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Photoelectrocatalytic disinfection of water and wastewater

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6   **Photoelectrocatalytic disinfection of water and wastewater: performance evaluation by qPCR**

7   **and culture techniques**

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10   Danae Venieri[1*], Efthalia Chatzisymeon[1], Eleonora Politi[1], Spiridon S. Sofianos[1], Alexandros

11   Katsaounis[2], Dionissios Mantzavinos[1]

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13   [1] Department of Environmental Engineering, Technical University of Crete, Polytechneioupolis,

14   GR-73100 Chania, Greece

15   [2] Department of Chemical Engineering, University of Patras, GR-26504 Patras, Greece

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17   **Short title**: Photoelectrocatalytic disinfection of water and wastewater

[*] Corresponding author:

Email: danae.venieri@enveng.tuc.gr; Tel.: +302821037801; Fax: +302821037858

18 **Abstract**

19 Photoelectrocatalytic oxidation (PEC) was evaluated as a disinfection technique using water and

20 secondary treated wastewater spiked with *Escherichia coli* and *Enterococcus faecalis*. PEC

21 experiments were carried out using a TiO_2/Ti-film anode and a zirconium cathode under simulated

22 solar radiation. Bacterial inactivation was monitored by culture and quantitative PCR (qPCR).

23 Inactivation was enhanced increasing the duration of the treatment and decreasing bacterial

24 population and the complexity of the aqueous matrix. *E. coli* cells were reduced approximately 6

25 orders of magnitude after 15min of PEC treatment in water at 2V of applied potential and an initial

26 concentration of 10^7 CFU/mL; pure photocatalysis led to about 5 log reduction, while

27 electrochemical oxidation alone resulted in negligible inactivation. The superiority of PEC relative

28 to PC can be attributed to a more efficient separation of the photogenerated charge carriers.

29 Regarding disinfection in mixed bacterial suspensions, *E.coli* was more susceptible than *E.faecalis*

30 at a potential of 2V. The complex composition of wastewater affected disinfection efficiency,

31 yielding lower inactivation rates compared to water treatment. qPCR yielded lower inactivation

32 rates at longer treatment times than culture techniques presumably due to the fact that the latter do

33 not take into account the viable but not culturable state of microorganisms.

34

35 **Keywords:** *E. coli* · *E. faecalis* · Disinfection · qPCR · TiO_2 · Voltage

INTRODUCTION

The growing demand for clean water and wastewater, free from disease-causing pathogenic microorganisms, poses new challenges in the development of effective disinfection techniques. Over the last decades, advanced oxidation processes (AOPs) have been recognised as an emerging group of techniques, highly effective for the decomposition and mineralization of organic compounds in aqueous samples (Malato *et al.* 2009; Chong *et al.* 2010). TiO_2 photocatalysis is an important member of AOPs and its benefits regarding water and wastewater disinfection have been demonstrated with respect to *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus faecalis* (Chen *et al.* 2010; Chatzisymeon *et al.* 2011; Rémy *et al.* 2011; Venieri *et al.* 2011). In most photocatalytic applications the catalyst is employed as slurry of fine particles in a photochemical reactor, resulting in certain difficulties, such as post-reaction catalyst recovery and low quantum efficiencies (Koivunen & Heinonen-Tanski 2005; Egerton *et al.* 2006; Marugán *et al.* 2006). These problems may be addressed by immobilization of TiO_2 on a conducting support and application of a potential bias, so as to reduce the recombination of charge carriers, which is the main limitation of the process photonic efficiency (Marugán *et al.* 2009; Egerton 2011).

These modifications have led to the development of photoelectrocatalysis (PEC), which consists of the application of a constant bias anodic potential usually to a TiO_2-based thin film acting as a photoanode. The photoinduced electrons are then continuously extracted from the anode by an external electrical circuit to be injected into the cathode (Sirés & Brillas 2011). The constant current density or bias potential applied to the semiconductor electrode promotes the efficient separation of electron-hole pairs and accelerates the production of photogenerated oxidizing species onto the catalyst surface (Martínez-Huitle & Brillas 2009). In this view, it is not surprising that PEC has recently found a wide range of environmental applications, including degradation of organic pollutants and pharmaceuticals, detoxicifation of aqueous samples, wastewater remediation and microbial inactivation (Fraga *et al.* 2009; Martínez-Huitle & Brillas 2009; Nissen *et al.* 2009; Egerton 2011; Frontistis *et al.* 2011; Li *et al.* 2011; Sirés & Brillas 2011).

62   PEC seems to be a promising and efficient tool, regarding the effective inactivation of

63   pathogens which are contained in water and wastewater, contributing in the control of waterborne

64   diseases. The bactericidal function of this technique can be attributed to the oxidation properties of

65   photocatalytically generated active oxygen species (AOS), which cause damage to cellular

66   membrane and further destruction of bacterial structure (Li *et al.* 2011). The studies referred to PEC

67   as means of disinfection highlight the importance of certain parameters like applied voltage,

68   bacterial concentration, treatment time and the aqueous matrix, which are considered determining

69   factors of microbial inactivation (Martínez-Huitle & Brillas 2009). Generally, the overall evaluation

70   of PEC, as a method for disinfection, is mainly performed with the use of a faecal bacterial

71   indicator, applying conventional microbiological protocols, based colony-forming units (CFU)

72   counts on selective culture media (Egerton *et al.* 2006; Philippidis *et al.* 2010; Egerton 2011; Li *et*

73   *al.* 2011). Yet, the environment of PEC treatment may be considered rather stressful for microbial

74   populations, inducing their entrance to the viable but not culturable (VBNC) state. Within this state,

75   bacteria demonstrate metabolic activity and maintain their pathogenic features but they are not

76   recoverable in culture media, leading to false negative results (Muela *et al.* 2008). The application

77   of molecular methods surpasses this limitation, based on nucleic acid identification and

78   quantification. Among them quantitative PCR (qPCR) has proven to be sensitive yielding accurate

79   quantitative results and allowing for new approaches in waterborne pathogen research (Lee *et al.*

80   2006; Shannon *et al.* 2007).

81   The aim of this work was to investigate the inactivation of faecal indicators in water and

82   wastewater by means of photoelectrocatalytic oxidation, applying conventional cultural methods

83   and qPCR. This was implemented using representative strains of faecal indicator bacteria, namely

84   *Escherichia coli* and *Enterococcus faecalis*. The effect of operational conditions such as bacterial

85   concentration, potential, treatment time, and aqueous matrix were also examined.

86

4

MATERIALS AND METHODS

Bacterial strains and wastewater

The bacterial strains used as reference faecal indicators in the present study were Gram-negative *E. coli* ATCC 23716 and Gram-positive *E. faecalis* ATCC 14506 (American Type Culture Collection, Rockville, Md. USA). Suspensions of bacterial indicators were prepared in sterile distilled water, which was used as sample for water disinfection experiments. The concentration of bacterial cells in the suspension was estimated measuring its optical density at 600 nm (Shimadzu UV1240 spectrophotometer). The bacterial concentrations used in this study were within the range of 10^4 - 10^8 CFU/mL. Plate counts were also performed for accurate bacterial count.

Wastewater disinfection experiments were carried out in real wastewater collected from the outlet of the activated sludge unit (prior to chlorination) of the municipal wastewater treatment plant of Chania, W. Crete, Greece. Its main characteristics were determined according to Standard Methods (1999) as follows: the chemical oxygen demand and dissolved organic carbon were 26 and 7.8 mg/L, respectively, the concentration of chlorides, sulfates, nitrates, nitrites, bicarbonates and total solids were 222.1, 60.3, 25.9, 57.1, 182.1 and 7 mg/L, respectively, while the pH was 7.8. Sterilization of the sample was performed prior to any photoelectrocatalytic treatment in order to adjust the initial bacterial concentration at the desired level.

Disinfection experiments

Photocatalytic experiments were performed using a solar radiation simulator (Newport, model 96000) equipped with a 150 W xenon ozone-free lamp and an Air Mass 1.5 Global Filter (Newport, model 81094), simulating solar radiation reaching the surface of the earth at a zenith angle of 48.2∘. According to the spectral irradiance data given by the manufacturer, simulated solar radiation contains about 5% UV-A radiation, and 0.1% UV-B radiation, while the filter cuts radiations with wavelengths lower than 280 nm. The incident radiation intensity on the

113 photochemical reactor in the UV region of the electromagnetic spectrum was measured

114 actinometrically using 2-nitrobenzaldehyde (Sigma-Aldrich) as the chemical actinometer (Willett &

115 Hites 2000; Galbavy *et al.* 2010) and it was found to be 5.8×10^{-7} einstein/(L×s), which corresponds

116 to an irradiance of 1.31×10^{-2} W/m^2. Reactions took place in an open, double-walled, cylindrical

117 glass vessel under continuous stirring.

118 Constant potentials (2 and 5V) were applied between the TiO_2/Ti anode and the zirconium

119 cathode using a galvanostat-potensiostat (Amel Instruments, model 2053). Details concerning the

120 experimental setup, as well as the preparation and characterization of the TiO_2/Ti-film are given

121 elsewhere (Frontistis *et al.* 2011). In brief, the anode surface was 5.8 cm^2 and had 75:25

122 anatase:rutile phase composition. The reactant mixture had a liquid holdup of 60 mL. The reaction

123 temperature was maintained constant at 25 ± 1°C with a temperature control unit. All experiments

124 were conducted in the presence of 1% (w/v) Na_2SO_4 aqueous solution serving as the electrolyte.

125 Water samples were processed applying (a) photoelectrocatalytic treatment, (b)

126 photocatalytic treatment (PC: solar radiation alone) and (c) electrochemical oxidation (EO:

127 application of voltage only in dark conditions). Photoelectrocatalytic treatment was also applied for

128 disinfection of wastewater samples. All disinfection experiments were performed in triplicate. In a

129 typical experiment, the inoculated aqueous solution was loaded to the cell, left in the dark under

130 stirring for 20 min in order to equilibrate and then exposed to solar radiation and/or potential. At

131 specific time intervals about 3 mL of the reaction solution were withdrawn. Half of the quantity was

132 immediately analyzed with respect to viable bacterial cells applying conventional culture method

133 and the rest was used for DNA extraction and PCR amplification.

134

135 **Culture method**

136 The detection and enumeration of *E. coli* and *E. faecalis* in the solution were performed

137 using the serial dilution streak plate procedure. The media used in the study were HiCrome

138 Coliform Agar (HiMedia Laboratories) and Slanetz & Bartley medium (OXOID) for *E. coli* and *E.*

139 *faecalis*, respectively. Incubation took place at 37°C. Bacterial counts were performed after 20-24 h

140 and 48 h for *E. coli* and *E. faecalis* determination, respectively.

141

DNA extraction

143 Genomic DNA was extracted performing chemical lysis and phenol/chloroform/isoamyl

144 alcohol (25:24:1) extraction. Namely, the cells were spun for 2 min and were lyzed for 1 h at 37°C

145 with 300 µL of lysozyme lysis buffer (100 mM NaCl, 500 mM Tris [pH 8], lysozyme 10 mg/mL)

146 and 3 µL of 20 mg/ml proteinase K. Then, 200 µL of SDS lysis buffer (100 mM NaCl, 500 mM Tris

147 [pH 8], 10% [w/v] SDS) were added, followed by incubation at 65°C for 10 min. The solution was

148 extracted with 750 µL of chloroform/isoamyl alcohol (24:1), spun, and the aqueous phase was re-

149 extracted with phenol/chloroform/isoamyl alcohol (25:24:1). Ethanol purification step was

150 performed and the quantity and purity of all DNA samples were determined measuring their

151 absorbance at 260 nm and estimating the ratio of absorbance values at 260 nm and 280 nm,

152 respectively.

153

Monitoring of *E. coli* and *E. faecalis* through qPCR

155 The SYBR green method was chosen for the quantification of both bacterial indicators using

156 the StepOne Plus System (Applied Biosystems Inc., Foster City, CA, USA). The gadAB gene was

157 used as a target for *E. coli* quantification through qPCR, and the primers were as follows: forward

158 primer 5´-GCG TTG CGT AAA TAT GGT TGC CGA-3´ (gadrt-1) and reverse primer 5´-CGT

159 CAC AGG CTT CAA TCA TGC GTT-3´ (gadrt-2) (Chen *et al.* 2006). The product size for this

160 primer set is 305 bp. The set of primers for *E. faecalis* detection and quantification was designed

161 according to the sequences of 16S rRNA, which are available in GenBank (Bartosch *et al.* 2004).

162 The primer pair contained a forward primer 5´-AACCTACCCATCAGAGGG -3´ (Efs130F) and a

163 reverse primer 5´-GACGTTCAGTTACTAACG -3´ (Efs490R), which yield a 360 bp PCR product.

164 Triplicate PCR reactions were carried out with Quantimix Easy SYG Kit (Biotools) to a final

165   volume of 20 μL. The mixed qPCR solution contained 2XPCR master mix, 0.5 mM of each primer,

166   30 nM of reference dye (Rox) all diluted to the final volume of the reaction mixture with

167   DNase/RNase free water. PCR reactions were carried out at a temperature profile of 10 min initial

168   denaturation at 95°C, followed by 40 cycles each of denaturation at 95°C for 0.5 min, annealing at

169   57°C for 1 min, and extension at 72°C for 0.5 min. Deionized water and DNase-treated *E. faecalis*

170   and *E. coli* served as negative controls. Also, melt curve analyses were conducted from 55 to 95°C.

171   To determine the detection sensitivity of the qPCR assay, a series of 10-fold diluted pure

172   culture genomic DNA of each bacterium was tested for qPCR amplification and cycle threshold

173   (C_T). Standard curves were generated with *E. coli* and *E. faecalis* DNA.

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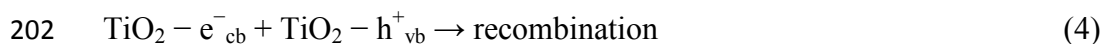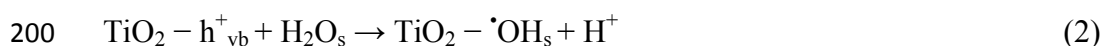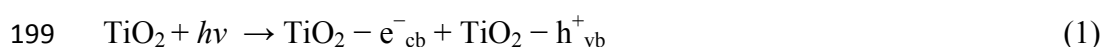
175   **RESULTS AND DISCUSSION**

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177   **Efficiency of photoelectrocatalysis in relation to photocatalysis and electrochemical oxidation**

178   The decrease of bacterial population in water samples was investigated under three distinct

179   conditions: (a) photoelectrocatalytic treatment (PEC: simultaneous application of solar radiation and

180   2V bias potential); (b) photocatalytic treatment (PC: solar radiation alone); (c) electrochemical

181   oxidation (EO: application of voltage only in dark conditions).

182   The results, summarized in Fig. 1, show that the application of a 2V potential during PEC increased

183   the extent of disinfection compared to PC. For instance, PEC led to a 5.9 log reduction after 15 min,

184   with the respective values of PC and EO being 4.9 and 0.2. However, both PEC and PC resulted in

185   an approximately 7 log reduction after 120 min of treatment, while in the absence of radiation

186   bacterial inactivation was inadequate (i.e. 1.5 log reduction); this clearly shows that EO is not a

187   suitable disinfection process at the conditions under consideration as it is not able to achieve total *E.*

188   *coli* inactivation in aqueous suspensions. Control runs were also performed in the absence of

189   radiation and potential showing no change in bacterial population after 120 min of contact time

190   (data not shown).

191   Illumination of a semiconductor-electrolyte interface with photons having energy greater

192   than its band gap energy generates electron-hole pairs at the anode electrode surface. The

193   simultaneous application of a bias positive to the flat-band potential produces a bending of the

194   conduction and valence bands which, in turn, causes a more effective separation of the

195   photogenerated carriers within the space charge layer (Morrison 1980). The potential gradient

196   forces the electrons towards the cathode, thus leaving the photogenerated holes to react at the anode

197   with H_2O and/or OH^- to yield hydroxyl radicals, i.e.:

198   *Anode (working electrode):*

199    $TiO_2 + hv \rightarrow TiO_2 - e^-_{cb} + TiO_2 - h^+_{vb}$ (1)

200    $TiO_2 - h^+_{vb} + H_2O_s \rightarrow TiO_2 - {}^\bullet OH_s + H^+$ (2)

201    $TiO_2 - h^+_{vb} + OH^-_s \rightarrow TiO_2 - {}^\bullet OH_s$ (3)

202    $TiO_2 - e^-_{cb} + TiO_2 - h^+_{vb} \rightarrow$ recombination   (4)

203   *Cathode (counter electrode):*

204    $2H_2O + 2e^- \rightarrow H_2 + 2OH^-$ (5)

205   where the subscripts cb and vb denote the conduction and valence bands, respectively, h^+ and e^-

206   denote the photogenerated holes and electrons, respectively, while the subscript s refers to species

207   adsorbed onto the photoanode surface.

208   Solar radiation has the potential to inactivate bacterial cells. Moreover, in the presence of a

209   catalyst, disinfection is mainly achieved by the action of oxidative radicals released from irradiated

210   TiO_2 (Chen *et al.* 2010). PEC under sunlight radiation has better performance than PC since the

211   application of potential is believed to suppress the rate of electron-hole recombination, thus

212   enhancing photocatalytic rates (Baram *et al.* 2009). The holes which have been left by electrons are

213   accelerated into the semiconductor surface, where they are available to react with organisms in the

214   water. The obtained results are in accordance to other studies which highlight PEC as an efficient

215   disinfection method, in terms of inactivating mainly *E. coli* and Gram-negative bacteria (Philippidis

216   *et al.* 2010; Rahmawati *et al.* 2011). Generally, the benefits of electric field enhancement have been

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217   demonstrated not only for coliforms but also for *Clostridium perfringens* spores and the recalcitrant

218   *Cryptospiridium parvum* (Egerton *et al.* 2006).

219

220   **Effect of initial bacterial concentration**

221   The influence of the initial *E. coli* concentration on PEC disinfection ability is presented in

222   Fig. 2. Conventional culture technique (Fig. 2a) showed that the rate of PEC disinfection was

223   inversely proportional to *E. coli* concentration in water samples. Total inactivation was achieved in

224   relatively short treatment time (i.e. within approximately 15 min) only when bacterial inoculum

225   contained 10^5 CFU/mL, while at higher concentrations residual *E. coli* cells were determined even

226   after 60 min. For instance and at an initial concentration of 10^8 CFU/mL, a 5.5 log reduction was

227   achieved within 30-60 min of treatment, beyond which no further inactivation was recorded.

228   SYBR green qPCR was performed to detect the potential presence of live non-culturable

229   bacterial cells. Our effort was to evaluate the consistency of *E. coli* cells and taking into account

230   that cultivability is not synonymous of viability (Rémy *et al.* 2011). qPCR showed different periods

231   of microbial inactivation (Fig. 2b) compared to plate counts. Inactivation rates were quite similar

232   when initial genome copies were of 10^7 and 10^5 /mL, as there was no enhancement of the process

233   after 40 min of treatment. During PEC, cell injury and loss of viability are achieved as cellular

234   membrane is destructed through direct contact with the photoanode (Li *et al.* 2011). Furthermore,

235   radiation induces DNA lesions, damaging nucleic acids and making them functionless. The

236   hydroxyl radical (and other AOS), directly generated by this process, is the main cause of DNA

237   destruction, which in turn leads to cell death (Sinha & Häder 2002; Gogniat & Dukan 2007).

238   According to our results, even after 120 min of treatment, genome copies were detected and

239   quantified in the water sample, reaching a plateau without any further decrease. This profile of

240   genome copies in the reaction mixture could be attributed to the detection sensitivity of the method.

241   qPCR is acknowledged as a reliable and sensitive molecular method, whose detection limit may be

242   in the region of 100 fg of *E. coli* genomic DNA (Lleo *et al.* 2005; Shannon *et al.* 2007). However,

243 in the present study environmental samples were processed which may inhibit qPCR or restrict its

244 detection limits. On the other hand, qPCR seems to be valuable in detecting the non-culturable

245 bacterial strains, which are induced under the stressed conditions of PEC. The so-called viable but

246 non culturable (VBNC) state of microorganisms is very common when dealing with environmental

247 samples or disinfection techniques, making plating counts inadequate for accurate bacterial

248 enumeration. Bacteria in this state retain their metabolic activity and pathogenic features, posing

249 danger for public health, while they are not recoverable in standard culture media. On the contrary,

250 molecular biology methods are capable of detecting these strains contributing in a more reliable

251 microbial evaluation of environmental samples (Wéry et al. 2008). Nevertheless, certain attention

252 should be paid on designing PCR experiments, including the amplicon length of the reactions, since

253 it is strongly correlated to the recorded disinfection efficiency. In the present study, SYBR green

254 PCR reactions yielded a 305 bp product for *E. coli* identification and quantification, resulting in

255 reliable and acceptable evaluation of PEC disinfection efficiency (Süb *et al.* 2009). Also, other

256 points under consideration concerning qPCR is that reactions can be affected by nucleic acid

257 contamination, leading to false positive results and the formation of primer - dimers. Some of the

258 possible sources of contamination are cross-contamination between samples, contamination from

259 laboratory equipment and carryover contamination of amplification products and primers from

260 previous PCRs. Therefore, appropriate measures should be taken to increase the reactions

261 specificity and all experiments should be performed with extra care to minimize contamination

262 risks.

263

264 **Effect of mixed bacterial populations**

265　　　In a set of experiments, an attempt was made to evaluate the disinfection efficiency of PEC

266 at 2V in water samples containing two bacterial populations. In this sense, sterile water samples

267 were inoculated with either 10^7 CFU/mL *E. coli* or 10^7 CFU/mL *E. faecalis* or both. The results

268 from culture technique and qPCR are summarized in Fig. 3.

269   Screening the inactivation profiles of the tested bacterial strains individually, *E. coli* seems

270   to be less resistant than *E. faecalis* at the experimental conditions in question. This outcome, which

271   is more obvious in qPCR results, was quite expected and in agreement with other studies dealing

272   with the inactivation of various bacterial populations (Cho *et al.* 2011; Li *et al.* 2011). Enterococci

273   are Gram positive bacteria, possessing a thick peptidoglycane cell wall and an additional outer

274   membrane containing two lipid bilayers, which provide them high complexity and potential of

275   preserving their viability during PEC treatment. On the other hand, *E. coli* cells require longer

276   treatment periods so as to achieve equal inactivation rates. In this sense, attention should be paid to

277   the differences among main aquatic microbial indicators, which may lead to different PEC

278   inactivation efficiency (Cho *et al.* 2011).

279   When *E. coli* and *E. faecalis* were inoculated together in water samples, the extent of

280   inactivation for either strain was lower than that of their individual treatment. This may be

281   attributed partially to the elevated initial bacterial concentration, whose influence on PEC has been

282   discussed previously (Fig. 2). Other possible explanations would include the presence of

283   competitive microorganisms and the interaction amongst them and/or the competition for AOS

284   between the bacteria and the organic by-products, released to the solution by the inactivated

285   bacteria (Baram *et al.* 2009). In the present study, although PEC seems to be capable of inactivating

286   the used faecal indicators to a certain extent, the residual cells raise concerns about the suitability of

287   the method for disinfection of complex samples, containing various bacterial populations.

288   These findings are more obvious in qPCR results (Fig. 3b), which reveal longer periods

289   required for bacterial decrease. In the present study, considerable genome copies/mL were recorded

290   after 90 min of treatment, which is in contrast to plate counts. These extended periods could reflect

291   the time necessary to mutate DNA to a point that can no longer be assayed. Cells may have been

292   completely inactivated long before that point. On the other hand, concerns are raised about the

293   suitability of the PCR method to establish a correlation between amplified bacterial DNA and viable

294   *E. coli* and *E. faecalis*. However, because the half-life of the DNA released in the environment is

295 considered to be very short owing to the presence of numerous nucleases it might be deduced that

296 the DNA detected is that contained in non-culturable cells (Lleo *et al.* 2005).

297

Disinfection in real wastewater

299   PEC was also employed to disinfect biologically treated effluents taken just before the

300 chlorination step. Sterilized samples were inoculated with approximately 10^7 CFU/mL or 10^4

301 CFU/mL *E. coli* (Fig. 4). In this set of experiments, a higher value of applied potential (5V) was

302 chosen to highlight its effect on bacterial inactivation. Given that (i) the aqueous matrix was real

303 wastewater, which is generally considered as a "complex" sample containing various organic and

304 inorganic components and (ii) raising the anodic potential enhances photocatalytic rates (Baram *et*

305 *al.* 2009), runs were performed at 5V.

306   As seen in Fig. 4a, total *E. coli* inactivation occurred in almost 15 min when the initial cell

307 density was 10^4 CFU/mL and this increased to 90 min at 10^7 CFU/mL. The degree of disinfection

308 was also determined by means of qPCR, estimating bacterial genome copies remained in the

309 aqueous solution after treatment (Fig. 4b). Comparing findings from both procedures there was a

310 significant contrast, as already seen in previous experiments conducted in the present study. qPCR

311 showed that even after 90 min of PEC treatment at 5V potential, genome copies of the bacterial

312 indicator decreased by only 5 orders of magnitude, while colonies of *E. coli* were not recoverable at

313 the same time. Apart from factors such as the detection limit of the method or VBNC cells, which

314 have been mentioned previously, an important parameter under consideration is the particulate

315 matter present in wastewater. This aids in the resistance of microorganisms to disinfection, as it

316 may interfere by physically shielding bacterial cells and protecting the integrity of the contained

317 DNA. Therefore, the extent of DNA damage is limited, which inevitably results in high numbers of

318 genome copies detected by qPCR, even after long periods of treatment. In the present study,

319 considerable genome copies/mL were recorded, highlighting the importance of wastewater

320 composition and its bacterial content with respect to PEC efficiency. In this sense, plate counts

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321 should be matched to qPCR results in order to perform a reliable and complete evaluation of

322 disinfection techniques, such as PEC and other photocatalytic treatments.

323   Furthermore, part of the photogenerated AOS may be wasted to attack the organic carbon of

324 the wastewater (about 8 mg/L which typically consists of highly resistant humic-type compounds

325 and biomass-associated products) and/or scavenged by bicarbonates, sulfates and chlorides (whose

326 collective concentration is about 460 mg/L) rather than inactivate pathogens. This could be

327 overcome increasing AOS concentration through raising the applied potential (Baram *et al.* 2009)

328 and would possibly justify the nearly identical *E. coli* profiles shown in Figs. 2a and 4a at an initial

329 concentration of 10^7 CFU/mL.

330

Conclusions

332 • Photocatalytic inactivation of *E. coli* in water samples is enhanced applying a positive potential

333   on TiO_2/Ti-films under simulated solar radiation. At the conditions in question, initial bacterial

334   density plays an important role, as it affects adversely the required treatment time for complete

335   microbial inactivation.

336 • *E. faecalis* are more resistant than *E. coli* bacteria during PEC treatment in water samples.

337   Furthermore, the disinfection efficiency is directly affected by the sample composition and the

338   contained mixed bacterial populations. In complex samples, bacteria are more physically

339   protected, resulting in lower inactivation rates.

340 • There was a considerable contrast between colony counts and qPCR results, concerning the

341   required time for total bacterial inactivation. qPCR data revealed longer periods required for

342   complete bacterial inactivation, compared to the time estimated by culture method. Stressed

343   conditions generated during PEC treatment may induce VBNC state of bacteria, which could

344   explain the discrepancy between the applied quantitative methods. Additionally, in order to

345   establish the accuracy and reliability of qPCR certain factors should be under consideration.

346   These include detection limits of the method, amplicon size, primer sequence, cycling conditions

347   and inhibitors present in the processed sample.

348   • For large scale applications, the use of sunlight as renewable energy source would undoubtedly

349   be beneficial to induce photocatalytic reactions, as well as provide the necessary electric field,

350   thus promoting the principle of sustainable development.

351

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Figure captions

456

457 **Figure 1**

458 *E. coli* inactivation in water samples during PEC, PC and EO. Conditions: 10^7 CFU/mL initial

459 bacterial concentration; 2V potential.

460 **Figure 2**

461 *E. coli* inactivation in water samples containing various initial bacterial concentrations during PEC

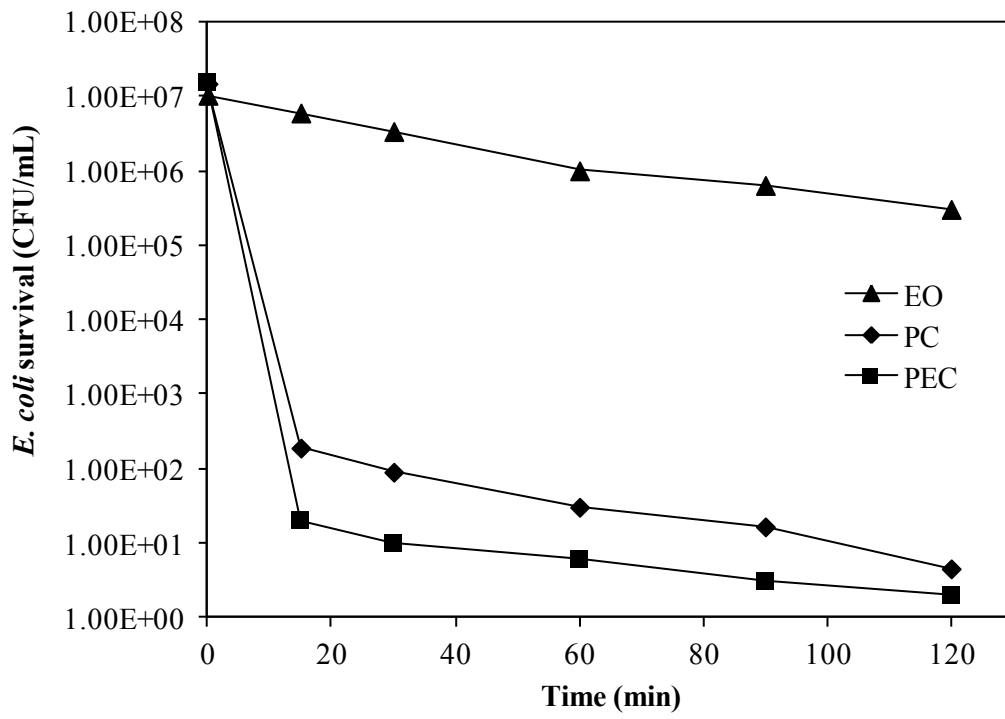462 (2V potential), assessed by (a) the culture technique and (b) qPCR.

463 **Figure 3**

464 *E. coli* and *E. faecalis* inactivation in water samples during PEC (2V potential), assessed by (a) the

465 culture technique and (b) qPCR.

466 **Figure 4**

467 *E. coli* inactivation in real wastewater during PEC (5V potential), assessed by (a) the culture

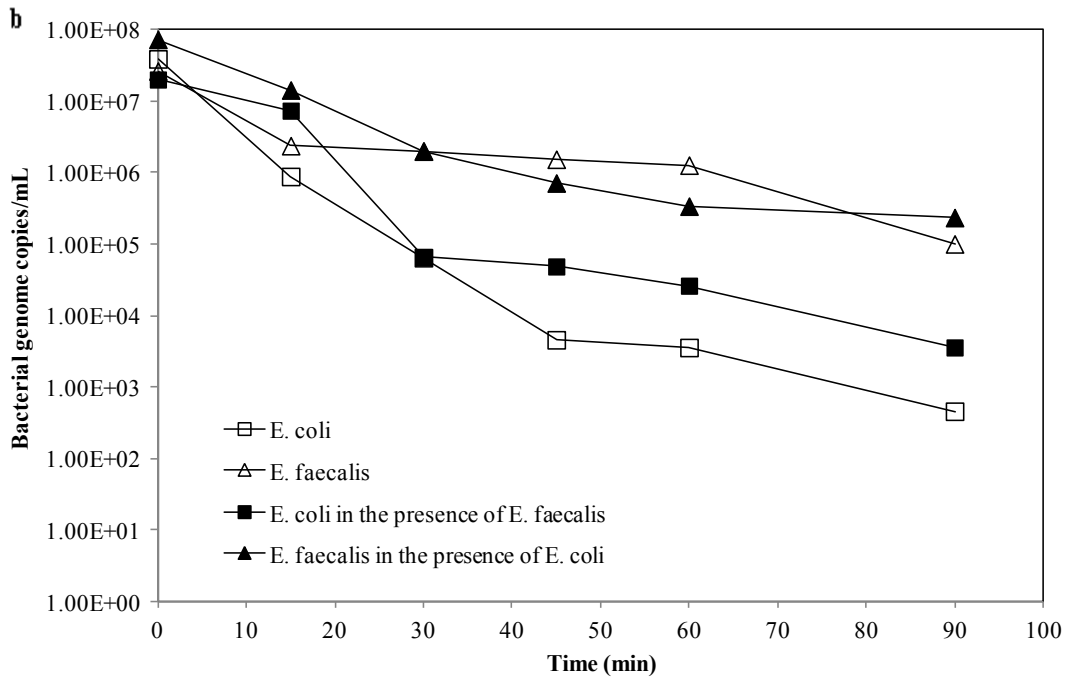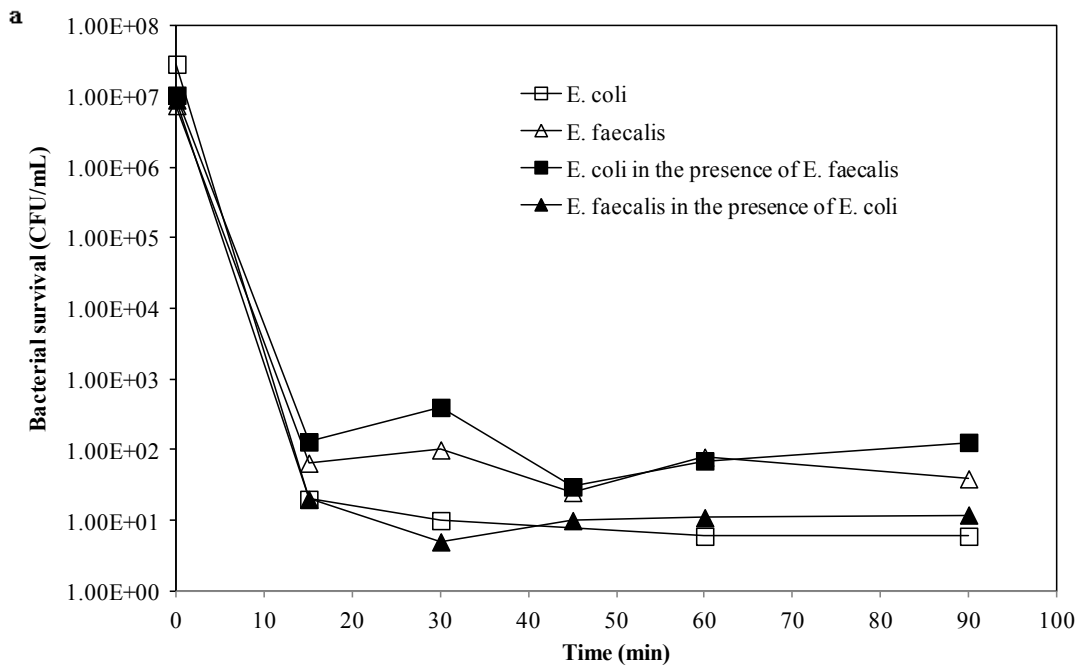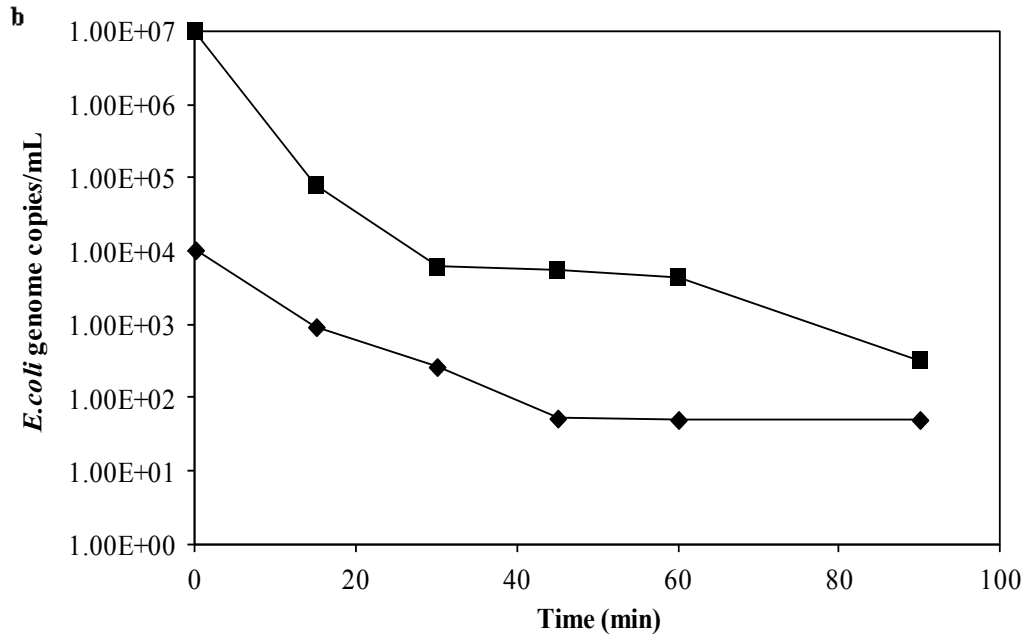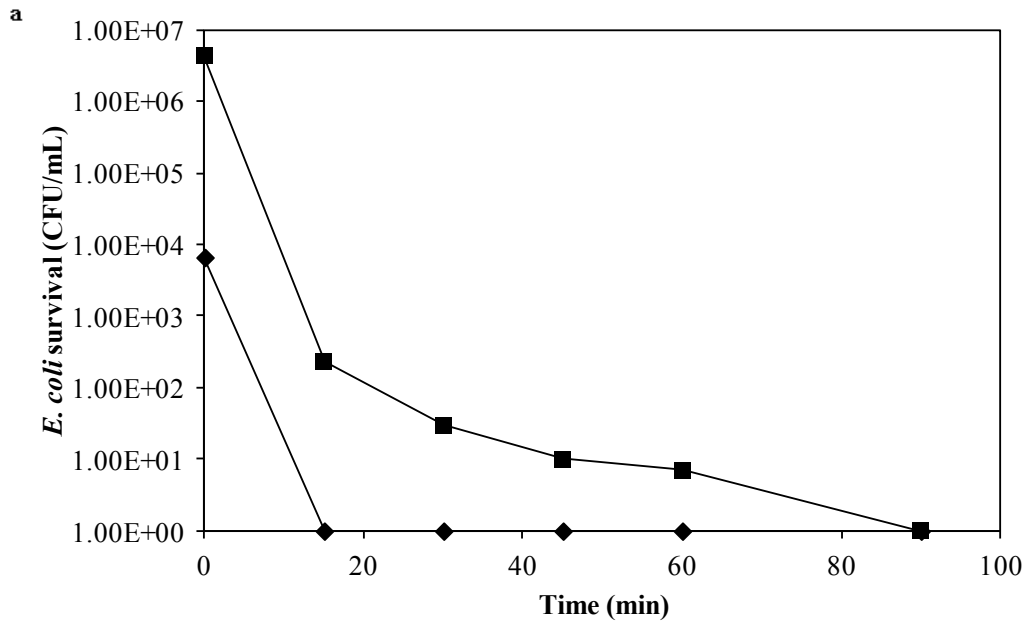468 technique and (b) qPCR.

469   **Figure 1**

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472   **Figure 2**
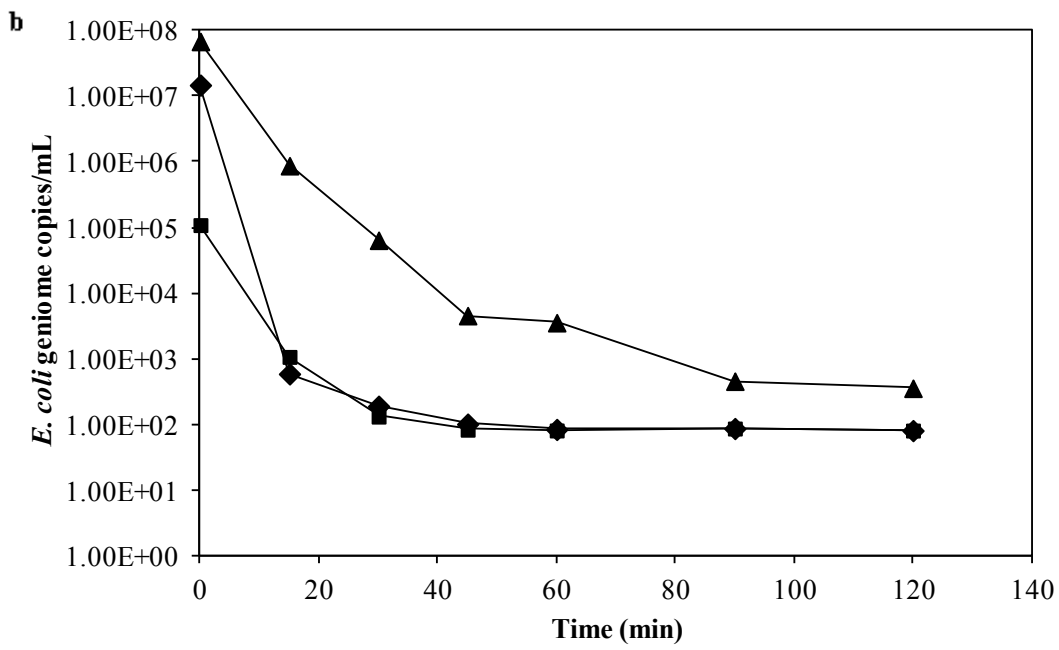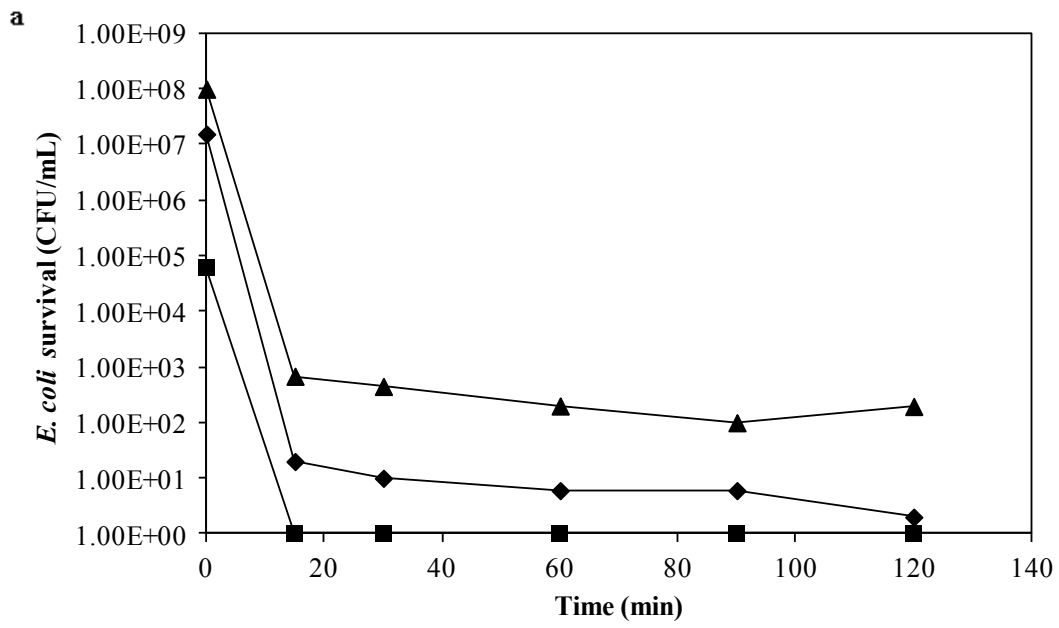
475   **Figure 3**

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478   **Figure 4**

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