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Photoelectrocatalytic disinfection of water and wastewater: performance evaluation by qPCR and culture techniques

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Abstract

Photoelectrocatalytic oxidation (PEC) was evaluated as a disinfection technique using water and secondary treated wastewater spiked with *Escherichia coli* and *Enterococcus faecalis.* PEC experiments were carried out using a TiO_2/Ti-film anode and a zirconium cathode under simulated solar radiation. Bacterial inactivation was monitored by culture and quantitative PCR (qPCR). Inactivation was enhanced increasing the duration of the treatment and decreasing bacterial population and the complexity of the aqueous matrix. *E. coli* cells were reduced approximately 6 orders of magnitude after 15min of PEC treatment in water at 2V of applied potential and an initial concentration of 10^7 CFU/mL; pure photocatalysis led to about 5 log reduction, while electrochemical oxidation alone resulted in negligible inactivation. The superiority of PEC relative to PC can be attributed to a more efficient separation of the photogenerated charge carriers. Regarding disinfection in mixed bacterial suspensions, *E.coli* was more susceptible than *E.faecalis* at a potential of 2V. The complex composition of wastewater affected disinfection efficiency, yielding lower inactivation rates compared to water treatment. qPCR yielded lower inactivation rates at longer treatment times than culture techniques presumably due to the fact that the latter do not take into account the viable but not culturable state of microorganisms.

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).

PEC seems to be a promising and efficient tool, regarding the effective inactivation of pathogens which are contained in water and wastewater, contributing in the control of waterborne diseases. The bactericidal function of this technique can be attributed to the oxidation properties of photocatalytically generated active oxygen species (AOS), which cause damage to cellular membrane and further destruction of bacterial structure (Li *et al.* 2011). The studies referred to PEC as means of disinfection highlight the importance of certain parameters like applied voltage, bacterial concentration, treatment time and the aqueous matrix, which are considered determining factors of microbial inactivation (Martínez-Huitle & Brillas 2009). Generally, the overall evaluation of PEC, as a method for disinfection, is mainly performed with the use of a faecal bacterial indicator, applying conventional microbiological protocols, based colony-forming units (CFU) counts on selective culture media (Egerton *et al.* 2006; Philippidis *et al.* 2010; Egerton 2011; Li *et al.* 2011). Yet, the environment of PEC treatment may be considered rather stressful for microbial populations, inducing their entrance to the viable but not culturable (VBNC) state. Within this state, bacteria demonstrate metabolic activity and maintain their pathogenic features but they are not recoverable in culture media, leading to false negative results (Muela *et al.* 2008). The application of molecular methods surpasses this limitation, based on nucleic acid identification and quantification. Among them quantitative PCR (qPCR) has proven to be sensitive yielding accurate quantitative results and allowing for new approaches in waterborne pathogen research (Lee *et al.* 2006; Shannon *et al.* 2007).

The aim of this work was to investigate the inactivation of faecal indicators in water and wastewater by means of photoelectrocatalytic oxidation, applying conventional cultural methods and qPCR. This was implemented using representative strains of faecal indicator bacteria, namely *Escherichia coli* and *Enterococcus faecalis.* The effect of operational conditions such as bacterial concentration, potential, treatment time, and aqueous matrix were also examined.

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

photochemical reactor in the UV region of the electromagnetic spectrum was measured actinometrically using 2-nitrobenzaldehyde (Sigma-Aldrich) as the chemical actinometer (Willett & Hites 2000; Galbavy *et al.* 2010) and it was found to be 5.8×10^{-7} einstein/(L×s), which corresponds to an irradiance of 1.31×10^{-2} W/m^2. Reactions took place in an open, double-walled, cylindrical glass vessel under continuous stirring.

Constant potentials (2 and 5V) were applied between the TiO_2/Ti anode and the zirconium cathode using a galvanostat-potensiostat (Amel Instruments, model 2053). Details concerning the experimental setup, as well as the preparation and characterization of the TiO_2/Ti-film are given elsewhere (Frontistis *et al.* 2011). In brief, the anode surface was 5.8 cm^2 and had 75:25 anatase:rutile phase composition. The reactant mixture had a liquid holdup of 60 mL. The reaction temperature was maintained constant at 25±1^{o}C with a temperature control unit. All experiments were conducted in the presence of 1% (w/v) Na_2SO_4 aqueous solution serving as the electrolyte.

Water samples were processed applying (a) photoelectrocatalytic treatment, (b) photocatalytic treatment (PC: solar radiation alone) and (c) electrochemical oxidation (EO: application of voltage only in dark conditions). Photoelectrocatalytic treatment was also applied for disinfection of wastewater samples. All disinfection experiments were performed in triplicate. In a typical experiment, the inoculated aqueous solution was loaded to the cell, left in the dark under stirring for 20 min in order to equilibrate and then exposed to solar radiation and/or potential. At specific time intervals about 3 mL of the reaction solution were withdrawn. Half of the quantity was immediately analyzed with respect to viable bacterial cells applying conventional culture method and the rest was used for DNA extraction and PCR amplification.

Culture method

The detection and enumeration of *E. coli* and *E. faecalis* in the solution were performed using the serial dilution streak plate procedure. The media used in the study were HiCrome Coliform Agar (HiMedia Laboratories) and Slanetz & Bartley medium (OXOID) for *E. coli* and *E.*

faecalis, respectively. Incubation took place at 37°C. Bacterial counts were performed after 20-24 h and 48 h for *E. coli* and *E. faecalis* determination, respectively.

DNA extraction

Genomic DNA was extracted performing chemical lysis and phenol/chloroform/isoamyl alcohol (25:24:1) extraction. Namely, the cells were spun for 2 min and were lyzed for 1 h at 37°C with 300 μL of lysozyme lysis buffer (100 mM NaCl, 500 mM Tris [pH 8], lysozyme 10 mg/mL) and 3 μL of 20 mg/ml proteinase K. Then, 200 μL of SDS lysis buffer (100 mM NaCl, 500 mM Tris [pH 8], 10% [w/v] SDS) were added, followed by incubation at 65°C for 10 min. The solution was extracted with 750 μL of chloroform/isoamyl alcohol (24:1), spun, and the aqueous phase was re-extracted with phenol/chloroform/isoamyl alcohol (25:24:1). Ethanol purification step was performed and the quantity and purity of all DNA samples were determined measuring their absorbance at 260 nm and estimating the ratio of absorbance values at 260 nm and 280 nm, respectively.

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

The SYBR green method was chosen for the quantification of both bacterial indicators using the StepOne Plus System (Applied Biosystems Inc., Foster City, CA, USA). The gadAB gene was used as a target for *E. coli* quantification through qPCR, and the primers were as follows: forward primer 5′-GCG TTG CGT AAA TAT GGT TGC CGA-3′ (gadrt-1) and reverse primer 5′-CGT CAC AGG CTT CAA TCA TGC GTT-3′ (gadrt-2) (Chen *et al*. 2006). The product size for this primer set is 305 bp. The set of primers for *E. faecalis* detection and quantification was designed according to the sequences of 16S rRNA, which are available in GenBank (Bartosch *et al.* 2004). The primer pair contained a forward primer 5′-AACCTACCCATCAGAGGG -3′ (Efs130F) and a reverse primer 5′-GACGTTCAGTTACTAACG -3′ (Efs490R), which yield a 360 bp PCR product. Triplicate PCR reactions were carried out with Quantimix Easy SYG Kit (Biotools) to a final

volume of 20 μL. The mixed qPCR solution contained 2XPCR master mix, 0.5 mM of each primer, 30 nM of reference dye (Rox) all diluted to the final volume of the reaction mixture with DNase/RNase free water. PCR reactions were carried out at a temperature profile of 10 min initial denaturation at 95^{o}C, followed by 40 cycles each of denaturation at 95^{o}C for 0.5 min, annealing at 57^{o}C for 1 min, and extension at 72^{o}C for 0.5 min. Deionized water and DNase-treated *E. faecalis* and *E. coli* served as negative controls. Also, melt curve analyses were conducted from 55 to 95°C.

To determine the detection sensitivity of the qPCR assay, a series of 10-fold diluted pure culture genomic DNA of each bacterium was tested for qPCR amplification and cycle threshold (C_T). Standard curves were generated with *E. coli* and *E. faecalis* DNA.

RESULTS AND DISCUSSION

Efficiency of photoelectrocatalysis in relation to photocatalysis and electrochemical oxidation

The decrease of bacterial population in water samples was investigated under three distinct conditions: (a) photoelectrocatalytic treatment (PEC: simultaneous application of solar radiation and 2V bias potential); (b) photocatalytic treatment (PC: solar radiation alone); (c) electrochemical oxidation (EO: application of voltage only in dark conditions).

The results, summarized in Fig. 1, show that the application of a 2V potential during PEC increased the extent of disinfection compared to PC. For instance, PEC led to a 5.9 log reduction after 15 min, with the respective values of PC and EO being 4.9 and 0.2. However, both PEC and PC resulted in an approximately 7 log reduction after 120 min of treatment, while in the absence of radiation bacterial inactivation was inadequate (i.e. 1.5 log reduction); this clearly shows that EO is not a suitable disinfection process at the conditions under consideration as it is not able to achieve total *E. coli* inactivation in aqueous suspensions. Control runs were also performed in the absence of radiation and potential showing no change in bacterial population after 120 min of contact time (data not shown).

Illumination of a semiconductor-electrolyte interface with photons having energy greater than its band gap energy generates electron-hole pairs at the anode electrode surface. The simultaneous application of a bias positive to the flat-band potential produces a bending of the conduction and valence bands which, in turn, causes a more effective separation of the photogenerated carriers within the space charge layer (Morrison 1980). The potential gradient forces the electrons towards the cathode, thus leaving the photogenerated holes to react at the anode with H_2O and/or OH^- to yield hydroxyl radicals, i.e.:

Anode (working electrode):

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

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

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

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

Cathode (counter electrode):

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

where the subscripts cb and vb denote the conduction and valence bands, respectively, h^+ and e^- denote the photogenerated holes and electrons, respectively, while the subscript s refers to species adsorbed onto the photoanode surface.

Solar radiation has the potential to inactivate bacterial cells. Moreover, in the presence of a catalyst, disinfection is mainly achieved by the action of oxidative radicals released from irradiated TiO_2 (Chen *et al.* 2010). PEC under sunlight radiation has better performance than PC since the application of potential is believed to suppress the rate of electron-hole recombination, thus enhancing photocatalytic rates (Baram *et al.* 2009). The holes which have been left by electrons are accelerated into the semiconductor surface, where they are available to react with organisms in the water. The obtained results are in accordance to other studies which highlight PEC as an efficient disinfection method, in terms of inactivating mainly *E. coli* and Gram-negative bacteria (Philippidis *et al.* 2010; Rahmawati *et al.* 2011). Generally, the benefits of electric field enhancement have been

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.

Screening the inactivation profiles of the tested bacterial strains individually, *E. coli* seems to be less resistant than *E. faecalis* at the experimental conditions in question. This outcome, which is more obvious in qPCR results, was quite expected and in agreement with other studies dealing with the inactivation of various bacterial populations (Cho *et al.* 2011; Li *et al.* 2011). Enterococci are Gram positive bacteria, possessing a thick peptidoglycane cell wall and an additional outer membrane containing two lipid bilayers, which provide them high complexity and potential of preserving their viability during PEC treatment. On the other hand, *E. coli* cells require longer treatment periods so as to achieve equal inactivation rates. In this sense, attention should be paid to the differences among main aquatic microbial indicators, which may lead to different PEC inactivation efficiency (Cho *et al.* 2011).

When *E. coli* and *E. faecalis* were inoculated together in water samples, the extent of inactivation for either strain was lower than that of their individual treatment. This may be attributed partially to the elevated initial bacterial concentration, whose influence on PEC has been discussed previously (Fig. 2). Other possible explanations would include the presence of competitive microorganisms and the interaction amongst them and/or the competition for AOS between the bacteria and the organic by-products, released to the solution by the inactivated bacteria (Baram *et al.* 2009). In the present study, although PEC seems to be capable of inactivating the used faecal indicators to a certain extent, the residual cells raise concerns about the suitability of the method for disinfection of complex samples, containing various bacterial populations.

These findings are more obvious in qPCR results (Fig. 3b), which reveal longer periods required for bacterial decrease. In the present study, considerable genome copies/mL were recorded after 90 min of treatment, which is in contrast to plate counts. These extended periods could reflect the time necessary to mutate DNA to a point that can no longer be assayed. Cells may have been completely inactivated long before that point. On the other hand, concerns are raised about the suitability of the PCR method to establish a correlation between amplified bacterial DNA and viable *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
298 **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

should be matched to qPCR results in order to perform a reliable and complete evaluation of disinfection techniques, such as PEC and other photocatalytic treatments.

Furthermore, part of the photogenerated AOS may be wasted to attack the organic carbon of the wastewater (about 8 mg/L which typically consists of highly resistant humic-type compounds and biomass-associated products) and/or scavenged by bicarbonates, sulfates and chlorides (whose collective concentration is about 460 mg/L) rather than inactivate pathogens. This could be overcome increasing AOS concentration through raising the applied potential (Baram *et al.* 2009) and would possibly justify the nearly identical *E. coli* profiles shown in Figs. 2a and 4a at an initial concentration of 10^7 CFU/mL.

Conclusions

- Photocatalytic inactivation of *E. coli* in water samples is enhanced applying a positive potential on TiO_2/Ti-films under simulated solar radiation. At the conditions in question, initial bacterial density plays an important role, as it affects adversely the required treatment time for complete microbial inactivation.
- *E. faecalis* are more resistant than *E. coli* bacteria during PEC treatment in water samples. Furthermore, the disinfection efficiency is directly affected by the sample composition and the contained mixed bacterial populations. In complex samples, bacteria are more physically protected, resulting in lower inactivation rates.
- There was a considerable contrast between colony counts and qPCR results, concerning the required time for total bacterial inactivation. qPCR data revealed longer periods required for complete bacterial inactivation, compared to the time estimated by culture method. Stressed conditions generated during PEC treatment may induce VBNC state of bacteria, which could explain the discrepancy between the applied quantitative methods. Additionally, in order to 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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455 **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

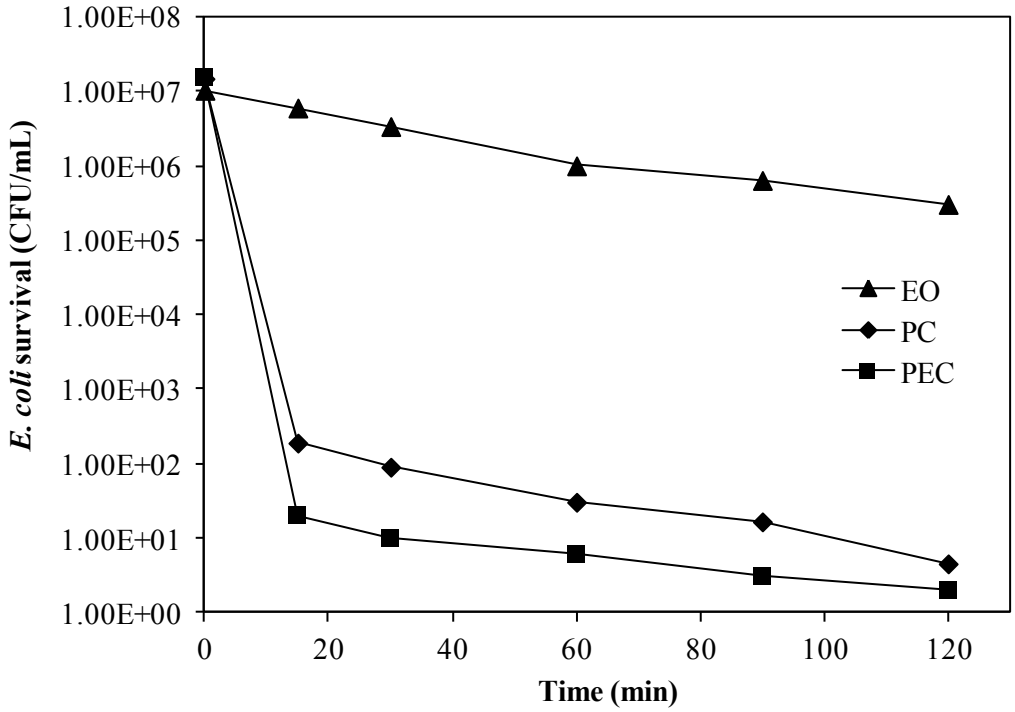
465 culture technique and (b) qPCR.

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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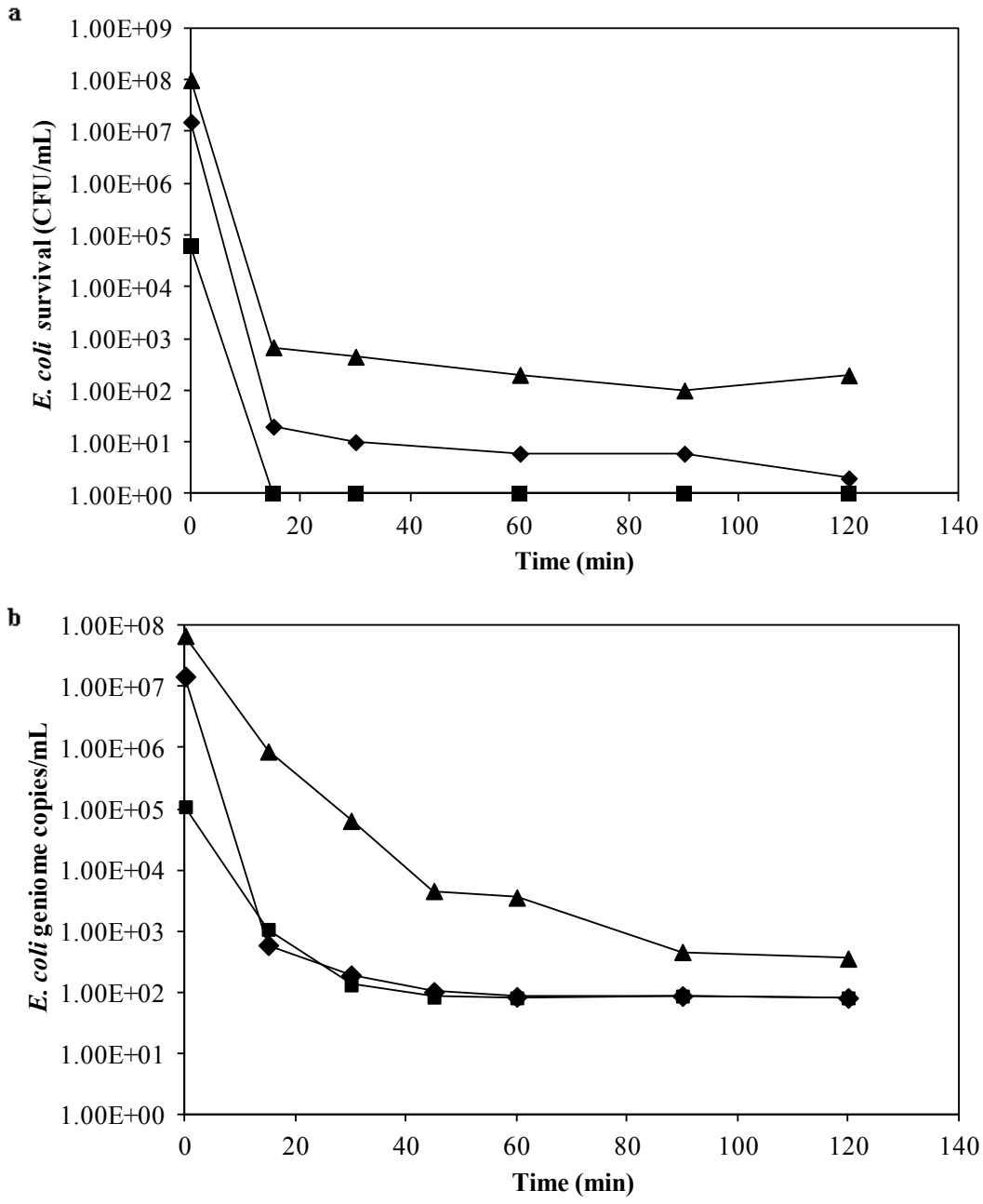
471

472 **Figure 2**



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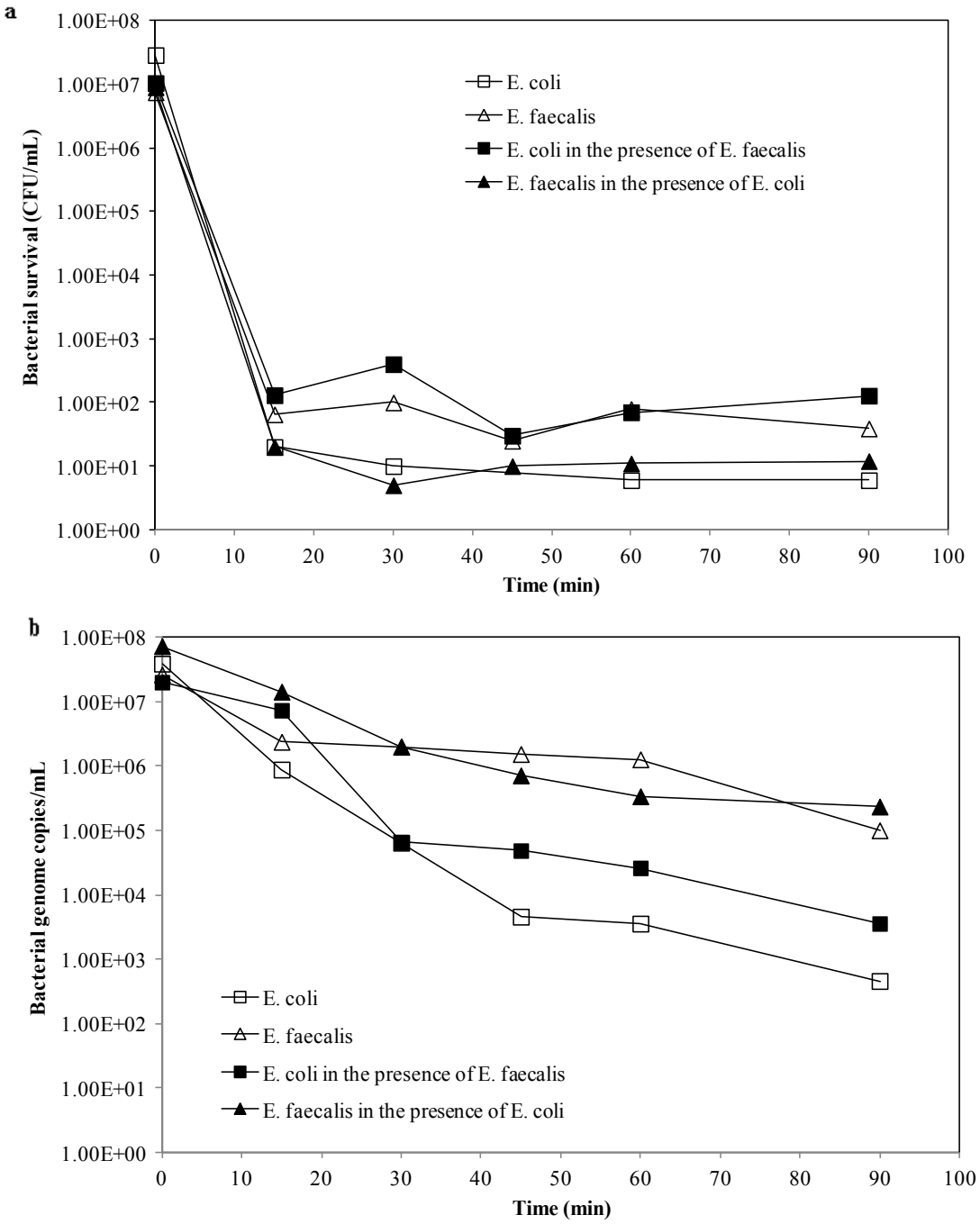
475 **Figure 3**



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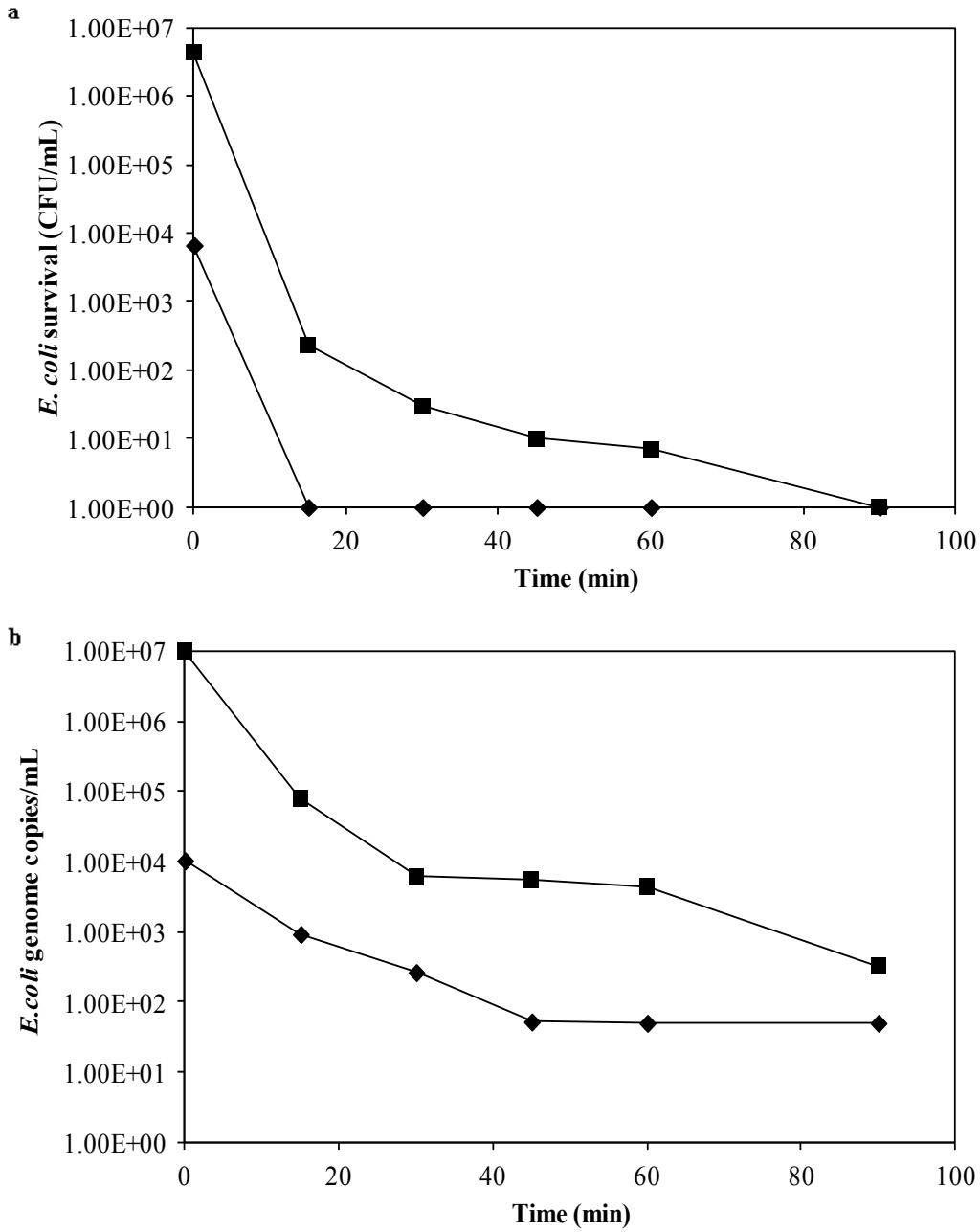
478 **Figure 4**



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