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PAPER

Inactivation of *Enterococcus faecalis* by TiO_2-mediated UV and solar irradiation in water and wastewater: culture techniques never say the whole truth

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In this work, the disinfection efficiency of water and secondary treated wastewater by means of UV-A, UV-C and solar irradiation in the presence or absence of TiO_2, using a reference strain of *Enterococcus faecalis* as faecal indicator, was evaluated. Operating parameters such as TiO_2 loading (0–1500 mg L^{-1}), initial bacterial concentration (2×10^2–10^8 CFU mL^{-1}) and treatment time (up to 120 min) were assessed concerning their impact on disinfection. *E. faecalis* inactivation was monitored by the conventional culture method and real-time PCR. Regarding photocatalytic treatment, disinfection efficiency was improved by increasing TiO_2 concentration and bacterial inactivation took place in relatively short treatment times. Comparing the three disinfection methods, it was observed that UV-C irradiation yielded a better efficiency during water treatment than UV-A and solar irradiation. Furthermore, UV-A was more efficient than solar irradiation in the presence of the same loading of TiO_2. Regarding real wastewater, it was observed that only UV-C irradiation was capable of totally inactivating *E. faecalis* population in a short time. Screening the results obtained from both applied techniques (culture method and real-time PCR), there was a discrepancy, regarding the recorded time periods of total bacterial inactivation. Real-time PCR data revealed that longer periods are needed for 100% bacterial reduction during the treatments tested compared to the estimated time by culture method. This is probably attributed to the phenomenon of "viable but not culturable bacteria", caused by stressed conditions induced during disinfection experiments. Taking into account the contrast of results and in order to perform a thorough evaluation of disinfection techniques, conventional culture method should be accompanied by a DNA-based method. According to our findings, real-time PCR proved to be a reliable and accurate molecular tool for the identification and quantification of bacterial indicators, like *E. faecalis*, in aquatic samples after disinfection treatment.

1. Introduction

Waterborne outbreaks recorded worldwide are mainly caused by exposure to pathogens *via* consumption of contaminated water. The destruction or prevention of growth of microorganisms is essential in the control infectious disease transmission. With the growing demand for clean water sources and the protection of public health, various disinfection methods have been adopted.

Conventional water treatment technologies, such as filtration, chemical and membrane methods involve high operating costs and could potentially generate toxic secondary pollutants.[1] Although chlorination has been the most widely used process, the potential formation of carcinogenic by-products is highly undesirable in terms of protecting human health.[2] As a disinfection method, it is quite efficient against many enteric bacteria, but it has lower efficiency against viruses, bacterial spores and protozoan cysts.[3,4] A significant proportion of these pathogens, carried in water/wastewater, develop resistance mechanisms and remain present either in water supplies, or in treated effluents.[4–6] As a result, there is a widespread danger to public health from these hardy environmental microorganisms.

In order to suppress the aforementioned drawbacks, innovative water disinfection methods have been developed, among which advanced oxidation processes (AOPs) have demonstrated high disinfection efficiency.[1,2] The rationale of AOPs is based primarily on the *in situ* generation of highly reactive transitory species, like hydroxyl radicals, which are able to inactivate water pathogens and to mineralize various organic compounds and disinfection by-products.[7] Among AOPs, heterogeneous photocatalysis employs semiconductor catalysts, like TiO_2, demonstrating high efficiency in decontamination and disinfection studies. TiO_2 is the most

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commonly used photocatalyst as it is highly active, non-toxic, inexpensive and photochemically stable.[1,2,6,7]

The effectiveness of UV irradiation regarding microbial inactivation is attributed to the fact that DNA molecules absorb UV photons between 200 and 300 nm, with peak absorption at 265 nm.[8] Specifically, UV-C light, when absorbed by the cell DNA, damages irradiated DNA directly, inducing pyrimidine and purine dimers and pyrimidine adducts.[1,9] 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, causing mutations and cell death.[10]

Solar radiation has also been identified as an important factor inactivating allochthonous microorganisms in water. The damage caused by solar and UV-A light is mainly due to its absorption by cellular components called intracellular chromophores. Damage by light absorption through chromophores is contributed to the generation of reactive oxygen species, resulting in oxidative stress and cell damages.[1]

The microbiological parameter that is the most frequently studied, regarding the efficacy of disinfection methodologies, is *Escherichia coli*.[11–14] It is a Gram-negative bacterium and serves as indicator of faecal contamination of water and effluent samples. However, considering the significance of cell structure, attention has been paid to the differences among main aquatic microbial indicators, which may lead to different photocatalytic inactivation efficiency.[15] In this sense, differences of wall structure between Gram-negative and Gram-positive bacteria are taken into account since the latter have a thick peptidoglycane cell wall with an additional outer membrane containing two lipid bilayers, which provide them with a higher complexity. Therefore, many studies dealing with bacterial inactivation use Gram-positive species like members of enterococci.[3,6,15–17] Moreover, enterococci (*e.g. Enterococcus faecalis*) are considered valuable bacterial indicators, as their die-off in water is less rapid than that of coliforms and their persistence patterns are similar to those of potential waterborne pathogenic bacteria.[18]

The evaluation of disinfection techniques in water/wastewater samples is mainly performed by applying conventional plate counting methods, which are based on microbiological cultures on selective media and on colony-forming units (CFU) counts. Significant limitations of bacterial cultures, among which the most important is their inability to detect viable but not culturable (VBNC) bacteria, make their reliability quite limited.[19] To match the results of conventional plate counting, molecular identification and quantification of bacteria is essential. Molecular methods based on nucleic acids, like real-time PCR (qPCR), are able to detect VBNC bacteria to a certain extent.[20] Therefore, they are considered valuable tools for the monitoring of microbiological quality of aquatic samples. Extreme conditions, like those of disinfection, often induce the VBNC state of bacteria, which makes them irrecoverable in standard culture media.[21] Nevertheless, microorganisms in this state demonstrate metabolic activity and maintain their pathogenic features.[22] Introduction of DNA-based techniques and particularly qPCR has contributed in surpassing the major drawbacks of culture methods, yielding accurate quantitative results.[23,24]

The investigation of the present study has been focused on the evaluation of disinfection efficiency of water and real wastewater by UV-A, UV-C and solar irradiation, using *Enterococcus faecalis* as faecal indicator and applying both conventional culture method and qPCR. Disinfection was measured in terms of *E. faecalis* removal from samples as a function of various operating parameters, namely TiO_2 loading, initial bacterial concentration and treatment time.

2. Experimental

2.1. Bacterial strain and wastewater

The bacterial strain used as reference in the present study was *E. faecalis* ATCC 14506 (American Type Culture Collection, Rockville, MD, USA). Colonies of *E. faecalis* 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 it was estimated by measuring its optical density at 600 nm (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. The chemical oxygen demand and dissolved organic carbon was 26 and 7.8 mg L^{-1}, 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^{-1}, respectively, while the effluent's inherent pH was 7.8. The concentration of *E. faecalis* was 2×10^2–10^3 CFU mL^{-1}. Wastewater characterization was performed according to standard methods.[25]

2.2. Disinfection experiments

UV-A irradiation was provided by a 9 W lamp (Radium Ralutec, 9W/78, 350–400 nm), while UV-C irradiation was provided by a 11 W lamp (Philips, TUV, 11 W, PL-S). Experiments were conducted in an immersion well, batch type, laboratory scale photoreactor, purchased from Ace Glass (Vineland, NJ, USA). Solar irradiation experiments were carried out in a Newport 67005–150 Watt Solar Simulator system.

In a typical run, 350 mL of the bacterial suspension were 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 0.2–1.5 g 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. faecalis* bacteria onto the catalyst surface and subsequently the light source was turned on. 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.

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 loss of bacteria during filtration. Half of the quantity was immediately analyzed with the conventional

plate count method and the other 1.5 mL of each sample was used for DNA extraction and PCR amplification.

2.3. Culture method

The detection and enumeration of *E. faecalis* in the 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 the inoculated sample prior to treatment) were pipetted onto Slanetz & Bartley medium (OXOID), which is a very selective culture medium for enterococci. The plates were incubated at 37 °C for 48 h before determination of viable counts.

2.4. Genomic DNA purification

Genomic DNA was extracted from treated bacterial suspensions and wastewater samples by the standard protocol, based on 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^{-1}). Then, 200 μL of SDS lysis buffer (100 mM NaCl, 500 mM Tris [pH 8], 10% [wt/vol] 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). 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. Due to the large amounts of impurities contained in the wastewater DNA samples, the protocol was repeated again from the protein precipitation step, to the ethanol purification step to further purify DNA. The quantity and purity of all DNA samples were determined by measuring their absorbance value at 260 nm and by estimating the ratio of absorbance values at 260 nm and 280 nm, respectively.

2.5. Real-time PCR method

The groES gene was used as a target for *E. faecalis* quantification through qPCR. 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.*[26] The TaqMan® probes were designed to possess a higher melting temperature than primers by about 10 °C in order to ensure binding at the target sites. The probe was labeled with a fluorescent reporter dye at the 5′ end (FAM), and a non-fluorescent quencher at the 3′ end (BBQ). The primer pair yielded an 85bp PCR product.

Template genomic DNA, PCR primers, and probes were added to TaqMan® Universal PCR Mastermix (Applied Biosystems) to a final volume of 20 μL. The mixed real-time qPCR solution contained 2XPCR master mix, 1 μM 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. Deionized water and DNase-treated *E. faecalis* served as negative controls.

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 was used to estimate the amount of DNA detected in each treated sample. The generated standard curve reflects the efficacy of the PCR assay as a quantification method, considering the values of linear correlation coefficient and slope, which were 0.993 and –3.4, respectively.[23] *E. faecalis* copy number was calculated from the known mass of *E. faecalis*, which has been estimated 3.5 fg per genome.[27] Detection sensitivity of this real-time PCR assay was determined to be in the region of 10 fg of *E. faecalis* pure culture genomic DNA, which is approximately 30 genome copies.

2.6. Durability experiments

Disinfection durability experiments were performed to determine the efficiency of the UV irradiation treatment. In order to evaluate the bacterial photoregeneration, 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 analyzed in terms of *E. faecalis* viability.

3. Results and discussion

3.1. Effect of TiO_2 concentration

Preliminary runs investigating the extent of bacteria adsorption/desorption onto TiO_2 surface were performed. This is a prerequisite for heterogeneous photocatalytic systems since the mechanism of heterogeneous catalysis involves three basic steps: (i) the transferring of the reactants from the liquid bulk towards the catalyst particles, (ii) their adsorption onto the catalyst surface, and (iii) the desorption and removal of the final products in the liquid phase.[28] It was found that bacteria adsorption onto 200 mg L^{-1} TiO_2 was 60% after 40 min in the dark (data not shown), when these were inoculated in 0.8% NaCl aqueous solution. However, when real wastewater samples were stirred in the presence of 1000 mg L^{-1} TiO_2 in the dark, adsorption of *Enterococcus faecalis* onto the catalyst surface was 20% after 40 min (data not shown). Beyond that time, bacteria population remained practically unchanged in the wastewater indicating that the adsorption/desorption equilibrium onto the catalyst had been

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

Primers and probes	Sequences (5′ → 3′)
Forward primer	TGTGGCAACAGGGATCAAGA
Reverse primer	TTCAGCGATTTGACGGATTG
TaqMan® probe	6FAM-TGTCGTTCGTGCATTAGAAGAACC-BBQ

already achieved. In all subsequent experiments, the suspension was magnetically stirred for 40 min in the dark to ensure complete equilibration of adsorption/desorption of *E. faecalis* bacteria onto the catalyst surface and subsequently the light source was turned on.

Concentration of TiO_2 in slurry photocatalytic processes is an important factor that affects the overall reaction rate. A linear dependency holds until certain extent when the reaction rate starts to aggravate and becomes independent of TiO_2 concentration.[2] In the present study, experiments were carried out using different concentrations of titania in aqueous solution in the range from 0.2 to 1.5 g L^{-1}. The results after application of the conventional culture method and qPCR are shown in Fig. 1.

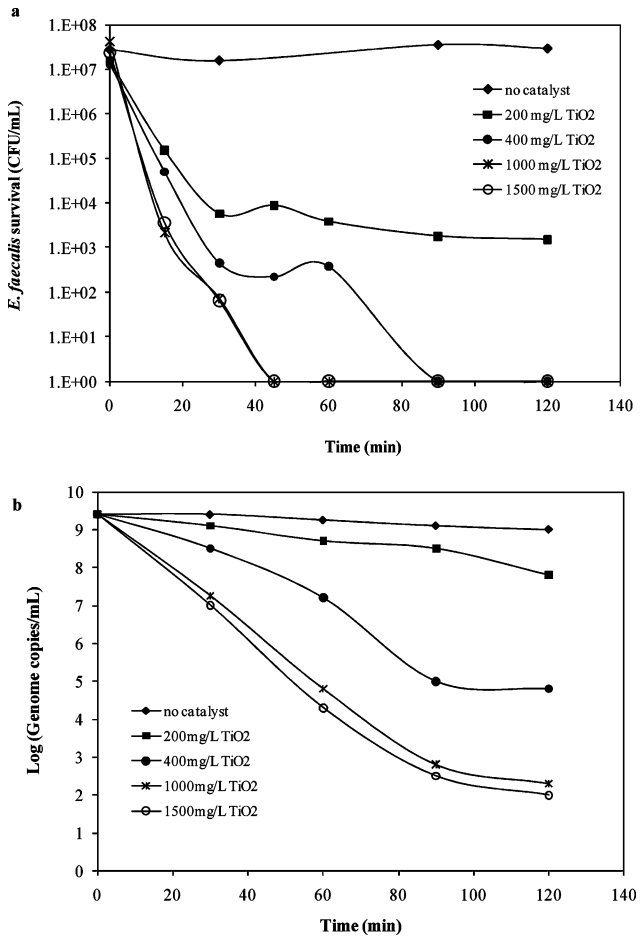

Fig. 1 *E. faecalis* inactivation in 0.8% (w/v) NaCl aqueous solution for various TiO_2 concentrations under UV-A irradiation, assessed by (a) the culture technique and (b) qPCR.

The effect of TiO_2 loading on the survival-time profiles of *E. faecalis* is similar to other studies, dealing with Gram-positive bacteria.[7,11] As seen in Fig. 1a, *E. faecalis* inactivation was sufficiently high (99.99%) after 45 min of treatment with the two higher catalyst loadings. The bactericidal effect of TiO_2 photocatalysis involves loss of membrane integrity and peroxidation of its phospholipids, which leads ultimately to cell death.[29] Therefore, the importance of catalyst loading should be underlined when disinfection concerns Gram-positive microorganisms, like *E. faecalis*, which possess complex wall structure.

Decreasing TiO_2 concentration led to significant presence of the bacterium in treated samples. It should be borne in mind that according to EU regulations, in water suitable for consumption, *E. faecalis* population must be equal to zero. Hence, considering the importance of treatment time and catalyst loading, the overall process can be regarded as efficient in the presence of 1–1.5 g L^{-1}, where total bacterial degradation is achieved within 45 min. In this sense, subsequent experiments regarding wastewater treatment were performed in the presence of 1 g L^{-1} TiO_2.

Comparing findings from both methods applied, there was a significant contrast. Real-time PCR showed that even after 120 min of irradiation, genome copies of the bacterial indicator were identified in the samples tested. In order for qPCR monitoring of enterococci to be effective, it is important to know the correspondence between qPCR values and viable cells. However, it has repeatedly been shown that VBNC cells, which may be present in stressed disinfection conditions, are only detected by molecular biology tools and can potentially cause underestimation by conventional culture methods.[30,31] Moreover, Lebuhn *et al.* concluded that DNA released from decayed cells is further degraded in the functioning digester ecosystems, making DNA a reliable parameter to quantify viable organisms in extreme environments.[30] In the present study, the only factor that could damage bacterial cells was irradiation (UV-A, UV-C and solar), which has long been recognized as capable of degrading microorganisms.[9] The molecular method was properly designed, with experimental parameters steady and controlled. Therefore, positive results obtained from qPCR were reliable, as far as the viability of the bacteria and the DNA integrity are concerned.

Another parameter that should be taken under consideration is the size/length of PCR product. According to research studies the longer the targeted DNA fragment then the more UV-induced DNA lesions inhibit the PCR.[32,33] Therefore, the molecular biology reduction rates of the present study would further increase when analyzing much longer DNA sections. This, however, is clearly limited by the nature of the technique used. One of the objectives of the present study was to apply a method which would provide highly sensitive and specific detection of DNA. A probe-based system which meets these criteria is TaqMan® qPCR, for which the ideal amplicon length should be within the range of 50–150 bases. In this sense, qPCR measurements should always be compared to conventional cell counts.

3.2. Effect of the type of UV light (UV-A and UV-C)

In the present study UV-A and UV-C irradiation were investigated in terms of their disinfection efficiency. 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. The results from the aforementioned experiments are shown in Fig. 2 for both monitoring methods applied (culture and qPCR).

Considering results obtained from the culture technique, it was observed that UV-A was less effective than UV-C irradiation, even in the presence of 200 mg L^{-1} of TiO_2. Total inactivation of *E. faecalis* was not achieved even after 90 min of UV-A/TiO_2 treatment. On the contrary, UV-C proved to be far more

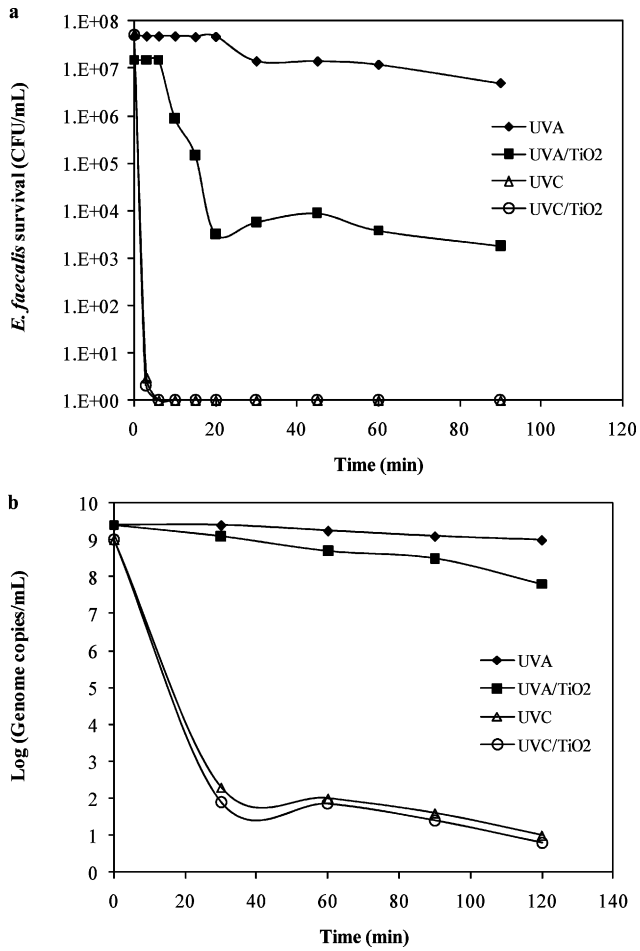

Fig. 2 *E. faecalis* inactivation in 0.8% (w/v) NaCl aqueous solution assessed by (a) the culture technique and (b) qPCR during treatment by UV-A/TiO_2, UV-A alone, UV-C/TiO_2 and UV-C alone. [TiO_2] = 200 mg L^{-1}.

efficient, as the concentration of bacteria was decreased to a non-detectable level within only 6 min of photolytic or photocatalytic treatment.

Comparing both methods used, qPCR revealed compatible results regarding the different efficiency of UV-A and UV-C irradiation but showed different periods of bacterial inactivation. Although UV-C was more powerful, the required treatment time for an approximately 90% microbial reduction was elevated to 120 min. More specifically, reduction of genome copies quantified by qPCR reached a plateau after 30 min of treatment, implying a threshold under the applied experimental conditions for maximum inactivation and degradation of *E. faecalis*. The primary mechanism responsible for cell injury and loss of viability by UV irradiation is damage to the structure and function of DNA.[10] UV-C light, when absorbed by the cell DNA, damages irradiated DNA, directly inducing pyrimidine and purine dimers and pyrimidine adducts.[1] These DNA lesions are the primer point for cell mutations and destruction. Moreover, DNA injuries inhibit the PCR reaction due to an obstruction of polymerase-mediated strand elongation at damaged DNA sites.[32]

The fact that results of the applied methods are not compatible may be explained by the VBNC state of bacteria to a certain extent. The disinfection stressing agent along with the oligotrophic environment of the samples tested induce this bacterial state, in which microorganisms form non-culturable cells, leading to false negative results. These findings are in compliance with other studies, dealing with these microbiological monitoring techniques.[24,32,34]

Furthermore, the specific molecular biology disinfection rates could be attributed to the amplicon length of the qPCR reaction. The correlation between amplicon size and obtained UV disinfection rate should always be taken into account in order to evaluate all possible DNA lesions occurred in the bacterial genome.[32]

3.3. Real wastewater disinfection – durability experiments

UV and simulated solar irradiation were also employed to disinfect biologically treated effluents taken just before the chlorination step. Photocatalytic experiments were performed with 1000 mg L^{-1} of titania. Culture technique showed that only UV-C irradiation was capable of totally inactivating *E. faecalis* population within just 3 min of treatment. Comparing UV-A and simulated solar irradiation, UV-A was more efficient in terms of bacterial removal in the presence of the same TiO_2 loading (Fig. 3). Real-time PCR revealed that genome copies of *E. faecalis* population contained in wastewater decreased approximately 60% within 3 min of irradiation (Fig. 3b). Interestingly and despite all

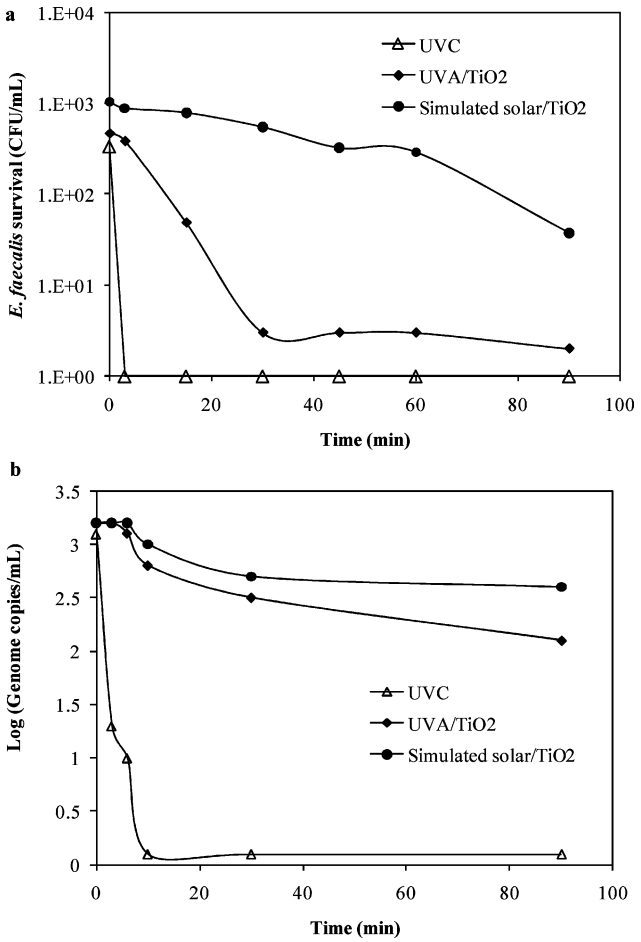

Fig. 3 *E. faecalis* inactivation in real wastewater assessed by (a) the culture technique and (b) qPCR during treatment by UV-A/TiO_2, UV-C and solar/TiO_2 irradiation. [TiO_2] = 1000 mg L^{-1}.

inhibitors included in wastewater samples, disinfection did occur to a satisfying degree, according to the results obtained from both applied methods (culture and qPCR). Particulate matter present in wastewater aids in the resistance of microorganisms to disinfection, as it may interfere by physically shielding bacterial cells. Furthermore, wastewater includes many PCR inhibitors, like particles or humic and fulvic acids, which interact and block PCR reactions.[35]

It should be pointed out though that the initial CFU values of wastewater samples were significantly lower (*i.e.* 4–5 orders of magnitude) than the seeded aqueous samples. However, when processing wastewater samples, bacterial detection limits are quite high, as the viability and culturability of bacteria in real wastewater can be affected by many other factors such as the presence of other competitive microorganisms and the interaction between them. In addition, environmental bacteria have been characterized as more resistant to UV irradiation than seeded strains of the same species.[36] Our findings indicate that the water matrix, including bacterial initial concentration affects disinfection efficiency to a considerable extent. The potential of a species to restore UV-induced DNA damage depends on the initial cell density. According to Süb *et al.*, when initial cell numbers are below a "critical" level the possibility of DNA repair and bacterial re-growth is reduced.[32] Large bacterial populations may lead to the formation of cellular aggregates, which may provide physical protection to internal cells and limit the extent of DNA damage. The more limited the DNA damage then the fewer repairs occur in bacterial populations.

When photolytic and photocatalytic treatments are applied it is important to verify the disinfection durability, as generated oxidative species have short half-life and microbial reactivation may occur. Photoreactivation is an enzymatic reaction where light energy (300–500 nm) is used to split the dimers which are formed as a consequence of irradiation.[32] This microbial reaction reduces the efficiency of UV disinfection and may lead to the proliferation of pathogens.

In the present study disinfection durability experiments were carried out in the dark, as well as under natural sunlight irradiation, so as to determine the efficiency of the photodegradation of the microorganisms. Regarding both water and wastewater samples, no reactivation was recorded either by UV-A/TiO_2 treatment or by UV-C, even after 72 h of exposure to sunlight, indicating that the oxidative species developed on the titania surface caused severe damage to the cells. These results seem quite promising, as *E. faecalis* is considered one of the most severe faecal bacteria, quite persistent in aquatic environments and resistant against various treatment procedures. Cell density has been recognized as determinant of bacterial potential to restore DNA damage after photodisinfection. According to other studies, an initial concentration of 10^6 CFU mL^{-1} seems critical in order not to record any amplifiable DNA targets or colony counts, even after 66 h of incubation after irradiation.[32,37] When initial bacterial concentration is beyond that limit, long periods of irradiation are mandatory for complete and permanent inactivation. In our study, initial *E. faecalis* densities were approximately 10^7 CFU mL^{-1} and 10^3 CFU mL^{-1} for seeded aqueous and wastewater samples, respectively. According to our findings, it would be possible to use UV-C in the presence of TiO_2 to disinfect water, taking into account initial cell densities.

Conclusions

–If properly designed, real-time PCR is a highly suitable, reliable and versatile tool for the quantification of microbial DNA in environmental samples, surpassing considerable drawbacks of conventional culture techniques.

–It has been shown that, under controlled experimental conditions, DNA may be used to quantify explicitly potentially infectious agents in samples with high intrinsic metabolic turnover.

–There was a significant contrast of results obtained from both techniques concerning the required time for total bacterial inactivation. Real-time PCR data revealed that longer periods are needed for 100% bacterial reduction, compared to the estimated time by culture method. To a certain extent, this is probably attributed to the phenomenon of VBNC cells, caused by stressed conditions induced during disinfection experiments. However, qPCR results should be noted with caution, taking into consideration the parameter of amplicon length.

–*E. faecalis* is a valuable microbial indicator of aquatic samples with a significant resistance to UV-A and solar irradiation. Generally, the bacterial strain studied is enlisted among the most resistant in water/wastewater treatment, as high survival fractions were recorded after several hours of irradiation.

–Both culture method and real-time PCR showed that UV-C irradiation is more effective regarding the disinfection of water and wastewater samples.

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