Disinfection of water and wastewater by UV-A and UV-C irradiation: application of real-time PCR method†

Efthalia Chatzisymeon, Ariadni Droumpali, Dionissios Mantzavinos and Danae Venieri*

Received 13th June 2010, Accepted 23rd August 2010
DOI: 10.1039/c0pp00161a

The disinfection efficiency of synthetic and real wastewater by means of UV-A and UV-C irradiation in the presence or absence of TiO_2 was investigated. A reference strain of *Escherichia coli* suspended in sterile 0.8% (w/v) NaCl aqueous solution was used as a synthetic wastewater, while real wastewater samples were collected from the outlet of the secondary treatment of a municipal wastewater treatment plant. *E. coli* inactivation was monitored both by the conventional culture technique and by the real-time PCR method. Culture method showed that UV-C irradiation (11 W lamp) achieved total *E. coli* inactivation of 100% within 3 min of photolytic treatment. On the other hand, UV-A (9 W lamp)/TiO_2; [TiO_2] = 200 mg L^{-1} (*i.e.* best operating conditions) required 60 min to achieve total disinfection of the synthetic wastewater. Real time PCR revealed compatible results, regarding the better efficiency of UV-C. However, it showed different times of bacterial inactivation, probably due to the phenomenon of "viable but not culturable bacteria". Disinfection durability tests in the dark and under natural sunlight irradiation showed that there is cell repair when UV-C irradiation is used for synthetic wastewater disinfection. Regarding real wastewater it was observed that only UV-C irradiation was capable of totally inactivating *E. coli* population in short time. Comparing results obtained from both methods, real time PCR proved to be more reliable and accurate, concerning the bacterial detection and enumeration in aquatic samples after the application of UV irradiation.

1. Introduction

Maintenance of the microbiological quality and safety of water systems is imperative, as their faecal contamination may exact high risks to human health and result in significant economic losses. Despite significant advances in water and wastewater treatment technology, waterborne diseases constitute a major worldwide threat to public health. Water-related outbreaks of disease are frequently caused by the consumption of water that is contaminated with human or animal faecal material.[1] Though many of these infections occur in developing countries with lower levels of sanitation and less public health awareness, outbreaks have occurred in developed countries as well. Human population growth, inadequate sewage systems, and management of animal waste (especially related to concentrated animal feeding operations) are some of the issues associated with maintenance of supplies of clean water. Generally, most water-related outbreaks are attributed to failures in some part of the water treatment process, indicating the importance of effective treatment and monitoring [2,3]

The conventional water and wastewater disinfection technologies include chlorination and ozonation. However, these methods can lead to the formation of harmful disinfection by-products (DBPs).[4] Chlorine reacts with the natural organic matter present in water and wastewater, leading to the production of DBPs, among the most dangerous of which are the trihalomethanes (THMs), well-known for their high carcinogenic and/or mutagenic potential.[5] Subsequently, intensive chemical treatments, such as those involving chlorine compounds or ozone can add to the problems of contamination of aquatic environment.[4] Therefore, ongoing research focuses on the development of alternative disinfection methods.

In recent years, advanced oxidation processes have received considerable attention for the disinfection of water and wastewater. Among them, UV-C irradiation has been extensively investigated yielding high disinfection performance.[4–7] However, it is well-known that UV-C is not a renewable source of energy since it does not form part of the solar irradiation. Therefore, research is turning to the use of solar (UV-A mainly, and UV-B) irradiation, an abundant source of energy, for water disinfection. In particular, there are many publications dealing with UV inactivation of *Escherichia coli* (a common and very popular indicator pathogen microorganism) in synthetic/purified or deionized water and real wastewater.[5,7–10]

The evaluation of solar irradiation as disinfection method is mainly performed by applying standard microbiological methods, which are based on colony-forming units (CFU) counts. Yet, these methods have limitations from both quantitative and qualitative points of view. They are time-consuming, laborious and allow detection only of bacteria capable of dividing.[11,12] However, a significant part of the microbial population in the aquatic environment, including enteric bacteria, has been described as non-culturable. Oligotrophic and extreme conditions may induce bacteria to enter into a state characterized as viable but non-culturable (VBNC). Although these microorganisms are not recoverable in standard culture media, they demonstrate metabolic activity

Department of Environmental Engineering, Technical University of Crete, 73100, Chania, Greece. E-mail: danae.venieri@enveng.tuc.gr; mantzavi@mred.tuc.gr; Fax: +30 282102821037858; Tel: +30 2821037801
† This paper is published as part of the themed issue of contributions from the 6th European Meeting on Solar Chemistry and Photocatalysis: Environmental Applications held in Prague, Czech Republic, June 2010.

and maintain their pathogenic features.[13] Stress factors which are responsible for the VBNC state may include UV exposure, antimicrobial agents, pH and temperature changes, and carbon and energy starvation.[14] Introduction of molecular techniques has contributed in surpassing the major drawbacks of culture methods. Specifically, PCR has allowed various new approaches in waterborne pathogen research because of its high sensitivity, specificity, and speed.[12] Moreover, applying the quantitative real-time PCR (qPCR), we are able to detect bacterial pathogenic DNA in water and wastewater samples in extremely low quantities and to assess the possible risk for public health. The technology of qPCR has proven to be sensitive, specific, and yields accurate quantitative results.[2] However, to our knowledge, there are no published papers that evaluate bacterial inactivation, during UV disinfection, using real-time PCR measurements.

Thus, the aim of the present work was to investigate the disinfection efficiency of water and real wastewater by UV-A and UV-C irradiation, using *E. coli* as faecal indicator and by applying qPCR and the conventional culture method. Disinfection rate was measured in terms of *E. coli* inactivation as a function of various operating parameters, namely TiO_2 loading, UV irradiation source, and treatment time. Furthermore, disinfection durability experiments were carried out under dark and natural sunlight irradiation conditions.

2. Methods and materials

2.1 Bacterial strain and wastewater

The bacterial strain used as reference in the present study was *E. coli* ATCC 23716 (American Type Culture Collection, Rockville, MD, USA). Colonies of *E. coli* were inoculated in a sterile 0.8% (w/v) sodium chloride aqueous solution, which was used as sample for the disinfection experiments. The concentration of bacterial cells in the suspension was 10^7–10^8 CFU mL^{-1}, as estimated by measuring its optical density at 600 nm on a Shimadzu UV1240 spectrophotometer.

Disinfection experiments were also carried out with real wastewater collected from the outlet of the secondary treatment of Chania (W. Crete, Greece) municipal wastewater treatment plant just before entering the chlorination step.

2.2 Disinfection experiments

Experiments were conducted in an immersion well, batch type, laboratory scale photoreactor. In a typical experimental run, 350 mL of the bacterial suspension was introduced in the reaction vessel and the appropriate amount of TiO_2, when required, was added to achieve the desirable catalyst loading in the range 50–400 mg L^{-1}. The catalyst used in this study was a commercially available TiO_2 (Degussa P-25) powder supplied by Degussa AG. Its physicochemical characteristics are anatase : rutile 75 : 25, particle size of 21 nm and its BET area is 50 m^2 g^{-1}.

The suspension was magnetically stirred for 40 min in the dark to ensure complete equilibration of adsorption/desorption of *E. coli* bacteria onto the catalyst surface and subsequently the UV lamp was turned on. UV-A irradiation was provided by a 9 W lamp (Radium Ralutec, 9W/78, 350–400 nm). UV-C irradiation was provided by a 11 W lamp (Philips, TUV, 11 W, PL-S). Air was continuously sparged into the liquid, the reaction mixture was continuously stirred and the temperature was maintained at 25 ± 1 °C with a temperature control unit. The external reaction vessel was covered with aluminium foil to reflect irradiation exerting the outer wall of the reaction vessel. Representative experiments were carried out at triplicates to check the reproducibility of the process.

At specific time intervals about 3 mL of the reaction solution were withdrawn. Prior to analysis, samples were not filtered to remove TiO_2 particles to avoid losses of bacteria during filtration. Half of the quantity was immediately analyzed with respect to viable *E. coli* cells applying the conventional culture method and the other 1.5 mL of each sample was used for DNA extraction.

2.3 Culture method

The detection and enumeration of *E. coli* in the reaction solution were performed using the serial dilution pour plate agar technique. Serial dilutions of the reaction solution were performed in sterile 0.8% (w/v%) NaCl aqueous solution and 200 μL of each dilution (including neat sample) were pipetted onto HiCrome Coliform Agar plates (HiMedia Laboratories), which is a selective *E. coli* culture medium. The plates were incubated at 37 °C for 20–24 h before viable counts were determined. The minimum detectable number of bacteria in these experiments was 1 CFU mL^{-1} (a reflection of the fact that 1000 mL of neat sample (5 × 200 μL) were plated out from each neat sample).

2.4 Genomic DNA purification

Genomic DNA was extracted by the standard protocol based on chemical lysis and phenol/chloroform/isoamyl alcohol (25 : 24 : 1) extraction.[15] Namely, the cells were spun for 2 min in a microcentrifuge, suspended in 567 μL of 50 μM Tris, 50 mM EDTA, pH 8.0 plus 30 μL 10% SDS and 3 μL of 20 mg ml^{-1} proteinase K and incubated for 1 h at 37 °C. Then 80 μL of 10% CTAB in 0.7% NaCl were added and the mixture was incubated for 10 min at 65 °C. 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). DNA was precipitated from the aqueous phase with 500 μL of isopropanol. The precipitate was washed with 70% ethanol, dried briefly and resuspended in 100 μL of 50 mM Tris, 50 mM EDTA, pH 8.0. The quantity of all DNA samples was determined by measuring their absorbance value at 260 nm. The purity of nucleic acid was also determined by the ratio of samples' absorbance values at 260 nm and 280 nm.

2.5 Real-time PCR method

The qPCR primers and fluorescent TaqMan® probes (Table 1) were designed using the computer software Primer Express® v 2.0 (Applied Biosystems), according to Lee *et al.* (2008).[16] The TaqMan® probes were designed to possess a higher melting temperature (T_m) than primers by about 10 °C in order to ensure binding at the target sites. The probe was labeled with a fluorescent reporter dye, FAM, at the 5′ end and a non-fluorescent quencher at the 3′ end. The overall PCR product size was smaller than 100 bases. Template genomic DNA, PCR primers, and probes were added to TaqMan® Universal PCR Mastermix (Applied Biosystems) to a final volume of 25 μL. The mixed real-time qPCR

Table 1 List of the real-time quantitative PCR primers and TaqMan® probes

Primers and probes	Sequences (5′ → 3′)	Gene	Function	GeneBank accession no.
Forward primer	GTCCAAAGCGGCGATTTG	*uidA*	Glucuronidase	S69414
Reverse primer	CCTGCCAGGCCAGAAGTT			
TaqMan® probe	F-AAACGGCAGAGAAGGTACTGGAA-BBQ			

solution contained 2XPCR master mix, 1μM of each primer and 250 nM of TaqMan® probes. Real time PCR reactions were carried out in a StepOne Plus System (Applied Biosystems Inc., Foster City, CA, USA) at a temperature profile of 50 °C for 2 min and 95 °C for 10 min, followed by 45 cycles of 95 °C for 15 s and 60 °C for 60 s. All samples were analyzed in triplicates to ensure the repeatability of the method.

To determine detection sensitivity, a series of 10-fold diluted *E. coli* genomic DNA (100 ng to 10 fg) was tested for threshold cycles (C_T) using the real-time qPCR assay.

2.6 Durability experiments

Disinfection durability experiments were performed to determine the efficiency of the UV irradiation treatment. Two recovery processes are known to exist for bacteria photoregeneration and these are dark repair and photoreactivation.[10] Therefore, after the disinfection treatment, 100 mL of the final effluent were kept in dark and other 100 mL were irradiated by natural sunlight under continuous stirring for 3 days. After this period of time the final sample was analysed in terms of *E. coli* viability.

3. Results and discussion

3.1 Application of real-time PCR method

Quantification of bacterial DNA was made by means of real-time PCR method. For this reason a standard curve presented in Fig. 1 was used. Genomic DNA from pure cultures of *E. coli* (reference strain) was prepared and purified as described previously. To determine the detection sensitivity of the TaqMan® real-time PCR assay, a series of 10-fold diluted pure culture genomic DNA was tested for real-time PCR amplification and cycle threshold (C_T). The standard curve shown in Fig. 1 was used to estimate the amount of DNA detected in each treated sample. Detection sensitivity of this real-time PCR assay was determined to be in the region of 100 fg of *E. coli* pure culture genomic DNA, which is approximately 21 copies of the *E. coli uidA* gene. This calculation is based on 4.8 fg being the average amount of DNA present in an *E. coli* cell (4.7 Mbp size genome).[17] This detection limit has been reported in other studies,[2,11] underlying the importance of molecular methods, regarding the accurate detection, isolation and quantification of environmental microorganisms.

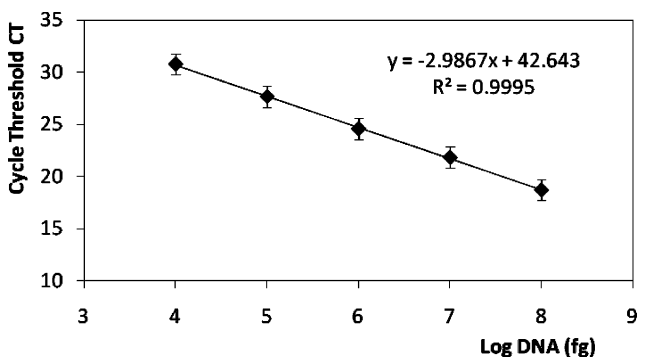

Fig. 1 Standard curve of the inverse relationship between cycle threshold (C_T) and *E. coli* genomic DNA quantity (fg).

3.2 Efficiency of photocatalysis in *E. coli* inactivation

In preliminary control experiments a 4.8×10^7 CFU mL^{-1} *E. coli* suspension in saline aqueous solution was stirred for 120 min in the dark in the presence of 100 mg L^{-1} TiO_2, at 25 °C (Fig. 2). It was observed that cultivable *E. coli* population decreased about 70% over the first 40 min beyond which the residual *E. coli* concentration remained stable. This implies that *E. coli* bacteria interacted with the catalyst surface (*i.e.* aggregation of titania clusters) and this interaction resulted in loss of bacteria viability. Moreover, an experiment was carried out in order to investigate the effect of the UV-A irradiation alone on water disinfection. It was found that UV-A irradiation alone resulted in 90% *E. coli* inactivation after 120 min. However, performing an experimental run in the presence of 100 mg L^{-1} TiO_2 under UV-A irradiation there were only 5 ± 1 CFU mL^{-1} left. These preliminary experiments pointed out that there is a synergistic effect of UV-A and supplementary oxidative species generated by the photoactivation of TiO_2. These results are consistent with other works[7,18] that showed that culturability started to decrease when cells started to interact with catalyst, strongly suggesting that adsorption onto TiO_2 aggregates is a prerequisite for bacterial inactivation.

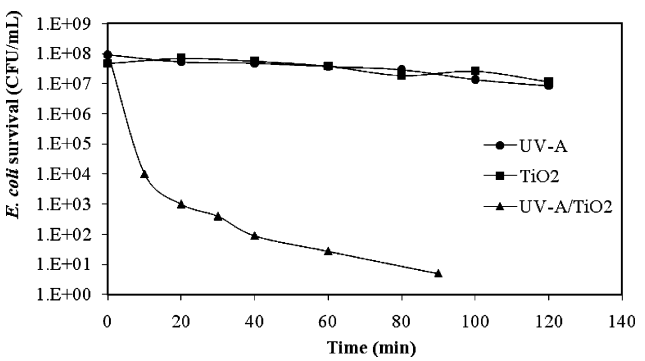

Fig. 2 *E. coli* inactivation in 0.8% (w/v) NaCl aqueous solution by UV-A alone, TiO_2 in dark and UV-A/TiO_2 photocatalysis. [TiO_2] = 100 mg L^{-1}.

3.3 Effect of TiO_2 loading

Catalyst loading in photocatalytic processes is an important factor that can highly influence the efficiency of the treatment. Experiments were carried out with TiO_2 Degussa P-25 catalyst at

concentrations ranging from 50–400 mg L^{-1} and the results after application of the conventional culture method and real time PCR are shown in Fig. 3 & 4, respectively.

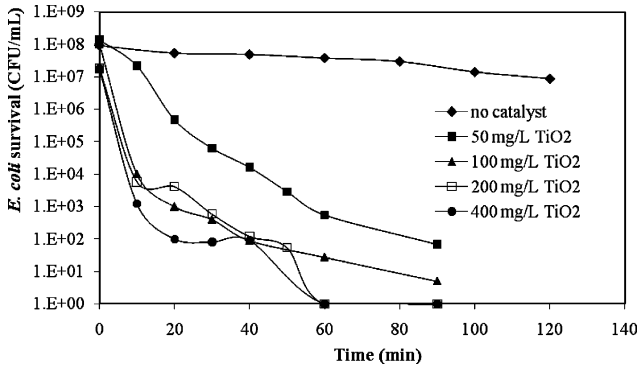

Fig. 3 *E. coli* inactivation in 0.8% (w/v) NaCl aqueous solution for various TiO_2 loadings under UV-A irradiation.

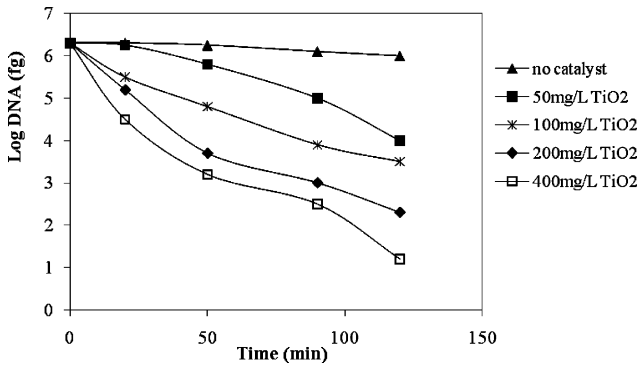

Fig. 4 Detection of *E. coli* genomic DNA in 0.8% (w/v) NaCl aqueous solution for various TiO_2 loadings under UV-A irradiation by the real-time qPCR assay.

Results obtained from the culture method showed that *E. coli* inactivation increases by increasing TiO_2 loading. Yet, it should be pointed out that according to current EU regulations *E. coli* population in water must be equal to zero. Hence, only in the presence of 200–400 mg L^{-1} TiO_2 and after 60 min of photocatalysis, where 100% of *E. coli* inactivation is achieved, the process can be regarded as efficient. Taking into account that the less TiO_2 loading is used, the more cost-effective is the photocatalytic process, all subsequent experiments were performed in the presence of 200 mg L^{-1} TiO_2.

Comparing findings from both methods applied, there was a significant differentiation. Real time PCR showed that even after 60 min of irradiation, genomic DNA was identified in the samples tested. The important issue of many studies dealing with molecular techniques is whether positive results are indicative of viable cells. However, in this study the only factor that could damage bacterial cells was UV-A and UV-C irradiation, which have long been recognized as capable of damaging microorganisms.[19] Considering the fact that all other parameters were steady and controlled, positive results obtained from real time PCR were reliable, as far as the viability of the bacteria and the DNA integrity are concerned.

3.4 Effect of the type of UV light used (UV-A and UV-C)

Photocatalytic processes are mainly affected by the irradiation source. In this study UV-A and UV-C irradiation were investigated in terms of their disinfection efficiency. Therefore, a series of experiments were conducted in the presence of UV-A, UV-A/TiO_2, UV-C and UV-C/TiO_2 for 0.8% (w/v) NaCl aqueous solution disinfection. The effect of the different UV lights was studied under similar intensities, which were 9 W and 11 W for UV-A and UV-C respectively.

Considering results obtained from the culture technique, it was observed that the UV-A was less effective than the UV-C irradiation, even in the presence of 200 mg L^{-1} where it yields its best efficiency (Fig. 5). Total inactivation was achieved after 60 min by UV-A/TiO_2 treatment. Moreover, *E. coli* inactivation is by far more efficient under UV-C irradiation in the presence or absence of TiO_2. The concentration of bacteria decreases to a non-detectable level within only 3 min of photocatalytic treatment.

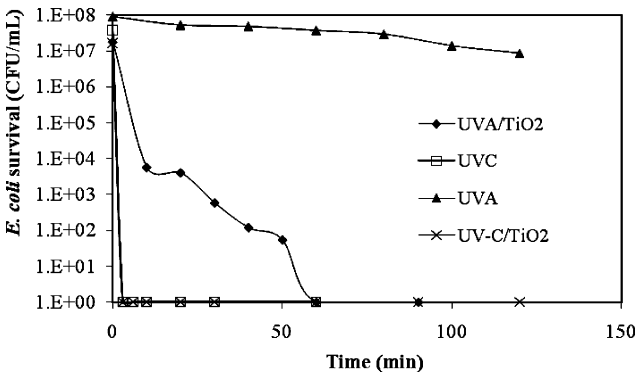

Fig. 5 *E. coli* inactivation in 0.8% (w/v) NaCl aqueous solution during its treatment by UV-A/TiO_2; [TiO_2] = 200 mg L^{-1}, UV-A alone, UV-C alone and UV-C/TiO_2; [TiO_2] = 25 mg L^{-1}.

Comparing both methods used, real-time PCR resulted in different periods of bacterial inactivation (Fig. 6). Nevertheless, there was a compatibility, regarding the efficiency of UV irradiation, as UV-C was more powerful. Within 3 min of treatment there was an 80% reduction of detected bacterial DNA, while after approximately 90 min, fluorescent signal in PCR was decreased by 95%. The primary mechanism responsible for cell injury and

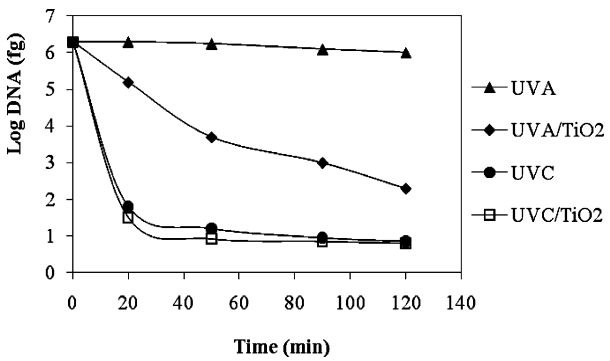

Fig. 6 Detection of *E. coli* genomic DNA in 0.8% (w/v) NaCl aqueous solution during its treatment by UV-A/TiO_2; [TiO_2] = 200 mg L^{-1}, UV-A alone, UV-C alone and UV-C/TiO_2; [TiO_2] = 25 mg L^{-1} by the real-time qPCR assay.

loss of viability by UV irradiation is damage to the structure and function of DNA.[20,21] Most studies dealing with photocatalysis and UV irradiation of microbial cells, have concluded that hydroxyl radical ($HO^{\bullet}$), directly generated by this process, is the main cause of the bactericidal effect of photocatalysis. The extremely reactive $HO^{\bullet}$, for which no defence exists, is able to damage DNA.[22] Specifically, UV-C light, when absorbed by the cell DNA, damages irradiated DNA, directly inducing pyrimidine and purine dimers and pyrimidine adducts.[4] Therefore, these DNA lesions, if unrepaired, may distort the DNA helix, interfere with DNA transcription and replication, and can lead to misreading of the genetic code and cause mutations and cell death.[21]

The differentiation of results of the applied methods is attributed to the "viable but not culturable" (VBNC) state of bacteria, as a consequence of the disinfection method. UV radiation can be considered as one of the most powerful stressing agents, which induces the VBNC state of bacteria.[23] Also, water constitutes an oligotrophic environment, in which large populations of bacteria form nonculturable cells, making it difficult for the researcher to quantify them properly.[14] The VBNC state leads to apparent loss of viability in bacteria and false negative results applying conventional culture methods. Nevertheless, microorganisms which enter this specific state maintain their pathogenic features and their detection and enumeration is mandatory, so as the microbial evaluation of aquatic environment to be accurate and reliable.

From our results it can be deduced that molecular methods, based on nucleic acid detection and quantification are capable of detecting bacterial populations present in waters including the nonculturable ones.[11]

3.5 Real wastewater disinfection

UV irradiation was also employed to disinfect biologically treated effluents collected from the outlet of the secondary treatment of Chania (W. Crete, Greece) municipal wastewater treatment plant just before chlorination. Results in terms of *E. coli* inactivation over time are shown in Fig. 7 & 8. Culture technique showed that only UV-C irradiation was capable of totally inactivating *E. coli* population in just 3 min. Moreover, it is interesting to note that in the presence of UV-A irradiation *E. coli* inactivation remains constant after 90 min of photocatalytic treatment, at about 99.7%, regardless TiO_2 loading. However, the bacteria inactivation curves seem to be not so regular (Fig. 7). There are some points that fall away from the trendline of the kinetic curves, in the presence of

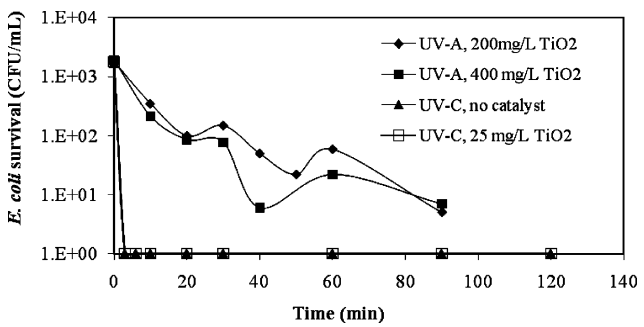

Fig. 7 *E. coli* inactivation in real wastewater during its treatment by UV-A/TiO_2; [TiO_2] = 200 mg L^{-1}, UV-A/TiO_2; [TiO_2] = 400 mg L^{-1}, UV-C alone and UV-C/TiO_2; [TiO_2] = 25 mg L^{-1}.

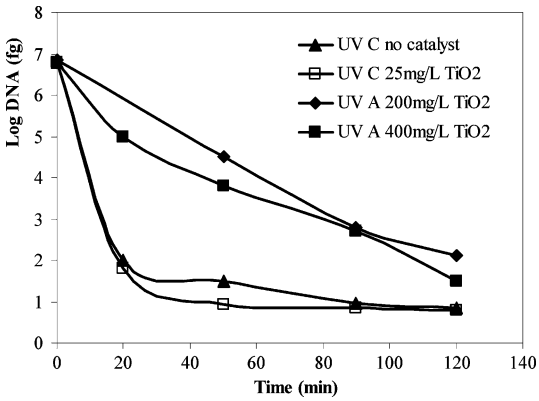

Fig. 8 Detection of *E. coli* genomic DNA in in 0.8% (w/v) NaCl aqueous solution during its treatment by UV-A/TiO_2; [TiO_2] = 200 mg L^{-1}, UV-A alone, UV-C alone and UV-C/TiO_2; [TiO_2] = 25 mg L^{-1} by the real-time qPCR assay.

UV-A irradiation, and this can be attributed both to (a) experimental error associated with the culture technique that was used and (b) to the fact that the viability and culturability of bacteria in real wastewater can be affected by many other factors such as the presence of other microorganisms. On the other hand, when quantification of *E. coli* was performed by real time PCR (Fig. 8) it was revealed that in approximately 100 min of treatment detected DNA was reduced by 95%.

Generally, wastewater requires more efficient and durable treatments, as it has complex composition and heavy bacterial load. In our study, UV-C was by far more efficient despite all inhibitors present in the samples tested.

Despite the fact that kinetics study was out of the scope of the present work it is worth noticing that there is a linear inactivation of *E. coli* bacteria in terms of their DNA reduction, in the presence of UV-A irradiation in many cases (Fig. 4, 6 and 8). This linear inactivation indicates first-order kinetics. For example, based on the experimental results of Fig. 4, the estimated first-order kinetics constants were 0.0051 min^{-1} (R^2 = 0.9053), 0.0583 min^{-1} (R^2 = 0.9595), 0.0842 min^{-1} (R^2 = 0.9173) and 0.1038 min^{-1} (R^2 = 0.8892), in the presence of 0, 50, 200 and 400 mg L^{-1} TiO_2, respectively.

3.6 Disinfection durability experiments

Disinfection durability experiments were performed to determine the efficiency of the photodegradation of the microorganisms. Bacteria have evolved four main mechanisms in the repair or damage tolerance of UV radiation-damaged DNA, including photoreactivation, nucleotide excision repair, mutagenic DNA repair, and recombinational DNA repair.[9] Photoreactivation is an enzymatic reaction where light energy (300–500 nm) is used to split the dimers. Once the damage sites in the DNA are repaired, the micro-organism will then be able to carry out reproduction and re-contaminate the disinfected water.[24,25] This reduces the efficiency of UV disinfection and may lead to the proliferation of pathogens. Therefore, to comply with requirements in the disinfection systems and assure that no bacterial re-growth will occur, the effective disinfection time (EDT) that ensures total killing of bacteria has to be determined during the evaluation of the UV disinfection process.

Table 2 *E. coli* survival after the end of disinfection durability experiments either in the dark or under natural sunlight irradiation for 3 days

Operating conditions								
Run	Lamp	[TiO_2] (mg L^{-1})	Water matrix	Initial [*E. coli*] (CFU mL^{-1})	*E. coli* survival after 90 min of phototreatment (CFU mL^{-1})	*E. coli* survival after 120 min of phototreatment (CFU mL^{-1})	*E. coli* survival after 3 d of durability experiment in the dark (CFU mL^{-1})	*E. coli* survival after 3 d of photoreactivation experiment under natural sunlight irradiation (CFU mL^{-1})
1	UV-A	200	0.8% (w/v) NaCl	10^7	0	—	0	0
2	UV-A	400	0.8% (w/v) NaCl	10^7	0	—	0	0
3	UV-C	—	0.8% (w/v) NaCl	10^7	—	0	nn[a]	376
4	UV-C	25	0.8% (w/v) NaCl	10^7	—	0	0	0
5	UV-A	400	Real wastewater	10^3	7	—	4	0
6	UV-C	—	Real wastewater	10^3	—	0	0	0
7	UV-C	25	Real wastewater	10^3	—	0	0	0

[a] nn: non-numerable colonies (>300).

Experiments were carried out at dark and natural sunlight irradiation under continuous stirring for 3 days and the results are shown in Table 2. Regarding the synthetic wastewater it was found that by UV-A/TiO_2 treatment there is no bacteria repair either at dark or at sunlight irradiation. However, when water is illuminated by UV-C in the absence of TiO_2 the EDT was not reached, even if no *E. coli* was detected (by plate count) after only 3 min of disinfection treatment, there was bacteria repair after 3 days both in dark and sunlight irradiation conditions. It is elsewhere suggested that during photocatalytic disinfection radicals and other oxidative species produced by illuminated TiO_2 induce damage that can in certain cases get worse in the dark, generating a "residual effect" of the photocatalytic treatment.[9] For this reason, in experimental run 4 (Table 2) in the presence of UV-C/TiO_2, the DNA repair mechanism becomes less active rendering the *E. coli* inactivation irreversible. For this reason, no dark or photo-repair of bacteria was reported in run 4, thus presenting an EDT of only 3 min. In other words, in the presence of TiO_2, which led to the same result, no recovery of the microorganisms was observed. This result indicates that the oxidative species developed at the titania surface caused severe damage to the cells. According to the results obtained, it would be possible to use UV-C in the presence of TiO_2 to disinfect water.

4. Conclusions

The use of TaqMan® real-time PCR proved to be effective in the detection and quantification of bacterial DNA in water and wastewater samples during disinfection experiments.

Both culture method and real time PCR showed that UV-C irradiation is more effective, regarding the disinfection of water and wastewater samples. However, regarding phototoreactivation experiments it was found that by UV-A/TiO_2 treatment there is no bacteria repair either at dark or at sunlight irradiation.

There was a significant differentiation of results obtained from both techniques, concerning the time needed for total bacterial inactivation. Real-time PCR data revealed that longer time is needed for 100% bacterial reduction, compared to the findings from culture method. This is probably attributed to the phenomenon of VBNC cells. UV irradiation causes physiological stress to bacterial populations, leading them to the VBNC state, which prohibits their detection by conventional techniques. This underlines the importance of molecular methods for the microbiological examination of environmental samples in terms of reliability, accuracy and protection of public health.

References

1 L. Heijnen and G. Medema, Method for rapid detection of viable *Escherichia coli* in water using real-time NASBA, *Water Res.*, 2009, **43**, 3124–3132.

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

3 M. Berney, H. U. Weilenmann, J. Ihssen, C. Bassin and T. Egli, Specific growth rate determines the sensitivity of *Escherichia coli* to thermal, UVA, and solar disinfection, *Appl. Environ. Microbiol.*, 2006, **72**, 2586–2593.

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

5 A. K. Benabbou, Z. Derriche, C. Felix, P. Lejeune and C. Guillard, Photocatalytic inactivation of *Escherischia coli*. Effect of concentration of TiO2 and microorganism, nature, and intensity of UV irradiation, *Appl. Catal., B*, 2007, **76**, 257–263.

6 M. D. Labas, R. J. Brandi, C. S. Zalazar and A. E. Cassano, Water disinfection with UVC radiation and H2O2. A comparative study, *Photochem. Photobiol. Sci.*, 2009, **8**, 670–676.

7 A. Paleologou, H. Marakas, N. P. Xekoukoulotakis, A. Moya, Y. Vergara, N. Kalogerakis, P. Gikas and D. Mantzavinos, Disinfection of water and wastewater by TiO2 photocatalysis, sonolysis and UV-C irradiation, *Catal. Today*, 2007, **129**, 136–142.

8 N. Lydakis-Simantiris, D. Riga, E. Katsivela, D. Mantzavinos and N. P. Xekoukoulotakis, Disinfection of spring water and secondary treated municipal wastewater by TiO2 photocatalysis, *Desalination*, 2010, **250**, 351–355.

9 A.-G. Rincón and C. Pulgarin, Field solar *E. coli* inactivation in the absence and presence of TiO2: Is UV solar dose an appropriate parameter for standardization of water solar disinfection?, *Sol. Energy*, 2004, **77**, 635–648.

10 A.-G. Rincón and C. Pulgarin, Bactericidal action of illuminated TiO_2 on pure *Escherichia coli* and natural bacterial consortia: Post-irradiation events in the dark and assessment of the effective disinfection time, *Appl. Catal., B*, 2004, **49**, 99–112.

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

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

13 A. Muela, C. Seco, E. Camafeita, I. Arana, M. Orruño, J. A. López and I. Barcina, Changes in *Escherichia coli* outer membrane subproteome under environmental conditions inducing the viable but nonculturable state, *FEMS Microbiol. Ecol.*, 2008, **64**, 28–36.
14 L. A. Bjergbæk and P. Roslev, Formation of nonculturable *Escherichia coli* in drinking water, *J. Appl. Microbiol.*, 2005, **99**, 1090–1098.
15 H. Zhu, F. Qu and L.-H. Zhu, Isolation of genomic DNAs from plants, fungi, and bacteria using benzyl chloride, *Nucleic Acids Res.*, 1993, **21**, 5279–5280.
16 D. Y. Lee, H. Lauder, H. Cruwys, P. Falletta and L. A. Beaudette, Development and application of an oligonucleotide microarray and real-time quantitative PCR for detection of wastewater bacterial pathogens, *Sci. Total Environ.*, 2008, **398**, 203–211.
17 Y. Koharaa, K. Akiyamaa and K. Isono, The physical map of the whole *E. coli* chromosome: Application of a new strategy for rapid analysis and sorting of a large genomic library, *Cell*, 1987, **50**, 495–508.
18 G. Gogniat, M. Thyssen, M. Denis, C. Pulgarin and S. Dukan, The bactericidal effect of TiO_2 photocatalysis involves adsorption onto catalyst and the loss of membrane integrity, *FEMS Microbiol. Lett.*, 2006, **258**, 18–24.
19 M. Guo, H. Hu, J. R. Bolton and M. G. El-Din, Comparison of low- and medium-pressure ultraviolet lamps: Photoreactivation of *Escherichia coli* and total coliforms in secondary effluents of municipal wastewater treatment plants, *Water Res.*, 2009, **43**, 815–821.
20 J. L. Zimmer-Thomas, R. M. Slawson and P. M. Huck, A comparison of DNA repair and survival of *Escherichia coli* O157:H7 following exposure to both low- and medium pressure UV irradiation, *J. Water Health*, 2007, **5**, 407–415.
21 R. P. Sinha and D. P. Häder, UV-induced DNA damage and repair: a review, *Photochem. Photobiol. Sci.*, 2002, **1**, 225–236.
22 G. Gogniat and S. Dukan, TiO2 Photocatalysis causes DNA damage *via* Fenton reaction-generated hydroxyl radicals during the recovery period, *Appl. Environ. Microbiol.*, 2007, **73**, 7740–7743.
23 Ö. Idil, R. Özkanca, C. Darcan and K. P. Flint, *Escherichia coli*: Dominance of red light over other visible light sources in establishing viable but nonculturable state, *Photochem. Photobiol.*, 2010, **86**, 104–109.
24 A. Locas, J. Demers and P. Payment, Evaluation of photoreactivation of *Escherichia coli* and enterococci after UV disinfection of municipal wastewater, *Can. J. Microbiol.*, 2008, **54**, 971–975.
25 P. H. Quek and J. Hu, Influence of photoreactivating light intensity and incubation temperature on photoreactivation of *Escherichia coli* following LP and MP UV disinfection, *J. Appl. Microbiol.*, 2008, **105**, 124–133.