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

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

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4 Waso, M.¹, Khan, S.², Singh, A.³, McMichael, S.³, Ahmed, W.⁴, Fernández-Ibáñez, P.³, Byrne, J.A.³
5 and Khan, W.^{1*}

6

7 ¹ Department of Microbiology, Faculty of Science, Stellenbosch University, Private Bag X1,
8 Stellenbosch, 7602, South Africa.

9 ² Faculty of Health Sciences, University of Johannesburg, PO Box 17011, Doornfontein, 2028, South
10 Africa.

11 ³ Nanotechnology and Integrated BioEngineering Centre, Ulster University, Jordanstown Campus,
12 Shore Road, Newtownabbey, Belfast, BT37 0QB, Northern Ireland.

13 ⁴ CSIRO Land and Water, Ecosciences Precinct, 41 Boggo Road, Queensland, 4102, Australia.

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22 *Address Correspondence to W. Khan; Email: wesaal@sun.ac.za; Tel: +27 21 808 5804; Fax: +27
23 21 808 5846

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 2.00×10^9 colony forming units (CFU)/mL to below the detection limit (BDL) (<1 CFU/100 μ 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 1.00×10^8 CFU/mL to BDL) and the gene copies were reduced by ~ 3.39 logs (from
35 2.09×10^6 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/m², 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., TiO₂ P25, TiO₂ PC500, TiO₂ Ruana and
83 Russelite (Bi₂WO₆)]. 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 TiO₂ P25, which was the most effective photocatalytic material. We have
86 also previously reported enhanced solar disinfection utilising TiO₂-reduced graphene oxide
87 composites (TiO₂-rGO) (Fernández-Ibáñez et al., 2015; Cruz-Ortiz et al., 2017). Adán et al. (2018)
88 then showed that TiO₂ 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 TiO₂-rGO
116 composite was synthesised using graphene oxide (GO; Nanoinnova, Spain) and TiO₂ P25 (Aeroxide
117 P25, Evonik, Germany) as previously described by Fernández-Ibáñez et al. (2015). The TiO₂-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 TiO₂-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 TiO₂-rGO was ca. 0.89 mg/cm².

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 (OD₆₀₀). The concentration of the bacterial cells was adjusted with PBS to obtain a final OD₆₀₀
139 of 1.00 (which corresponded to approximately 10⁹ 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 TiO₂-
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* (OD₆₀₀ = 1.00) (section 2.2.1). Subsequently, each
160 sample was inoculated with 100 mL of the *B. bacteriovorus* stock lysate (OD₆₀₀ < 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 (OD₆₀₀ = 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 TiO₂-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 10⁻⁶) for each sample ($n = 40$), and 100 μ L 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 μ L 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*[™] Fecal/Soil Microbe Miniprep kit (Zymo
203 Research, Inqaba Biotech, South Africa) as per the manufacturer’s instructions.

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

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

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

2.3 Data Analysis

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

3. Results

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

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

230 of *B. bacteriovorus* correspondingly increased by 0.202 logs from 6.53×10^5 PFU/mL (before
231 predation) to 1.04×10^6 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 2.95×10^8 (before predation) to
234 9.20×10^4 GC/mL, while the concentration of *B. bacteriovorus* increased by 0.430 logs from
235 7.96×10^3 (before predation) to 2.14×10^4 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 1.97×10^7 CFU/mL and 7.50×10^8 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 2.40×10^7 CFU/mL (at 0 min) to BDL (<1 CFU/100 μ 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 2.00×10^9 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 2.95×10^8 GC/mL (before predation) to 4.19×10^2 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 1.04×10^6 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 2.14×10^4 GC/mL to 5.49×10^1 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 2.40×10^7 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 2.00×10^9 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 2.95×10^8 GC/mL (before predation)
259 to 1.14×10^3 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 1.04×10^6 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 2.14×10^4 GC/mL to 1.12×10^2 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 7.33×10^8 CFU/mL
267 to 3.33×10^2 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 6.41×10^7 GC/mL
269 (initial concentration 0 min) to 1.39×10^5 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 7.33×10^8 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 6.41×10^7 GC/mL (initial concentration 0 min) to 2.24×10^4 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 3.57×10^9 (before predation) to
279 9.00×10^8 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 8.24×10^5 GC/mL (before
281 predation) to 1.60×10^5 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 1.08×10^4 (before predation) to 7.34×10^3 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 4.17×10^8 CFU/mL and 1.63×10^8 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 9.00×10^8 CFU/mL (at 0 min) to 1.40×10^5 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 3.57×10^9 CFU/mL (Table 2). The EMA-qPCR analysis
292 then indicated that the *E. faecium* concentration was reduced by 1.57 logs from 1.60×10^5 GC/mL
293 (at 0 min) to 4.35×10^3 GC/mL (at 240 min) (Fig. 3B), with an overall reduction of 2.28 logs recorded
294 from an initial concentration of 8.24×10^5 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 7.34×10^3 GC/mL (at 0 min) to 8.13×10^2 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 9.00×10^8 CFU/mL (at 0 min) to 1.67×10^2 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 1.60×10^5 GC/mL (at 0 min) to 1.29×10^3 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 8.24×10^5 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 7.34×10^3 GC/mL (at 0 min) to 8.95×10^2 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

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

4. Discussion

While disinfection methods are effective in significantly reducing the concentration of microbial contaminants in water sources, various pathogens and opportunistic pathogens employ survival strategies and persist after treatment (Strauss et al., 2018; Clements et al., 2019). It was thus proposed in the current study that a combination of physical, chemical and biological treatments, could prove effective in eliminating disinfection resistant species. *Bdellovibrio bacteriovorus* is known to attach to the cell wall of Gram-negative prey, such as *K. pneumoniae*, through an unknown mechanism or receptor, whereafter the predator rotates to create a pore in the prey cell wall and enters the prey cell's periplasmic space forming a structure called the bdelloplast (Sockett, 2009). Once the predator has invaded the prey cell, it secretes various hydrolytic enzymes to break down the prey cell's constituents and produce progeny (Sockett, 2009). Correspondingly, as *K. pneumoniae* is sensitive to predation, the pre-treatment with *B. bacteriovorus* aided in effectively reducing the concentration of this organism in the seeded water samples. Furthermore, the addition of the photocatalytic material enhanced the disinfection efficiency as the treatment time required to reduce the *K. pneumoniae* CFU to BDL was decreased from 240 min (solar disinfection) to 120 min (solar photocatalysis). Under solar UV-visible exposure, the TiO₂-rGO composite photocatalytic material produces reactive oxygen species (ROS), which significantly disrupts the cell membrane structures and damages DNA and RNA, ultimately leading to cell death (Byrne et al., 2011).

We previously investigated the mechanisms behind the antimicrobial activity of TiO₂-rGO in water 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/cm²) 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 TiO₂ 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 TiO₂ 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 TiO₂ in suspension to TiO₂ 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 TiO₂ coated raschig
359 rings, was comparable to the disinfection obtained in the reactor with TiO₂ 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

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

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

Correspondingly, the most efficient treatment strategy for the reduction of *E. faecium* was the use of solar disinfection or solar photocatalytic disinfection without *B. bacteriovorus* pre-treatment. While it is generally theorised that *B. bacteriovorus* does not prey on Gram-positive bacteria, studies have indicated that this predator can prey on *Staphylococcus aureus* (Iebba et al., 2014; Pantanella et al., 2018). The lytic enzymes produced by *B. bacteriovorus* have also been shown to disrupt biofilms produced by Gram-positive bacteria, while proteases produced by *B. bacteriovorus* can decrease the efficiency of *S. aureus* invasion into human epithelial cells (Monnappa et al., 2014). Furthermore, 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 TiO₂ doped with zinc; and a glass reactor (9 L) with a TiO₂ 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 TiO₂ 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 TiO₂ 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

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

5. Conclusions

Based on the results obtained, *B. bacteriovorus* may be applied to decrease the concentration of Gram-negative bacteria, such as *K. pneumoniae*, prior to solar disinfection. This is crucial as many pathogenic Gram-negative bacteria have been found to persist after the implementation of various disinfection strategies. Solar disinfection or solar photocatalytic treatment successfully reduced the concentration of *E. faecium* and it is likely that forced convection in a solar photocatalytic system may further enhance the effect of the photocatalytic material on the disinfection of Gram-positive 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 TiO₂.
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 TiO₂ 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 TiO₂ 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 TiO₂ 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., Quirynen, 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 TiO₂ to eliminate water-transmitted bacterial
605 pathogens: Photocatalyst characterization and disinfection performance. *Applied Catalysis B: Environmental* 154-155, 93–101.

607 Veneiri, 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.