradiation) must be applied to prepare the culture of all dead bacteria cells. Finally, mixtures of the 100% living and dead cell cultures at several ratios are made and analyzed by different detection methods.

5. Practical applications of multiplex detection methods

Depending on the primary disinfection mechanisms (i.e., membrane and/or DNA damage), different multiplex detection methods can be employed to quantify viable cells, VBNC cells, dead cells, the degree of DNA damage, and the repair potential (Fig. 5).

When UV at a wavelength less than 300 nm (i.e., UVB and UVC) is applied, bacterial genetic materials is the first to be damaged by adsorbing UV light, inhibiting cell replication. The expression level of certain genes, quantified by RT-qPCR, can effectively and sensitively reflect bacterial cell viability, or quantify DNA-damaged cells if DNA damage repair gene is targeted. For example, four primers were used in the analysis of mRNA by RT-qPCR for analyzing *E. coli* viability during UV disinfection (Xu et al., 2018): single-stranded DNA-binding protein (*ssb*), chromosomal replication initiator protein (*dnaA*), glutamate decarboxylase (*gadA*), and SOS response and DNA repair (*RecA*) primers. The study found that the damage detected by *RecA* primer was the most substantial, followed by *ssb, dnaA* and *gadA*, so the authors suggest the primer for DNA repair gene (*RecA*) as a useful tool indicator for bacterial viability (Xu et al., 2018). Similar to solar and UV disinfection, photo Fenton and photo/H_2O_2 are both essentially intracellular processes (Giannakis et al., 2018a; Feng et al., 2020; Jin et al., 2020), in which oxidative damages happen to DNA, cytoplasmic proteins and cell wall components, so methods sensitive to the change of nucleic acids (e.g., RT-qPCR) would be suitable for viability tests. In practice, the use of RT-qPCR to quantify bacterial viability would permit the preparation of a standard curve between viability indicators such as gene copy number and culturable cells quantified by CDM, then further VBNC cells can be quantified as the difference between viable and culturable cells. Therefore, the multiplex detection method **(1)** (Fig. 5), combination of RT-qPCR and CDM, is proposed and expected to be helpful in quantifying viable/DNA-damaged, culturable and VBNC cells during disinfection by light irradiation, photo-Fenton or photo/H_2O_2.

In case of chemical disinfections (e.g., photocatalysis and photo/chlorination) that primarily damage cell membrane, the multiplex detection methods **(2)** and **(3)**, combing CDM with CIMs based on membrane integrity test, are proposed. For instance, using EMA-qPCR and plate count method for serial 10-fold diluted *Legionella*, the standard curve between cycle threshold (C_T) and cell equivalent (CE) mL^{-1} given by colony forming units (CFU mL^{-1}) was determined, so that membrane damaged cells in water treated by photocatalysis could be quantified (Polo-López et al., 2017). One possible extension of that study is to further use the standard curve and plate count method for the quantification of different bacterial subpopulations including VBNC cells. It should be noted that when cell membrane remains unaffected during disinfection (e.g., low dose UVC irradiation), EMA-qPCR and LIVE/DEAD viability test are not useful for cell quantification.

The concept of multiplex detection methods for different disinfection techniques is based on the method principles and primary disinfection mechanism. However, it is also noteworthy to mention that disinfection mechanisms may change case by case, so it is highly recommended to implement a selection procedure for the suitable multiplex detection method especially when a complex disinfection system is targeted (e.g., complicated water matrix or multiple disinfection processes). Specifically, to implement the selection procedure for a suitable multiplex detection method, the three options **(1), (2)** and **(3)** shown in Fig. 5 may be considered as options. For a complex disinfection system, three options may be applied, then based on the results from those analytical methods, which reveal the changes of bacterial membrane integrity or gene copies, the selection can be achieved. For example, when the membrane integrity measured by PMA/EMA-qPCR or LIVE/DEAD viability test remains unchanged, these methods should not be further considered. Note that when the disinfection mechanism is well understood, the selection does not need to go through the experiment-based procedure unless for the sake of being rigorous. Once the selection is completed, the suitable multiplex detection method can be further applied in extensive experiments of regrowth tests, to understand the changes in the abundance of various bacterial subpopulations throughout the period of disinfection and post-disinfection storage.

Although multiplex detection methods are more time-consuming, the various combinations of CDM and CIM allow for not only reliable estimation of regrowth but also a more comprehensive understanding of the underlying mechanisms. Subsequently, it would allow us to develop models of different bacterial subpopulations for the purpose of regrowth evaluation and prediction. Moreover, the deep understanding of post-disinfection bacterial regrowth would provide a basis for the control of

bacterial regrowth in water and wastewater treatment, so as to reduce the potential risks posing to public health and the aquatic environments. While caution should be taken in clarifying the mechanisms of disinfection, we strongly recommend transitioning from a single method to the proposed multiplex detection method.

6. Conclusions

Bacterial regrowth occurs through three mechanisms: 1) reactivation from a VBNC state; 2) repair of photo-induced DNA damage, and 3) reproduction of surviving bacteria in disinfected water. The literature shows that bacterial regrowth occurs at different degrees, depending on disinfection processes and post-disinfection storage conditions. Recent studies on light-based disinfection have revealed the effectiveness of AOPs such as photocatalysis for suppressing bacterial regrowth compared with UV or solar disinfection. However, regrowth tests were performed in only a limited number of studies and did not adequately recreate realistic conditions (e.g., storage duration and temperature). In addition, conventional culture-based methods (i.e., plate count) for counting bacteria failed to quantify VBNC bacteria and bacteria with photo-induced DNA damage, both of which have the potential for reactivation and recovery, thereby resulting in inaccurate estimates of the degree of bacterial regrowth. Thus, more effective evaluation approaches involving carefully considered experimental designs for regrowth tests are urgently required, to reduce microbial risk to public health and aquatic environments via better understanding of bacterial regrowth process. Based on state-of-the-art techniques and the knowledge gap that we described in this review, our recommendations for moving forward are as follows:

- Multiplex detection methods for counting bacteria should be chosen based on inactivation and regrowth mechanisms, thereby providing a credible basis for regrowth estimation with minimal analytical bias.
- Regrowth tests should be designed to simulate actual conditions, especially in cases where the target water is well characterized and the disinfection conditions are well established.
- A comprehensive assessment of various major influential factors (e.g., organic components, inorganic species, light/dark conditions) in regrowth tests is necessary to provide basic scientific knowledge on the underlying mechanisms of bacterial regrowth, particularly for advanced light-based disinfection processes.
- The ultimate goal is to control undesirable regrowth after disinfection, which we believe is possible via implementation of the above-mentioned.

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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References

Amarasiri, M., Sano, D., Suzuki, S., 2019. Understanding human health risks caused by antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARG) in water environments: current knowledge and questions to be answered. Crit. Rev. Environ. Sci. Technol. 1–44. https://doi.org/10.1080/10643389.2019.1692611.

Ayrapetyan, M., Williams, T., Oliver, J.D., 2018. Relationship between the viable but nonculturable state and antibiotic persister cells. J. Bacteriol. 200, e00249, 18.

Bennett, A., 2008. Drinking water: pathogen removal from water - technologies and techniques. Filtr. Sep. 45, 14–16. https://doi.org/10.1016/S0015-1882(08)70495-6.

Biancullo, F., Moreira, N.F.F., Ribeiro, A.R., Manaia, C.M., Faria, J.L., Nunes, O.C., Castro-Silva, S.M., Silva, A.M.T., 2019. Heterogeneous photocatalysis using UVA-LEDs for the removal of antibiotics and antibiotic resistant bacteria from urban wastewater treatment plant effluents. Chem. Eng. J. 367, 304–313. https://doi.org/10.1016/j.cej.2019.02.012.

Chen, S., Li, Xi, Wang, Y., Zeng, J., Ye, C., Li, Xianping, Guo, L., Zhang, S., Yu, X., 2018. Induction of Escherichia coli into a VBNC state through chlorination/chloramination and differences in characteristics of the bacterium between states. Water Res. 142, 279–288. https://doi.org/10.1016/j.watres.2018.05.055.

Crittenden, J.C., Reddy, P.S., Arora, H., Trynoski, J., Hand, D.W., Perram, D.L., Summers, R.S., 1991. Predicting GAC performance with rapid small-scale column tests. J. Am. Water Works Assoc. 83, 77–87. https://doi.org/10.1002/j.1551-8833.1991.tb07088.x.

Das, S., Misra, A.J., Habeeb Rahman, A.P., Das, B., Jayabalan, R., Tamhankar, A.J., Mishra, A., Lundborg, C.S., Tripathy, S.K., 2019. Ag@SnO2@ZnO core-shell nanocomposites assisted solar-photocatalysis downregulates multidrug resistance in Bacillus sp.: a catalytic approach to impede antibiotic resistance. Appl. Catal. B Environ. 259, 118065. https://doi.org/10.1016/j.apcatb.2019.118065.

de la Obra Jimenez, I., Casas Lopez, J.L., Rivas Ibanez, G., Esteban García, B., Sanchez Prez, J.A., 2019. Kinetic assessment of antibiotic resistant bacteria inactivation by solar photo-Fenton in batch and continuous flow mode for wastewater reuse. Water Res. 159, 184–191. https://doi.org/10.1016/j.watres.2019.04.059.

Deng, S., Yan, X., Zhu, Q., Liao, C., 2019. The utilization of reclaimed water: possible risks arising from waterborne contaminants. Environ. Pollut. 254, 113020. https://doi.org/10.1016/j.envpol.2019.113020.

Destiani, R., Templeton, M.R., 2019. Chlorination and ultraviolet disinfection of antibiotic-resistant bacteria and antibiotic resistance genes in drinking water. AIMS Environ. Sci. 6, 222–241. https://doi.org/10.3934/environsci.2019.3.222.

Ding, T., Suo, Y., Xiang, Q., Zhao, X., Chen, S., Ye, X., Liu, D., 2017. Significance of viable but nonculturable Escherichia coli: induction, detection, and control. J. Microbiol. Biotechnol. 27, 417–428. https://doi.org/10.4014/jmb.1609.09063.

Dodd, M.C., 2012. Potential impacts of disinfection processes on elimination and deactivation of antibiotic resistance genes during water and wastewater treatment. J. Environ. Monit. 14, 1754–1771. https://doi.org/10.1039/c2em00006g.

Emerson, J.B., Adams, R.I., Román, C.M.B., Brooks, B., Coil, D.A., Dahlhausen, K., Ganz, H.H., Hartmann, E.M., Hsu, T., Justice, N.B., Paulino-Lima, I.G., Luongo, J.C., Lymperopoulou, D.S., Gomez-Silvan, C., Rothschild-Mancinelli, B., Balk, M., Huttenhower, C., Nocker, A., Vaishampayan, P., Rothschild, L.J., 2017. Schrödinger's microbes: tools for distinguishing the living from the dead in microbial ecosystems. Microbiome 5, 86. https://doi.org/10.1186/s40168-017-0285-3.

Feng, L., Peillex-Delphe, C., Lü, C., Wang, D., Giannakis, S., Pulgarin, C., 2020. Employing bacterial mutations for the elucidation of photo-Fenton disinfection: focus on the intracellular and extracellular inactivation mechanisms induced by UVA and H2O2. Water Res. 182, 116049. https://doi.org/10.1016/j.watres.2020.116049.

Fiorentino, A., Ferro, G., Alferez, M.C., Polo-López, M.I., Fernández-Ibañez, P., Rizzo, L., 2015. Inactivation and regrowth of multidrug resistant bacteria in urban wastewater after disinfection by solar-driven and chlorination processes. J. Photochem. Photobiol. B Biol. 148, 43–50. https://doi.org/10.1016/j.jphotobiol.2015.03.029.

Giannakis, S., Darakas, E., Escalas-Cañellas, A., Pulgarin, C., 2015a. Solar disinfection modeling and post-irradiation response of Escherichia coli in wastewater. Chem. Eng. J. 281, 588–598. https://doi.org/10.1016/j.cej.2015.06.077.

Giannakis, S., Darakas, E., Escalas-Cañellas, A., Pulgarin, C., 2015b. Temperature-dependent change of light dose effects on E. coli inactivation during simulated solar treatment of secondary effluent. Chem. Eng. Sci. 126, 483–487. https://doi.org/10.1016/j.ces.2014.12.045.

Giannakis, S., Darakas, E., Escalas-Cañellas, A., Pulgarin, C., 2015c. Environmental considerations on solar disinfection of wastewater and the subsequent bacterial (re)growth. Photochem. Photobiol. Sci. 14, 618–625. https://doi.org/10.1039/c4pp00266k.

Giannakis, S., Merino Gamo, A.I., Darakas, E., Escalas-Cañellas, A., Pulgarin, C., 2014. Monitoring the post-irradiation E. coli survival patterns in environmental water matrices: implications in handling solar disinfected wastewater. Chem. Eng. J. 253, 366–376. https://doi.org/10.1016/j.cej.2014.05.092.

Giannakis, S., Polo López, M.I., Spuhler, D., Sánchez Pérez, J.A., Fernández Ibáñez, P., Pulgarin, C., 2016a. Solar disinfection is an augmentable, in situ-generated photo-Fenton reaction-Part 2: a review of the applications for drinking water and wastewater disinfection. Appl. Catal. B Environ. 198, 431–446. https://doi.org/10.1016/j.apcatb.2016.06.007.

Giannakis, S., Polo López, M.I., Spuhler, D., Sánchez Pérez, J.A., Fernández Ibáñez, P., Pulgarin, C., 2016b. Solar disinfection is an augmentable, in situ-generated photo-Fenton reaction—Part 1: a review of the mechanisms and the fundamental aspects of the process. Appl. Catal. B Environ. 199, 199–223. https://doi.org/10.1016/j.apcatb.2016.06.009.

Giannakis, S., Rtimi, S., Darakas, E., Escalas-Cañellas, A., Pulgarin, C., 2015d. Light wavelength-dependent E. coli survival changes after simulated solar disinfection of secondary effluent. Photochem. Photobiol. Sci. 14, 2238–2250. https://doi.org/10.1039/c5pp00110b.

Giannakis, S., Rtimi, S., Pulgarin, C., 2017. Light-assisted advanced oxidation processes for the elimination of chemical and microbiological pollution of wastewaters in developed and developing countries. Molecules 22. https://doi.org/10.3390/molecules22071070.

Giannakis, S., Voumard, M., Rtimi, S., Pulgarin, C., 2018a. Bacterial disinfection by the photo-Fenton process: extracellular oxidation or intracellular photo-catalysis? Appl. Catal. B Environ. 227, 285–295. https://doi.org/10.1016/j.apcatb.2018.01.044.

Giannakis, S., Watts, S., Rtimi, S., Pulgarin, C., 2018b. Solar light and the photo-Fenton process against antibiotic resistant bacteria in wastewater: a kinetic study with a Streptomycin-resistant strain. Catal. Today 313, 86–93. https://doi.org/10.1016/j.cattod.2017.10.033.

Gong, M., Xiao, S., Yu, X., Dong, C., Ji, J., 2019. Research progress of photocatalytic sterilization over semiconductors. RCS Adv 9. https://doi.org/10.1039/c9ra01826c, 19278–19284.

Grob, C., Pollet, B.G., 2016. Regrowth in ship's ballast water tanks: think again! Mar. Pollut. Bull. 109, 46–48. https://doi.org/10.1016/j.marpolbul.2016.04.061.

Guo, M.-T., Kong, C., 2019. Antibiotic resistant bacteria survived from UV disinfection: safety concerns on genes dissemination. Chemosphere 224, 827–832. https://doi.org/10.1016/j.chemosphere.2019.03.004.

Guo, M.T., Yuan, Q. Bin, Yang, J., 2015. Distinguishing effects of ultraviolet exposure and chlorination on the horizontal transfer of antibiotic resistance genes in municipal wastewater. Environ. Sci. Technol. 49, 5771–5778. https://doi.org/10.1021/acs.est.5b00644.

Hallmich, C., Gehr, R., 2010. Effect of pre- and post-UV disinfection conditions on photoreactivation of fecal coliforms in wastewater effluents. Water Res. 44, 2885–2893. https://doi.org/10.1016/j.watres.2010.02.003.

Hiller, C.X., Hübner, U., Fajnorova, S., Schwartz, T., Drewes, J.E., 2019. Antibiotic microbial resistance (AMR) removal efficiencies by conventional and advanced wastewater treatment processes: a review. Sci. Total Environ. 685, 596–608. https://doi.org/10.1016/j.scitotenv.2019.05.315.

Hou, A. ming, Yang, D., Miao, J., Shi, D. yang, Yin, J., Yang, Z., wei, Shen, qiang, Z., Wang, H., ran Qiu, Z., Liu, gang, li, W., Li, J., wen Jin, M., 2019. Chlorine injury enhances antibiotic resistance in Pseudomonas aeruginosa through over expression of drug efflux pumps. Water Res. 156, 366–371. https://doi.org/10.1016/j.watres.2019.03.035.

Hu, Y., Zhang, T., Jiang, L., Yao, S., Ye, H., Lin, K., Cui, C., 2019. Removal of sulfonamide antibiotic resistant bacterial and intracellular antibiotic resistance genes by UVC-activated peroxymonosulfate. Chem. Eng. J. 368, 888–895. https://doi.org/10.1016/j.cej.2019.02.207.

Huang, J.J., Hu, H.Y., Tang, F., Li, Y., Lu, S.Q., Lu, Y., 2011. Inactivation and reactivation of antibiotic-resistant bacteria by chlorination in secondary effluents of a municipal wastewater treatment plant. Water Res. 45, 2775–2781. https://doi.org/10.1016/j.watres.2011.02.026.

Huo, Z.Y., Du, Y., Chen, Z., Wu, Y.H., Hu, H.Y., 2020. Evaluation and prospects of nanomaterial-enabled innovative processes and devices for water disinfection: a state-of-the-art review. Water Res. 173 https://doi.org/10.1016/j.watres.2020.115581.

Ioannou-Ttofa, L., Raj, S., Prakash, H., Fatta-Kassinos, D., 2019. Solar photo-Fenton oxidation for the removal of ampicillin, total cultivable and resistant E. coli and ecotoxicity from secondary-treated wastewater effluents. Chem. Eng. J. 355, 91–102. https://doi.org/10.1016/j.cej.2018.08.057.

Jin, Y., Shi, Y., Chen, Z., Chen, R., Chen, X., Zheng, X., Liu, Y., 2020. Combination of sunlight with hydrogen peroxide generated at a modified reticulated vitreous carbon for drinking water disinfection. J. Clean. Prod. 252, 119794. https://doi.org/10.1016/j.jclepro.2019.119794.

Karaolia, P., Michael-Kordatou, I., Hapeshi, E., Drosou, C., Bertakis, Y., Christo, D., Armatas, G.S., Sygellou, L., Christofilos, D., Armatas, G.S., Sygellou, L., Schwartz, T., Xekoukoulotakis, N.P., Fatta-Kassinos, D., 2018. Removal of antibiotics, antibiotic-resistant bacteria and their associated genes by graphene-based TiO2 composite photocatalysts under solar radiation in urban wastewaters. Appl. Catal. B Environ. 224, 810–824. https://doi.org/10.1016/j.apcatb.2017.11.020.

Kashimada, K., Kamiko, N., Yamamoto, K., Ohgaki, S., 1996. Assessment of photoreactivation following ultraviolet light disinfection. Water Sci. Technol. 33, 261–269. https://doi.org/10.2166/wst.1996.0683.

Kielbassa, C., Roza, L., Epe, B., 1997. Wavelength dependence of oxidative DNA damage induced by UV and visible light. Carcinogenesis 18, 811–816. https://doi.org/10.1093/carcin/18.4.811.

Li, G.Q., Wang, W.L., Huo, Z.Y., Lu, Y., Hu, H.Y., 2017. Comparison of UV-LED and low pressure UV for water disinfection: photoreactivation and dark repair of Escherichia coli. Water Res. 126, 134–143. https://doi.org/10.1016/j.watres.2017.09.030.

Li, L., Mendis, N., Trigui, H., Oliver, J.D., Faucher, S.P., 2014. The importance of the viable but non-culturable state in human bacterial pathogens. Front. Microbiol. 5 (1), 1. https://doi.org/10.3389/fmicb.2014.00258.

Li, X., Cai, M., Wang, L., Niu, F., Yang, D., Zhang, G., Niu, F., Wang, L., Li, X., Cai, M., Wang, L., Niu, F., Yang, D., Zhang, G., 2019. Evaluation survey of microbial disinfection methods in UV-LED water treatment systems. Sci. Total Environ. 659, 1415–1427. https://doi.org/10.1016/j.scitotenv.2018.12.344.

Li, Y., Yang, M., Zhang, X., Jiang, J., Liu, J., Yau, C.F., Graham, N.J.D., Li, X., 2017a. Two-step chlorination: a new approach to disinfection of a primary sewage effluent. Water Res. 108, 339–347. https://doi.org/10.1016/j.watres.2016.11.019.

Li, Y., Zhang, X., Yang, M., Liu, J., Li, W., Graham, N.J.D., Li, X., Yang, B., 2017b. Three-step effluent chlorination increases disinfection efficiency and reduces DBP formation and toxicity. Chemosphere 168, 1302–1308. https://doi.org/10.1016/j.chemosphere.2016.11.137.

Liltved, H., Landfald, B., 1996. Influence of liquid holding recovery and photoreactivation on survival of ultraviolet-irradiated fish pathogenic bacteria. Water Res. 30, 1109–1114. https://doi.org/10.1016/0043-1354(95)00276-6.

Lin, H., Ye, C., Chen, S., Zhang, S., Yu, X., 2017. Viable but non-culturable E. coli induced by low level chlorination have higher persistence to antibiotics than their culturable counterparts. Environ. Pollut. 230, 242–249. https://doi.org/10.1016/j.envpol.2017.06.047.

Lin, W., Zhang, M., Zhang, S., Yu, X., 2016. Can chlorination co-select antibiotic-resistance genes? Chemosphere 156, 412–419. https://doi.org/10.1016/j.chemosphere.2016.04.139.

Linden, K.G., Darby, J.L., 1997. Estimating effective germicidal dose from medium pressure UV lamps. J. Environ. Eng. 123, 1142–1149 (ASCE)0733-9372(1997)123:11(1142), 10.1061/.

Lindenauer, K.G., Darby, J.L., 1994. Ultraviolet disinfection of wastewater: effect of dose on subsequent photoreactivation. Water Res. 28, 805–817. https://doi.org/10.1016/0043-1354(94)90087-6.

Liu, S.S., Qu, H.M., Yang, D., Hu, H., Liu, W.L., Qiu, Z.G., Hou, A.M., Guo, J., Li, J.W., Shen, Z.Q., Jin, M., 2018. Chlorine disinfection increases both intracellular and extracellular antibiotic resistance genes in a full-scale wastewater treatment plant. Water Res. 136, 131–136. https://doi.org/10.1016/j.watres.2018.02.036.

Liu, Y., Wang, C., Tyrrell, G., Hrudey, S.E., Li, X.F., 2009. Induction of Escherichia coli O157:H7 into the viable but non-culturable state by chloraminated water and river water, and subsequent resuscitation. Environ. Microbiol. Rep. 1, 155–161. https://doi.org/10.1111/j.1758-2229.2009.00024.x.

Lyons, M.M., Aas, P., Pakulski, J.D., Van Waasbergen, L., Miller, R.V., Mitchell, D.L., Jeffrey, W.H., 1998. DNA damage induced by ultraviolet radiation in coral-reef microbial communities. Mar. Biol. 130, 537–543. https://doi.org/10.1007/s002270050274.

Malvestiti, J.A., Dantas, R.F., 2019. Influence of industrial contamination in municipal secondary effluent disinfection by UV/H2O2. Environ. Sci. Pollut. Res. 26, 13286–13298. https://doi.org/10.1007/s11356-019-04705-1.

Malvestiti, J.A., Dantas, R.F., 2018. Disinfection of secondary effluents by O3, O3/H2O2 and UV/H2O2: influence of carbonate, nitrate, industrial contaminants and regrowth. J. Environ. Chem. Eng. 6, 560–567. https://doi.org/10.1016/j.jece.2017.12.058.

Mao, G., Song, Y., Bartlam, M., Wang, Y., 2018. Long-term effects of residual chlorine on Pseudomonas aeruginosa in simulated drinking water fed with low AOC medium. Front. Microbiol. 9, 1–10. https://doi.org/10.3389/fmicb.2018.00879.

McGuigan, K.G., Conroy, R.M., Mosler, H.J., du Preez, M., Ubomba-Jaswa, E., Fernandez-Ibañez, P., 2012. Solar water disinfection (SODIS): a review from bench-top to roof-top. J. Hazard Mater. 235–236, 29–46. https://doi.org/10.1016/j.jhazmat.2012.07.053.

McKinney, C.W., Pruden, A., 2012. Ultraviolet disinfection of antibiotic resistant bacteria and their antibiotic resistance genes in water and wastewater. Environ. Sci. Technol. 46, 13393–13400. https://doi.org/10.1021/es303652q.

Mecha, A.C., Onyango, M.S., Ochieng, A., Momba, M.N.B., 2017. Evaluation of synergy and bacterial regrowth in photocatalytic ozonation disinfection of municipal wastewater. Sci. Total Environ. 601– 602, 626–635. https://doi.org/10.1016/j.scitotenv.2017.05.204.

Michael-Kordatou, I., Karaolia, P., Fatta-Kassinos, D., 2018. The role of operating parameters and oxidative damage mechanisms of advanced chemical oxidation processes in the combat against antibiotic-resistant bacteria and resistance genes present in urban wastewater. Water Res. 129, 208–230. https://doi.org/10.1016/j.watres.2017.10.007.

Michael, S.G., Michael-kordatou, I., Nahim-granados, S., Polo-lópez, I., Rocha, J., Martínez-piernas, A.B., 2020. Investigating the impact of UV-C/H2O2 and sunlight/H2O2 on the removal of antibiotics, antibiotic resistance determinants and toxicity present in urban wastewater. Chem. Eng. J. 124383 https://doi.org/10.1016/j.cej.2020.124383.

Miklos, D.B., Remy, C., Jekel, M., Linden, K.G., Drewes, J.E., Hübner, U., 2018. Evaluation of advanced oxidation processes for water and wastewater treatment – a critical review. Water Res. 139, 118–131. https://doi.org/10.1016/j.watres.2018.03.042.

Moles, S., Valero, P., Escuadra, S., Mosteo, R., Gómez, J., Ormad, M.P., 2020. Performance comparison of commercial TiO2: separation and reuse for bacterial photo-inactivation and emerging pollutants photo-degradation. Environ. Sci. Pollut. Res. 27, 9099–9113. https://doi.org/10.1007/s11356-019-07276-3.

Moreira, N.F.F., Narciso-da-Rocha, C., Polo-López, M.I., Pastrana-Martínez, L.M., Faria, J.L., Manaia, C.M., Fernández-Ibáñez, P., Nunes, O.C., Silva, A.M.T., 2018. Solar treatment (H2O2, TiO2-P25 and GO-TiO2photocatalysis, photo-Fenton) of organic micropollutants, human pathogen indicators, antibiotic resistant bacteria and related genes in urban wastewater. Water Res. 135, 195–206. https://doi.org/10.1016/j.watres.2018.01.064.

Moreira, N.F.F., Sousa, J.M., Macedo, G., Ribeiro, A.R., Barreiros, L., Pedrosa, M.,

Faria, J.L., Pereira, M.F.R., Castro-Silva, S., Segundo, M.A., Manaia, C.M., Nunes, O.C., Silva, A.M.T., 2016. Photocatalytic ozonation of urban wastewater and surface water using immobilized TiO 2 with LEDs: micropollutants, antibiotic resistance genes and estrogenic activity. Water Res. 94, 10–22. https://doi.org/10.1016/j.watres.2016.02.003.

Moreno-Andrés, J., Rios Quintero, R., Acevedo-Merino, A., Nebot, E., 2019. Disinfection performance using a UV/persulfate system: effects derived from different aqueous matrices. Photochem. Photobiol. Sci. 18, 878–883. https://doi.org/10.1039/c8pp00304a.

Nowakowska, J., Oliver, J.D., 2013. Resistance to environmental stresses by Vibrio vulnificus in the viable but nonculturable state. FEMS Microbiol. Ecol. 84, 213–222. https://doi.org/10.1111/1574-6941.12052.

Nyangaresi, P.O., Qin, Y., Chen, G., Zhang, B., Lu, Y., Shen, L., 2019a. Comparison of UV-LED photolytic and UV-LED/TiO2 photocatalytic disinfection for Escherichia coli in water. Catal. Today 335, 200–207. https://doi.org/10.1016/j.cattod.2018.11.015.

Nyangaresi, P.O., Qin, Y., Chen, G., Zhang, B., Lu, Y., Shen, L., 2019b. Comparison of the performance of pulsed and continuous UVC-LED irradiation in the inactivation of bacteria. Water Res. 157, 218–227. https://doi.org/10.1016/j.watres.2019.03.080.

Nyangaresi, P.O., Qin, Y., Chen, G., Zhang, B., Lu, Y., Shen, L., 2018. Effects of single and combined UV-LEDs on inactivation and subsequent reactivation of E. coli in water disinfection. Water Res. 147, 331–341. https://doi.org/10.1016/j.watres.2018.10.014.

Pei, M., Zhang, B., He, Y., Su, J., Gin, K., Lev, O., Shen, G., Hu, S., 2019. State of the art of tertiary treatment technologies for controlling antibiotic resistance in wastewater treatment plants. Environ. Int. 131, 105026. https://doi.org/10.1016/j.envint.2019.105026.

Pichel, N., Vivar, M., Fuentes, M., 2019. The problem of drinking water access: a review of disinfection technologies with an emphasis on solar treatment methods. Chemosphere 218, 1014–1030. https://doi.org/10.1016/j.chemosphere.2018.11.205.

Polo-López, M.I., Castro-Alférez, M., Nahim-Granados, S., Malato, S., Fernández-Ibáñez, P., 2017. Legionella jordanis inactivation in water by solar driven processes: EMA-qPCR versus culture-based analyses for new mechanistic insights. Catal. Today 287, 15–21. https://doi.org/10.1016/j.cattod.2016.10.029.

Price, P.B., Sowers, T., 2004. Temperature dependence of metabolic rates for microbial growth, maintenance, and survival. Proc. Natl. Acad. Sci. U.S.A. 101, 4631–4636. https://doi.org/10.1073/pnas.0400522101.

Rachmadi, A.T., Kitajima, M., Watanabe, K., Okabe, S., Sano, D., 2018a. Disinfection as a selection pressure on RNA virus evolution. Environ. Sci. Technol. https://doi.org/10.1021/acs.est.8b00590.

Rachmadi, A.T., Kitajima, M., Watanabe, K., Yaegashi, S., Serrana, J., Nakamura, A., Nakagomi, T., Nakagomi, O., Katayama, K., Okabe, S., Sano, D., 2018b. Free-chlorine disinfection as a selection pressure on norovirus. Appl. Environ. Microbiol. 84, 1–14. https://doi.org/10.1128/AEM .00244-18.

Robben, C., Fister, S., Witte, A.K., Schoder, D., Rossmanith, P., Mester, P., 2018. Induction of the viable but non-culturable state in bacterial pathogens by household cleaners and inorganic salts. Sci. Rep. 8, 1–9. https://doi.org/10.1038/s41598-018-33595-5.

Salcedo, D.E., Kim, S., 2017. Fate of tetracycline resistance in synthetic livestock carcass leachate for two years. J. Environ. Manag. 187, 220–228. https://doi.org/10.1016/j.jenvman.2016.11.048.

Salcedo, I., Andrade, J.A., Quiroga, J.M., Nebot, E., 2007. Photoreactivation and dark repair in UV-treated microorganisms: effect of temperature. Appl. Environ. Microbiol. 73, 1594–1600. https://doi.org/10.1128/AEM.02145-06.

Sánchez-Montes, I., Salmerón García, I., Rivas Ibañez, G., Aquino, J.M., Polo-López, M.I., Malato, S., Oller, I., 2020. UVC-based advanced oxidation processes for simultaneous removal of microcontaminants and pathogens from simulated municipal wastewater at pilot plant scale. Environ. Sci. Water Res. Technol. 6, 2553–2566. https://doi.org/10.1039/d0ew00279h.

Sanders, R.W., Macaluso, A.L., Sardina, T.J., Mitchell, D.L., 2005. Photoreactivation in two freshwater ciliates: differential responses to variations in UV-B flux and temperature. Aquat. Microb. Ecol. 40, 283–292. https://doi.org/10.3354/ame040283.

Sanz, E.N., Dávila, I.S., Balao, J.A.A., Alonso, J.M.Q., 2007. Modelling of reactivation after UV disinfection: effect of UV-C dose on subsequent photoreactivation and dark repair. Water Res. 41, 3141–3151. https://doi.org/10.1016/j.watres.2007.04.008.

Serna-Galvis, E.A., Salazar-Ospina, L., Jiménez, J.N., Pino, N.J., Torres-Palma, R.A., 2020. Elimination of carbapenem resistant Klebsiella pneumoniae in water by UV-C, UV-C/persulfate and UV-C/H2O2. Evaluation of response to antibiotic, residual effect of the processes and removal of resistance gene. J. Environ. Chem. Eng. 8, 102196. https://doi.org/10.1016/j.jece.2018.02.004.

Serna-Galvis, E.A., Vélez-Peña, E., Osorio-Vargas, P., Jiménez, J.N., Salazar-Ospina, L., Guaca-González, Y.M., Torres-Palma, R.A., 2019. Inactivation of carbapenem-resistant Klebsiella pneumoniae by photo-Fenton: residual effect, gene evolution and modifications with citric acid and persulfate. Water Res. 161, 354–363. https://doi.org/10.1016/j.watres.2019.06.024.

Shafaei, S., Klamerth, N., Zhang, Y., McPhedran, K., Bolton, J.R., Gamal El-Din, M., 2017. Impact of environmental conditions on bacterial photoreactivation in wastewater effluents. Environ. Sci. Process. Impacts 19, 31–37. https://doi.org/10.1039/c6em00501b.

Sharma, V.K., Johnson, N., Cizmas, L., McDonald, T.J., Kim, H., 2016. A review of the influence of treatment strategies on antibiotic resistant bacteria and antibiotic resistance genes. Chemosphere 150, 702–714. https://doi.org/10.1016/j.chemosphere.2015.12.084.

Shekoohiyan, S., Rtimi, S., Moussavi, G., Giannakis, S., Pulgarin, C., 2019. Enhancing solar disinfection of water in PET bottles by optimized in-situ formation of iron oxide films. From heterogeneous to homogeneous action modes with H2O2 vs. O2 – Part 1: iron salts as oxide precursors. Chem. Eng. J. 358, 211–224. https://doi.org/10.1016/j.cej.2018.09.219.

Shen, L., Griffith, T.M., Nyangaresi, P.O., Qin, Y., Pang, X., Chen, G., Li, M., Lu, Y., Zhang, B., 2020. Efficacy of UVC-LED in water disinfection on Bacillus species with consideration of antibiotic resistance issue. J. Hazard Mater. 386, 121968. https://doi.org/10.1016/j.jhazmat.2019.121968.

Shimizu, Y., Ateia, M., Wang, M., Awfa, D., Yoshimura, C., 2019. Disinfection mechanism of E. coli by CNT-TiO2 composites: photocatalytic inactivation vs. physical separation. Chemosphere 235, 1041–1049.

Sinha, R.P., Häder, D.P., 2002. UV-induced DNA damage and repair: a review. Photochem. Photobiol. Sci. 1, 225–236. https://doi.org/10.1039/b201230h.

Song, J., Wu, X., Zhang, M., Liu, C., Yu, J., Sun, G., Si, Y., Ding, B., 2020. Highly flexible, core-shell heterostructured, and visible-light-driven titania-based nanofibrous membranes for antibiotic removal and E. coil inactivation. Chem. Eng. J. 379, 122269. https://doi.org/10.1016/j.cej.2019.122269.

Song, K., Mohseni, M., Taghipour, F., 2016. Application of ultraviolet light-emitting diodes (UV-LEDs) for water disinfection: a review. Water Res. 94, 341–349. https://doi.org/10.1016/j.watres.2016.03.003.

Sousa, J.M., Macedo, G., Pedrosa, M., Becerra-Castro, C., Castro-Silva, S., Pereira, M.F.R., Silva, A.M.T., Nunes, O.C., Manaia, C.M., 2017. Ozonation and UV254nm radiation for the removal of microorganisms and antibiotic resistance genes from urban wastewater. J. Hazard Mater. 323, 434–441. https://doi.org/10.1016/j.jhazmat.2016.03.096.

Stephanie, K., Ursula, O., Thomas, S., 2011. Immunological detection of UV induced cyclobutane pyrimidine dimers and (6-4) photoproducts in DNA from reference bacteria and natural aquatic populations. J. Microbiol. Methods 84, 435–441. https://doi.org/10.1016/j.mimet.2011.01.004.

Stokell, J.R., Steck, T.R., 2012. Viable but nonculturable bacteria. eLS 1–8. https://doi.org/10.1002/9780470015902.a0000407.pub2.

Takayama, K., Kjelleberg, S., 2000. The role of RNA stability during bacterial stress responses and starvation. Environ. Microbiol. https://doi.org/10.1046/j.1462-2920.2000.00119.x.

Thayanukul, P., Kurisu, F., Kasuga, I., Furumai, H., 2013. Evaluation of microbial regrowth potential by assimilable organic carbon in various reclaimed water and distribution systems. Water Res. 47, 225–232. https://doi.org/10.1016/j.watres.2012.09.051.

Ugwuja, C.G., Adelowo, O., Ogunlaja, A., Omorogie, M.O., Olukanni, O., Ikhimiukor, O., Iermak, I., Kolawole, G., Guenter, C., Taubert, A., Bodede, O., Moodley, R., Inada, N., de Camargo, A.S.S., Unuabonah, E.I., 2019. Visible-light mediated photodynamic water disinfection @ bimetallic doped hybrid clay nanocomposites. ACS Appl. Mater. Interfaces 11, 25483–25494. https://doi.org/10.1021/acsami.9b01212.

Umar, M., Roddick, F., Fan, L., 2019. Moving from the traditional paradigm of pathogen inactivation to controlling antibiotic resistance in water - role of ultraviolet irradiation. Sci. Total Environ. 662, 923–939. https://doi.org/10.1016/j.scitotenv.2019.01.289.

Uyguner Demirel, C.S., Birben, N.C., Bekbolet, M., 2018. A comprehensive review on the use of second generation TiO2 photocatalysts: microorganism inactivation. Chemosphere 211, 420–448. https://doi.org/10.1016/j.chemosphere.2018.07.121.

Valero, P., Giannakis, S., Mosteo, R., Ormad, M.P., Pulgarin, C., 2017. Comparative effect of growth media on the monitoring of E. coli inactivation and regrowth after solar and photo-Fenton treatment. Chem. Eng. J. 313, 109–120. https://doi.org/10.1016/j.cej.2016.11.126.

Wang, D., Pillai, S.C., Ho, S.H., Zeng, J., Li, Y., Dionysiou, D.D., 2018. Plasmonic-based nanomaterials for environmental remediation. Appl. Catal. B Environ. https://doi.org/10.1016/j.apcatb.2018.05.094.

Wen, G., Wan, Q., Deng, X., Cao, R., Xu, X., Chen, Z., Wang, J., Huang, T., 2019. Reactivation of fungal spores in water following UV disinfection: effect of temperature, dark delay, and real water matrices. Chemosphere 237, 124490. https://doi.org/10.1016/j.chemosphere.2019.124490.

WHO, 2017a. Antibacterial Agents in Clinical Development: an Analysis of the Antibacterial Clinical Development Pipeline, Including Tuberculosis. https://doi.org/10.1016/S0065-7743(08)61279-8.

WHO, 2017b. Guidelines for Drinking-Water Quality: Fourth Edition Incorporating the First Addendum.

Wilson, M.R., Jiang, Y., Villalta, P.W., Stornetta, A., Boudreau, P.D., Carrá, A., Brennan, C.A., Chun, E., Ngo, L., Samson, L.D., Engelward, B.P., Garrett, W.S., Balbo, S., Balskus, E.P., 2019. The human gut bacterial genotoxin colibactin alkylates DNA. Science (80-.) 363, eaar7785. https://doi.org/10.1126/science.aar7785.

Xu, H.S., Roberts, N., Singleton, F.L., Attwell, R.W., Grimes, D.J., Colwell, R.R., 1982. Survival and viability of nonculturable Escherichia coli and Vibrio cholerae in the estuarine and marine environment. Microb. Ecol. 8, 313–323. https://doi.org/10.1007/BF02010671.

Xu, L., Zhang, C., Xu, P., Wang, X.C., 2018. Mechanisms of ultraviolet disinfection and chlorination of Escherichia coli: culturability, membrane permeability, metabolism, and genetic damage. J. Environ. Sci. (China) 65, 356–366. https://doi.org/10.1016/j.jes.2017.07.006.

You, J., Guo, Y., Guo, R., Liu, X., 2019. A review of visible light-active photocatalysts

for water disinfection : features and prospects. Chem. Eng. J. 373, 624–641. https://doi.org/10.1016/j.cej.2019.05.071.

Zeng, F., Cao, S., Jin, W., Zhou, X., Ding, W., Tu, R., Han, S.F., Wang, C., Jiang, Q., Huang, H., Ding, F., 2020. Inactivation of chlorine-resistant bacterial spores in drinking water using UV irradiation, UV/Hydrogen peroxide and UV/Peroxymonosulfate: efficiency and mechanism. J. Clean. Prod. 243, 118666. https://doi.org/10.1016/j.jclepro.2019.118666.

Zhang, C., Li, Y., Shuai, D., Shen, Y., Xiong, W., Wang, L., 2019. Graphitic carbon nitride (g-C3N4)-based photocatalysts for water disinfection and microbial control: a review. Chemosphere 214, 462–479. https://doi.org/10.1016/j.chemosphere.2018.09.137.

Zhang, S., Ye, C., Lin, H., Lv, L., Yu, X., 2015. UV disinfection induces a vbnc state in escherichia coli and pseudomonas aeruginosa. Environ. Sci. Technol. 49, 1721–1728. https://doi.org/10.1021/es505211e.