

1 **Predatory bacteria in combination with solar disinfection and solar photocatalysis for the**  
2 **treatment of rainwater**

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24 **Abstract**

25 The predatory bacterium, *Bdellovibrio bacteriovorus*, was applied as a biological pre-treatment to  
26 solar disinfection and solar photocatalytic disinfection for rainwater treatment. The photocatalyst  
27 used was immobilised titanium-dioxide reduced graphene oxide. The pre-treatment followed by solar  
28 photocatalysis for 120 min under natural sunlight reduced the viable counts of *Klebsiella pneumoniae*  
29 from  $2.00 \times 10^9$  colony forming units (CFU)/mL to below the detection limit (BDL) (<1 CFU/100  $\mu$ L).  
30 Correspondingly, ethidium monoazide bromide quantitative PCR analysis indicated a high total log  
31 reduction in *K. pneumoniae* gene copies (GC)/mL (5.85 logs after solar photocatalysis for 240 min).  
32 In contrast, solar disinfection and solar photocatalysis without the biological pre-treatment were more  
33 effective for *Enterococcus faecium* disinfection as the viable counts of *E. faecium* were reduced by  
34 8.00 logs (from  $1.00 \times 10^8$  CFU/mL to BDL) and the gene copies were reduced by ~3.39 logs (from  
35  $2.09 \times 10^6$  GC/mL to  $\sim 9.00 \times 10^2$  GC/mL) after 240 min of treatment. Predatory bacteria can be  
36 applied as a pre-treatment to solar disinfection and solar photocatalytic treatment to enhance the  
37 removal efficiency of Gram-negative bacteria, which is crucial for the development of a targeted  
38 water treatment approach.

39

40 **Keywords:** Harvested rainwater; *Bdellovibrio bacteriovorus*; Biological pre-treatment; Solar  
41 disinfection; Photocatalysis

## 42 1. Introduction

43 Domestic rainwater harvesting is employed as a supplementary water source, particularly in water  
44 scarce regions. However, the quality of harvested rainwater does not always comply with drinking  
45 water standards, and some bacteria of public health concern such as *Pseudomonas*, *Klebsiella*,  
46 *Campylobacter* and *Staphylococcus* spp., have been detected in rainwater samples (De  
47 Kwaadsteniet et al., 2013). While various treatment methods have been investigated and applied to  
48 disinfect rainwater (Dobrowsky et al., 2015; Reyneke et al., 2016), the World Health Organisation  
49 (WHO) recognises solar disinfection (SODIS) as a cost-effective, household-based technology,  
50 which can be employed to decrease the number of viable pathogenic organisms in contaminated  
51 water sources and reduce the incidence of diarrheal disease (Byrne et al., 2011). The protocol  
52 involves exposing water in UV-visible transparent containers to direct sunlight for a minimum of 6 h  
53 (48 h in cloudy conditions). Nalwanga et al. (2018) investigated the use of SODIS with 2 L  
54 polyethylene-terephthalate (PET) bottles for the treatment of harvested rainwater in Uganda. While  
55 the viable counts of *Escherichia coli* and faecal enterococci exceeded drinking water standards in  
56 the majority of the untreated samples analysed, culture-based analysis indicated that after SODIS,  
57 the concentrations of these bacteria were significantly reduced (detailed information on counts not  
58 presented) (Nalwanga et al., 2018). The major limitations associated with the use of a simple SODIS  
59 system are, however, the small volume of treated water generated (1 to 5 L) and the treatment time  
60 required for sufficient disinfection of the water. It is also recommended that the treated water should  
61 be used within 24 h as regrowth of bacteria may occur (Makwana et al., 2015).

62 Different approaches have subsequently been investigated to improve the efficiency of solar  
63 disinfection. Ubomba-Jaswa et al. (2010) used a 25 L methacrylate batch reactor fitted with a  
64 compound parabolic collector (CPC; concentrates diffuse solar irradiation onto a reactor vessel in  
65 order to increase the dose of solar irradiation) to disinfect well water. Complete inactivation of *E. coli*  
66 was achieved within 5 h on sunny days and a 3-log reduction was achieved within this time period  
67 during overcast conditions (cloudy days). However, some organisms are more resistant to solar  
68 disinfection than others. For example, Strauss et al. (2018) reported that while a solar-CPC treatment  
69 system effectively reduced the *E. coli* and total coliform counts to below the detection limit (BDL) at

70 temperatures exceeding 39°C and UV-A radiation exceeding 20 W/m<sup>2</sup>, ethidium monoazide  
71 quantitative polymerase chain reaction (EMA-qPCR) analysis indicated that viable *Legionella* and  
72 *Pseudomonas* were detected in all the SODIS-CPC treated samples throughout the sampling period.  
73 Clements et al. (2019) used EMA-qPCR to screen solar pasteurized (SOPAS) harvested rainwater  
74 for potentially viable bacteria and found that *Klebsiella* spp., amongst others, survived at  
75 temperatures > 90°C. It is hypothesised that the survival of bacteria in solar disinfection systems  
76 could be due to the possession of heat shock proteins, DNA repair mechanisms (such as *recA*) and  
77 their ability to form associations with protozoa (Strauss et al., 2018). Additional treatment techniques  
78 are thus required to overcome this bacterial resistance to disinfection strategies and effectively  
79 eliminate these pathogens and opportunistic pathogens from water sources.

80 Advanced oxidative processes (AOP), such as heterogeneous photocatalysis with semiconductor  
81 materials (Byrne et al., 2011), have also been explored and Helali et al. (2014) investigated the solar  
82 inactivation of *E. coli* with different photocatalysts [i.e., TiO<sub>2</sub> P25, TiO<sub>2</sub> PC500, TiO<sub>2</sub> Ruana and  
83 Russelite (Bi<sub>2</sub>WO<sub>6</sub>)]. With only solar irradiation, 3 to 5 h were required for complete inactivation of  
84 *E. coli*. In contrast, the treatment time required for the inactivation of *E. coli* was significantly reduced  
85 to between 5 to 30 min for TiO<sub>2</sub> P25, which was the most effective photocatalytic material. We have  
86 also previously reported enhanced solar disinfection utilising TiO<sub>2</sub>-reduced graphene oxide  
87 composites (TiO<sub>2</sub>-rGO) (Fernández-Ibáñez et al., 2015; Cruz-Ortiz et al., 2017). Adán et al. (2018)  
88 then showed that TiO<sub>2</sub> immobilised on borosilicate glass raschig rings effectively reduced *E. coli*  
89 concentrations, in co-culture with *Acanthamoeba* trophozoites, by 3 logs in distilled water after  
90 60 min, while a 2-log reduction was recorded after 180 min for synthetic wastewater. It was thus  
91 concluded that immobilised photocatalysts might be practical for water treatment as the post-  
92 treatment removal of the photocatalytic material is not required.

93 An interesting approach to the inactivation of resistant strains is the use of predatory bacteria such  
94 as *Bdellovibrio*-and-like-organisms (known as BALOs). These have been identified as potential “live  
95 antibiotics” as they are able to prey on and reduce the concentration of predominantly Gram-negative  
96 bacteria in co-culture experiments (Socket, 2009). This group of predatory bacteria include species  
97 such as *Bdellovibrio bacteriovorus* and *Micavibrio aeruginosavorus*. Kadouri et al. (2013)

98 investigated whether *B. bacteriovorus* and *M. aeruginosavorus* could prey on clinically significant  
99 multidrug-resistant Gram-negative bacteria and found that *B. bacteriovorus* HD100 was able to prey  
100 on all the host organisms (100%), while *B. bacteriovorus* 109J was able to prey on 93% and  
101 *M. aeruginosavorus* ARL-13 was only able to prey on 35% of the host bacteria. Limited research has  
102 however been conducted on the application of these predatory bacteria as biocontrol agents for  
103 potable water treatment, with most studies focussing on their application as probiotics in aquaculture  
104 (Chu & Zhu, 2010; Willis et al., 2016) or as bioremediation agents in wastewater treatment plants  
105 (Yu et al., 2017; Ökzan et al., 2018).

106 Based on the survival of pathogenic microorganisms in treated rainwater, a need exists to investigate  
107 a combination of technologies that incorporate biocontrol, physical and chemical treatment. This  
108 study thus aimed to apply *B. bacteriovorus* in combination with solar-CPC reactors and solar-CPC  
109 treatment with photocatalysis to disinfect rainwater. *Klebsiella pneumoniae* S1 43 (isolated from solar  
110 pasteurized rainwater at a treatment temperature above 70°C) (Clements et al., 2019) and  
111 *Enterococcus faecium* 8D (isolated from untreated harvested rainwater) (Dobrowsky et al., 2014)  
112 were included as test organisms.

## 113 **2. Materials and Methods**

### 114 **2.1 Coating of Raschig Rings**

115 The design and construction of the CPC is outlined in the Supplementary Information. The TiO<sub>2</sub>-rGO  
116 composite was synthesised using graphene oxide (GO; NanoInnova, Spain) and TiO<sub>2</sub> P25 (Aeroxide  
117 P25, Evonik, Germany) as previously described by Fernández-Ibáñez et al. (2015). The TiO<sub>2</sub>-rGO  
118 was immobilised on borosilicate glass raschig rings [5 mm (length) × 5 mm (outer diameter) × 1 mm  
119 (glass thickness); Sigma-Aldrich, Germany] for application in the designed small-scale solar-CPC  
120 systems. The raschig rings were cleaned as described by Cunha et al. (2018). The TiO<sub>2</sub>-rGO (1.5 g)  
121 was added to 100 mL absolute methanol to obtain a final concentration of 1.5% w/v. The suspension  
122 was sonicated for 15 min and the raschig rings were submerged in the suspension. To evaporate  
123 the methanol, the rings in the suspension were added to a rotary evaporator (Heidolph Instruments,  
124 Schwabach GmbH, Germany) with the water bath temperature set to 65°C and the rotary speed set

125 to 120 rpm. Once the methanol was evaporated, the coated raschig rings were dried at 80°C for  
126 90 min and annealed at 400°C for 2 h (with a heating rate of 2°C per min) in air (Cunha et al., 2018).  
127 The rings were weighed before and after the coating, and it was determined that the loading of  
128 TiO<sub>2</sub>-rGO was ca. 0.89 mg/cm<sup>2</sup>.

## 129 **2.2 Solar Treatment Experiments**

### 130 **2.2.1 Prey Bacterial Strains**

131 *Klebsiella pneumoniae* S1 43 and *E. faecium* 8D were obtained from the Water Resource Laboratory  
132 Culture Collection at Stellenbosch University (Department of Microbiology). These bacteria were  
133 inoculated into 500 mL Luria Bertani (LB) broth (Biolab, Merck, South Africa) and were incubated at  
134 37°C for 24 to 48 h with shaking at 200 rpm. The bacterial cells were harvested by centrifugation at  
135 11 305 x g for 15 min. The bacterial biomass was washed and re-suspended in phosphate buffered  
136 saline (PBS) and the optical density (OD) of the re-suspended pellets was measured using the T60  
137 UV-Visible Spectrophotometer (PG Instruments Limited, Thermo Fisher Scientific, South Africa) at  
138 600 nm (OD<sub>600</sub>). The concentration of the bacterial cells was adjusted with PBS to obtain a final OD<sub>600</sub>  
139 of 1.00 (which corresponded to approximately 10<sup>9</sup> cells/mL) (Feng et al., 2016).

### 140 **2.2.2 Preparation of the Predatory Bacteria Stock Lysate**

141 *Bdellovibrio bacteriovorus* PF13 was isolated from wastewater collected from the influent point of  
142 the Stellenbosch Wastewater Treatment Plant (GPS co-ordinates: 33° 59' 21.13"S 18° 47' 47.75"E)  
143 as described by Waso et al. (2019). The predatory bacterium was stored as plaques on double-layer  
144 agar plates, with *Pseudomonas fluorescens* ATCC 13525 used as prey cells at 4°C until further  
145 experimentation commenced (Dashiff et al., 2011). To apply *B. bacteriovorus* PF13 as a pre-  
146 treatment to SODIS, a predator stock lysate (used as the predator inoculum in the pre-treatment  
147 experiments) was prepared as described by Dashiff et al. (2011) in the presence of *P. fluorescens*  
148 ATCC 13525 as prey cells (Supplementary Information).

149

### 150 2.2.3 Experimental Set Up

151 Synthetic rainwater was used to ensure that the composition of the medium remained constant  
152 throughout the study and was prepared by the method reported by Jones and Edwards (1993). For  
153 each test organism (*K. pneumoniae* S1 43 and *E. faecium* 8D), two experimental groups were  
154 analysed as follows: for one experimental group (two systems) the test organisms were pre-treated  
155 with *B. bacteriovorus*; while for the second experimental group (two systems) no pre-treatment  
156 occurred (Fig. 1). Additionally, for each experimental group, one solar-CPC system contained TiO<sub>2</sub>-  
157 rGO coated raschig rings, while the second system contained uncoated raschig rings (solar  
158 disinfection only) (Fig. 1). For the pre-treated samples, 800 mL of synthetic rainwater was seeded  
159 with 100 mL of *K. pneumoniae* or *E. faecium* (OD<sub>600</sub> = 1.00) (section 2.2.1). Subsequently, each  
160 sample was inoculated with 100 mL of the *B. bacteriovorus* stock lysate (OD<sub>600</sub> < 0.2). The co-culture  
161 was incubated for 72 h at 30°C with shaking at 200 rpm to allow for the predation of *B. bacteriovorus*  
162 on the respective prey cells. For the samples which were not subjected to *B. bacteriovorus* pre-  
163 treatment, 900 mL of synthetic rainwater was seeded with 100 mL of *K. pneumoniae* or *E. faecium*  
164 cells (OD<sub>600</sub> = 1.00) (section 2.2.1) (Fig. 1) on the day of solar treatment.

165 The four solar-CPC reactors were filled with approximately 390 mL of the pre-treated or untreated  
166 seeded synthetic rainwater samples and were exposed to natural sunlight for 4 h (Fig. 1). The  
167 remaining volume of each sample was kept in the dark and served as dark controls (Fig. 1). Samples  
168 (10 mL) were collected from each solar-CPC system at 0, 30, 60, 90, 120, 150, 180, 210, and  
169 240 min. For each of the collected samples, the pH, temperature, total dissolved solids (TDS), and  
170 electrical conductivity (EC) were measured with a hand-held Milwaukee Instruments MI806 meter  
171 (Spraytech, South Africa), and the dissolved oxygen (DO) was measured using a Milwaukee  
172 Instruments M600 meter (Spraytech). The solar irradiance data [maximum UV-A and UV-B radiation  
173 and the maximum direct normal irradiance (DNI)] were obtained from the Stellenbosch Weather  
174 Services [Stellenbosch University, Faculty of Engineering ([http:// weather.sun.ac.za/](http://weather.sun.ac.za/))], and the  
175 ambient temperature data were obtained from the South African Weather Services (Supplementary  
176 Information Tables A.1 and A.2). The results for the conductivity, temperature, pH, TDS, and DO  
177 collected for the different solar treatment time points (0 to 240 min) for each test organism and

178 experimental design, are summarised in the Supplementary Information Tables A.1 and A.2.  
179 Throughout the text the term “solar disinfection” will refer to solar treatment using only the designed  
180 solar-CPC system (with uncoated raschig rings), while “solar photocatalytic disinfection” or “solar  
181 photocatalysis” will refer to the solar treatment using the designed solar-CPC system in combination  
182 with the immobilised TiO<sub>2</sub>-rGO. Furthermore, “solar treatment” will be used to refer simultaneously  
183 to both disinfection strategies.

#### 184 **2.2.3.1 Culture-based Analysis**

185 To enumerate the *K. pneumoniae* and *E. faecium* cells during the solar treatments [in colony forming  
186 units per mL (CFU/mL)], samples (10 mL) were collected as described in section 2.2.3. In addition,  
187 for the samples subjected to *B. bacteriovorus* pre-treatment, 10 mL samples were collected before  
188 (0 h) and after pre-treatment (72 h). A further 10 mL sample was collected from each of the dark  
189 control samples after 240 min (to confirm that the changes in viable organisms occurred as a result  
190 of solar or solar photocatalytic disinfection). A 10-fold serial dilution was prepared (ranging from  
191 undiluted to 10<sup>-6</sup>) for each sample (*n* = 40), and 100 µL of each dilution was spread plated onto LB  
192 agar in triplicate. The plates were incubated at 30°C for 12 to 18 h (overnight).

193 In order to verify that the solar treatment effectively removed the predatory bacteria from the pre-  
194 treated samples, double-layer agar overlays (as described by Yu et al., 2017) were also prepared  
195 using the serial dilutions from the *B. bacteriovorus* pre-treated samples. The plates were incubated  
196 at 30°C for up to 7 days and the predatory bacteria were enumerated in plaque forming units per mL  
197 (PFU/mL).

#### 198 **2.2.3.2 Molecular Analysis**

199 For the molecular analysis of the solar-CPC samples collected at each time point (0 to 240 min) as  
200 well as the samples collected before (0 h) and after (72 h) *B. bacteriovorus* pre-treatment, 500 µL of  
201 each sample was EMA treated as described by Reyneke et al. (2016). The EMA-treated aliquots  
202 were subjected to DNA extractions using the *Quick-DNA*<sup>™</sup> Fecal/Soil Microbe Miniprep kit (Zymo  
203 Research, Inqaba Biotech, South Africa) as per the manufacturer’s instructions.



204 Quantitative real-time PCR was subsequently performed to quantify the gene copies (GC) of  
205 *B. bacteriovorus*, *K. pneumoniae* and *E. faecium* during the various solar treatments. All qPCR  
206 assays were performed using the LightCycler® 96 Instrument (Roche Diagnostics, Mannheim,  
207 Germany) and the FastStart Essential DNA Green Master (Roche Diagnostics). All the qPCR primers  
208 and cycling parameters are outlined in Table 1, while the qPCR mixture as described by Waso et al.  
209 (2018) was utilised. Additionally, the standard curves utilised for GC quantification in the qPCR  
210 assays were generated as described by Waso et al. (2019), using conventional PCR and the cycling  
211 parameters defined in Table 1.

212 All the qPCR results were analysed using the Roche LightCycler® 96 Software Version 1.1 and  
213 Microsoft Excel 2016. In addition, the lower limit of detection (LLOD) for each qPCR assay was  
214 determined as the lowest concentration (GC/μL) consistently detected in the standard curve  
215 samples. Furthermore, the lower limit of quantification (LLOQ) for each qPCR sample was  
216 determined as the lowest number of GC/μL that could reliably be quantified in the standard curve  
217 samples. All GC numbers were converted to GC/mL using the following modified equation (which  
218 excludes compensation for sample filtration) (Eq. 1) as described by Rajal et al. (2007):

219 
$$\left( \frac{\text{mL Original Sample}}{\text{mL DNA eluted}} \right) \times (\text{mL used per qPCR assay}) = \text{mL original sample per qPCR assay} \dots \dots \dots (1)$$

## 220 **2.3 Data Analysis**

221 All graphs were generated using GraphPad Prism 7.04 (2018). Two-way Analysis of Variance  
222 (ANOVA) for Multiple Comparisons with Dunnett's tests (alpha value of 0.05) was utilised to  
223 determine whether the concentration of the prey bacteria (*K. pneumoniae* and *E. faecium*) and  
224 *B. bacteriovorus* changed significantly during the various solar treatments. Significance was  
225 observed at  $p < 0.05$ .

## 226 **3. Results**

### 227 **3.1 Impacts of Different Disinfection Strategies on the Survival of *Klebsiella pneumoniae***

228 For the *B. bacteriovorus* pre-treated samples, the CFU of *K. pneumoniae* were reduced by 1.92 logs  
229 during the 72-h pre-treatment, from  $2.00 \times 10^9$  (before predation) to  $2.40 \times 10^7$  CFU/mL. The PFU

230 of *B. bacteriovorus* correspondingly increased by 0.202 logs from  $6.53 \times 10^5$  PFU/mL (before  
231 predation) to  $1.04 \times 10^6$  PFU/mL. Additionally, EMA-qPCR analysis (characteristics summarised in  
232 Table A.3) confirmed that the concentration of *K. pneumoniae* was reduced after predation as the  
233 GC of *K. pneumoniae* decreased by 3.51 logs from  $2.95 \times 10^8$  (before predation) to  
234  $9.20 \times 10^4$  GC/mL, while the concentration of *B. bacteriovorus* increased by 0.430 logs from  
235  $7.96 \times 10^3$  (before predation) to  $2.14 \times 10^4$  GC/mL. Overall, for the dark controls, the plate counts  
236 indicated that the concentration of *K. pneumoniae* remained relatively constant with an average of  
237  $1.97 \times 10^7$  CFU/mL and  $7.50 \times 10^8$  CFU/mL recorded (after 240 min) for the 72-h *B. bacteriovorus*  
238 pre-treated sample and non-pre-treated sample, respectively.

239 For the *K. pneumoniae* pre-treated sample subsequently exposed to solar photocatalytic treatment,  
240 culture-based enumeration indicated that within 120 min the *K. pneumoniae* cell counts were  
241 reduced by 7.38 logs from  $2.40 \times 10^7$  CFU/mL (at 0 min) to BDL ( $<1$  CFU/100  $\mu$ L) ( $p < 0.0001$ ) (Fig.  
242 2A; Table 2). Thus, considering the reduction in CFU/mL recorded after the *B. bacteriovorus* pre-  
243 treatment as well as after the solar photocatalytic treatment, the CFU counts of *K. pneumoniae* were  
244 reduced by a total of 9.30 logs ( $p < 0.0001$ ) from the initial concentration of  $2.00 \times 10^9$  CFU/mL  
245 (Table 2). Correspondingly, EMA-qPCR analysis indicated that in total a 5.85 log reduction in the  
246 *K. pneumoniae* GC was obtained [from  $2.95 \times 10^8$  GC/mL (before predation) to  $4.19 \times 10^2$  GC/mL  
247 (after 240 min of solar exposure)] ( $p < 0.0001$ ) (Fig. 2B; Table 2). The culture-based enumeration of  
248 the *B. bacteriovorus* cells analysed indicated that in the samples exposed to solar photocatalytic  
249 treatment, the PFU of *B. bacteriovorus* was reduced by 6.02 logs from  $1.04 \times 10^6$  PFU/mL (at 0 min)  
250 to BDL ( $p < 0.0001$ ) within 120 min (Fig. A.8A). Similarly, the EMA-qPCR analysis indicated that the  
251 concentration of *B. bacteriovorus* was reduced by 2.59 logs ( $p < 0.0001$ ) after solar exposure from  
252 an initial concentration of  $2.14 \times 10^4$  GC/mL to  $5.49 \times 10^1$  GC/mL after 240 min (Fig. A.8B).

253 For the sample pre-treated with *B. bacteriovorus* and subsequently exposed to solar disinfection, the  
254 cell counts of *K. pneumoniae* were reduced by 7.38 logs from  $2.40 \times 10^7$  CFU/mL (at 0 min) to BDL  
255 ( $p < 0.0001$ ), after 240 min of solar exposure (Fig. 2A; Table 2). Thus, the cell counts of  
256 *K. pneumoniae* were also reduced by a total of 9.30 logs ( $p < 0.0001$ ) from the initial concentration  
257 of  $2.00 \times 10^9$  CFU/mL (Table 2). In addition, the EMA-qPCR analysis indicated that overall the

258 *K. pneumoniae* concentration was reduced by 5.41 logs from  $2.95 \times 10^8$  GC/mL (before predation)  
259 to  $1.14 \times 10^3$  GC/mL (after 240 min of solar exposure) ( $p < 0.0001$ ) (Fig. 2B; Table 2). The  
260 *B. bacteriovorus* was reduced by 6.02 logs from  $1.04 \times 10^6$  PFU/mL (at 0 min) to BDL ( $p < 0.0001$ )  
261 within 150 min in the samples exposed to solar disinfection (Fig. A.8A). Accordingly, the EMA-qPCR  
262 analysis indicated that the concentration of *B. bacteriovorus* was reduced by 2.28 logs ( $p < 0.0001$ )  
263 after solar exposure, from an initial concentration of  $2.14 \times 10^4$  GC/mL to  $1.12 \times 10^2$  GC/mL (Fig.  
264 A.8B).

265 In comparison, the cell counts of *K. pneumoniae* in the non-pre-treated sample exposed to solar  
266 photocatalytic treatment were reduced by a total of 6.34 logs after 240 min (from  $7.33 \times 10^8$  CFU/mL  
267 to  $3.33 \times 10^2$  CFU/mL) ( $p < 0.0001$ ) (Fig. 2A; Table 2), while the molecular analysis indicated that  
268 the GC of *K. pneumoniae* in this sample were reduced by a total of 2.67 logs [from  $6.41 \times 10^7$  GC/mL  
269 (initial concentration 0 min) to  $1.39 \times 10^5$  GC/mL (after 240 min of solar exposure)] ( $p < 0.0001$ ) (Fig.  
270 2B; Table 2). Furthermore, for the non-pre-treated sample exposed to only solar disinfection, the cell  
271 counts of *K. pneumoniae* were reduced by 8.87 logs from  $7.33 \times 10^8$  CFU/mL (at 0 min) to BDL within  
272 210 min ( $p < 0.0001$ ) (Fig. 2A; Table 2). The EMA-qPCR analysis confirmed a reduction in the  
273 concentration of the *K. pneumoniae* cells as the GC were reduced by 3.46 logs [from  
274  $6.41 \times 10^7$  GC/mL (initial concentration 0 min) to  $2.24 \times 10^4$  GC/mL (after 240 min of solar exposure)]  
275 ( $p < 0.0001$ ) in this sample (Table 2).

### 276 **3.2 Impacts of Different Disinfection Strategies on the Survival of *Enterococcus faecium***

277 For the *B. bacteriovorus* pre-treated samples, the culture-based enumeration indicated that the  
278 *E. faecium* cell counts were reduced by 0.598 logs from  $3.57 \times 10^9$  (before predation) to  
279  $9.00 \times 10^8$  CFU/mL (after 72 h of predation). The EMA-qPCR analysis then confirmed that the  
280 concentration of *E. faecium* was reduced after 72 h of predation from  $8.24 \times 10^5$  GC/mL (before  
281 predation) to  $1.60 \times 10^5$  GC/mL with a log reduction of 0.712 recorded. While, *B. bacteriovorus* did  
282 not produce any plaques on the double-layer agar overlays when *E. faecium* was utilised as prey,  
283 the EMA-qPCR analysis indicated that the concentration of *B. bacteriovorus* decreased by 0.167  
284 logs from  $1.08 \times 10^4$  (before predation) to  $7.34 \times 10^3$  GC/mL (after 72 h of predation). Overall, for

285 the dark controls, the plate counts indicated that the concentration of *E. faecium* remained relatively  
286 constant with an average of  $4.17 \times 10^8$  CFU/mL and  $1.63 \times 10^8$  CFU/mL recorded (after 240 min) for  
287 the 72-h *B. bacteriovorus* pre-treated sample and non-pre-treated sample, respectively.

288 Subsequently, the cell counts of *E. faecium* recorded for the pre-treated sample exposed to solar  
289 photocatalysis were reduced by 3.81 logs from  $9.00 \times 10^8$  CFU/mL (at 0 min) to  $1.40 \times 10^5$  CFU/mL  
290 (at 240 min) ( $p < 0.0001$ ) (Fig. 3A). Thus, the CFU/mL of *E. faecium* was reduced by a total of  
291 4.41 logs from an initial concentration of  $3.57 \times 10^9$  CFU/mL (Table 2). The EMA-qPCR analysis  
292 then indicated that the *E. faecium* concentration was reduced by 1.57 logs from  $1.60 \times 10^5$  GC/mL  
293 (at 0 min) to  $4.35 \times 10^3$  GC/mL (at 240 min) (Fig. 3B), with an overall reduction of 2.28 logs recorded  
294 from an initial concentration of  $8.24 \times 10^5$  GC/mL (Table 2). As mentioned previously,  
295 *B. bacteriovorus* did not produce any plaques on the double-layer agar overlays when *E. faecium*  
296 was utilised as prey and the EMA-qPCR analysis indicated that the concentration of *B. bacteriovorus*  
297 was reduced from an initial concentration of  $7.34 \times 10^3$  GC/mL (at 0 min) to  $8.13 \times 10^2$  GC/mL (0.956  
298 log reduction;  $p < 0.0001$ ) after 240 min of solar photocatalysis (Fig. A.9).

299 The cell counts of *E. faecium* recorded for the pre-treated sample exposed to solar disinfection, were  
300 reduced by 6.73 logs from  $9.00 \times 10^8$  CFU/mL (at 0 min) to  $1.67 \times 10^2$  CFU/mL (at 240 min)  
301 ( $p < 0.0001$ ) (Fig. 3A; Table 2). Therefore, an overall log reduction of 7.33 in *E. faecium* CFU/mL  
302 was recorded after the *B. bacteriovorus* pre-treatment and solar disinfection (Table 2). The EMA-  
303 qPCR analysis indicated that the concentration of *E. faecium* was reduced by 2.09 logs from  
304  $1.60 \times 10^5$  GC/mL (at 0 min) to  $1.29 \times 10^3$  GC/mL (at 240 min) ( $p < 0.0001$ ) (Fig. 3B). Overall, the  
305 concentration of *E. faecium* was thus reduced by a total of 2.81 logs in the pre-treated sample  
306 exposed to solar disinfection, from an initial concentration of  $8.24 \times 10^5$  GC/mL (Table 2). For the  
307 predatory bacteria, the EMA-qPCR analysis indicated that the concentration of *B. bacteriovorus* was  
308 reduced from an initial concentration of  $7.34 \times 10^3$  GC/mL (at 0 min) to  $8.95 \times 10^2$  GC/mL (0.914 log  
309 reduction;  $p < 0.0001$ ) after 240 min of solar exposure (Fig. A.9).

310 For the samples which were not pre-treated with *B. bacteriovorus* but exposed to solar disinfection  
311 and solar photocatalytic treatment, the culture-based enumeration of *E. faecium* indicated that for

312 both treatment methods, the cell counts were reduced by 8.00 logs from an initial concentration of  
313  $1.00 \times 10^8$  CFU/mL to BDL ( $<1$  CFU/100  $\mu$ L) within 210 min of solar exposure ( $p < 0.0001$ ) (Fig. 3A;  
314 Table 2). The EMA-qPCR analysis then indicated that during solar disinfection the concentration of  
315 *E. faecium* was reduced by 3.39 logs from  $2.09 \times 10^6$  GC/mL (at 0 min) to  $8.53 \times 10^2$  GC/mL (at 240  
316 min) ( $p < 0.0001$ ) (Fig. 3B; Table 2). Similarly, for the sample exposed to solar photocatalytic  
317 treatment, the concentration of *E. faecium* was reduced by 3.38 logs from  $2.09 \times 10^6$  GC/mL (at  
318 0 min) to  $8.74 \times 10^2$  GC/mL (at 240 min) ( $p < 0.0001$ ) (Fig. 3B; Table 2).

#### 319 **4. Discussion**

320 While disinfection methods are effective in significantly reducing the concentration of microbial  
321 contaminants in water sources, various pathogens and opportunistic pathogens employ survival  
322 strategies and persist after treatment (Strauss et al., 2018; Clements et al., 2019). It was thus  
323 proposed in the current study that a combination of physical, chemical and biological treatments,  
324 could prove effective in eliminating disinfection resistant species. *Bdellovibrio bacteriovorus* is known  
325 to attach to the cell wall of Gram-negative prey, such as *K. pneumoniae*, through an unknown  
326 mechanism or receptor, whereafter the predator rotates to create a pore in the prey cell wall and  
327 enters the prey cell's periplasmic space forming a structure called the bdelloplast (Sockett, 2009).  
328 Once the predator has invaded the prey cell, it secretes various hydrolytic enzymes to break down  
329 the prey cell's constituents and produce progeny (Sockett, 2009). Correspondingly, as  
330 *K. pneumoniae* is sensitive to predation, the pre-treatment with *B. bacteriovorus* aided in effectively  
331 reducing the concentration of this organism in the seeded water samples. Furthermore, the addition  
332 of the photocatalytic material enhanced the disinfection efficiency as the treatment time required to  
333 reduce the *K. pneumoniae* CFU to BDL was decreased from 240 min (solar disinfection) to 120 min  
334 (solar photocatalysis). Under solar UV-visible exposure, the TiO<sub>2</sub>-rGO composite photocatalytic  
335 material produces reactive oxygen species (ROS), which significantly disrupts the cell membrane  
336 structures and damages DNA and RNA, ultimately leading to cell death (Byrne et al., 2011).

337 We previously investigated the mechanisms behind the antimicrobial activity of TiO<sub>2</sub>-rGO in water  
338 using *E. coli* as the model organism (Fernández-Ibáñez et al., 2015; Cruz-Ortiz et al., 2017).

339 Fernández-Ibáñez et al. (2015) reported that *E. coli* was reduced by 6 logs (within 10 min, less than  
340 2 J/cm<sup>2</sup>) under natural sunlight with a photocatalyst loading of 500 mg/L. Probes were used to  
341 investigate the primary ROS produced during the disinfection experiments and we found that under  
342 UV-visible light, hydrogen peroxide, hydroxyl radicals and singlet oxygen were mainly responsible  
343 for the reduction in *E. coli* concentrations. Under visible light irradiation, only singlet oxygen was  
344 produced which resulted in the reduction of the *E. coli* concentration (Fernández-Ibáñez et al., 2015;  
345 Cruz-Ortiz et al., 2017). Lin et al. (2014) investigated the cytotoxic effects of UV excited TiO<sub>2</sub> on  
346 Gram-negative bacteria by also employing *E. coli* as the test organism. With the use of transmission  
347 electron microscopy, the authors found that the TiO<sub>2</sub> nanoparticles attached to the outside of the  
348 *E. coli* cells, while some microbial cells were also observed to contain internalised nanoparticles. It  
349 was concluded that the nanoparticles attached to the cell surface, induced cell distortion, plasmolysis  
350 and extensive cell wall and membrane damage. In addition, the authors hypothesised that the  
351 attachment of the nanoparticles to the cells resulted in decreased movement of substances into and  
352 out of the bacterial cells, ultimately resulting in homeostatic imbalances and cellular metabolic  
353 disturbances, which would eventually result in cell death (Lin et al., 2014).

354 However, while Lin et al. (2014) evaluated the photocatalyst in suspension, in the current study, the  
355 photocatalyst was immobilised onto glass raschig rings and exposed to real solar irradiation. Sordo  
356 et al. (2010) compared the use of TiO<sub>2</sub> in suspension to TiO<sub>2</sub> immobilised onto a glass tube (used  
357 as the reactor vessel) and raschig rings for the disinfection of *E. coli* in a recirculating solar treatment  
358 system. The authors found that the disinfection of *E. coli* in the reactor with the TiO<sub>2</sub> coated raschig  
359 rings, was comparable to the disinfection obtained in the reactor with TiO<sub>2</sub> in suspension, while  
360 disinfection efficiency was not enhanced in the glass tube reactor vessel coated with the  
361 photocatalyst. It was hypothesised that the high disinfection efficiency obtained with the raschig ring  
362 immobilised photocatalyst was due to the greater contact area generated, which increased exposure  
363 of the *E. coli* cells to hydroxyl radicals produced during the photocatalytic process. However, the  
364 authors also noted that the flow rate generated in the recirculating system containing the raschig  
365 rings, greatly enhanced the disinfection efficiency of the reactor as strong mechanical stress was  
366 exerted on the bacterial cells (Sordo et al., 2010). The use of raschig rings as support materials for

367 the immobilisation of photocatalysts is thus advantageous as post-treatment removal of the material  
368 is not required. In addition, immobilising the photocatalyst creates a greater contact area which may  
369 increase the exposure of the cells to the photocatalytic material. Furthermore, if a flow rate is applied,  
370 mechanical stress is exerted on the cells.

371 Apart from using photocatalytic material in two of the solar-CPC reactors in the current study, all the  
372 water samples (pre-treated with *B. bacteriovorus* and non-pre-treated) were exposed to solar  
373 treatment under CPC concentrated solar UV-A radiation. The CPC mirrors were used for the solar  
374 treatment reactors as it significantly enhances any kind of solar water treatment by improving the  
375 solar UV energy income by a concentration factor of 1 (Keane et al., 2014). Navntoft et al. (2008)  
376 demonstrated that the use of a CPC accelerated the reduction of 6-log *E. coli* K12 under solar  
377 disinfection by 90 minutes as compared to a PET plastic bottle. Based on the solar UV-A dose  
378 calculated for the *K. pneumoniae* trials (Supplementary Information), a similar UV-A dose was  
379 obtained within 120 min of solar exposure ( $25.83 \text{ J cm}^{-2}$ ), to the dose reported in literature ( $27 \text{ J/cm}^2$ )  
380 to achieve a 5-log reduction in *E. coli* K12 by solar disinfection in a 2 L-PET bottle filled with clear  
381 transparent water (Castro-Alf3rez et al., 2018). Additionally, the dose obtained in the current study  
382 was 10 times higher than the  $1.8 \text{ J/cm}^2$  (10 min at  $30 \text{ W/m}^2$  of solar UV-A) required to achieve a 6-  
383 log reduction of *E. coli* K 12 using the same catalyst ( $\text{TiO}_2\text{-rGO}$ ) suspended as a slurry at a  
384 concentration of 500 mg/L (Fern3andez-Ib3a3ñez et al., 2015). Similar solar dose values were obtained  
385 for the *E. faecium* trial. Thus, sufficient solar irradiation was obtained to reduce the concentration of  
386 *K. pneumoniae* and *E. faecium* during the current study.

387 Correspondingly, the most efficient treatment strategy for the reduction of *E. faecium* was the use of  
388 solar disinfection or solar photocatalytic disinfection without *B. bacteriovorus* pre-treatment. While it  
389 is generally theorised that *B. bacteriovorus* does not prey on Gram-positive bacteria, studies have  
390 indicated that this predator can prey on *Staphylococcus aureus* (Iebba et al., 2014; Pantanella et al.,  
391 2018). The lytic enzymes produced by *B. bacteriovorus* have also been shown to disrupt biofilms  
392 produced by Gram-positive bacteria, while proteases produced by *B. bacteriovorus* can decrease  
393 the efficiency of *S. aureus* invasion into human epithelial cells (Monnappa et al., 2014). Furthermore,  
394 using culture-based methods and EMA-qPCR, we have recently reported that *B. bacteriovorus* PF13

395 can reduce the concentration of *S. aureus* and *E. faecium* in co-culture experiments (Waso et al.,  
396 2019). Thus, while it is warranted to investigate the effect of *B. bacteriovorus* pre-treatment on the  
397 disinfection of Gram-positive bacteria, in this study pre-treatment with *B. bacteriovorus* PF13 did not  
398 significantly reduce the concentration of *E. faecium*.

399 Based on the results obtained for the *E. faecium* trials, the addition of the photocatalyst also did not  
400 significantly enhance the disinfection efficiency. Gutiérrez-Alfaro et al. (2015) compared three  
401 systems to disinfect potable water inoculated with wastewater containing *E. coli*, *Enterococcus* spp.  
402 and *Clostridium perfringens*: a 2 L PET bottle; a 2 L PET bottle with an internal cylinder coated with  
403 TiO<sub>2</sub> doped with zinc; and a glass reactor (9 L) with a TiO<sub>2</sub> coated inner cylinder. In all the systems  
404 analysed, *E. coli* was readily reduced to BDL, while *Enterococcus* spp. and *C. perfringens* were more  
405 resistant to disinfection. In addition, the immobilised photocatalyst used in the 2 L PET bottles only  
406 enhanced the disinfection efficiency of the SODIS bottles by 0.43 logs for *E. coli*, 0.45 logs for  
407 *Enterococcus* spp. and 0.28 logs for *C. perfringens* under natural sunlight (Gutiérrez-Alfaro et al.,  
408 2015). The authors ultimately concluded that Gram-positive bacteria, which have more complex cell  
409 walls, are more resistant to disinfection in comparison to Gram-negative bacteria. However, they  
410 found that recirculating the water in the solar photocatalytic systems, increased turbulence and  
411 contact between the catalyst and the bacteria, significantly enhancing the disinfection efficiency,  
412 especially for Gram-positive bacteria (Gutiérrez-Alfaro et al., 2015). Veneiri et al. (2014) also  
413 investigated the disinfection of *Enterococcus faecalis* using TiO<sub>2</sub> P25 (200 mg/L to 1500 mg/L) and  
414 SODIS under simulated sunlight, using culture-based methods and qPCR. The culturing results  
415 indicated that at the highest TiO<sub>2</sub> concentration (1500 mg/L), *E. faecalis* was reduced by 7 logs to  
416 BDL after approximately 40 min of treatment. Similarly, while qPCR analysis indicated that a 7-log  
417 reduction in the GC of *E. faecalis* was obtained after 120 min of treatment, the GC were not reduced  
418 to BDL in any of the treated samples. The authors concluded that viable but non-culturable (VBNC)  
419 *E. faecalis* cells were still present in the treated samples and that the SODIS treatment time should  
420 be extended in order to eradicate *E. faecalis* (Veneiri et al., 2014).

421 Similarly, in the current study, for all the treatment combinations analysed, EMA-qPCR results  
422 indicated that the GC of *K. pneumoniae* and *E. faecium* were not reduced to BDL, signifying that



423 VBNC cells may still have persisted. While numerous research groups have detected *Klebsiella* spp.  
424 in untreated harvested rainwater (De Kwaadsteniet et al., 2013), the *K. pneumoniae* strain (S1 43)  
425 employed in the current study was isolated from SOPAS rainwater at a treatment temperature above  
426 70°C (Clements et al., 2019). The thermal tolerance of *Klebsiella* spp. has been associated with the  
427 expression of heat shock proteins or can be acquired through plasmids encoding for ClpK ATPase  
428 (Bojer et al., 2011). Moreover, *K. pneumoniae* have prominent capsules which have been  
429 hypothesised to protect this organism from bactericidal stressors such as UV irradiation and  
430 antibiotic agents (Veneiri et al., 2017; Dorman et al., 2018). In contrast, the *E. faecium* strain (8D)  
431 employed was isolated from untreated harvested rainwater (Dobrowsky et al., 2014).  
432 *Enterococcus* spp. are known to tolerate a wide range of environmental conditions and they have  
433 been found to exhibit increased resistance to UV disinfection (McKinney & Pruden, 2012; Maraccini  
434 et al., 2012). Some strains of enterococci have been found to possess intracellular carotenoids which  
435 may act as quenchers of intracellularly produced ROS upon exposure to sunlight, ultimately  
436 protecting the cell from increasing oxidative stress and providing *Enterococcus* spp. with a  
437 competitive advantage against sunlight-induced inactivation (Maraccini et al., 2012). Gram-negative  
438 and Gram-positive bacteria also possess DNA repair mechanisms, which can repair damage  
439 induced by UV irradiation, and allow bacterial cells to persist and survive after UV disinfection  
440 (McGuigan et al., 2012). Thus, while the molecular analysis results obtained in the current study  
441 indicated that significant reductions ( $p < 0.0001$ ) in GC were recorded (Fig. 2B and 3B), further work  
442 may include extending the solar disinfection and solar photocatalytic treatment time.

## 443 5. Conclusions

444 Based on the results obtained, *B. bacteriovorus* may be applied to decrease the concentration of  
445 Gram-negative bacteria, such as *K. pneumoniae*, prior to solar disinfection. This is crucial as many  
446 pathogenic Gram-negative bacteria have been found to persist after the implementation of various  
447 disinfection strategies. Solar disinfection or solar photocatalytic treatment successfully reduced the  
448 concentration of *E. faecium* and it is likely that forced convection in a solar photocatalytic system  
449 may further enhance the effect of the photocatalytic material on the disinfection of Gram-positive  
450 bacteria.

451 Furthermore, as hydroxyl radicals produced during photocatalysis significantly disrupts the cell  
452 membrane of bacteria (Polo-López et al., 2017), the use of EMA-qPCR is recommended to  
453 supplement culture-based analysis and should therefore be included in future studies monitoring  
454 such water treatment systems. As natural water sources will contain mixed bacterial communities,  
455 future research should investigate the effect of predatory bacteria pre-treatment on mixed bacterial  
456 communities in natural water sources, to assess the overall effect of *B. bacteriovorus* pre-treatment.

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476 **Declaration of Interest**

477 The authors declare no conflict of interest.

478 **References**

- 479 Adán, C., Magnet, A., Fenot, S., Pablos, C., Del Águila, C., Marugán, J., 2018. Concomitant  
480 inactivation of *Acanthamoeba* spp. and *Escherichia coli* using suspended and immobilized TiO<sub>2</sub>.  
481 Water Research 144, 512–521.
- 482 Bojer, M. S., Krogfelt, K. A., Struve, C., 2011. The newly discovered ClpK protein strongly promotes  
483 survival of *Klebsiella pneumoniae* biofilm subjected to heat shock. Journal of Medical Microbiology  
484 60(10), 1559-1561.
- 485 Brisse, S., Verhoef, J., 2001. Phylogenetic diversity of *Klebsiella pneumoniae* and *Klebsiella oxytoca*  
486 clinical isolates revealed by randomly amplified polymorphic DNA, *gyrA* and *parC* gene sequencing  
487 and automated ribotyping. International Journal of Systematic and Evolutionary Microbiology 51,  
488 915-924.
- 489 Byrne, J.A., Fernández-Ibáñez, P.A., Dunlop, P.S., Alrousan, D.M., Hamilton, J.W., Abdel-Mottaleb,  
490 M.S., 2011. Photocatalytic enhancement for solar disinfection of water: A review. International  
491 Journal of Photoenergy 2011, 1-12.
- 492 Castro-Alfárez, M., Polo-López, M.I., Marugán, J., Fernández-Ibáñez, P., 2018. Validation of a solar-  
493 thermal water disinfection model for *Escherichia coli* inactivation in pilot scale solar reactors and real  
494 conditions. Chemical Engineering Journal 331, 831-840.
- 495 Chu, W.H., Zhu, W., 2010. Isolation of *Bdellovibrio* as biological therapeutic agents used for the  
496 treatment of *Aeromonas hydrophila* infection in fish. Zoonoses and Public Health 57(4), 258–264.
- 497 Clements, T.L., Reyneke, B., Strauss, A., Khan, W., 2019. Persistence of viable bacteria in solar  
498 pasteurised harvested rainwater. Water Air Soil Pollution 230(130), 1–13.
- 499 Cruz-Ortiz, B.R., Hamilton, J., Pablos, C., Díaz-Jiménez, L., Cortés-Hernández, D.A., Sharma, P.K.,  
500 Castro-Alfárez, M., Fernández-Ibáñez, P., Dunlop, P.S.M., Byrne, J.A., 2017. Mechanism of

501 photocatalytic disinfection using titania-graphene composites under UV and visible irradiation.  
502 Chemical Engineering Journal 316, 179–186.

503 Cunha, D.L., Kuznetsov, A., Achete, C.A., Da Hora Machado, A.E., Marques, M., 2018. Immobilized  
504 TiO<sub>2</sub> on glass spheres applied to heterogeneous photocatalysis: Photoactivity, leaching and  
505 regeneration process. PeerJ Environmental Science 6, e4464.

506 Dashiff, A., Junka, R.A., Libera, M., Kadouri, D.E., 2011. Predation of human pathogens by the  
507 predatory bacteria *Micavibrio aeruginosavorus* and *Bdellovibrio bacteriovorus*. Journal of Applied  
508 Microbiology 110(2), 431–444.

509 De Kwaadsteniet, M., Dobrowsky, P.H., Van Deventer, A., Khan, W., Cloete, T.E., 2013. Domestic  
510 rainwater harvesting: Microbial and chemical water quality and point-of-use treatment systems.  
511 Water Air Soil Pollution 224(7), 1-19.

512 Dobrowsky, P.H., De Kwaadsteniet, M., Cloete, T.E., Khan, W., 2014. Distribution of indigenous  
513 bacterial pathogens and potential pathogens associated with roof-harvested rainwater. Applied and  
514 Environmental Microbiology 80 (7), 2307-2316.

515 Dobrowsky, P.H., Lombard, M., Cloete, W.J., Saayman, M., Cloete, T.E., Carstens, M., Khan, S.,  
516 Khan, W., 2015. Efficiency of microfiltration systems for the removal of bacterial and viral  
517 contaminants from surface and rainwater. Water Air Soil Pollution 226, 1-14.

518 Dorman, M.J., Feltwell, T., Goulding, D.A., Parkhill, J., Short, F.L., 2018. The capsule regulatory  
519 network of *Klebsiella pneumoniae* defined by density-TraDISort. Molecular Biology and Physiology  
520 9(6), 1-19.

521 Feng, S., Tan, C.H., Cohen, Y., Rice, S.A., 2016. Isolation of *Bdellovibrio bacteriovorus* from a  
522 tropical wastewater treatment plant and predation of mixed species biofilms assembled by the native  
523 community members. Environmental Microbiology 18(11), 3923–3931.

524 Fernández-Ibáñez, P., Polo-López, M.I., Malato, S., Wadhwa, S., Hamilton, J.W.J., Dunlop, P.S.M.,  
525 D'Sa, R., Magee, E., O'Shea, K., Dionysiou, D.D., Byrne, J.A., 2015. Solar photocatalytic disinfection  
526 of water using titanium dioxide graphene composites. Chemical Engineering Journal 261, 36-44.

527 Frahm, E., Obst, U., 2003. Application of the fluorogenic probe technique (TaqMan PCR) to the  
528 detection of *Enterococcus* spp. and *Escherichia coli* in water samples. Journal of Microbiological  
529 Methods 52(1), 123-131.

530 Gutiérrez-Alfaro, S., Acevedo, A., Rodríguez, J., Carpio, E.A., Manzano, M.A., 2015. Solar  
531 photocatalytic water disinfection of *Escherichia coli*, *Enterococcus* spp. and *Clostridium perfringens*  
532 using different low-cost devices. Journal of Chemical Technology and Biotechnology 91(7), 2026–  
533 2037.

534 Helali, S., Polo-López, M.I., Fernández-Ibáñez, P., Ohtanu, B., Amano, F., Malato, S., Guillard, C.,  
535 2014. Solar photocatalysis: A green technology for *E. coli* contaminated water disinfection. Effect of  
536 concentration and different types of suspended catalyst. Journal of Photochemistry and  
537 Photobiology A: Chemistry 276, 31-40.

538 Iebba, V., Totino, V., Santangelo, F., Gagliardi, A., Ciotoli, L., Virga, A., Ambrosi, C., Pompili, M., De  
539 Biase, R.V., Selan, L., Artini, M., Pantanella, F., Mura, F., Passariello, C., Nicoletti, M., Nencioni, L.,  
540 Trancassini, M., Quattrucci, S., Schippa, S., 2014. *Bdellovibrio bacteriovorus* directly attacks  
541 *Pseudomonas aeruginosa* and *Staphylococcus aureus* cystic fibrosis isolates. Frontiers in  
542 Microbiology 5(280), 1–9.

543 Jones, D.L., Edwards, A.C., 1993. Evaluation of polysulfone hollow fibres and ceramic suction  
544 samplers as devices for the *in situ* extraction of soil solution. Plant and Soil 150(2), 157–165.

545 Kadouri, D.E., To, K., Shanks, R.M.Q., Doi, Y., 2013. Predatory bacteria: a potential ally against  
546 multi-drug resistant Gram-negative pathogens. PLOS One 8 (5), e63397.

547 Keane, D.A., McGuigan, K.G., Fernández-Ibáñez, P., Polo-López, M.I., Byrne, J.A., Dunlop, P.S.M,  
548 O'Shea, K., Dionysiou, D.D., Pillai, S.C., 2014. Solar photocatalysis for water disinfection: Materials  
549 and reactor design. Catalysis Science & Technology 4, 1211-1226.

550 Lin, X., Li, J., Ma, S., Liu, G., Yang, K., Tong, M., Lin, D., 2014. Toxicity of TiO<sub>2</sub> nanoparticles to  
551 *Escherichia coli*: Effects of particle size, crystal phase and water chemistry. PLOS One 9 (10),  
552 e110247.

553 Makwana, N.M., Hazael, R., McMillan, P.F., Darr, J.A., 2015. Photocatalytic water disinfection by  
554 simple and low-cost monolithic and heterojunction ceramic wafers. *Photochemical and*  
555 *Photobiological Sciences* 14, 1190-1196.

556 Maraccini, P.A., Ferguson, D.M., Boehm, A.B., 2012. Diurnal variation in *Enterococcus* species  
557 composition in polluted ocean water and a potential role for the enterococcal carotenoid in protection  
558 against photoinactivation. *Applied and Environmental Microbiology* 78, 305–310.

559 McGuigan, K.G., Conroy, R.M., Mosler, H., Du Preez, M., Ubomba-Jaswa, E., Fernández-Ibáñez,  
560 P., 2012. Solar water disinfection (SODIS): A review from bench-top to roof-top. *Journal of*  
561 *Hazardous Materials* 235-236, 29–46.

562 McKinney, C.W., Pruden, A., 2012. Ultraviolet disinfection of antibiotic resistant bacteria and their  
563 antibiotic resistance genes in water and wastewater. *Environmental Science and Technology* 46(24),  
564 13393-13400.

565 Monnappa, A.K., Dwidar, M., Seo, J.K., Hur, J., Mitchel, R.J., 2014. *Bdellovibrio bacteriovorus*  
566 inhibits *Staphylococcus aureus* biofilm formation and invasion into human epithelial cells. *Nature*  
567 *Scientific Reports* 4 (3811), 1-8.

568 Nalwanga, R., Muyanja, C.K., McGuigan, K.G., Quilty, B., 2018. A study of the bacteriological quality  
569 of roof-harvested rainwater and an evaluation of SODIS as a suitable treatment technology in rural  
570 Sub-Saharan Africa. *Journal of Environmental Chemical Engineering* 6(3), 3648–3655.

571 Navntoft, C., Ubomba-Jaswa, E., McGuigan, K.G., Fernández-Ibáñez, P., 2008. Effectiveness of  
572 solar disinfection using batch reactors with non-imaging aluminium reflectors under real conditions:  
573 Natural well water and solar light. *Journal of Photochemistry and Photobiology B*: 93, 155-161.

574 Özkan, M., Yilmaz, H., Çelik, M.A., Şengezer, Ç., Erhan, E., 2018. Application of *Bdellovibrio*  
575 *bacteriovorus* for reducing fouling of membranes used for wastewater treatment. *Turkish Journal of*  
576 *Biochemistry* 43, 296-305.

577 Pantanella, F., Iebba, V., Mura, F., Dini, L., Totino, V., Neroni, B., Bonfiglio, G., Maria, T., Passariello,  
578 C., Schippa, S., 2018. Behaviour of *Bdellovibrio bacteriovorus* in the presence of Gram-positive  
579 *Staphylococcus aureus*. *The New Microbiologica* 41(2),145–152.

580 Polo-López, M.I., Castro-Alfárez, M., Nahim-Granados, S., Malato, S., Fernández-Ibáñez, P., 2017.  
581 *Legionella jordanis* inactivation in water by solar driven processes: EMA-qPCR versus culture-based  
582 analyses for new mechanistic insights. *Catalysis Today* 287, 15–21.

583 Rajal, V., McSwain, B., Thompson, D., Leutenegger, C., Wuertz, S., 2007. Molecular quantitative  
584 analysis of human viruses in California stormwater. *Water Research* 41(19), 4287–4298.

585 Reyneke, B., Dobrowsky, P.H., Ndlovu, T., Khan, S., Khan, W., 2016. EMA-qPCR to monitor the  
586 efficiency of a closed-coupled solar pasteurization system in reducing *Legionella* contamination of  
587 roof-harvested rainwater. *Science of the Total Environment* 553, 662–670.

588 Sockett, R.E., 2009. Predatory Lifestyle of *Bdellovibrio bacteriovorus*. *Annual Review of Microbiology*  
589 63, 523-539.

590 Sordo, C., Van Grieken, R., Marugán, J., Fernández-Ibáñez, P., 2010. Solar photocatalytic  
591 disinfection with immobilised TiO<sub>2</sub> at pilot-plant scale. *Water Science and Technology* 61(2), 507–  
592 512.

593 Strauss, A., Reyneke, B., Waso, M., Ndlovu, T., Khan, S., Khan, W. 2018. Compound parabolic  
594 collector solar disinfection for the treatment of harvested rainwater. *Environmental Science: Water*  
595 *Research & Technology* 4, 976 – 991.

596 Ubomba-Jaswa, E., Fernández-Ibáñez, P., Navntoft, C., Polo-López, M.I., McGuigan, K.G., 2010.  
597 Investigating the microbial inactivation efficiency of a 25 L batch solar disinfection (SODIS) reactor  
598 enhanced with a compound parabolic collector (CPC) for household use. *Journal of Chemical*  
599 *Technology and Biotechnology* 85(8), 1028-1037.

600 Van Essche, M., Sliepen, I., Loozen, G., Van Eldere, J., Quiryneen, M., Davidov, Y., Jurkevitch, E.,  
601 Boon, N., Teughels, W., 2009. Development and performance of quantitative PCR for the  
602 enumeration of *Bdellovibrionaceae*. *Environmental Microbiology Reports* 1(4), 228–233.

603 Venieri, D., Fraggadaki, A., Kostadima, M., Chatzisyneon, E., Binas, V., Zachopoulos, A., Kiriakidis,  
604 G., Mantzavinos, D., 2014. Solar light and metal-doped TiO<sub>2</sub> to eliminate water-transmitted bacterial  
605 pathogens: Photocatalyst characterization and disinfection performance. *Applied Catalysis B:  
606 Environmental* 154-155, 93–101.

607 Venieri, D., Gounaki, I., Bikouvaraki, M., Binas, V., Zachopoulos, A., Kiriakidis, G., Mantzavinos, D.,  
608 2017. Solar photocatalysis as disinfection technique: Inactivation of *Klebsiella pneumoniae* in  
609 sewage and investigation of changes in antibiotic resistance profile. *Journal of Environmental  
610 Management* 195(2), 140–147.

611 Waso, M., Khan, S., Khan, W., 2018. Microbial source tracking markers associated with domestic  
612 rainwater harvesting systems: Correlation to indicator organisms. *Environmental Research* 161,  
613 446–455.

614 Waso, M., Khan, S., Khan, W., 2019. Assessment of predatory bacteria and prey interactions using  
615 culture-based methods and EMA-qPCR. *Microbiological Research* 228, 1 – 10.

616 Willis, A.R., Moore, C., Mazon-Moya, M., Krokowski, S., Lambert, C., Till, R., Mostowy, S., Sockett,  
617 R.E., 2016. Injections of predatory bacteria work alongside host immune cells to treat *Shigella*  
618 infection in zebrafish larvae. *Current Biology* 26, 3343-3351.

619 Yu, R., Zhang, S., Chen, Z., Li, C., 2017. Isolation and application of predatory *Bdellovibrio*-and-like  
620 organisms for municipal waste sludge biolysis and dewaterability enhancement. *Frontiers of  
621 Environmental Science & Engineering* 11(10), 1-11.