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Inactivation of bacteria in seafood processing water by means of UV treatment

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ABSTRACT

Seafood processing is a large-scale food industrial activity, in the UK and worldwide, which requires substantial quantities of clean water for washing purposes. Therefore, the aim of this study is to assess the feasibility of ultraviolet (UV) treatment to disinfect water coming from shellfish washing process, as to safely recycle it in the process. For this reason, different operating parameters that typically affect UV treatment efficiency, namely the power output of the UV lamp (5W, 9W, and 11W), the turbidity of the washing water (0 – 52 NTU), and the initial bacterial concentration (10^4 , 10^5 , 10^6 CFU mL⁻¹) were studied. Water disinfection was monitored by following changes in the concentration of the *Escherichia coli* (*E. coli*) bacteria. Photoreactivation of bacteria after UV disinfection was also investigated. Results showed that the UV treatment can efficiently inactivate bacteria in shellfish processing water, since *E. coli* (10^6 CFU mL⁻¹) in turbid (i.e. 0.074 – 35 NTU) seafood processing water were inactivated within the first 15 sec of treatment, by means of an 11 W germicidal lamp. Under these conditions, no bacteria photoreactivation was observed after 2 h of exposure to natural light. The disinfection efficiency was decreased when the initial bacterial concentration and water turbidity were increased. In addition, the increase of UV power output resulted in a substantial increase of bacterial inactivation. Furthermore, *E. coli* were reactivated after 2 h of exposure to natural light when the turbidity of the washing water was ≥ 42 NTU or when the initial bacterial concentration was high (i.e. 10^5 and 10^6 CFU mL⁻¹).

KEYWORDS: shellfish; seafood processing industry; water disinfection; aquaculture; water recycling

30 **1. Introduction**

31 Shellfish farming and packaging is a large-scale food industrial activity in the UK and
32 worldwide. The UK exports most of the seafood it harvests, thus resulting to high economic
33 gains (e.g. in 2011 just over 435,000 tonnes of seafood, worth £1.46 billion, were exported
34 from the UK (Seafish, 2012a)). High value shellfish, such as langoustine, crab and scallops,
35 are exported to the French, Spanish and Italian markets (Seafish, 2012a). Moreover, Scotland
36 dominates the UK seafood processing industry, while secondary processing units are found in
37 the North England and Wales, thus providing 11,864 full-time jobs in 325 units throughout
38 the UK (data for 2011) (Seafish, 2012a, b). To maintain the high quality and profitability of
39 the UK shellfish species, domestic suppliers have focused on improving the sustainability of
40 their farming, as well as their packaging process.

41 Shellfish packaging requires vigorous washing and scrubbing with clean water, as to ensure
42 maximum removal of sediments and other debris. Water should be taken from an appropriate
43 source, which is usually sea or tap water (MassachusettsGeneralLaws, 2015). Nonetheless,
44 seawater pumping is an energy intensive process, while also it may be inappropriate due to
45 high pollution levels. It has been extensively reported that seawater in the European continent
46 and worldwide face great challenges due to heavy metal (Besada et al., 2011; Kallithrakas-
47 Kontos and Foteinis, 2015; Wang et al., 2013) and oil pollution (Cohen, 2013). Moreover,
48 many seafood processing industries are sited inland, therefore seawater utilization is
49 unpractical. In these cases, tap water is the only solution, but its use can significantly increase
50 operational costs and negatively affect the sustainability of the process. Furthermore,
51 shellfish processing machinery consumes large amounts of water (e.g. for shellfish washing,
52 equipment and floor cleaning), while water reclamation and recycling is not applied.
53 Therefore, water minimization and reuse strategies should be introduced in such industries, as
54 to make seafood washing more efficient and sustainable; thus improving their overall
55 environmental footprint, competitiveness and profitability.

56 Ultraviolet (UV) irradiation is a well-established treatment technology for bacterial
57 inactivation in water, air and solid surfaces and is one of the approved technologies used for
58 food processing and preservation (EPA, 2006; Gardner and Shama, 1999; Quek and Hu,
59 2013; Venieri et al., 2013). The efficiency of UV treatment is attributed to the hazardous
60 effects of UV-C radiation, which can destroy directly the DNA and the outer cell membrane
61 of pathogenic microorganisms (Chatzisyneon et al., 2011; Venieri et al., 2013). UV-C
62 irradiation between 250 nm and 270 nm, where the maximum absorbance of nucleotide bases

63 of the genome occurs, including thymine, cytosine and uracil, can induce damages in DNA
64 and RNA, thus inhibiting cell transcription and replication (Vélez-Colmenares et al., 2012).
65 Specifically, the major DNA lesion, induced by germicidal UV-C irradiation at 254 nm, is the
66 formation of pyrimidine dimers. The presence of these lesions inhibits the normal replication
67 of DNA, and therefore results in inactivation of the microorganisms in short time periods
68 (Nebot Sanz et al., 2007). In addition, UV disinfection does not require chemical reagents,
69 thus another advantage is that there is no formation of hazardous disinfection by-products
70 after treatment (Summerfelt, 2003). However, its main drawback is that many
71 microorganisms, including bacteria, are known to possess the ability to repair their DNA
72 damage in the presence (photoreactivation) or absence (dark repair) of light (EPA, 2006;
73 Nebot Sanz et al., 2007; Quek and Hu, 2013; Sinha and Hader, 2002). This can lead to the
74 reactivation of bacteria, after UV treatment, hence affecting disinfection efficiency and
75 rendering UV treatment unsafe. Till now, few studies have dealt with the use of UV
76 irradiation for food processing, including the inactivation of bacteria on raspberries and
77 strawberries (Bialka et al., 2008), in fruit juices (Gayán et al., 2012; Müller et al., 2011;
78 Santhirasegaram et al., 2015), apple cider (Unluturk et al., 2004), goat milk (Kasahara et al.,
79 2015), and in liquid egg products (Unluturk et al., 2008). However, to the best of the author's
80 knowledge, there is no study dealing with the application of UV for the treatment of seafood
81 processing waters.

82 Therefore, the aim of this study is to investigate the feasibility of the UV method to disinfect
83 shellfish washing water, thus being able to safely recycle treated water in the process. For this
84 purpose, washing water from a shellfish processing industry was used and various operating
85 parameters that typically affect UV efficiency were studied. These were the lamp power
86 output, the initial bacterial concentration, water turbidity and treatment time. The effect of
87 bacterial photoreactivation on treatment durability was also examined, as to ensure the
88 feasibility of the process.

89

90 **2. Materials and Methods**

91 **2.1. Shellfish processing water**

92 Shellfish processing water was collected from an industry that uses tap water for shellfish
93 washing, located in the UK. The processing water originates from the industry's shellfish

94 washing line, where tap water is initially used and then it is collected in tanks (about 250 L)
95 and reused, if appropriate, in the washing process. However, shellfish-associated bacteria,
96 including potential pathogens and spoilage organisms, build up in the tanks, thus rendering
97 the used water inadequate for recycling purposes after a short period of time. Therefore, this
98 water has to be disposed of, about every 10 min, when the bacterial concentration becomes
99 too high. thus preventing the efficient water recycling in the washing stage, and fresh tap
100 water needs to be introduced in the system. Shellfish-associated bacteria can include *Vibrio*
101 and *Shigella* species, *Salmonella*, or other toxin-forming bacteria (Iwamoto et al., 2010). In
102 this work, water disinfection was monitored by following changes in the *E. coli* bacteria,
103 which is a common and very popular indicator pathogenic microorganism for potable water
104 (Chatzisyneon et al., 2011), since according to current legislation the quality of seafood
105 washing water should follow the standards of drinking water (MassachusettsGeneralLaws,
106 2015).

107 In order to measure bacterial contamination in the used washing water and assess the
108 feasibility of the UV treatment, tap water was continuously (i.e. every 10 min) recycled in the
109 shellfish washing line for up to 40 min. Washing water samples were withdrawn after 10, 20,
110 30, and 40 min of washing, as to measure their physicochemical and microbiological
111 characteristics. The water samples were collected in sterilized sampling bottles of 1 L, kept at
112 4 °C and immediately dispatched for further analyses. After measuring their characteristics,
113 samples were sterilized at 121 °C for 15 min and kept in the fridge (4 – 8 °C).

114

115 **2.2. Bacterial strain**

116 The bacterial strain of *Escherichia coli*, which was used in this work as a water quality
117 indicator, was isolated from the shellfish washing waters by membrane filtration. From the
118 collected samples 200 µL were passed through a 0.45 µm pore-sized filter (cellulose
119 acetate/nitrate membranes by Sigma-Aldrich), using a vacuum pump VP series (KNF Lab).
120 These membranes were aseptically placed up on plates with Brilliance *E. coli*/Coliform Agar
121 (Oxoid) selective media, thus ensuring that no air bubbles were trapped. The plates were
122 incubated at 37 °C for 20 – 24 hours and *E. coli* colonies with purple-blue colour were picked
123 for further use. Specifically, the isolated *E. coli* were spiked into the sterile industrial washing
124 water to achieve the desired initial bacterial loading for each experimental run. The standard
125 *E. coli* ATCC 23716 (American Type Culture Collection, Rockville, MD, USA) strain was

126 also used. The freeze-dried cultures were rehydrated and reactivated according to the
127 manufacturer's instructions. The concentration of bacterial cells in the shellfish processing
128 water ranged from 10^4 – 10^6 CFU mL⁻¹, as estimated by measuring its optical density at 600
129 nm on a Cary100 UV-Vis double-beam (Varian, Inc.) spectrophotometer.

130

131 **2.3. UV experiments**

132 Experiments were conducted in an immersion well, batch type, laboratory scale photoreactor
133 shown in Schematic 1. This is a two compartment apparatus and consists of an inner quartz
134 glass housing the lamp and an exterior cylindrical reaction vessel made of borosilicate glass.
135 The reaction mixture was placed in the exterior cylindrical reaction vessel (compartment 1)
136 and the inner quartz glass was immersed inside the reaction mixture. The UV lamp was
137 placed inside the inner glass tube (compartment 2). It should be noted that this apparatus was
138 constructed and assembled in the workshop of the University of Edinburgh, UK. In a typical
139 experimental run, 300 mL of the shellfish processing water were introduced in the reaction
140 vessel. The bacterial suspension was magnetically stirred, to ensure complete mixing of *E.*
141 *coli* with the processing water, and then the UV lamp was turned on. UV-C irradiation, with
142 emission wavelength at 254 nm, was provided by an 11 W (11TUV, PL-S, Philips) or a 9 W
143 (PL, 2 PIN, Philips) or a 5 W (5TUV, PL-S, 2G7 base, Philips) germicidal lamp. The
144 temperature was constant at 18 ± 1 °C (i.e. ambient temperature), during each experimental
145 run, since in the shellfish processing industry the washing process takes place at ambient
146 conditions. The exterior reaction vessel was covered with aluminium foil to reflect back UV
147 irradiation. Representative experiments were carried out in triplicates to check the
148 reproducibility of the process. At specific time intervals, 2 mL of the reaction solution were
149 withdrawn and immediately analysed with respect to viable *E. coli* cells, by the serial dilution
150 culture method.

151

152 Schematic 1

153

154 **2.4. Microbiological and chemical analyses**

155 The detection and quantification of *E. coli* in the processing water was performed using the
156 serial dilution pour plate agar technique. Serial dilutions of the reaction solution were
157 performed in sterile 0.8% (w/v%) NaCl (Fisher Scientific, UK) aqueous solution and 200 μ L
158 of each dilution (including neat sample) were pipetted and spread onto Brilliance *E.*
159 *coli*/Coliform Agar (Oxoid) plates, a selective culture medium. The plates were incubated at
160 37 °C for 20–24 h before viable counts were determined. *E. coli* colonies appeared with
161 purple colour, while coliforms colonies had a pinkish colour. For the undiluted samples, 1
162 mL of sample was spread over five 90 mm Petri dishes (i.e. 200 μ L of sample per Petri dish).
163 This was done to reduce the detection limit to 1 CFU mL⁻¹ for the undiluted samples
164 (Paleologou et al., 2007; Rincón and Pulgarin, 2004).

165 The turbidity was measured on a HACH 2100N turbidity meter, while conductivity and pH
166 were measured by a portable conductivity and pH meter (\pm 0.1 pH accuracy), respectively, by
167 Hanna Instruments.

168

169 **2.5. Photoreactivation experiments**

170 Bacteria are known to be capable of repairing their damaged DNA after UV treatment, either
171 by dark repair or by photoreactivation mechanisms (Chatzisymeon et al., 2011; Venieri et al.,
172 2011). The latter is considered to be the most important mechanism (Nebot Sanz et al., 2007).
173 In addition, in the seafood processing industry, under study, the tanks, where the shellfish
174 washing water is collected and it is then recycled into the washing process, are open and
175 exposed to natural light. Therefore, in this case, the investigation of bacterial
176 photoreactivation is of major importance. Most photoreactivation studies involve the use of
177 visible light from artificial sources, such as fluorescent lamps, which emit light at 360 nm and
178 halogen lamps emitting between 400 nm and 800 nm. However, very few have dealt with
179 natural light (Chatzisymeon et al., 2011; Vélez-Colmenares et al., 2012; Venieri et al., 2011),
180 as is the case of the present work. Specifically, *E. coli* photoreactivation experiments in UV
181 treated shellfish washing water were carried out under natural light. For this reason, 100 mL
182 of the final treated effluent were transferred into a sterile conical flask, which was then sealed
183 up to prevent air getting in and potentially contaminating the effluent. The flasks were kept
184 under continuous stirring for about a day (22 h) and under natural light conditions. After this
185 period the final sample was analysed in terms of *E. coli* viability.

186

187 3. Results and Discussion

188 3.1. Physicochemical and microbiological characteristics of the shellfish washing water

189 The physicochemical and microbiological characteristics of the collected washing water are
190 shown in Table 1, where it can be observed that conductivity is increasing with washing time,
191 from the initial value of 0.05 to 0.52 mS/cm, after 40 min of shellfish washing. This increase
192 in conductivity can be attributed mainly to the increased water salinity, deriving from
193 dissolved salts coming out from shellfish washing. Interestingly, turbidity is increased from
194 0.079 to 42.7 NTU during the first 10 min of washing; while further washing (i.e. from 10
195 min to 40 min) does not considerably affect turbidity. This sharp increase of turbidity from
196 the first 10 min of washing is attributed to solid particles that are washed out from the
197 shellfish; these may include cracked shells, seaweed residuals, etc.. Moreover, turbidity
198 values remained at the same order of magnitude for the rest of the washing time, e.g. 42.7
199 NTU at 10 min to 52 NTU at 40 min. Although, it was expected that water turbidity would be
200 rapidly increased, due to the high loads of solids, which are washed out during the washing
201 process, this is not the case here. This can be attributed to the fact that a sieve to hold all large
202 solid particles coming out of the washing process was installed at the end of the shellfish
203 washing line, and therefore this is the main reason that turbidity is increased up to a value of
204 about 42 – 52 NTU and after that it remains almost constant with time. Finally, a slight
205 increase of pH values by time is also observed, which can be attributed to the increase of
206 conductivity and turbidity. Conductivity (i.e. content of salts in water) and turbidity (i.e.
207 suspended solids coming from cracked shells and residual seaweeds) can have neutral or
208 alkaline pH values, thus slightly increasing the pH of the washing water from 5.76 to 6.14.

209

210 Table 1.

211

212 As far as the microbiological characteristics are concerned, it was observed (Table 1) that
213 pathogen microorganisms, namely *E. coli* along with other coliforms, were increased up to
214 the order of 10^3 and 10^4 CFU mL⁻¹, respectively, after 20 min of washing. Surprisingly,
215 further processing did not cause any greater increase of bacterial concentration in the washing

216 water. This can be explained by the increased (≥ 0.42 mS/cm) conductivity (i.e. salinity) of
217 the water, which prevented the further growth of bacteria in water (Kaspar and Tamplin,
218 1993). In general, enteric bacteria, when released into saline water, are subjected to an
219 immediate osmotic shock, and their ability to overcome this by means of several
220 osmoregulatory systems could largely influence their subsequent survival in the marine
221 environment (Rozen and Belkin, 2001). Specifically, the survival of *E. coli* bacteria in saline
222 water depends, at least partly, on whether they possess certain genes which enable them to
223 regulate osmotic pressure and whether they can be stimulated to express those genes before
224 or after their release into the saline aquatic environment (Munro et al., 1989). For example, in
225 a previous study it was observed that survival of *E. coli* in seawater/distilled water mixtures
226 at different ratios (0, 25, 50, 75 and 100% seawater) for 48 h showed an optimal survival
227 (74%) at the 25% seawater mixture (Carlucci and Pramer, 1960). Moreover, Anderson et al.
228 (1979) who studied the survival of an *E. coli* isolate for 8 days in seawater at selected
229 salinities (1, 1.5, 2.5, and 3%), observed that decreasing salinity was accompanied by
230 increasing survival (Anderson et al., 1979). Finally, the slight decrease in bacteria counts
231 (Table 1) from 30 min to 40 min of washing can be assumed as negligible, since this is within
232 the same logarithmic order of magnitude.

233

234 **3.2. Effect of UV power**

235 The effect of UV power on inactivation of bacteria was also studied. For this purpose, three
236 UV lamps, with different power outputs of 5 W, 9 W, and 11W, were used. It should be noted
237 that, in this case, turbidity can be assumed as constant, since there is a similar effect on
238 disinfection efficiency when turbidity values are ≥ 42 NTU (see section 3.4). Results are
239 shown in Figure 1, where it is observed that the inactivation of bacteria is rapidly increasing
240 with increasing the power output. Thus, the 11 W UV lamp achieved total inactivation of
241 bacteria after 30 sec of treatment, which was not the case for either the 5 W or the 9 W lamp.
242 Specifically, when initial bacterial concentrations of the order of 10^6 CFU mL⁻¹ are
243 concerned, the 5 W and 9 W germicidal lamps did not achieve water disinfection, not even
244 after 4 min of treatment. In general, photolysis in real water samples occurs directly through
245 light absorption by the organic molecules of the bacterial cells (Chatzisyneon et al., 2011;
246 Nebot Sanz et al., 2007; Vélez-Colmenares et al., 2012; Venieri et al., 2013). Therefore, the
247 higher performance of the 11 W UV system can be attributed to the higher photon flux that

248 finally reaches the reactant solution and causes the rapid photolytic degradation of bacteria.
249 In addition, the treatment time obtained here is comparable with previous studies, where *E.*
250 *coli* inactivation in biologically treated municipal effluents occurred after 3 min of UV
251 irradiation with an 11 W germicidal lamp (Chatzisymeon et al., 2011). It should be noted that
252 experiments were performed with initial bacterial concentration of 10^6 CFU mL⁻¹, which is
253 above the real bacterial concentration (i.e. 10^4 CFU mL⁻¹), as shown in Table 1. This was
254 done to ensure that UV treatment can work under stressed (high bacterial load) conditions.
255 Summing up, a UV germicidal lamp with power output ≥ 11 W can become a feasible option
256 for disinfecting shellfish processing washing waters, thus improving the overall sustainability
257 of the industrial process.

258

259 Figure 1.

260

261 3.3. Effect of bacterial concentration

262 The effect of bacterial initial concentration on process efficiency was investigated and the
263 results are presented in Figure 2. Three different initial bacterial concentrations, i.e. 10^4 , 10^5 ,
264 and 10^6 CFU mL⁻¹, were tested; which are substantially above the *E. coli* loadings (i.e. 10^3 –
265 10^4 CFU mL⁻¹ as shown in Table 1) in real washing waters. It was observed that inactivation
266 of bacteria occurs more rapidly when their initial concentration is lower. For example, when
267 the initial *E. coli* concentration was 10^4 CFU mL⁻¹, water was disinfected after 240 sec of
268 treatment, while for initial concentration of 10^6 CFU mL⁻¹, a substantial amount of *E. coli*
269 (10^3 CFU mL⁻¹) survived after 240 sec of treatment. Results in Figure 2 show that the amount
270 of photons emitted from the 9 W germicidal lamp were not adequate to disinfect *E. coli* of
271 10^5 and 10^6 CFU mL⁻¹ initial concentrations, within the first 240 sec of treatment. On the
272 other hand, for initial bacterial concentrations of $\leq 10^4$ CFU mL⁻¹, results show that the 9 W
273 germicidal lamp can be a feasible and applicable option for shellfish processing water
274 disinfection and recycling. Nonetheless, since initial bacterial concentrations are not always
275 $\leq 10^4$ CFU mL⁻¹, disinfection cannot at all times be secured in shellfish processing water, and
276 therefore a germicidal lamp of 11 W, or higher, is proposed as a feasible alternative for
277 recycling shellfish washing water.

278

279 Figure 2.

280

281 **3.4. Effect of water turbidity**

282 Water turbidity is a parameter that can negatively affect the efficiency of UV treatment, and
283 thus its investigation is of major importance. Process efficiency may be inhibited by the
284 presence of suspended solids in the water (Gullian et al., 2012). Inhibition is mainly
285 attributed to the facts that (a) turbidity prevents light from penetrating the whole water
286 matrix, and (b) bacteria can be shielded by solids, thus protecting them from exposure to UV
287 light and therefore preventing their inactivation. Therefore, a series of experiments was
288 performed to assess the effect of water turbidity on process efficiency. The range of turbidity
289 that was examined corresponds to the ones observed in the real shellfish washing water, i.e.
290 35 – 52 NTU, and the results are shown in Figure 3. It can be observed that for turbidity
291 lower than 35 NTU, bacteria are rapidly inactivated after the first 15 sec of treatment. When
292 turbidity values are 42.7 NTU and 52 NTU, results show that *E. coli* appear to have been
293 inactivated during the first 2 min of UV treatment. However, there is a bacterial increase to
294 15 CFU mL⁻¹ after 4 minutes of treatment and this reappearance can be explained by the fact
295 that the high turbidity of the water (i.e. 42.7 and 52 NTU) can both shield bacteria and hinder
296 the penetration of UV irradiation into the whole liquid volume, thus preventing its effective
297 disinfection. Therefore, it is highly recommended that turbidity should be decreased (\leq 35
298 NTU) before UV treatment, as to optimize the treatment time and process efficiency.

299

300 Figure 3.

301

302 **3.5. Effect of bacterial strain**

303 All the aforementioned experimental series were carried out by spiking *E. coli*, initially
304 isolated from the fresh shellfish washing water, into the same matrix, as to obtain the
305 desirable initial bacterial concentration. In order to confirm and generalize the feasibility of
306 the UV treatment for disinfecting such type of waters, experiments were also performed by
307 using the standard *E. coli* strain ATCC 23716. Results are shown in Figure 4, where it is
308 evident that the disinfection efficiency of the standard strain is slightly higher, than in the

309 case of the bacteria isolated from the real environmental samples. This indicates that isolated
310 bacteria are more persistent to UV treatment than standard strains, thus highlighting the
311 importance of this work which deals with the inactivation of isolated bacteria in real
312 industrial shellfish washing waters. There can be many causes for the difference in the
313 resistance of *E. coli* bacteria to UV treatment. Firstly, during evolution these are possibly
314 exposed to various kinds of environmental stresses, such as temperature, water medium, UV
315 irradiation or chemical agents. Each of these stresses can act differently on the bacterial cell
316 and cause lethality that can vary from strain to strain (Chintagari et al., 2015). Moreover, UV
317 is absorbed by nucleic acids producing several types of damage that interfere with replication
318 and transcription of DNA. If UV-induced damage is not repaired or eliminated from DNA, it
319 may lead to mutagenesis and cell death. Mutations not only promote genetic divergence of
320 populations living in different environments, but even in identical environments parallel
321 populations may diverge, if they find alternative adaptive solutions. To prevent the lethal
322 effects of this and other DNA damaging agents, different repair mechanisms have developed
323 through evolutionary history. Therefore, during adaptation of *E. coli* to UV irradiation,
324 mutations induced in DNA repair or replication genes can be indiscriminately selected
325 (Alcántara-Díaz et al., 2004; Chintagari et al., 2015).

326

327 Figure 4.

328

329 **3.6. Photoreactivation of bacteria**

330 Bacterial photoreactivation experiments were carried out, as to determine the efficiency of
331 UV treatment. At the premises of the seafood processing industry under study, shellfish
332 washing water is exposed to visible light before its further use. Thus the investigation of
333 bacterial photoreactivation is imperative in order to ensure the safe UV treated water
334 recycling supply. The results are shown in Table 2 and it is shown that in all cases *E. coli*
335 photoreactivation occurs after 22 h of exposure to natural light. However, no reactivation was
336 recorded after exposure to light for 2 h, at low initial bacterial concentration (i.e. 10^4 CFU
337 mL^{-1}) (Run 1, Table 2), at low turbidity value of 35 NTU (Run 5, Table 2), and during the
338 treatment of standard *E. coli* strains (Run 7, Table 2). Therefore, these results indicate that
339 UV-C irradiation can cause severe damage to bacterial cells. Comparing the effect of initial

340 bacterial concentration on photoreactivation (Runs 1 – 3), it is observed that in cases where
341 the initial *E. coli* concentrations are high, i.e. 10^5 CFU mL⁻¹ and 10^6 CFU mL⁻¹,
342 photoreactivation takes place after only 2 h of exposure to natural light. This shows that when
343 increasing the initial bacterial concentration at 10^5 CFU mL⁻¹ and above, photoreactivation is
344 favoured. It should be noted that, in this case, turbidity can be assumed as constant, since as it
345 was proved in section 3.4 there is a similar effect on disinfection efficiency, when turbidity
346 values are ≥ 42 NTU. Moreover, as shown in runs 3, 4, and 6, photoreactivation is not
347 affected by the different UV doses and occurs at all UV power outputs (11, 9, and 5 W). This
348 is in contrast with previous studies, where it was observed that an increase in UV dose is
349 valuable in minimizing photoreactivation events, since reduced UV dose causes reduced
350 DNA damages on targeted bacteria, thus increasing the risk of subsequent photoreactivation
351 (Lindenauer and Darby, 1994; Nebot Sanz et al., 2007). However, in this case it should be
352 noted that runs 3, 4, and 6 are carried out at high turbidity values (i.e. 42 – 52 NTU) that has
353 been proved to decrease disinfection efficiency. Not only this but, if runs 5 and 6 are
354 compared, it is observed that at low turbidity values (i.e. 35 NTU) photoreactivation of
355 bacteria does not occur for at least 2 h after UV treatment, while when turbidity is 52 NTU
356 (run 6) photoreactivation takes place within the first 2 h after UV treatment. Furthermore,
357 from runs 5 and 7, it can be concluded that bacterial strain has an effect on photoreactivation
358 of *E. coli*, since, although both strains were reactivated after 22 hours of exposure, the cell
359 count was higher for isolated bacteria. This is consistent with the results described in Figure 4
360 and enhances the fact that isolated bacteria are more resistant to UV treatment than standard
361 strains, such as the ATCC 23716, which also highlights the significance of this work.

362

363 Table 2.

364

365 **4. Conclusions**

366 In this work the feasibility of UV treatment to disinfect shellfish processing water was
367 assessed. For this purpose, the effect of important operating parameters, such as the initial
368 bacterial concentration, UV power output, water turbidity and treatment time, on process
369 efficiency was investigated. It should be noted that although this is a pressing problem for

370 seafood industry, it has received very little attention till now. The main findings of this work
371 can be summarized as follows:

- 372 - Shellfish washing waters are turbid and saline with values ranging between 35 – 52 NTU
373 and 0.28 – 0.52 mS/cm, respectively. Regarding their microbiological characteristics, there
374 is a built-up of *E. coli* and other coliforms of the order of 10^3 CFU ml⁻¹ and 10^4 CFU mL⁻¹,
375 respectively.
- 376 - UV treatment can be efficiently applied to disinfect shellfish washing water, since it was
377 observed that, at optimal operating conditions (i.e. UV power output at 11 W, water
378 turbidity ≤ 35 NTU and initial *E. coli* concentration up to 10^6 CFU mL⁻¹) the total
379 inactivation of bacteria is achieved after only 15 sec of treatment.
- 380 - Bacterial photoreactivation experiments were carried out and showed that no *E. coli*
381 photoreactivation occurs, after exposure to light for 2 h, at low initial bacterial
382 concentration (i.e. 10^4 CFU mL⁻¹), at low turbidity value of 35 NTU, and during the
383 treatment of standard *E. coli*. Hence, it can be concluded that UV disinfection of shellfish
384 washing waters, with initial bacterial loading of up to 10^4 CFU mL⁻¹, can be a very
385 efficient treatment process in the presence of a UV lamp with power output of 11 W and
386 when turbidity of the washing water is decreased to ≤ 35 NTU.

387

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390

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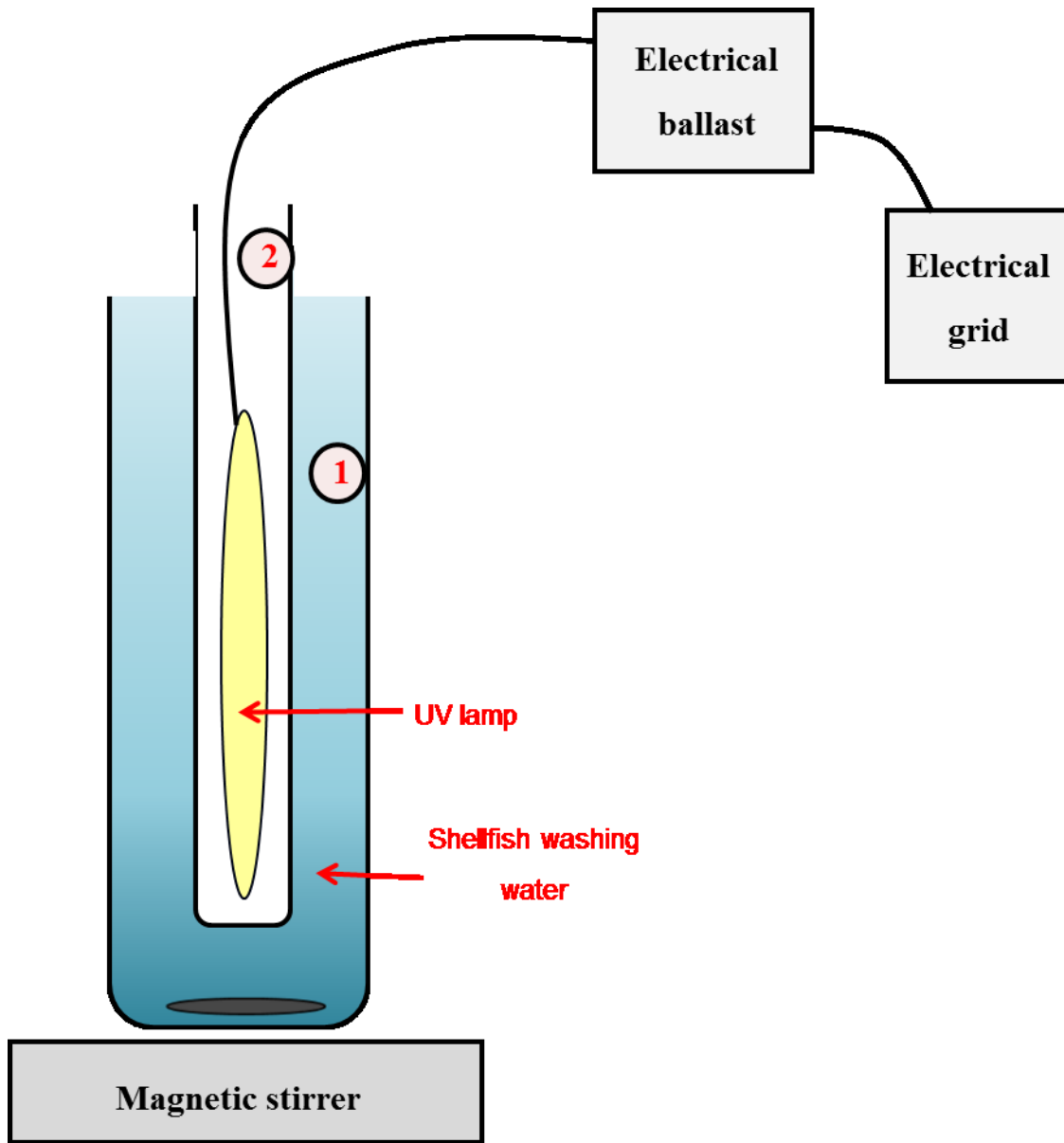
488 **List of Schematics**

489

490 Schematic 1. Experimental set up of the UV reactor. Compartments: (1) exterior cylindrical
491 reaction vessel made of borosilicate glass, and (2) inner quartz glass tube housing the lamp.

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496 Schematic 1. Experimental set up of the UV reactor. Compartments: (1) exterior cylindrical
497 reaction vessel made of borosilicate glass, and (2) inner quartz glass tube housing of the
498 lamp.

499 **List of Tables**

500

501 Table 1. Physicochemical and microbiological characteristics of shellfish washing water
502 samples. The standard deviation (SD) is shown in brackets.

503

504 Table 2. *E. coli* photoreactivation, under natural light, in UV treated shellfish processing
505 water.

506

507 Table 1. Physicochemical and microbiological characteristics of shellfish washing water
 508 samples. The standard deviation (SD) is shown in brackets.

Characteristics	Sample (shellfish washing time)				
	1 (0 min)	2 (10min)	3 (20 min)	4 (30 min)	5 (40 min)
Conductivity, mS/cm	0.05	0.28	0.42	0.46	0.52
pH	5.76	5.87	5.98	6.2	6.14
Turbidity, NTU	0.079	42.7	42	35	52
<i>Escherichia coli</i> , CFU mL ⁻¹	0 (SD=0)	510 (SD=14)	1235 (SD=230)	7530 (SD=1010)	2420 (SD=380)
Coliforms, CFU mL ⁻¹	0	750	20000	33583	15375

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511

512 Table 2. *E. coli* photoreactivation, under natural light, in UV treated shellfish processing
 513 water.

Operating conditions of UV treatment					<i>E. coli</i> survival after 240 sec of UV treatment, CFU mL ⁻¹	<i>E. coli</i> survival after 2h of phototreatment , CFU mL ⁻¹	<i>E. coli</i> survival after 22h of phototreatment, CFU mL ⁻¹
Run	Lamp power, W	Turbidity, NTU	Initial <i>E. coli</i> concentration , CFU mL ⁻¹	Bacterial strain			
1	9	52	10 ⁴	Isolated	0	0	100
2	9	52	10 ⁵	Isolated	57	18	>100
3	9	42	10 ⁶	Isolated	2600	>100	>100
4	5	42	10 ⁶	Isolated	7500	>100	>100
5	11	35	10 ⁶	Isolated	0	0	>100
6	11	52	10 ⁶	Isolated	15	>100	>100
7	11	35	10 ⁶	ATCC23716	0	0	20

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516 **List of Figures**

517

518 Figure 1. Inactivation of bacteria under different UV power outputs. Conditions: Initial
519 bacterial concentration = 10^6 CFU mL⁻¹; water turbidity = 42 – 52 NTU.

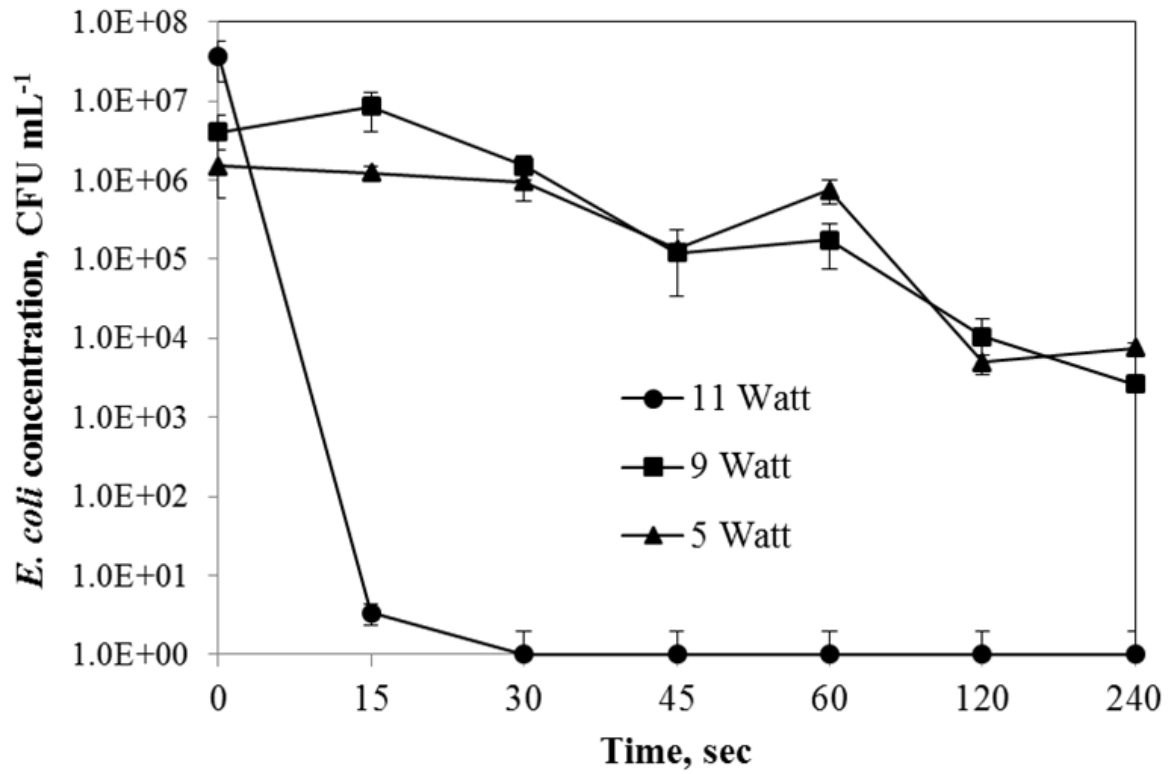
520 Figure 2. Inactivation of bacteria under different initial bacterial concentrations. Conditions:
521 UV power = 9 W; water turbidity = 52 NTU.

522 Figure 3. Inactivation of bacteria under different water turbidity values. Conditions: UV
523 power = 11 W; initial bacterial concentration = 10^6 CFU mL⁻¹.

524 Figure 4. Inactivation of bacteria in the presence of different *E. coli* strains. Conditions: UV
525 power= 11 W; initial bacterial concentration = 10^6 CFU mL⁻¹; water turbidity = 35 NTU.

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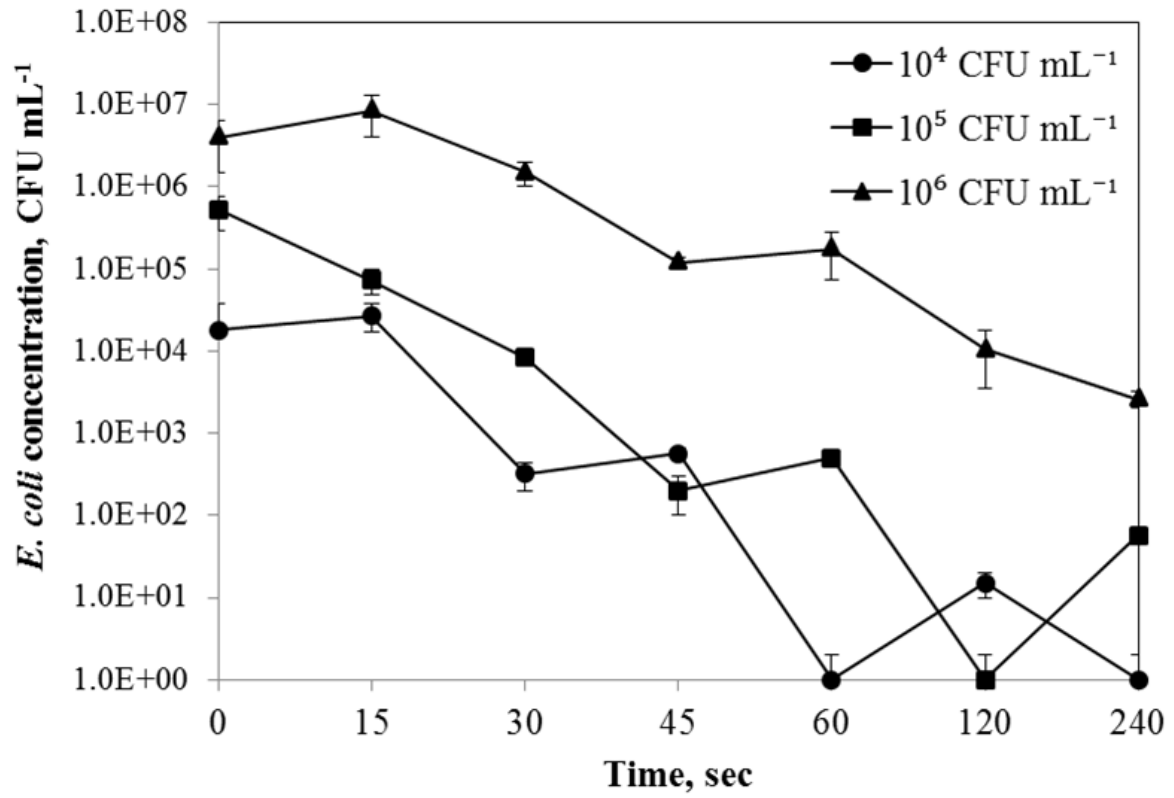


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530 Figure 1. Inactivation of bacteria under different UV power outputs. Conditions: Initial
 531 bacterial concentration = 10^6 CFU mL⁻¹; water turbidity = 42 – 52 NTU.

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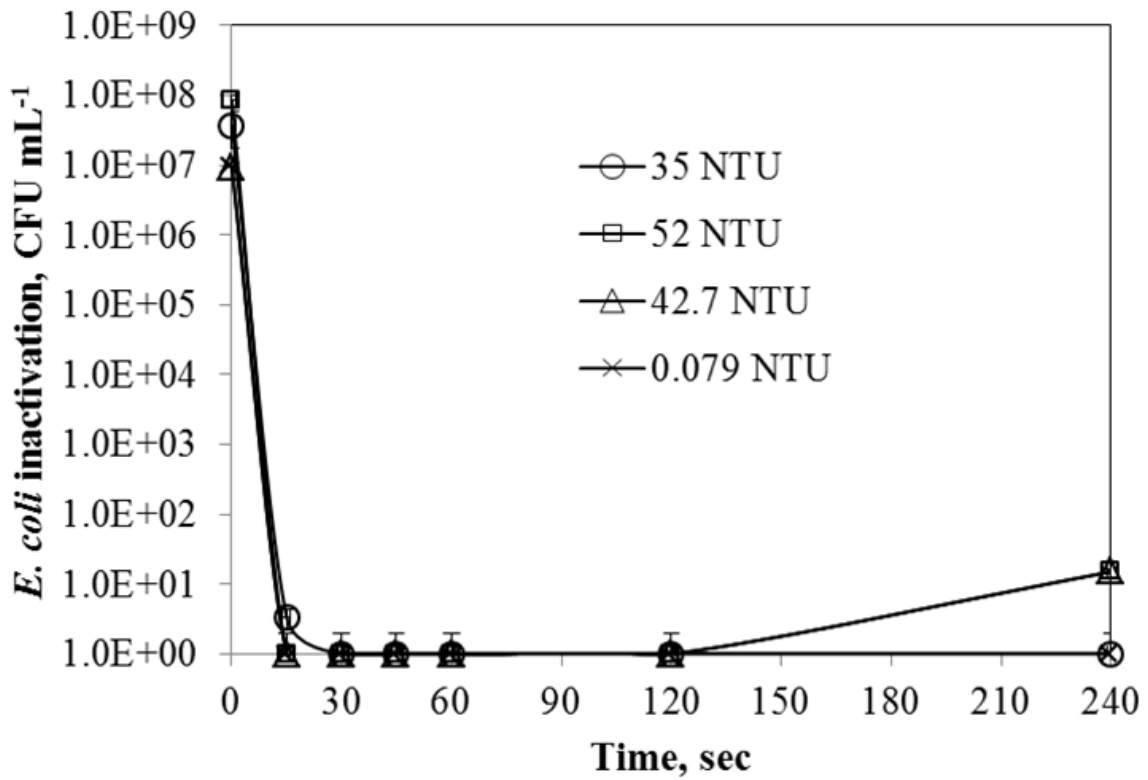
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535 Figure 2. Inactivation of bacteria under different initial bacterial concentrations. Conditions:
 536 UV power = 9 W; water turbidity = 52 NTU.

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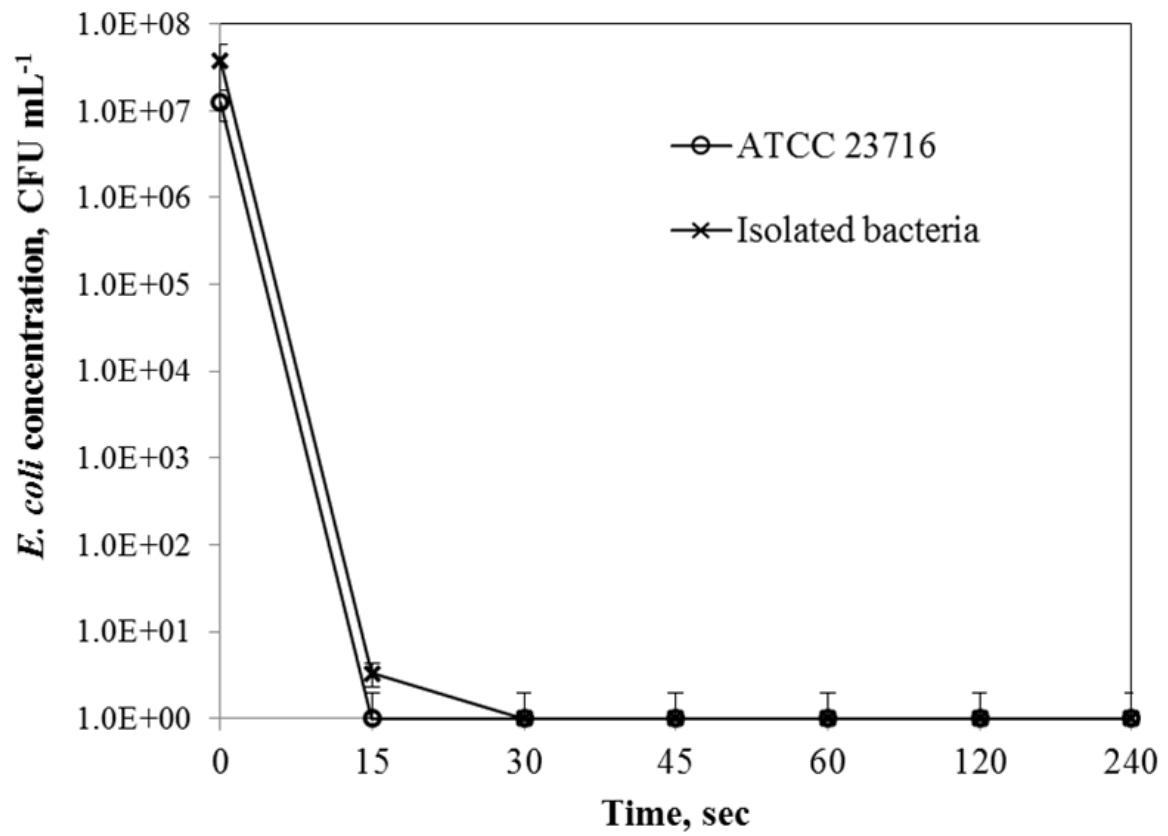


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539 Figure 3. Inactivation of bacteria under different water turbidity values. Conditions: UV
 540 power = 11 W; initial bacterial concentration = 10⁶ CFU mL⁻¹.

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544 Figure 4. Inactivation of bacteria in the presence of different *E. coli* strains. Conditions: UV
 545 power= 11 W; initial bacterial concentration = 10⁶ CFU mL⁻¹; water turbidity = 35 NTU.