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

Danae Venieri, Efthalia Chatzisymeon, Eleonora Politi, Spiridon S. Sofianos, Alexandros Katsaounis and Dionissios Mantzavinos

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 polymerase chain reaction (qPCR). Inactivation rates were enhanced when the duration of the treatment was prolonged and when the bacterial density and the complexity of the water matrix were decreased. *E. coli* cells were reduced by approximately 6 orders of magnitude after 15 min of PEC treatment in water at 2V of applied potential and an initial concentration of 10^7 CFU/mL; pure photocatalysis (PC) 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.

Key words | disinfection, *E. coli*, *E. faecalis*, qPCR, TiO_2, voltage

Danae Venieri (corresponding author)
Efthalia Chatzisymeon
Eleonora Politi
Spiridon S. Sofianos
Dionissios Mantzavinos
Department of Environmental Engineering,
Technical University of Crete,
Polytechneioupolis,
GR-73100 Chania,
Greece
E-mail: *danae.venieri@enveng.tuc.gr*

Alexandros Katsaounis
Department of Chemical Engineering,
University of Patras,
GR-26504 Patras,
Greece

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 recent decades, advanced oxidation processes (AOPs) have been recognized 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 the 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 a 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, detoxification 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 to 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 the cellular membrane and further destruction of bacterial structure (Li *et al.* 2011). The studies which refer to PEC as a 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 fecal bacterial indicator, applying conventional microbiological protocols, based on 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 polymerase chain reaction (qPCR) has proven to be sensitive,

yielding accurate quantitative results and allowing 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 fecal indicators in water and wastewater by means of photoelectrocatalytic oxidation, applying conventional cultural methods and qPCR. This was implemented using representative strains of fecal indicator bacteria, namely *E. coli* and *E. 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 fecal 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 a 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* (APHA 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 was 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 to 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% ultraviolet (UV)-A radiation and 0.1% UV-B radiation, while the filter cuts radiation 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-potentiostat (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 °C with a temperature control unit. All experiments were conducted in the presence of 1% (w/v) Na$_2$SO$_4$ 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 into 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 was withdrawn. Half of the quantity was immediately analyzed with respect to viable bacterial cells applying the 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 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 lysed 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). An ethanol purification step was performed and the quantity and purity of all DNA samples were determined by measuring their absorbance at 260 nm and estimating the ratio of absorbance values at 260 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'-GACGTTCAGTTAC-TAACG -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 and 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 °C, followed by 40 cycles each of denaturation at 95 °C for 0.5 min, annealing at 57 °C for 1 min, and extension at 72 °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; (b) photocatalytic treatment; (c) electrochemical oxidation.

The results, summarized in Figure 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 in the conditions under consideration as it is not able to achieve total *E. coli* inactivation in aqueous suspensions.
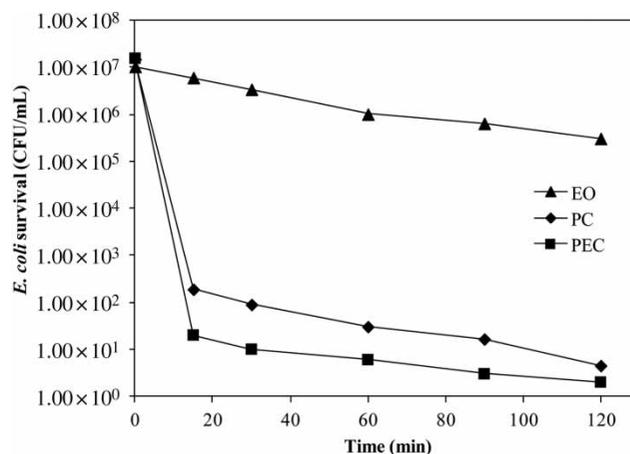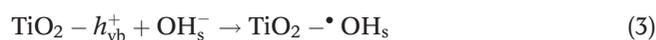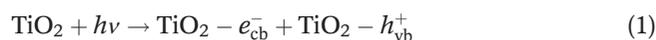

Figure 1 │ *E. coli* inactivation in water samples during PEC, PC and EO. Conditions: 10^7 CFU/mL initial bacterial concentration; 2V potential.

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):

$$TiO_2 + h\nu \rightarrow TiO_2 - e_{cb}^- + TiO_2 - h_{vb}^+ \qquad (1)$$

$$TiO_2 - h_{vb}^+ + H_2O_s \rightarrow TiO_2 - {}^\bullet OH_s + H^+ \qquad (2)$$

$$TiO_2 - h_{vb}^+ + OH_s^- \rightarrow TiO_2 - {}^\bullet OH_s \qquad (3)$$

$$TiO_2 - e_{cb}^- + TiO_2 - h_{vb}^+ \rightarrow recombination \qquad (4)$$

Cathode (counter electrode):

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

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 with 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 demonstrated not only for coliforms but also for Clostridium perfringens spores and the recalcitrant Cryptosporidium parvum (Egerton et al. 2006).

Effect of initial bacterial concentration

The influence of the initial E. coli concentration on PEC disinfection ability is presented in Figure 2. Conventional culture technique (Figure 2(a)) showed that the rate of PEC disinfection was inversely proportional to E. coli concentration in water samples. Total inactivation was achieved in a relatively short treatment time (i.e. within approximately 15 min) only when bacterial inoculum contained 10^5 CFU/mL, while at higher concentrations residual E. coli cells were determined even after 60 min. For instance, at an initial concentration of 10^8 CFU/mL, a 5.5 log reduction was achieved within 30–60 min of treatment, beyond which no further inactivation was recorded.

SYBR green qPCR was performed to detect the potential presence of live non-culturable bacterial cells. Our effort was to evaluate the consistency of E. coli cells, taking into account that cultivability is not synonymous with viability (Rémy et al. 2011). qPCR showed different periods of microbial inactivation (Figure 2(b)) compared to plate
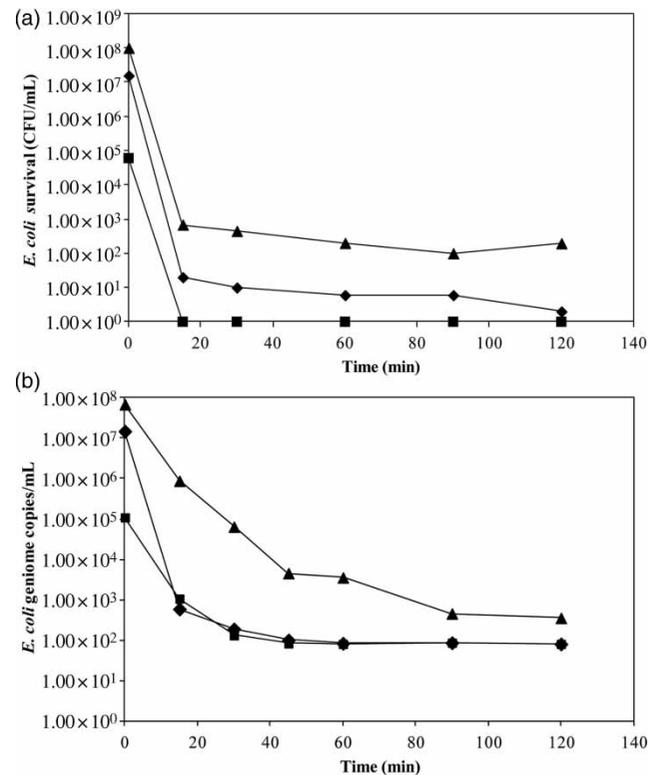
Figure 2 | E. coli inactivation in water samples containing various initial bacterial concentrations during PEC (2V potential), assessed by (a) the culture technique and (b) qPCR.

counts. Inactivation rates were quite similar when initial genome copies were 10^7 and 10^5/mL, as there was no enhancement of the process after 40 min of treatment. During PEC, cell injury and loss of viability are achieved as the cellular membrane is destroyed through direct contact with the photoanode (Li et al. 2011). Furthermore, radiation induces DNA lesions, damaging nucleic acids and making them functionless. The hydroxyl radical (and other AOS), directly generated by this process, is the main cause of DNA destruction, which in turn leads to cell death (Sinha & Häder 2002; Gogniat & Dukan 2007). According to our results, even after 120 min of treatment, genome copies were detected and quantified in the water sample, reaching a plateau without any further decrease. This profile of genome copies in the reaction mixture could be attributed to the detection sensitivity of the method. qPCR is acknowledged as a reliable and sensitive molecular method, whose detection limit may be in the region of 100 fg of E. coli genomic DNA (Lleo et al. 2005; Shannon et al. 2007). However,

in the present study environmental samples were processed which may inhibit qPCR or restrict its detection limits. On the other hand, qPCR seems to be valuable in detecting the non-culturable bacterial strains, which are induced under the stressed conditions of PEC. The so-called VBNC state of microorganisms is very common when dealing with environmental samples or disinfection techniques, making plating counts inadequate for accurate bacterial enumeration. Bacteria in this state retain their metabolic activity and pathogenic features, posing danger for public health, while they are not recoverable in standard culture media. On the contrary, molecular biology methods are capable of detecting these strains, contributing to a more reliable microbial evaluation of environmental samples (Wéry *et al.* 2008). Nevertheless, certain attention should be paid to the design of PCR experiments, including the amplicon length of the reactions, since it is strongly correlated to the recorded disinfection efficiency. In the present study, SYBR green PCR reactions yielded a 305 bp product for *E. coli* identification and quantification, resulting in reliable and acceptable evaluation of PEC disinfection efficiency (Süß *et al.* 2009). Also, another point under consideration concerning qPCR is that reactions can be affected by nucleic acid contamination, leading to false positive results and the formation of primer-dimers. Some of the possible sources of contamination are cross-contamination between samples, contamination from laboratory equipment and carryover contamination of amplification products and primers from previous PCRs. Therefore, appropriate measures should be taken to increase the reaction's specificity and all experiments should be performed with extra care to minimize contamination risks.

Effect of mixed bacterial populations

In a set of experiments, an attempt was made to evaluate the disinfection efficiency of PEC at 2V in water samples containing two bacterial populations. In this sense, sterile water samples were inoculated with either 10^7 CFU/mL *E. coli* or 10^7 CFU/mL *E. faecalis* or both. The results from culture technique and qPCR are summarized in Figure 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
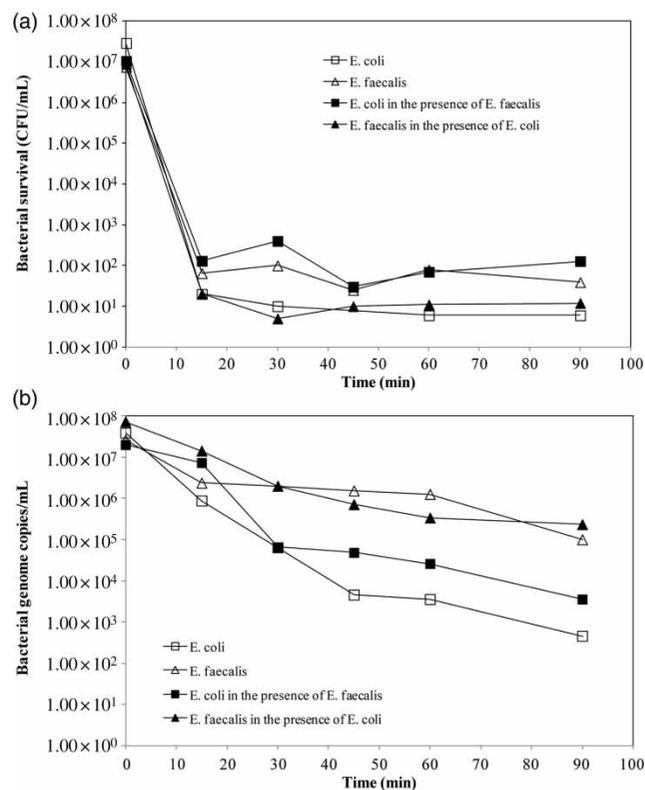

Figure 3 | *E. coli* and *E. faecalis* inactivation in water samples during PEC (2V potential), assessed by (a) the culture technique and (b) qPCR.

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 peptidoglycan cell wall and an additional outer membrane containing two lipid bilayers, which provide them with 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 efficiencies (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 (Figure 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 fecal indicators used 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 (Figure 3(b)), 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 considered to be very short owing to the presence of numerous nucleases, it might be deduced that the DNA detected is that contained in non-culturable cells (Lleo *et al.* 2005).

Disinfection in real wastewater

PEC was also employed to disinfect biologically treated effluents taken just before the chlorination step. Sterilized samples were inoculated with approximately 10^7 CFU/mL or 10^4 CFU/mL *E. coli* (Figure 4). In this set of experiments, a higher value of applied potential (5V) was chosen to highlight its effect on bacterial inactivation. Given that (i) the aqueous matrix was real wastewater, which is generally considered as a 'complex' sample containing various organic and inorganic components and (ii) raising the anodic potential enhances photocatalytic rates (Baram *et al.* 2009), runs were performed at 5V.

As seen in Figure 4(a), total *E. coli* inactivation occurred in almost 15 min when the initial cell density was 10^4 CFU/mL and this increased to 90 min at 10^7 CFU/mL. The degree of disinfection was also determined by means of qPCR, estimating bacterial genome copies remaining in the aqueous
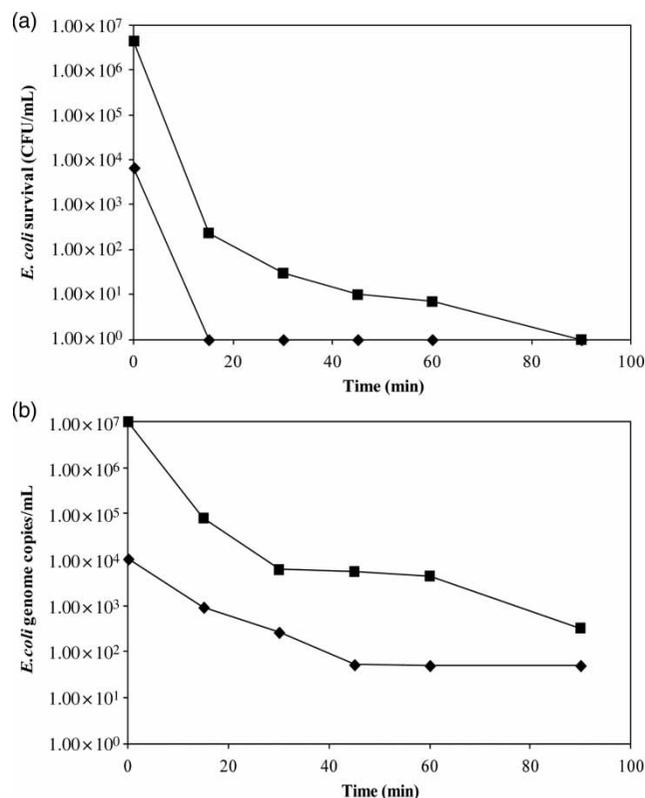
Figure 4 | *E. coli* inactivation in real wastewater during PEC (5V potential), assessed by (a) the culture technique and (b) qPCR.

solution after treatment (Figure 4(b)). Comparing findings from both procedures there was a significant contrast, as already seen in previous experiments conducted in the present study. qPCR showed that, even after 90 min of PEC treatment at 5V potential, genome copies of the bacterial indicator decreased by only five orders of magnitude, while colonies of *E. coli* were not recoverable at the same time. Apart from factors such as the detection limit of the method or VBNC cells, which have been mentioned previously, an important parameter under consideration is the particulate matter present in wastewater. This aids in the resistance of microorganisms to disinfection, as it may interfere by physically shielding bacterial cells and protecting the integrity of the contained DNA. Therefore, the extent of DNA damage is limited, which inevitably results in high numbers of genome copies detected by qPCR, even after long periods of treatment. In the present study, considerable genome copies/mL were recorded, highlighting the importance of wastewater 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 in attacking 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 inactivating pathogens. This could be overcome by increasing AOS concentration through raising the applied potential (Baram *et al.* 2009) and would possibly justify the nearly identical *E. coli* profiles shown in Figures 2(a) and 4(a) at an initial concentration of 10^7 CFU/mL.

CONCLUSIONS

- Photocatalytic inactivation of *E. coli* in water samples is enhanced by 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. These include detection limits of the method, amplicon size, primer sequence, cycling conditions and inhibitors present in the processed sample.

- For large scale applications, the use of sunlight as a renewable energy source would undoubtedly be beneficial to induce photocatalytic reactions, as well as provide the necessary electric field, thus promoting the principle of sustainable development.

REFERENCES

APHA 1999 *Standard Methods for the Examination of Water and Wastewater*, 20th edition, American Public Health Association, Washington, DC.

Baram, N., Starosvetsky, D., Starosvetsky, J., Epshtein, M., Armon, R. & Ein-Eli, Y. 2009 Enhanced inactivation of *E. coli* bacteria using immobilized porous TiO_2 photoelectrocatalysis. *Electrochim. Acta.* **54**, 3381–3386.

Bartosch, S., Fite, A., Macfarlane, G. T. & McMurdo, M. E. T. 2004 Characterization of bacterial communities in feces from healthy elderly volunteers and hospitalized elderly patients by using real-time PCR and effects of antibiotic treatment on the fecal microbiota. *Appl. Environ. Microbiol.* **70**, 3575–3581.

Chatzisymeon, E., Droumpali, A., Mantzavinos, D. & Venieri, D. 2011 Disinfection of water and wastewater by UV-A and UV-C irradiation: application of real-time PCR method. *Photochem. Photobiol. Sci.* **10**, 389–395.

Chen, Y. C., Higgins, M. J., Maas, N. A. & Murthy, S. N. 2006 DNA extraction and *Escherichia coli* quantification of anaerobically digested biosolids using the competitive touchdown PCR method. *Water Res.* **40**, 3037–3044.

Chen, C. Y., Wu, L. C., Chen, H. Y. & Chung, Y. C. 2010 Inactivation of *Staphylococcus aureus* and *Escherichia coli* in water using photocatalysis with fixed TiO_2. *Water Air Soil Pollut.* **212**, 231–238.

Cho, M., Cates, E. L. & Kim, J. H. 2011 Inactivation and surface interactions of MS-2 bacteriophage in a TiO_2 photoelectrocatalytic reactor. *Water Res.* **45**, 2104–2110.

Chong, M. N., Jin, B., Chow, C. W. K. & Saint, C. 2010 Recent developments in photocatalytic water treatment technology: a review. *Water Res.* **44**, 2997–3027.

Egerton, T. A. 2011 Does photoelectrocatalysis by TiO_2 work? *J. Chem. Technol. Biotechnol.* **86**, 1024–1031.

Egerton, T. A., Kosa, S. A. M. & Christensen, P. A. 2006 Photoelectrocatalytic disinfection of *E. coli* suspensions by iron doped TiO_2. *Phys. Chem. Chem. Phys.* **8**, 398–406.

Fraga, L. E., Anderson, M. A., Beatriz, M. L. P. M. A., Paschoal, F. M. M., Romão, L. P. & Zanoni, M. V. B. 2009 Evaluation of the photoelectrocatalytic method for oxidizing chloride and simultaneous removal of microcystin toxins in surface waters. *Electrochim. Acta.* **54**, 2069–2076.

Frontistis, Z., Daskalaki, V. M., Katsaounis, A., Poulios, I. & Mantzavinos, D. 2011 Electrochemical enhancement of solar photocatalysis: degradation of endocrine disruptor bisphenol-A on Ti/TiO_2 films. *Water Res.* **45**, 2996–3004.

Galbavy, E. S., Ram, K. & Cort Anastasio, C. 2010 2-Nitrobenzaldehyde as a chemical actinometer for solution and ice photochemistry. *J. Photochem. Photobiol. A-Chem.* **209**, 186–192.

Gogniat, G. & Dukan, S. 2007 TiO_2 Photocatalysis causes DNA damage via fenton reaction-generated hydroxyl radicals during the recovery period. *Appl. Environ. Microbiol.* **73**, 7740–7743.

Koivunen, J. & Heinonen-Tanski, H. 2005 Inactivation of enteric microorganisms with chemical disinfectants, UV irradiation and combined chemical/UV treatments. *Water Res.* **39**, 1519–1526.

Lee, D. Y., Shannon, K. & Beaudette, L. A. 2006 Detection of bacterial pathogens in municipal wastewater using an oligonucleotide microarray and real-time quantitative PCR. *J. Microbiol. Methods* **65**, 453–467.

Li, G., Liu, X., Zhang, H., An, T., Zhang, S., Carroll, A. R. & Zhao, H. 2011 *In situ* photoelectrocatalytic generation of bactericide for instant inactivation and rapid decomposition of Gram-negative bacteria. *J. Catal.* **277**, 88–94.

Lleo, M. M., Bonato, B., Tafi, M. C., Signoretto, C., Pruzzo, C. & Canepari, P. 2005 Molecular vs culture methods for the detection of bacterial faecal indicators in groundwater for human use. *Lett. Appl. Microbiol.* **40**, 289–294.

Malato, S., Fernandez-Ibanez, P., Maldonado, M. I., Blanco, J. & Gernjak, W. 2009 Decontamination and disinfection of water by solar photocatalysis: recent overview and trends. *Catal. Today* **147**, 1–59.

Martínez-Huitle, C. A. & Brillas, E. 2009 Decontamination of wastewaters containing synthetic organic dyes by electrochemical methods: a general review. *Appl. Catal. B: Environ.* **87**, 105–145.

Marugán, J., Christensen, P., Egerton, T. & Purnama, H. 2009 Synthesis, characterization and activity of photocatalytic sol–gel TiO_2 powders and electrodes. *Appl. Catal. B: Environ.* **89**, 273–283.

Marugán, J., Hufschmidt, D., Sagawe, G., Selzer, V. & Bahnemann, D. 2006 Optical density and photonic efficiency of silica-supported TiO_2 photocatalysts. *Water Res.* **40**, 833–839.

Morrison, S. R. 1980 *Electrochemistry at Semiconductors and Oxidized Metal Electrodes*. Plenum Press, New York.

Muela, A., Seco, C., Camafeita, E., Arana, I., Orruño, M., López, J. A. & Barcina, I. 2008 Changes in *Escherichia coli* outer membrane subproteome under environmental conditions inducing the viable but nonculturable state. *FEMS. Microbiol. Ecol.* **64**, 28–36.

Nissen, S., Alexander, B. D., Dawood, I., Tillotson, M., Wells, R. P. K., Macphee, D. E. & Killham, K. 2009 Remediation of a chlorinated aromatic hydrocarbon in water by photoelectrocatalysis. *Environ. Pollut.* **157**, 72–76.

Philippidis, N., Nikolakaki, E., Sotiropoulos, S. & Poulios, I. 2010 Photoelectrocatalytic inactivation of *E. coli* XL-1 blue colonies in water. *J. Chem. Technol. Biotechnol.* **85**, 1054–1060.

Rahmawati, F., Kusumaningsih, T. & Hastuti, A. 2011 Photo- and electro-catalysis for solar disinfection of *Escherichia coli*-contaminated water using Ag–TiO_2/graphite. *Toxicol. Environ. Chem.* **93**, 1602–1612.

Rémy, S. P., Simonet, F., Cerda, E. E., Lazzaroni, J. C., Atlan, D. & Guillard, C. 2011 Photocatalysis and disinfection of water: identification of potential bacterial targets. *Appl. Catal. B: Environ.* **104**, 390–398.

Shannon, K. E., Lee, D. Y., Trevors, J. T. & Beaudette, L. A. 2007 Application of real-time quantitative PCR for the detection of selected bacterial pathogens during municipal wastewater treatment. *Sci. Total. Environ.* **382**, 121–129.

Sinha, R. P. & Häder, D. P. 2002 UV-induced DNA damage and repair: a review. *Photochem. Photobiol. Sci.* **1**, 225–236.

Sirés, I. & Brillas, E. 2011 Remediation of water pollution caused by pharmaceutical residues based on electrochemical separation and degradation technologies: a review. *Environ. Int.* **40**, 212–229.

Süß, J., Volz, S., Obst, U. & Schwartz, T. 2009 Application of a molecular biology concept for the detection of DNA damage and repair during UV disinfection. *Water Res.* **43**, 3705–3716.

Venieri, D., Chatzisymeon, E., Gonzalo, M. S., Rosal, R. & Mantzavinos, D. 2011 Inactivation of *Enterococcus faecalis* by UV and solar irradiation in water and wastewater: culture techniques never say the whole truth. *Photochem. Photobiol. Sci.* **10**, 1744–1750.

Wéry, N., Lhoutellier, C., Ducray, F., Delgenès, J. P. & Godon, J. J. 2008 Behaviour of pathogenic and indicator bacteria during urban wastewater treatment and sludge composting, as revealed by quantitative PCR. *Water Res.* **42**, 53–62.

Willett, K. L. & Hites, R. A. 2000 Chemical actinometry: using *o*-nitrobenzaldehyde to measure light intensity in photochemical experiments. *J. Chem. Educ.* **77**, 900–902.