1 **Determining disinfection efficiency on *E. faecalis* in saltwater by photolysis of**
2 **H_2O_2: Implications for ballast water treatment**
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14 **Abstract:**

15 Organisms carried with ballast water can find a way that enables them to spread into a

16 new habitat, becoming invasive species. This can generate large impacts threatening the

17 ecosystem and human activities. The effectiveness of microbiological disinfection by

18 UV/H_2O_2 treatment on *E. faecalis* has been evaluated in this study at laboratory scale, in

19 both buffered distilled water (DW) and saltwater (SW). A Collimated Beam Reactor

20 was used to determine optimal H_2O_2 concentration with DW and a Continuous Flow

21 Reactor was tested with DW and SW. The optimal concentration of hydrogen peroxide

22 found was 5 mg/L. The improvement of adding H_2O_2 increased efficacy by 28.9 % in

23 SW compared with UV alone; while results indicated that water salinity did not induce

24 strong interference in treatment. In addition, re-growth of surviving bacteria was

25 prevented 24 h after the treatment; even an additional one-log inactivation was obtained.

26 The results suggest that the addition of small concentrations of H_2O_2 leads to an

27 improvement in UV treatment. Finally, the operational costs were estimated for typical

28 cargo vessels; UV/H_2O_2 treatment was considered to be competitive for ballast water

29 treatment, since it could improve the effectiveness of the process with similar costs per

30 1000 m^3 of treated water: 14 €for UV treatment and 16 €for UV/H_2O_2 treatment.

31
32 **Key Words**

33 UV/H_2O_2, Ballast Water Treatment, *E. faecalis,* Inactivation, Collimated beam reactor,

34 Continuous reactor

35

36   **Nomenclature**

37   A_{254}: UV absorbance at 254 nm (unitless)

38   AOP: Advanced Oxidation Process

39   BWM: Ballast Water Management

40   BWTS: Ballast Water Treatment System

41   CBR: Collimated Beam Reactor

42   CFR: Continuous Flow Reactor

43   CFU: Colony-Forming Unit

44   D_{CBR}: UV dose for CBR (mJ/cm^2)

45   D_{CFR}: UV dose for CFR (mJ/cm^2)

46   d: Depth of water suspension in CBR (cm)

47   DW: Buffered Distilled Water

48   e: quartz sleeve width(cm)

49   Es: Average UV intensity measured for CBR (mW/cm)

50   f: fraction of initial organisms that follows the first inactivation rate constant on

51   biphasic model (unitless)

52   Im: mean intensity in CFR (mW/cm)

53   IMO: International Maritime Organization

54   k_1, k_2: inactivation rates of biphasic model (cm^2/mJ)

55   k_{max}: first order inactivation rate for log-linear model (cm^2/mJ)

56   L: Distance from lamp centerline to solution surface (cm)

57   L_L: lamp length (cm)

58   N: concentration of viable bacteria after disinfection treatment (CFU/mL)

59   N_0: concentration of viable bacteria before treatment disinfection (CFU/mL)

60   N_r: concentration of viable bacteria of reactivated sample (CFU/mL)

61   P: lamp power (W)

62   Pf: Petri Factor (unitless)

63   R: Reflectance at the air-water interface at 254 nm (unitless)

64   r_q: external quartz sleeve radius (cm)

65   r_r: internal reactor radius (cm)

66   SW: Saltwater

67   t: Exposure time (s)

68   Tq: quartz transmittance (unitless)

69   TRT: Theoretical Retention Time (s)

70   Tw: water transmittance (unitless)

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1. Introduction

Ballast water is pumped into ships in order to provide stability and maneuverability when unloaded vessels are moving or the cargo is not heavy enough [1]. Current data shows that ships move about 90% of total world merchandise [2,3] and they transfer about 3-5 billion tons of ballast water yearly [4].

In ballast tanks, besides water, there are sediment particles and organisms [5, 6], which can be released through pumping systems and pipelines into the destination port when the ballast water is being unloaded. Since almost all marine organisms in the different stages of their lives are in free form, either swimming or planktonic [5], any aquatic species is capable of being transported and released into a far-off new geographic area that is not connected by natural routes. Once organisms are released, their evolution depends on their own characteristics and the receiving environment conditions [7,8]. Frequently, organisms carried with ballast water find a way that enables them to develop and spread into the new habitat, becoming invasive. This often results in ecosystem threats or a significant loss in economic value [7,9] and generates huge impacts on environment, economy and public health [10]. In fact, aquatic invasive species are among the four major global threats to the oceans [8, 11]. Shipping is the main pathway for alien species introduction [7,12], and it is estimated that ships transport thousands of species every day in ballast water [5].

Therefore, the International Maritime Organization (IMO) developed regulations to prevent or reduce these problems in the future, reflected in the Ballast Water Management Convention adopted in 2004 [13]. Once it enters into force, all cargo ships must have a system for treating ballast water that meets a number of parameters set out in Rule D2. It includes two indicators related to the size of planktonic organisms, as well as three indicator microbes: *Vibrio cholerae*, Intestinal *Enterococci* and *Escherichia coli*.The entry into force (Article 18) of this Convention will take place 12 months after 30 States have ratified it, and they represent at least 35% of world merchant shipping tonnage. As of April 2015, it has been ratified by 44 states, which represent 32.86 % of total tonnage (status of BWM Convention ratification at www.imo.org). It has not entered into force yet but its admission is imminent.

Because of these problems, it is necessary to develop treatment technologies that are efficient in both freshwater and saltwater; as well as being environmentally friendly and

109 cost-effective [14]. Furthermore, these treatments must be faster, adapting to the high

110 water-flow when charging and discharging ballast in vessels. So, it is necessary to find

111 alternative processes to achieve marine water disinfection without the generation of

112 toxic by-products. Currently, disinfection treatments in seawater have become a major

113 interest due to these demands [11,15–17].

114 Ultraviolet (UV) irradiation is a well-established treatment for disinfection purposes,

115 with the advantage of not generating by-products [18,19] but with the disadvantage of

116 both the subsequent dark and photo-repair of microorganisms [20,21] during the storage

117 of treated water. UV treatment efficiency can be improved by combining it with some

118 oxidants (H_2O_2, O_3, etc.) or photocatalysts (TiO_2) resulting in an Advanced Oxidation

119 Process (AOP). These kinds of processes generate radicals such as hydroxyl radical

120 ($\cdot OH$), which is an extremely powerful oxidizing agent that is short-lived and does not

121 cause environmental damage [22]. Some of these UV-based technologies have been

122 studied as alternatives to marine water disinfection treatment: UV/TiO_2 [23,24], UV/O_3

123 [16,25], etc. However, there are some disadvantages such as the need for catalyst

124 cleaning in the case of TiO_2 [25] or the generation of by-products in the case of O_3 [19].

125 The photolysis of H_2O_2 is used as a hydroxyl radical generator according to Equation (1)

126 [22]. The effectiveness of the application of UV/H_2O_2 technology is guaranteed in both

127 natural water and wastewater for organic pollutants degradation, including water

128 disinfection [26,27]. Nevertheless, it is still uncertain whether this process is a viable

129 option for marine water disinfection because it has special particularities, such as a high

130 concentration of ions, which are able to interfere with AOP applications [15].

131 $$H_2O_2 + h\nu \rightarrow 2 \cdot OH \qquad\qquad (1)$$
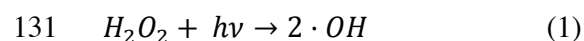
132 Some authors, such as Lanao *et al*. 2012, Koivunen *et al*. 2005, or Mamane *et al*. 2007,

133 achieved slight additional effects in fresh or wastewater disinfection under different

134 kinds of UV sources emitting in different UV ranges (UV-A and UV-B) or with

135 insignificant concentrations of H_2O_2 [28–30]. On the other hand, Penru *et al*. 2012 and

136 Rubio *et al*. 2013, achieved high disinfection rates in marine water, obtaining results

137 able to disinfect marine waters [15,31]. However, these previous studies have been

138 conducted in batch conditions, i.e. in the absence of water flow. In the specific case of

139 ballast water treatment, it is necessary to consider some key aspects such as maximum

140 and minimum ballasting and de-ballasting rates or ballast capacity [4]. The large

141 volumes of ballast water together with the need to establish high ballasting flow rates

142 make treatment in batch mode unfeasible, so it is necessary for these treatments to be

143 applied in order to meet flow rate requirements.

144 Among the microbiological indicators regulated by International standards (BWM

145 Convention, Rule D2), *E. coli* is a Gram-negative bacterium widely studied for

146 disinfection purposes with UV-based treatments[23,30,32]. This study is focused on

147 *Enterococcus faecalis*, Gram-positive bacteria that are also regulated by the BWM

148 Convention under the subgroup of intestinal *Enterococci*. The main difference between

149 these microbiological indicators is the thickness of their bacterial wall: *E. faecalis* have

150 a thick peptidoglycan layer (bacterial wall) which provides major protection for their

151 nucleus from incident UV light [33–35]. The discharge limit, according to D2 of the

152 BWM Convention, must not exceed 99 Colony-Forming Unit (CFU) per 100 mL for

153 Intestinal *Enterococci;* while in the case of *E. coli* it is set at less than 250 CFU/100 mL,

154 and less than 1 CFU/100 mL for *Vibrio cholerae*.

155 The inactivation of microorganisms depends on the UV dose they receive [34]; this UV-

156 C dose can be considered as a function of exposure time and UV-C intensity, taking into

157 account several specific factors of the reactor and water matrix [36]. For flow

158 conditions, the exposure time will be the hydraulic retention time [23,37].

159 Disinfection efficiency of the treatments can be determined through dose-response

160 curves. A typical dose-response curve of UV microbial inactivation follows first-order

161 kinetics with log-linear yield, which could be adapted with an initial baseline region, a

162 so-called shoulder, where little or no inactivation occurs. Moreover, at the end,

163 asymptotic decay could appear; which is called the tailing effect. The specific kinetic

164 could be determined through application of microbial inactivation kinetic models that fit

165 the experimental data, thus making it possible to correctly interpret the obtained results

166 [38].

167 The aim of this study is to research the disinfectant power of UV-C irradiation

168 combined with H_2O_2 (UV/H_2O_2 treatment) and its application for ballast water

169 treatment. To achieve this aim, the specific objectives are as follows:

170 - To determine the efficacy of UV-C treatment at different concentrations of hydrogen

171 peroxide in order to acquire an optimal H_2O_2 concentration, using a batch reactor.

172 - To evaluate UV/H_2O_2 treatment in different water matrices with a continuous reactor.

173   - To estimate the capacity of bacterial recovery in darkness conditions 24 h after the

174   treatment was applied (simulated as a ballast water tank).

175   - To assess economic parameters: reagent and electrical consumption to estimate the

176   operating cost of the process.

2. Material and Methods

2.1 Determination of hydrogen peroxide

The treatments were carried out employing hydrogen peroxide (30% by weight, Merck). The H_2O_2 concentration was measured via colorimetric method based on [39] by using a spectrophotometer (Genesys 20 – Thermo Fisher Scientific-4001/4) to measure the absorbance at 410 nm. To relate absorbance vs. concentration, two calibration curves of ten points were used (R^2>0.99) from 0 to 20 mg H_2O_2/L and from 10 to 100 mg H_2O_2/L; and the relationship was linear [40]. Additional H_2O_2 measurements were performed right after the treatment with peroxide tests (colorimetric test strips method, 0.5–25 and 1-100 mg/L H_2O_2 Merckoquant-Merck).

2.2 Water matrices and microbiological procedures

The experiments were carried out with two kinds of water: (i) phosphate-buffered distilled water (DW) and (ii) saltwater (SW) prepared by adding 35g/L of sea salt (natural sea salt from the "Unión Salinera de España, S.A." salt works) to Milli-Q® water. In table 1 some physicochemical characteristics of the waters used in the experiments are shown. UV_{254} transmittance (Jenway 7315 spectrophotometer), pH and conductivity at 20ºC of the samples were measured before and after each experiment (Crison Multimeter MM41).

The pure bacterial strain used was *Enterococcus faecalis* (ATCC 27285) provided by the Spanish Type Culture Collection (CECT) and preserved in glycerol at -20ºC. According to previous studies [23, 41]: 1 mL of a pure bacterial strain was shaken for 10 s, then it was added to Brain and Heart Infusion Broth (Scharlab) and incubated at 37ºC. After 24 h of incubation, 1 mL from suspension was sub-cultured for 48 h at 37ºC, obtaining the exponential phase for bacterial inoculums. The cells were centrifuged during 10 min at 3000 rpm; the pellet obtained was washed with peptone solution (10 %) and it was re-suspended in 50 mL of sterile Milli-Q® water, which resulted in the final inoculant to be added to the water matrix. The inoculated matrix was prepared by suspended bacterial cells in different water matrices obtaining a concentration between 10^6-10^7 CFU/mL. The solution was kept under stirring for 40 min in order to provide a time for bacteria acclimatization before starting the experiment.

208 The membrane filtration method was applied for determining bacterial concentration
209 after treatments [23,41]. In order to obtain the appropriate number of CFUs, decimal
210 serial dilutions from each sample were plated in triplicate. Slanetz & Barley Agar Base
211 (Scharlab) was used as a selective medium with TTC indicator for Petri dishes. The
212 samples were incubated at 37 ºC during 48 h; after this time the developed CFUs were
213 counted and those whose values were between 20 and 150 CFUs were considered as
214 valid data. Possible changes in bacterial population were taken into account during all
215 disinfection procedures. Sterile conditions were monitored through plating blank
216 samples during the microbiological analysis process.

217 ***2.3 Reactor description and UV Dose calculation***

218 *2.3.1 Collimated Beam Reactor*

219 A Collimated Beam Reactor (CBR) was designed and built (Fig. 1-A) [23,37,42]: the
220 light source was a 10 W UV-C low-pressure lamp (Wedeco Rex UV systems),
221 considering UV-C output (254 nm) of 2.9 W as provided by manufacturer and
222 according to the specific percentage applied to input power [43]. The distance from the
223 lamp to solution surface (L) was 20 cm with 5 cm of outer diameter.

224 The dose is considered as the product of exposure time and UV-C light intensity;
225 including several factors that affect it in the collimated beam according to Equation (2)
226 [36,42]. UV_{254} intensity on the sample surface was measured before and after irradiating
227 the sample during each test by a radiometer (PCE-UV36, PCE-Iberica).

228 $$D_{CBR} = E_s \cdot P_f \cdot (1\text{-R}) \cdot \frac{L}{(\text{d} + \text{L})} \cdot \frac{(1 - 10^{-A_{254} \cdot d})}{A_{254} \cdot d \cdot \ln (10)} \cdot t \quad (\text{mJ/cm}^2) \qquad (2)$$

229 *2.3.2 Continuous Flow Reactor*

230 Dynamic experiments were carried out in a UV Continuous Flow Reactor (CFR) with a
231 volume of 0.31 L and a reactor diameter of 4.4 cm (Fig. 1-B)[23]. The UV-C lamp used
232 was the same as for the CBR and was isolated from water by a quartz sleeve (2.4 cm of
233 external diameter).

234 The UV dose (D_{CFR}) was applied in a single exposure (Eq. (3)), as a function of mean
235 intensity (I_m) and Theoretical Retention Time (TRT) [44]. Previous experiments with
236 salt tracer indicated that this system works similarly to plug-flow using flow rates over
237 162 L/h [23].The integral term is for transverse section of the CFR. The values in all

238 experimental ranges with flow rate of 170-1200 L/h correspond to a TRT of 1.63-0.24 s

239 and doses of $35.00 - 4.50$ mJ/cm^2.

240 $$D_{CFR} = \text{TRT} \cdot I_m = \text{TRT} \cdot \frac{P \cdot T_q^e}{2\, L_L \pi^2 (r_r^2 - r_q^2)} \iint^{r - r_q} \frac{T_w}{r} \, dx \, dy \, (mJ/cm^2)$$ (3)

241 ***2.4 Experimental***

242 The experimental procedure consisted of applying UV and UV/H$_2$O$_2$ treatment, at

243 different UV-C doses in two kinds of water matrices inoculated with pure cultures of *E.*

244 *faecalis* at laboratory scale. The treatments were applied in several ways with two

245 different reactors. First, in the CBR (DW) the improvement of UV/H$_2$O$_2$ treatment was

246 compared to UV alone, adding different concentrations of hydrogen peroxide with the

247 aim to optimize the quantity of chemical. Then, a CFR (DW and SW) with UV alone

248 and UV/H$_2$O$_2$ treatment was used, with the aim to optimize the UV dose. Finally, the

249 possible dark repair after the treatment was monitored.

250 *2.4.1 Collimated Beam Reactor: Determining the optimum hydrogen peroxide*
251 *concentration*

252 Inoculated matrices of DW were subjected to the CBR at different doses of UV light

253 (either with or without H$_2$O$_2$). The addition of hydrogen peroxide was conducted in a

254 single dosage before the UV irradiation to reach different H$_2$O$_2$ concentrations in

255 solution: 5 mg/L, 10 mg/L, 30 mg/L, and 100 mg/L.

256 A 20 mL volume of inoculated matrix was transferred onto a glass Petri dish and the

257 light source was initiated immediately to start the test. Once the radiance on the water

258 surface reached a steady state (0.130 mW/cm^2), the samples were exposed to a UV

259 beam during exposition times between 20 s and 5 min; this corresponds to UV doses up

260 to 40 mJ/cm^2. All samples were gently stirred during collimated beam exposure.

261 The H$_2$O$_2$ present in treated samples was neutralized with catalase (Catalase, from

262 bovine liver; Sigma-Aldrich). The samples obtained from the CBR at specific UV dose

263 and H$_2$O$_2$ concentration were analyzed to obtain the microbiological concentration as

264 explained in subsection 2.2. In the same way, two untreated samples were analyzed in

265 each set of experiments for initial microbial concentration.

266 *2.4.2 Continuous Flow Reactor*

267   The optimal concentrations of H_2O_2 obtained from the CBR were tested in a CFR. UV

268   treatment was applied to a continuous water flow with and without H_2O_2, both in DW

269   and in SW.

270   Inoculated matrices were pumped into a single pass from a 10 L tank through the

271   reactor and immediately after, the samples were collected in sterile 500 mL flasks for

272   bacteria quantification. During all experimental procedure, the water in the tank was

273   well mixed before sampling and with rate of flow control to ensure that it did not

274   change. In order to prevent bacterial contamination at the reactor outlet, the collection

275   samples from each experiment started at low flow rates which means in high UV doses;

276   and all sampling procedure was performed in a period not exceeding 20 min.

277   The H_2O_2 present in the samples after treatment was neutralized with catalase. Samples

278   collected in 500 mL sterile flasks at specific UV dose and H_2O_2 concentration were

279   analyzed to obtain the microbiological concentration as explained in subsection 2.2.

280   Two untreated samples for each experiment were analyzed to obtain the initial

281   concentration for the survival curves.

282   *2.4.3 Evaluation of dark repair after treatment*

283   In this study, reactivation of bacteria after 1 h, 3 h and 24 h in the dark was measured

284   for UV alone and UV/H_2O_2 treatments in the CFR. Once the treatment was performed,

285   samples were placed in the dark at room temperature (18-25 ºC) and concentration of

286   bacteria was determined after 1 h, 3 h and 24 h.In these experiments the residual H_2O_2

287   was not neutralized in order to evaluate the possible effects after the treatment.

288   **2.5 Data treatment**

289   The raw data obtained from the CBR and CFR were similarly processed.

290   Microbiological concentration of each sample was measured by three replicates, using a

291   variation coefficient of less than 30 % as acceptance criteria. Data were rejected when

292   this coefficient was higher.

293   Disinfection efficiency was determined by logarithmic reduction of the survival of

294   microorganisms: log (N/N_0). The detection limit for disinfection efficiency was found to

295   be from a -6.3 to a -7.3 log reduction, since the starting concentration was between 10^6-

296   10^7 CFU/mL.

297   The model fitting was carried out by GinaFiT, a tool of Microsoft© Excel "for testing

298   different types of microbial survival models on experimental data" [38]. The Root Mean

299   Square Error (RMSE) (Eq. 4) is an informative measure of goodness of fit for

300   experimental data and can be applied to both linear and non-linear models. It is

301   calculated with experimental (y) and predicted values (x), degrees of freedom of

302   equation (k) and total number of data (n) [38,45]. RMSE was evaluated together with

303   the coefficient of determination (r^2), which was calculated on the basis of the sum of

304   squared errors (SSE) and the total sum of squares (SSTO) (Eq. 5) [38].

305      $$RMSE = \frac{\sqrt{\sum(x-y)^2}}{n-k} \qquad (4)$$

306      $$r^2 = 1 - \frac{SSE}{SSTO} \qquad (5)$$

307   Values between 0.25 and 0.50 for RMSE, and values above 0.90 for r^2, are considered

308   acceptable-fitting models [38,46]. The model parameters provided by GinaFiT, such as

309   disinfection rate constants, were evaluated to compare inactivation between the different

310   treatment tests. An important parameter is the estimated dose required to reduce the

311   viable bacteria by "n" magnitude orders (D_n). Parameters D_3 and D_4 were considered as

312   a way to easily compare disinfection efficiency between different conditions or sources.

313   To analyze dark repair assays, the % of bacterial repair was calculated according to

314   Equation (6); based on [47] and applied for these purposes [48].

315      $$\% \, repair = \frac{N_r - N}{N_0 - N} \cdot 100\% \qquad (6)$$

3. Results and discussion

The goal of this study was to evaluate the inactivation of $E.$ $faecalis$ with comparison of UV and UV/H_2O_2 treatment and the effects of different water matrices on disinfection efficiency.

3.1 Control tests: H_2O_2 and salinity effects.

Firstly, the effects of salinity and H_2O_2 alone on inactivation of $E.$ $faecalis$ were evaluated. For these purposes, different samples were taken as control tests for both DW and SW with a H_2O_2 dose of 10 mg/L, and were analyzed at regular intervals within 60 min. The same procedure was applied to a saltwater matrix without hydrogen peroxide.

Figure 2 represents the disinfection efficiency of these parameters within the time evaluated. Results obtained indicated slight bacterial mortality after SW matrix inoculation in comparison with DW, a little bit more pronounced in the presence of H_2O_2; but being in all cases less than one log reduction. Acclimatization was observed after 40 min of matrix inoculation, thus all disinfection assays were carried out after this stabilization time. According to these experiments, it is assured that all the effects found in the next steps are exclusively a result of UV/H_2O_2 treatment.

The results do not show relevant inactivation of $E.$ $faecalis$ by either H_2O_2 or salinity at 60 min contact time. They agree with previous works which established that neither hydrogen peroxide nor mechanical stress showed any significant inactivation of $Enterococcus$ sp [28,49]; this weak bactericidal activity of H_2O_2 was tested in other microorganisms using different concentrations and times with similar results [29,30,50].

3.2 H_2O_2 concentration: dose optimization

Different concentrations of hydrogen peroxide were chosen in order to evaluate them: 5, 10, 30 and 100 mg/L and were compared to no H_2O_2 concentration (0 mg/L), which means a UV treatment.

For these assays a CBR was used with DW. The results obtained from different H_2O_2 concentrations are showed in Figure 3 in which the disinfection efficiency versus the UV dose received is represented.

The best model to fit the experimental data obtained in the CBR was the biphasic model (Eq. (7)) [51]. This model assumes that within the microbiological disinfection process there are two phases: one phase which is easily inactivated and follows first-order

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347   kinetics and the other one which is more resistant to inactivation and describes a tailing

348   deviation with a small kinetic constant.

349   The corresponding model parameters were determined: first and second disinfection rate

350   constants (k_1, k_2) and the fraction of initial organisms that follows a fast disinfection

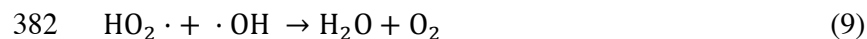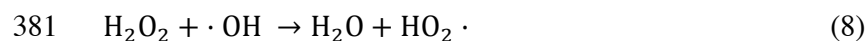351   rate constant (f). The RMSE was always between 0.25 - 0.30.

352    $$N=N_0\left[f \cdot e^{-k_1 D_{CBR}} + (1 - f)e^{-k_2 D_{CBR}}\right] \qquad (7)$$

353   In all of the cases the majority of the population (f > 99%) was inactivated by the first

354   (fast) order kinetic independently of the H_2O_2 concentration.The values of the first

355   kinetic constant (k_1) show that the addition of H_2O_2 improves the treatment in

356   comparison to the UV treatment alone (0 mg/L) i.e., the k_1 increases, as the

357   concentration of H_2O_2 is greater. In Figure 4 it can be observed that there is an

358   important increase in the k_1 as the H_2O_2 concentration is increasing until 10 mg/L with a

359   gradient value of 0.0470. On the other hand, when the H_2O_2 concentration is increasing

360   from 10 mg/L to 100 mg/L this value of the gradient is much lower (0.0015).Therefore,

361   the addition of more chemical is not associated with a major improvement of the

362   treatment. So, henceforth and considering the results obtained, 5 and 10 mg/L of H_2O_2

363   were considered as optimum concentrations.

364   The use of UV-C as a light source implies greater effectiveness in the process, in

365   contrast with other studies using UV-A and UV-B [28,49], with even the shoulder

366   phenomenon disappearing at initial times [49]. One reason for this is that the basis of

367   H_2O_2 breakdown is produced under wavelengths of less than 320 nm [28].The results

368   state that the addition of H_2O_2 improves the disinfection efficiency on *E. faecalis*; that

369   could be mainly because of the hydroxyl radical formation in the process, which directly

370   attack the cell wall [15,31]. It is also possible that the diffusion of H_2O_2 into the cell

371   increases cell permeability [52] with the consequence of a major effect of the treatment

372   that could result in bacterial sensitivity.

373   On the other hand, the results obtained from the biphasic model show a gradient value

374   reduction on k_1 (Fig. 4) at concentrations over 10 mg/L, which means inhibitory effects

375   of H_2O_2. This could be described because while hydrogen peroxide is at high

376   concentrations i.e., above optimal concentrations; additional reactions can appear (Eq.

377   (8), (9), (10)). H_2O_2 in excess can form $\cdot HO_2$ and O_2 with less oxidizing power

378   according to Equation (8) and Equation (9). Furthermore, when $\cdot OH$ is in high

379   concentrations, it can produce H_2O_2 again because of a recombination process (Eq.

380   (10)), which is unfavorable [53,54].

381    $H_2O_2 + \cdot OH \rightarrow H_2O + HO_2 \cdot$ (8)

382    $HO_2 \cdot + \cdot OH \rightarrow H_2O + O_2$ (9)

383    $\cdot OH + \cdot OH \rightarrow H_2O_2$ (10)

384   Therefore, it is necessary to find an optimal concentration of H_2O_2 for it to be able to act

385   as a promoter and not as scavenger of $\cdot OH$. In this respect, in order to avoid this

386   negative effect we found optimal concentrations up to 10 mg/L of H_2O_2 for disinfection

387   goals. These optimal concentrations agree with other experiments for disinfection

388   purposes, which determine the optimal concentration in the range 10-25 mg/L

389   [30,31,55]; lower than those experiments with degradation of organic compounds [27].

390   This low optimal concentration could be explained because microorganisms have

391   comparatively larger sizes than organic compounds, and could be more susceptible to

392   being attacked with less H_2O_2 [32].

393   *3.3 UV/H₂O₂ treatment in a Continuous Flow Reactor*

394   In this case, the UV/H_2O_2 treatment was evaluated in both DW and SW with a CFR.

395   The aim was to evaluate the behavior of the radiation using a dynamic system and to

396   optimize the best UV dose for the treatment with H_2O_2 concentration equal to: 0 mg/L

397   (UV alone), 5 mg/L and 10 mg/L; which were selected in subsection 3.2. The results are

398   represented in Figure 5.

399   It was observed that the addition of H_2O_2 improves the efficacy of the treatment, as in

400   CBR, but the kinetics is changed in relation to batch experiments. For a continuous

401   reactor the best fitting model is a log-linear regression [56] according to the Equation

402   (11). The parameters obtained from the model are shown in table 2, such as disinfection

403   rate constant (k_{max}), D_3 and D_4.

404    $N = N_0 \cdot e^{(-k_{max} \cdot D_{CFR})}$ (11)

405   In the best of the cases, the improvement of adding H_2O_2 increased efficacy by 33.3%

406   with 5 mg/L, 36.1% with 10 mg/L in DW and 28.9% with 5 and 10 mg/L in SW (based

407   on the increase in k_{max}). Parameters D_3 and D_4 reflect that a smaller UV dose is needed

408   to reach the same disinfection level.

409   Water salinity and H_2O_2 concentration (5 mg/L and 10 mg/L) did not produce any strong

410   interference in treatments (UV or UV/H_2O_2), obtaining similar results in both water

411   matrices and H_2O_2 concentrations. Some studies reflect that AOP-based treatments lost

412   effectiveness in sea water, because of the high ion concentrations [15,57]; in this case

413   there is a slight decrease in the improvement percentage (based on the increase in k_{max})

414   in SW with respect to DW, not more than 6 %. So, according to the results obtained, the

415   ions present in saltwater apparently did not affect the UV/H_2O_2 treatment. The same

416   case was reflected in other studies with *E. coli* (typical bacterial indicator)[31].

417   The fact of adding hydrogen peroxide results in an improvement of the disinfection

418   efficiency derived from hydroxyl radicals generated in the photolytic process; but in

419   these experiments it was observed that the increases of H_2O_2 concentration do not

420   influence the efficacy of the treatment at concentrations used, i.e. the same H_2O_2

421   concentration shows equal disinfection efficacy; unlike the CBR. That could be because

422   of the time of contact: one main distinction between CBR and CFR reactors is the

423   exposure time, since in batch experiments a longer exposure time with a weaker UV

424   light intensity is necessary in order to obtain the same UV dose as in CFR [46].

425   The application for ballast water involves water flow in the treatment, which

426   significantly reduces the exposure time in comparison with batch mode. However, the

427   volume of water exposed receives powerful light intensity, and it enhances the

428   formation of $\cdot OH$ via H_2O_2 photo-dissociation [58].

429   The results suggest that the UV/H_2O_2 process is a viable option for ballast water

430   treatment, with UV light intensity and contact time being the factors with major

431   influence on the photolysis process and the subsequent $\cdot OH$ formation.

432   ***3.4 Post-irradiation effects***

433   Due to the capacity of dark repair that some microorganisms have [20,31,41]; the

434   viability of growth after treatment was evaluated, saving samples in the dark in order to

435   simulate storage in a ballast tank. The growth results of *E. faecalis* surviving after

436   treatment in both DW and SW are presented in Figure 6, showing the evolution of the

437   bacteria concentration (Log N) up to 24 h after treatment.

16

438   The results obtained in both water matrices show that after UV treatment there is a

439   slightly bacterial growth in 24 h (about 1.5%- 4%). In relation to the UV/H_2O_2 process,

440   it can be observed that the bacterial concentration after treatment decreases by an order

441   of magnitude after 24 h. The main bacterial repair mechanism could be the catalase

442   enzymatic activity which detoxifies H_2O_2 [52, 59]. Nevertheless, the level of cellular

443   aggressions is apparently high in this treatment, and because of this the repair process

444   should be difficult after treatment [28]. On the other hand, this damage could allow the

445   residual H_2O_2 to enter into the bacterial cell [31]. The H_2O_2 can accumulate in *E.*

446   *faecalis*, causing a growth defect. This could be a major cause of cell damage after

447   treatment has been applied, resulting in growth inhibition [59]. Therefore, the reasons of

448   the behavior after UV/H_2O_2 treatment could be the severe damage produced in the cell

449   due to hydroxyl radicals or the accumulation of H_2O_2 in the cell.

450   These results are interesting with respect to a possible real application of ballast water

451   disinfection, since 24 h after the treatment the population could be reduced to one

452   additional logarithm, and this is critical for ballast water treatment, taking into account

453   that waters are kept in dark conditions during the journey.

454   ***3.5 Preliminary estimation of operation costs (Economic considerations)***

455   Once the effectiveness of the UV/H_2O_2 treatment was evaluated, the economic balance

456   of the process was estimated. To evaluate the best economic option, we used the

457   parameter D_4 as the disinfection goal, comparing UV treatment versus UV/H_2O_2

458   ([H_2O_2] =5 mg/L) treatment. In order to incorporate the benefits of the UV/H_2O_2 after

459   treatment; another kind of treatment assuming one log reduction after 24 hours was also

460   considered.

461   For simulation purposes, a hypothetical scenario evaluated for typical cargo vessels with

462   ballast water for vessel control with a standard pumping rate of 1000 m^3/h [1] was

463   considered. In order to calculate electrical consumption, a classic industrial lamp system

464   with 18 lamps (2650 W per lamp) [4] was simulated. The price of kWh is estimated at

465   0.3004€/kWh [60]. The chemical consumption was estimated as well; based on [61,62],

466   the price of H_2O_2 (30 %) is 0.39 €/kg. The data used for economic evaluation is shown

467   in Table 3.The price of a one-hour treatment has been estimated at 14 € for UV

468   treatment, 16 € for UV/H_2O_2 treatment and 14 € for UV/H_2O_2 + 24 h treatment.

469   According to these results and data, the cost of water treatment is not very significant

470   and the extra cost of using H_2O_2 in the treatment is not excessive plus.

471   *3.6 Considerations for full-scale application*

472   This study was conducted at laboratory scale to evaluate the process and mechanisms of

473   disinfection with UV/H_2O_2 treatment. Any Ballast Water Treatment System (BWTS)

474   has to be approved according to the G8 guideline of the BWM Convention [13], in

475   order to assess whether BWTSs meet the standard as set out in Rule D2. In this sense,

476   the results show that the treatment met the IMO D2 standards for BWTSs.

477   Furthermore, for systems using active compounds, an extra procedure is established

478   concerning human health, ship safety and aquatic environment, which is the G9

479   guideline of the BWM convention [13]. Ideally, BWTSs limit the use of active

480   compounds; however, the proposed treatment involves the use of active substances:

481   Hydrogen Peroxide. The aim of this study is to optimize the treatment in order to

482   achieve high efficacy with the smallest quantity of chemical possible. It was estimated

483   that 15 kg of H_2O_2 (30%) per 1000 m^3 is needed, which is a practical volume with the

484   added value that it is easy to store, readily available and relatively safe to handle [22].

485   Currently, there are already approved BWTSs in which H_2O_2 appears as active

486   substance e.g., Peraclean® [4].

487   In order for the process to be applied at full-scale with real seawater, it must take into

488   account the chemical composition of the water and some parameters that could interfere

489   with the treatment.

490   The presence of different planktonic organisms and the enzymatic mechanisms they

491   have, such as catalase, could exert a self-protecting action against H_2O_2. Catalase

492   detoxifies H_2O_2, it accelerates the dissociation into $H_2O + O_2$. However, the light

493   induces catalase inactivation [63], with the possibility to make plankton more

494   susceptible to peroxide at high UV doses. Some studies reported high inactivation rates

495   for both phytoplankton and zooplankton in the presence of H_2O_2 under UV light

496   [63,64].

497   On the other hand, water composition will interfere with bacterial inactivation [49]:

498   alkalinity and organic matter present in seawater are the main factors for UV

499   absorption/scattering and $\cdot OH$ scavenging [26,31]; this can result in a decrease in the

500   bactericidal effect. Some studies show that the process of competition between organic

501 compounds and microorganisms may decrease the effectiveness of the treatment by

502 approximately 20% [31], although the application of UV/H_2O_2 in all cases involves an

503 improvement compared with UV treatment alone [15,31]. However, some studies reflect

504 that dissolved organic carbon remained constant under $UV-H_2O_2$ treatments [49]; this

505 could result in non-significant changes in the physical-chemical parameters of the water

506 matrix [26], especially under optimal concentrations of H_2O_2. In future studies with real

507 seawater, it will be necessary to evaluate the process taking into account the amount and

508 nature of the organic matter in the water matrix; since there does not appear to be a

509 direct correlation between bacterial inactivation and the oxidation of organic matter

510 [26,31,49].

511

512   ## 4. Conclusions

513   A UV/H_2O_2 treatment with *E. faecalis* as indicator microorganism was evaluated and

514   optimized in laboratory scale as a viable treatment for ballast water. The main

515   conclusions obtained are shown below:

516   1. Increased disinfection efficiency of UV treatment is observed when hydrogen

517   peroxide is added. The UV/H_2O_2 treatment produces higher disinfection efficiency in all

518   cases. This means an increase in kinetic constants, which imply more speed and

519   effectiveness of disinfection and achieving similar results with a lower UV dose. The

520   application of an optimal concentration of hydrogen peroxide is necessary, because the

521   excess of H_2O_2 concentration makes it act as a scavenger. The optimal concentration

522   was found to be 5 mg H_2O_2/L.

523   2. The results for CBR evolution show different kinetics in relation to CFR. The

524   difference in dynamic disinfection for both treatments may be caused by the way that

525   the UV dose is applied, UV light intensity and contact time being the factors with major

526   influence on the photolysis process: in the CBR, the UV dose entails a longer exposition

527   time (minutes) with a weak UV light intensity, compared with CFR where the

528   exposition time is very short (seconds) but the UV intensity is greater. A ballast water

529   treatment must be done in continuous flow, thus the results obtained in the CFR are

530   more relevant in order to apply them to industrial scale.

531   3. According to the results, salinity does not have a major negative impact on treatment

532   and on the inactivation process. In addition, the effect of residual H_2O_2 present after the

533   treatment prevents subsequent growth of bacteria in stored water (as in a ballast tank);

534   reaching an additional one log reduction of population after 24 h.

535   4. Finally, the operational costs were estimated, and from the economic point of view,

536   UV/H_2O_2 treatment was considered to be competitive, since it could improve the

537   effectiveness of the process with similar costs: 14 €/1000 m^3 for UV treatment and 16

538   €/1000 m^3 for UV/H_2O_2 treatment; acquiring safer disinfection without excessive extra

539   costs.

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6. References

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7. Tables and Figure Captions

Tables

Table 1. Some physicochemical characteristics of water matrixes used in the experimentation.

Parameter	BufferedDistilledWater (DW)*	Saltwater (SW)*
pH	7.72 ± 0.15	8.00 ± 0.17
Conductivity at 20ºC (μS/cm)	82.61 ± 7.95	50443.30 ± 1107.06
Temperature (ºC)	22.66 ± 0.75	22.79 ± 0.19
Transmittance at 254 nm (%) **	96.68 ± 1.85	83.89 ± 0.65

*Average of samples
** Measurements compared with Milli-Q® Water

Table 2. Kinetic and statistical parameters predicted by fitting of disinfection experimental data for continuous flow reactor (CFR). Parameters were obtained for UV/H_2O_2 treatment applied in buffered distilled water (DW) or saltwater (SW).

[H_2O_2] (mg/L)	k_{max} (cm^2/mJ) ± S.E.	RMSE	R^2	D_4 (mJ/cm^2)	D_3 (mJ/cm^2)
DW					
0	0.36 ± 0.02	0.407	0.962	26.16	21.52
5	0.48 ± 0.06	0.471	0.919	19.21	15.09
10	0.49 ± 0.04	0.501	0.905	18.69	14.37
SW					
0	0.38 ± 0.01	0.366	0.980	24.64	22.44
5	0.49 ± 0.02	0.385	0.989	18.72	14.04
10	0.49 ± 0.03	0.255	0.974	18.74	16.57

Table 3. Consumption parameters and economical costs of the treatment in a theoretical possible scenario

Treatment		UV	UV/H_2O_2	UV/H_2O_2 + 24 h
UV-C Dose required (mJ/cm^2)*		24.64	18.72	14.04
Dose reduction (%)		--	24.03	43.02
Consumption	Lampconsumption (kWh/m^3)	0.047	0.034	0.026
	Chemical- H_2O_2 (kg/m^3)	0.000	0.015	0.015
Costs	Lamp (€/m^3)	0.014	0.010	0.008
	Chemical (€/m^3)	0.000	0.006	0.006
Total Costs (€/m^3)		**0.014**	**0.016**	**0.014**

*UV-C dose required to reach D_4 according to results shown in table 2.

Figure Captions

Figure 1. Schematic representation of the reactor set up. IW_m: Inoculated Water matrix; P: Pump; R: UV-Reactor; S_p: Sampling point. Intensity measuring point for CBR (A) was on S_p.

Figure 2. *E. faecalis* control tests, in both buffered distilled water (DW) and saltwater (SW) with presence and absence of hydrogen peroxide ($[H_2O_2]$ =10 mg/L). Time-survival curves.

Figure 3. Disinfection profiles for a collimated beam reactor on *E. faecalis* under UV and UV/H_2O_2 treatment, in buffered distilled water (DW). Symbols represent the average of experimental points at different H_2O_2 concentrations (mg/L) and lines show a fit by biphasic model. The RMSE was always in the range 0.25-0.30 and $R^2 > 0.92$.

Figure 4. Evolution of k_1 obtained for *E. faecalis* from biphasic model at different H_2O_2 concentration under UV-C irradiance. Standard Error (SE) is represented by error bars.

Figure 5. Disinfection profiles for a continuous flow reactor on *E. faecalis* under UV and UV/H_2O_2 treatment, in buffered distilled water, DW (A) and saltwater, SW (B). Symbols represent the average of experimental points at different H_2O_2 concentrations (mg/L) and lines show a fit by log-linear model.
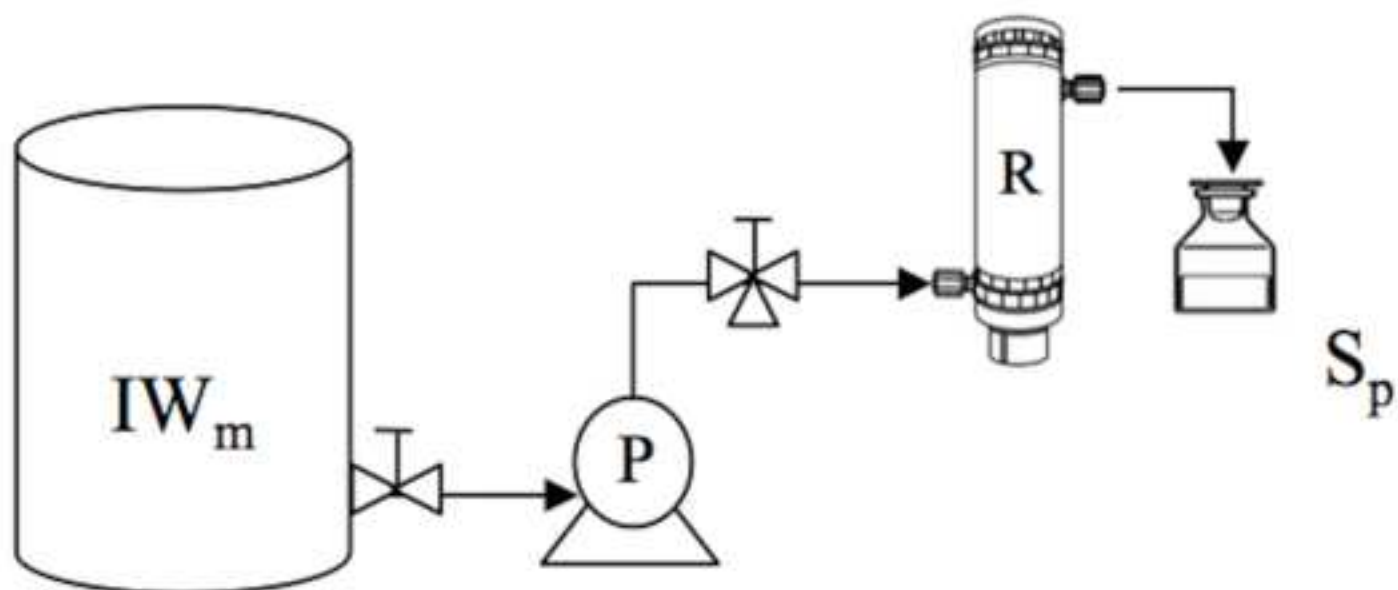
Figure 6. Results on dark growth for surviving *E. faecalis* after UV-treatment (A) and UV/H_2O_2 treatment (B). Circles represent buffered distilled water (DW) and triangles represent saltwater (SW).

A. Collimated Beam Reactor

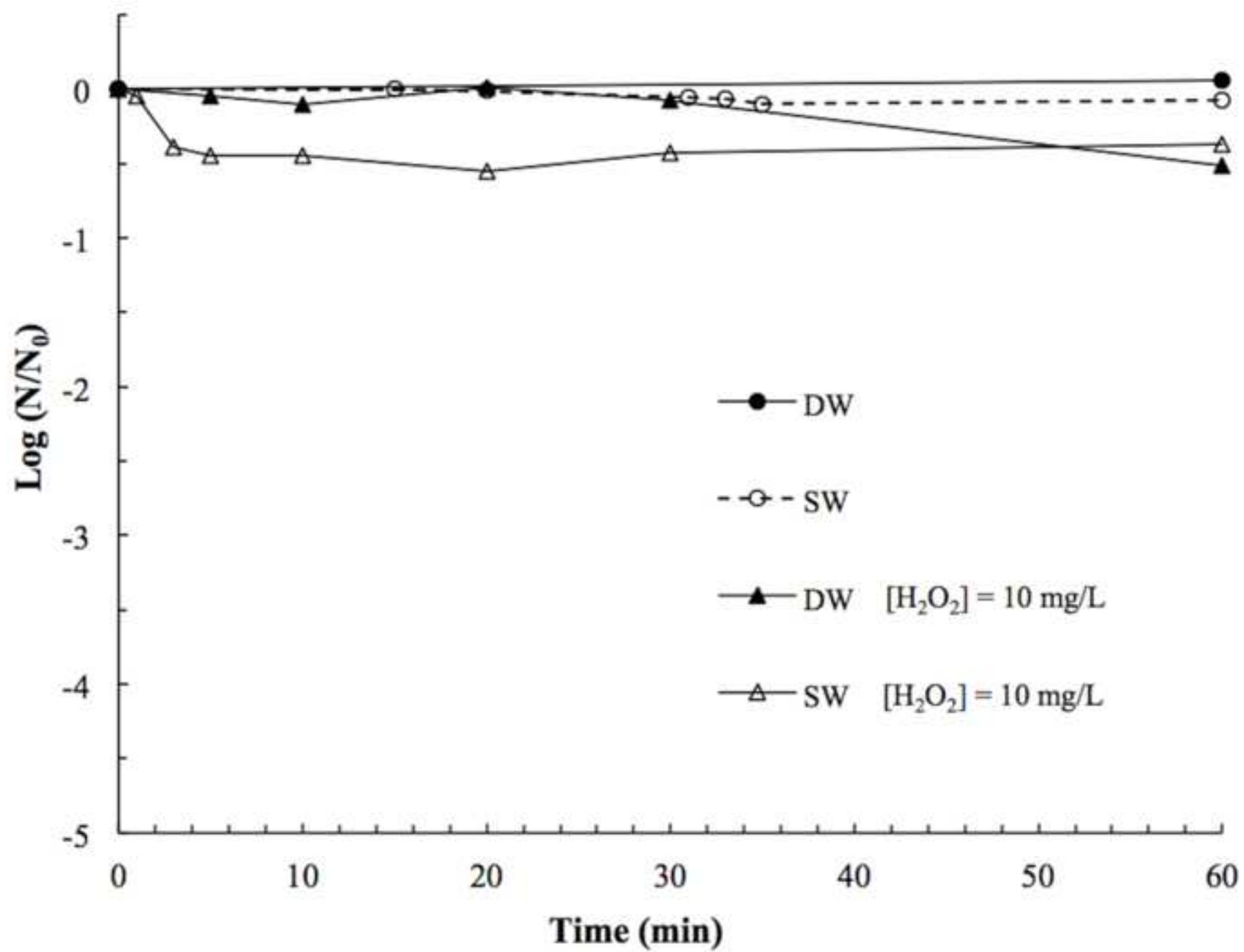UV-C lamp

R

S_p

IW_m
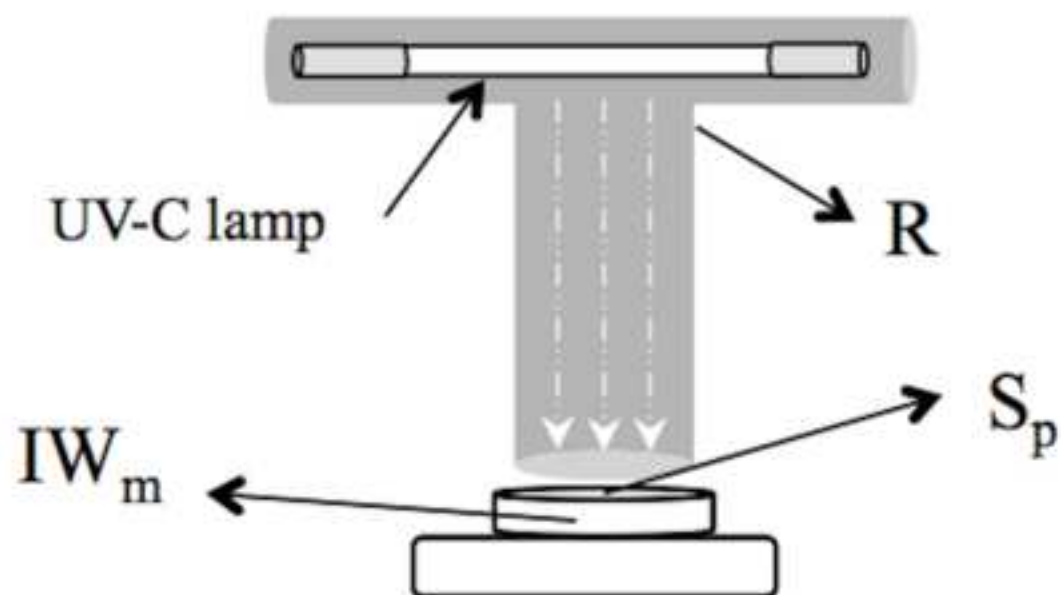
B. Continuous Flow Reactor

R

S_p

IW_m

P

FIG3_TIFF
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