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Use of coaxial photocatalytic reactor (CAPHORE) in the TiO_2 photo-assisted treatment of mixed *E. coli* and *Bacillus* sp. and bacterial community present in wastewater

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Abstract

This paper reports the photocatalytic disinfection of water contaminated by a mixture of *Escherichia coli* and *Bacillus* sp. as well as that of wastewater containing a larger microbial community. The photocatalytic reactions were carried out in a coaxial photocatalytic reactor called CAPHORE, using TiO_2 P-25 of Degussa. *E. coli* is more sensitive than *Bacillus* sp. to photocatalytic treatment. Bacterial inactivation was dependent on organic matter and dissolved oxygen (DO) concentration.

Of the bacterial community present in partially treated wastewater, *E. coli* appears to be more sensitive to the treatment than *Enterococcus* sp., coliforms (other than *E. coli*), and Gram-negative (other than coliforms). After photocatalytic treatment, no bacterial recovery of previous groups was observed for 24 h in the dark. However a very low bacterial inactivation rate was observed for the whole bacterial population present in wastewater and detected by non-selective media. The effective disinfection time (EDT), the time necessary for total inactivation of bacteria without re-growth in a subsequent dark period referenced at 24 h (or 48 h), was reached only for *Enterococcus* sp., and coliform groups. EDT_{24} was not reached for the whole population.

Keywords: Water disinfection; TiO_2; Photocatalysis; Solar disinfection; UV-irradiation; *E. coli*; *Bacillus* sp.; *Entreococcos* sp.; Total coliform; EDT

1. Introduction

TiO_2 photocatalysis generates strong oxidizing power when illuminated with UV light at wavelengths of less than 385 nm. With holes (h^+) and hydroxyl radicals ($OH^\bullet$) generated in the valence band (VB), and electrons and superoxide ions ($O_2^{\bullet}-$) generated in the conduction band (CB), illuminated TiO_2 photocatalysis can decompose and mineralize organic compounds by participating in a series of oxidation reactions leading to carbon dioxide.

It is known that oxidative stress in bacteria is caused by exposure to reactive oxygen intermediates, such as superoxide anions ($O_2^{\bullet}-$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($HO^\bullet$), which can damage proteins, nucleic acids and cell membranes. Increasing evidence suggests that the cumulative damage caused by these reactive oxygen species (ROS) may lead to cell death [1,2].

Much work has been done dealing with the bactericidal effect of TiO_2 photocatalyst over a wide range of organisms including viruses, bacteria, fungi, algae and cancer cells [3]. However, the understanding of the photochemical mechanism of the biocidal action largely remains unclear. In particular, the identity of the main photooxidants and their roles in the mechanism of killing microorganisms is under active investigation. However, it is generally accepted that ROS such as $HO^\bullet$ play an important role.

In this paper a coaxial photocatalytic reactor (CAPHORE), presented in Fig. 1, was chosen to study the photocatalytic disinfection of water. CAPHORE contains a black lamp as the source of light. Fluorescent black light with an emission maximum at about 365 nm has commonly been used for photocatalytic experiments, since it matches well the band gap of anatase. Ten years ago, Ireland et al. [4] reported the *Escherichia coli* disinfection of 12 l of

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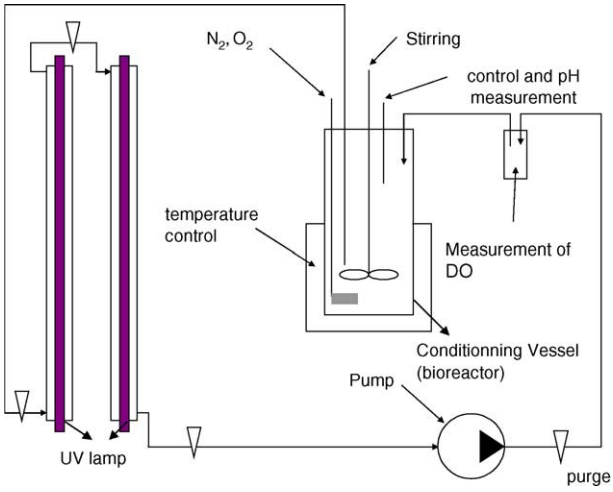

Fig. 1. Schematic representation of CAPHORE pilot system. The triangles on the tubing are fittings.

water using a reactor consisting of four coaxial black lamps. The lamps were covered with a fiberglass-mesh sleeve coated with a firmly bonded layer of TiO_2.

On the other hand, the reuse of wastewater after adequate disinfection constitutes a potential water resource, which could be of great interest to several sectors, such as agriculture, especially in countries suffering from a deficiency in water resources. Conventional wastewater processing (pre-primary and secondary treatments) allows satisfactory removal of the organic load (suspended solids, biological oxygen demand and chemical oxygen demand) but appears to be less efficient in removing pathogenic micro-organisms. Humans could be contaminated by water contact, digestion of spoiled foods and even by inhalation. In this context, photocatalytic disinfection of wastewater could be considered complementary to biological and/or physical (filtration) wastewater treatment. All the same, the use of chlorination after photocatalytic treatment could be necessary to maintain residual disinfecting activity if the water is subsequently stored for some time [5].

A few studies on the durability of photocatalytic disinfection using real water sources have been reported [6–8]. In this paper, photocatalytic disinfection by TiO_2 was tested in wastewater from an activated sludge treatment plant in Lausanne, in Switzerland.

The main purpose of this study is to evaluate the sensitivity of selected specific bacteria to photocatalytic treatment using a CAPHORE system. This reactor allows us to ask and discuss some questions related to scale-up of photocatalytic inactivation systems, and evaluate the influence and evolution of some parameters such as pH, dissolved oxygen (DO) and organic matter.

Two main topics studied in this paper are: (a) disinfection of water containing a mixed synthetic culture of *E. coli* and *Bacillus* sp., and (b) disinfection of wastewater containing a broad bacterial community.

2. Experimental details

2.1. Materials

The photocatalyst was TiO_2 Degussa P-25 (mainly anatase, specific surface area 50 $m^2\,g^{-1}$).

The catalyst was used as received without previous photo-activation or washing. TiO_2 concentrations of between 0.2 and 1.5 g/l were tested to study the photocatalytic inactivation of *E. coli*. The optimal TiO_2 concentration was 1 g/l.

Other experiments were conducted under the same conditions using partially treated wastewater. Water samples were taken at the same time, at the outlet of a biological wastewater treatment plant (Vidy, Lausanne, Switzerland). 0.5 g/l was found to be the optimal TiO_2 concentration for these experiments. The water qualities of these samples were not modified before phototreatment.

2.2. Reactors and procedures

Photochemical experiments were carried out using a pilot system called coaxial photocatalytic reactor (CAPHORE). Fig. 1 represents the CAPHORE system and Table 1 shows the experimental conditions of CAPHORE. The illuminated part of the set-up is connected with a non-illuminated bioreactor (Biolafitte). The illuminated part of the system is composed of two glass tubes which contain neon lamps. Two Philips 36-W (1.20 m long and 26 mm in diameter, TLD 36 W/08) black actinic lights are employed for irradiation in such a way that their center passes through the reactor axis. The distance between the inner tube surface and the external lamp surface is 0.9 cm. The lamp radiation distribution is between 330 and 390 nm centered at 366 nm. The coaxial tubes are connected by silicone tubes to a 2-l hermetic 15-cm-diameter bioreactor (Biolafitte). The sterile bioreactor was inoculated with bacterial culture in demineralized water; both parts of the system were sterilized before and after each experiment.

The bacterial suspension and the suspended TiO_2 circulate in a 4-l closed circuit. A small pump (Little Giant Pump Comp.) ensures circulation of water through the system; the pipe is a PVC–Nylon Solaflex. Temperature and

Table 1
Experimental conditions of CAPHORE

Parameter	Value
Recirculation rate (l/min)	1
Total volume (l)	4
Volume of water exposed to irradiation (ml)	2752
UV lamps, λ_{max} at 366 nm (W)	36
Residence time of water exposed to UV (min)	2.55
Temperature (°C)	20–25
Total residence time (min)	3.70
TiO_2 P-25 (g/l)	0.2–1
Agitation (rpm)	185
Initial concentration of bacteria (CFU/ml)	10^4–10^7

pH measurements, addition of gas (O_2 or N_2) as well as the sampling are done in the bioreactor. Agitation is ensured by a PolyMix system connected to the reactor propeller. The dissolved oxygen (DO) is measured by a sensor cell (WTW) at the outlet of the coaxial tubes. A pH probe (Mettler-Ingold) and a temperature probe (PT100) are connected to a bioreactor regulation system. The bioreactor is equipped with a pH and temperature control unit. The pH adjustment was made using either HCl or NaOH 0.1N.

2.3. Bacterial strain and growth media

The bacteria strain used was *E. coli* K12 (ATCC 23716) and was supplied by DSM. *E. coli* K12 was grown overnight in a rich medium (Nutrient Broth No. 2, Oxoid, Switzerland). Aliquots of the overnight cultures were inoculated into fresh medium and incubated aerobically at 37 °C until the exponential growth phase was reached. Growth was monitored by optical density (OD) at 600 nm. Bacterial cells were collected by centrifugation and the bacterial pellet was re-suspended in a tryptone solution (1% w/v). The cells suspended in tryptone solution were diluted with demineralized water to the required cell density (corresponding to 10^4–10^7 colony forming units per milliliter, CFU/ml). Bacterial suspension was irradiated and samples were taken at regular intervals. Dilutions were prepared if necessary in tryptone solution and the samples plated on agar PCA (Plate Count Agar, Merck, Germany) plates.

For some experiments, of phototreatment of wastewater samples, a commercial chromogenic medium CHROMagarECC (CHROMagar Microbiology, France) was used for detection of *E. coli* and coliforms. Gram-negative bacteria were also detected using ECD Agar Mug (Biolife, Italy). For detection of Gram-positive bacteria, such as *Enterococcus* sp., Enterococcus agar Slanetz and Bartley medium (Merck, Germany) was used. Bacterial counting was performed immediately on medium by the plate technique.

The mixed culture (*E. coli* plus *Bacillus* sp.), was grown overnight in a rich medium (Nutrient Broth No. 2, Oxoid, Switzerland). Aliquots of the overnight cultures were inoculated into fresh medium and incubated aerobically at 37 °C by 4 h. For detection of bacteria present in a mixed culture, two media were used, PCA for the counting of whole population and ECD (selective medium) for *E. coli*. Bacterial cells were collected by centrifugation and the bacterial pellet was re-suspended in a tryptone solution (1% w/v). The cells suspended in tryptone solution were diluted with demineralized water to the required cell density corresponding to 10^4–10^7 CFU/ml).

The plates were incubated at 37 °C for 24 h before counting. All experiments were repeated three times. Each point on the graphs represents an average of the three experiments. The error bars represent the standard deviation (S.D.) of each point.

A control without TiO_2 but with UV-A was carried out for the experiments described in this paper: *E. coli* pure culture, mixed culture (*E. coli* plus *Bacillus* sp.) and wastewater samples. In all cases, the low bacterial inactivation by solely UV-A was strongly enhanced by the presence of TiO_2.

2.4. Dark repair experiments

After predetermined exposure times, the samples were removed from the photoreactor and wrapped with aluminum foil immediately after light exposure. The exposure times were determined as a result of preliminary photocatalytic experiments (not shown here), as well as from results obtained in previous work [5]. One sample was immediately plated on agar as an experimental non-repair control. Duplicate samples were transferred to an incubator under mechanical agitation at 37 °C for 24 h. The duplicate samples were plated for counting after 24 h incubation (see Section 3.3).

2.5. Chemical and physical analysis

The chemical oxygen demand (COD) was carried out via a Hach-2400 spectrophotometer using dichromate solution as the oxidant in strong media (H_2SO_4) [9]. Turbidity of pretreated wastewater samples was measured with a HACH 2100A turbidimeter using formazin solution as a primary standard [10].

3. Results and discussion

3.1. Inactivation of E. coli in CAPHORE

The control experiment in Fig. 2 shows that, *E. coli* was not inactivated by TiO_2 in the dark. Using CAPHORE, bacterial inactivation by light was possible in the absence of the photocatalyst. However, when combined UVA and TiO_2, a marked increase in the disinfection rate was observed. Fig. 2 describes sharp photocatalytic disinfection for the first 30 min, followed by a lower inactivation rate up 225 min.

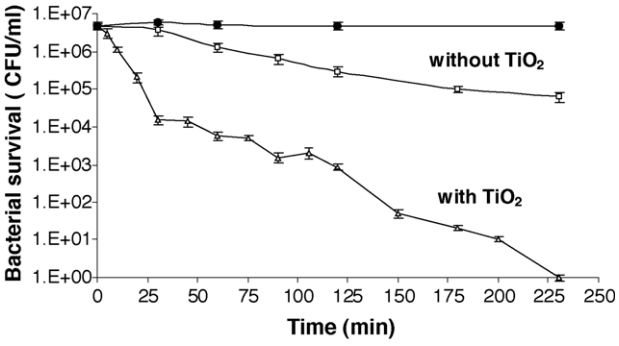

Fig. 2. *E. coli* K12 inactivation by photocatalysis in CAPHORE system in the presence (△) and absence (□) of TiO_2. Control experiments in the dark (●). *E. coli* was taken from a culture in exponential state. Initial concentration 10^7 CFU/ml, TiO_2 0.5 g/l.

Disinfection rates were calculated using the data points between 0 and 225 min according to Chick's law:

$$\mathrm{Log}\frac{N}{N_0} = -kt$$

where N means the bacterial concentration at the time of treatment (t) and N_0 the bacterial concentration at the start. Then the inactivation rates were: 0.1909 min^{-1} ($R^2 = 0.9809$) for the period between 0 and 30 min; 0.0605 min^{-1} ($R^2 = 0.9437$) for the period between 0 and 225 min; and 0.0492 min^{-1} ($R^2 = 0.9724$) for the period from 30 to 225 min, respectively.

The last stage of photocatalytic inactivation, in Fig. 2, is marked by strong attenuation of the bacterial inactivation rate. This could be due to:

(a) The competition for catalyst, light and $^{\bullet}$OH radicals of dead bacteria and by-products generated by the bacterial lysis.
(b) The probability of $^{\bullet}$OH and other radicals coming in contact with an active *E. coli* is lower at the end than at the beginning of photocatalytic treatment, since at the end of treatment very few active bacteria remain. In other experiments, using the same reactor (not shown here), it was observed that the concentration of active *E. coli* diminishes from 10^7 to 10 CFU/ml during the first 2.5 h of photocatalytic treatment and the latter value continues decreasing very slowly during the subsequent 3.5 h.
(c) The system may have reached a state where *E. coli* photocatalytic inactivation is built up by the bacterial growth rate. This could be favored by the fact that bacteria probably adsorb on the surfaces of non-illuminated parts of the system, becoming less accessible to the oxidative stress generated by the photocatalytic process. Moreover, the intermittency in the illumination, due to the recirculation of bacterial suspension through the non-illuminated part of the experimental set up (Fig. 1), negatively affects the *E. coli* inactivation rate as reported in a previous paper [5].

Experiments carried out at 1 g/l of TiO_2 presented higher disinfection kinetics: 0.4830 min^{-1} for the first stage and of 0.3000 min^{-1} for the period between 0 and 150 min. Consequently, this concentration was used for the following experiments carried out on a mixed culture. Bacterial culture and counting was done in a non-selective medium (PCA).

3.2. Inactivation of a mixed culture of *E. coli* and *Bacillus sp.*

Fig. 3 shows bacterial survival in water containing 70% *Bacillus* sp. and 30% *E. coli* and detected by a non-selective medium. *E. coli* were detected in parallel by a selective medium (ECC). Two kinetic steps in the bactericidal effect of illuminated TiO_2 on the mixture of bacteria have been

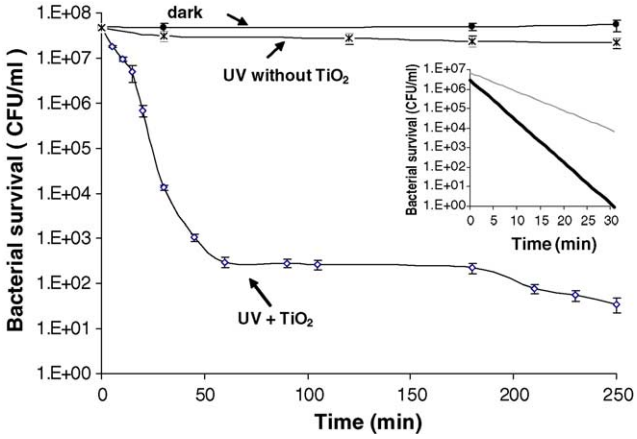

Fig. 3. Photocatalytic deactivation of bacterial culture containing *E. coli* and *Bacillus* sp. (◇). Control experiment, mixed culture under UV-A irradiation without TiO_2 (✕). Bacteria were detected by a non-selective medium (PCA). The inset illustrates deactivation of *E. coli* (dark line) and *Bacillus* sp. (thin line). TiO_2 1g/l.

observed, a first very fast step followed by a second slower one. The fast step is due to the massive photocatalytic $^{\bullet}$OH generation, which attacks bacteria causing a strong reduction in bacterial survival. In the second step of phototreatment, inactivation of bacteria becomes much slower because, as mentioned above, the few active bacteria still remaining in the irradiated water are in competition for $^{\bullet}$OH with both inactivated bacteria and metabolites released during the photo process.

The mixed culture inactivation rate calculated by Chick's law at a period of 60 min was 0.1997 min^{-1}. The contribution of *E. coli* to this constant was small, as after 30 min there is hardly any *E. coli* in the solution and their inactivation rate in the mixture was calculated as 0.0300 min^{-1}. Consequently, for the following experiments, the results obtained by non-selective PCA media were interpreted as representative of solely *Bacillus* sp. Thus, the last part of the curve (lag phase), in Fig. 3, is due to the strong resistance to inactivation of *Bacillus* sp. The different sensitivity of *E. coli and Bacillus* sp. to the photocatalytic treatment observed may be due to the morphological differences between these bacterial groups. In the mixed culture containing *E. coli* and *Bacillus* sp., it was observed by the microscope the spore formation by *Bacillus* sp. Resistance of *Bacillus* sp. may be then explained by their ability to sporulate [11]. In fact, sporulation is a form of resistance developed by some bacteria in order to withstand unfavorable conditions. Thus, when the *Bacillus* are subjected to stressful conditions such as temperature and pressure changes or lack of substrate, they are able to form spores [12]. These small oval or spherical units are characterized by a cycle alternating between vegetative growth, sporulation and germination. Spores are very resistant to high pressure; heat (5 min with 120 °C) as well as to UV radiation. The wall of the spores has a protein called keratin, rich in sulfide links, which are responsible for

the strong resistance to chemical agents and physical changes, such as the stress generated by photocatalysis. Spore UV resistance is the result of a complex set of molecular interactions which occur during sporulation, dormancy and germination [13].

The relative resistance of *Bacillus* sp. and *E. coli* to UV light has been studied elsewhere [14]. Our results concerning the stronger resistance of *Bacillus* sp. to photocatalytic treatment heads in the same direction as those reported by Haslay and Leclerc [15] on water exposed to UV treatment even though the bacterial strain and experimental conditions are different from those in this study.

It was observed that photocatalytic inactivation of *E. coli* in the mixed culture also containing *Bacillus* sp. is much faster than that observed in the pure *E. coli* culture. Competition between the two species for nutrients could partially explain this. It is also known [11] that antibiotics may be produced by microorganisms such as *Bacillus* sp. leading to the inhibition, or killing, of other microorganisms. For instance, *Bacillus* species produce polypeptide antibiotics such as polymyxin and bacitracin. Polymyxin produced by *Bacillus poly* affect Gram-negative bacteria by inducing damage on the cytoplasmic membranes [11]. Therefore, antibiotics generated by *Bacillus* sp. could make the cell wall of *E. coli* more sensitive to photocatalytic action. So acceleration of photocatalytic *E. coli* disinfection in the presence of *Bacillus* sp. is probably due to the combined action of these antibiotics and the TiO_2-photocatalysis. Note that, in the present experiment, the concentration of TiO_2 is doubled compared to the experiment shown in Fig. 2, this could be a supplementary reason for the increased rate k too.

On the other hand, exposure of microorganisms to UV radiation causes several effects on cellular systems. The most energetic fraction of the ultraviolet spectra corresponds to the UV-C range (200–290 nm). All cell types are susceptible to UV-C induced damage since many important biological molecules, including nucleic acids and proteins, absorb strongly at these wavelengths. In contrast with the destructive action of UV-C, light effects at longer wavelengths, UV-A (320–400 nm) and UV-B (290–320 nm), are mainly mediated by sensitizers rather than by direct absorption of the energy by the light-sensitive cellular components [16].

However, UV-B (near UV), radiation causes both direct and mediated action generating DNA photoproducts, of which the cyclobutane pyrimidine dimmer (CPD) and the pyrimidine (6–4) pyrimidinone (6–4PP) are the most common [17]. UV-A (or far-UV), wavelengths typically cause only indirect damage to cellular DNA through inducing the formation of chemical intermediates such as reactive oxygen species such as $^{\bullet}O_2^-$, H_2O_2 and $^{\bullet}OH$. In addition to pyrimidine dimmers, UV-A radiation, in common with UV-C and UV-B radiation, also induces single-strand breakes. Energy is indirectly transferred to DNA via intermediates called photosensitizers, producing reactive oxygen species [16]. The accumulation of DNA photoproducts can be lethal to cells through the blockage of DNA replication and RNA transcription [14,18]. However, bacteria have evolved mechanisms in the repair or damage tolerance of UV radiation damage DNA [19].

In the present experiment, a control without TiO_2 but with mainly UV-A, showed a low bacterial inactivation in the mixed culture containing *E. coli* and *Bacillus* sp. (Fig. 3 trace $*$).

3.2.1. pH, temperature and oxygen evolution during the photocatalytic process

pH, temperature and dissolved oxygen were continuously measured during photocatalytic disinfection in the CAPHORE. The initial pH of 7.0 stabilized quickly between 5.5 and 6.0 during the treatment. The initial temperature of 20 °C, increased progressively to 25 °C. In a previous paper we demonstrated that modifications in the initial pH and temperature in these ranges do not affect to a significant extent the photocatalytic deactivation of *E. coli* [5].

It can be observed in Fig. 4 (dashed line d) that during photocatalytic disinfection of the mixed culture, the dissolved oxygen (DO) decreases from a saturation value of 8 to 5 mg/l of O_2 in 30 min. This oxygen decay is probably due to two reasons. The first is associated with oxygen consumption by microorganism respiration. The second may be linked with O_2 consumption by photo-activated TiO_2 (Eqs. (1)–(7)). Molecular oxygen is the acceptor species in an electron-transfer reaction with the CB of the photocatalyst in a reductive reaction (Eq. (1)). Superoxide anions and its protonated form subsequently dismute to yield hydrogen peroxide or peroxide anions (Eqs. (2)–(4)). It has also been shown that hydrogen peroxide addition considerably enhances the photodegradation rate, most probably by Eq. (7), where H_2O_2 is reduced

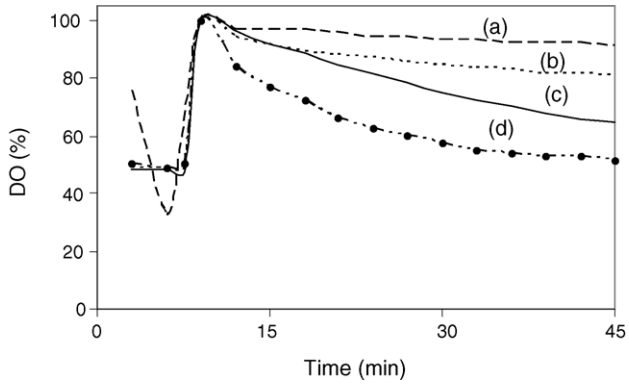

Fig. 4. Evolution of oxygen concentration in water containing a mixed culture of *E. coli* and *Bacillus* sp. in the systems (a) only light, (b) only bacteria, (c) TiO_2 + light, (d) bacteria + TiO_2 + light. 100% = 8 mg O_2/l. The water temperature increased during the treatment from 20 to 25 °C. The system was initially saturated by O_2 from the air. Thereafter (at 10 min) the air supply was turned off to make the system hermetic. Dashed line d corresponds to the experiment shown in Fig. 3.

by CB electrons, or by surface-catalyzed dismutation of H_2O_2. H_2O_2 is also generated by Eqs. (5) and (6). Organic pollutants or bacteria adsorbed on TiO_2 particle surface will then be attacked by $^{\bullet}OH$ radicals.

$$TiO_2(e^-) + O_2 \rightarrow TiO_2 + O_2^{\bullet -} \quad (1)$$

$$O_2^{\bullet -} + H^+ \rightarrow HO_2^{\bullet} \quad (2)$$

$$H_2O + O_2^{\bullet -} + HO_2^{\bullet} \rightarrow {}^{\bullet}OH + O_2 + H_2O_2 \quad (3)$$

$$2HO_2^{\bullet} \rightarrow O_2 + H_2O_2 \quad (4)$$

$$^{\bullet}OH + {}^{\bullet}OH \rightarrow H_2O_2 \quad (5)$$

$$HO_2^{\bullet} + TiO_2(e^-) + H^+ \rightarrow H_2O_2 + TiO_2 \quad (6)$$

$$TiO_2(e^-) + H_2O_2 \rightarrow TiO_2 + {}^{-}OH + {}^{\bullet}OH \quad (7)$$

In order to confirm these assumptions, we measured the evolution of oxygen in water under the following conditions: (a) light alone (b) bacteria alone, (c) TiO_2 + light without bacteria.

Fig. 4 confirms that the DO drop is due to both bacterial respiration and the reaction of oxygen with TiO_2. It was found (Line c) that the most significant O_2 consumption is due to the TiO_2 photo-assisted process through the mechanisms mentioned above (Eqs. (1)–(7)). However, it was not possible to carry out the O_2 mass balance by adding the values of Lines b and c in order to obtain the value of Line d. Indeed, the bacterial respiration would be overestimated this way because over the course of time, parallel bacterial inactivation takes place leading to the decrease in bacterial O_2 consumption. Another O_2 consuming process is the photooxidation of the bacterial debris. However, the values of DO presented in Fig. 4 must to be taken with caution, and only used to illustrate a relative tendency, since absolute values are influenced by a rise in temperature from 20 to 25 °C.

3.2.2. Influence of oxygen in the disinfection process

As mentioned above, dissolved oxygen molecules accept electrons from the conduction band of TiO_2 and are transformed into superoxide anion radicals, which react with H_2O and generate other oxidative species such as $^{\bullet}OH$ or H_2O_2 (Eqs. (1)–(6)). The concentration of the oxidative species generated directly influences the bacteria inactivation rate. Some experiments were performed in order to determine the effect of additional oxygen supply as pure O_2 on the photocatalytic disinfection of the mixed culture. Fig. 5a shows that bacteria were sensitive to additional oxygenation because after 75 min less than 1 CFU/ml were detected, while when DO was about 8 mg/l, 10^4 CFU/ml were still detected.

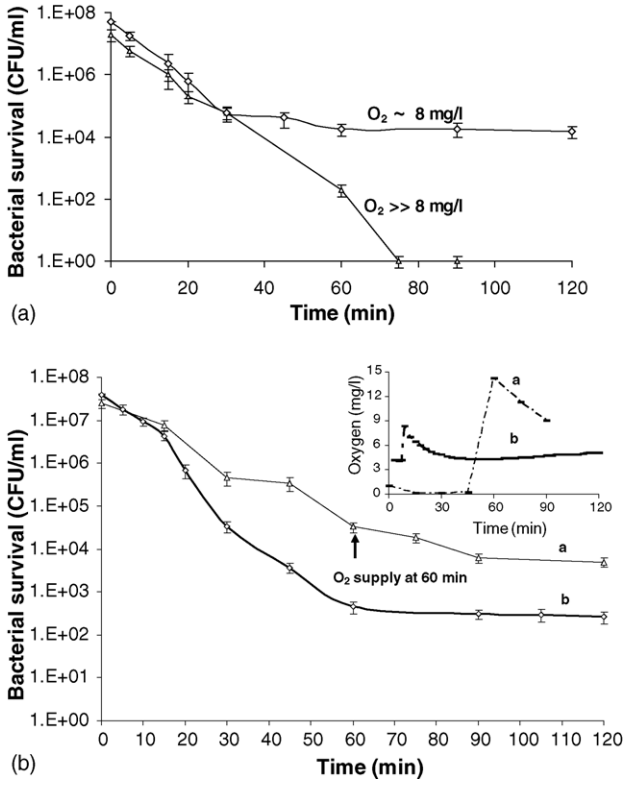

Fig. 5. (a) Influence of additional oxygenation (DO concentration > 8 mg/l) during photocatalytic treatment of a mixed culture (△) *E. coli* and *Bacillus* sp. Bacteria detected by a non-selective medium. Normal conditions of oxygenation ~8 mg/l (◇). TiO_2 1 g/l. In the experiment where DO concentration was >8 mg/l, oxygen was supplied as bubbled pure O_2. (b) Influence of a successive supply of O_2 during photocatalytic bacterial inactivation of the mixed culture. Curve b, normal conditions of oxygenation with air, ~8 mg/l (◇). Curve a, low O_2 concentration until 60 min and supply of O_2 thereafter (△). The inset contains the evolution of dissolved oxygen for the corresponding runs a and b. TiO_2 1 g/l.

3.2.3. Influence of a successive break-supply of O_2

Photocatalytic disinfection was also carried out in nitrogen-purged water and during the subsequent strong sharp supply of O_2. Results thus obtained were compared with those obtained with an initial DO concentration of 8 mg/l.

It was noticed in Fig. 5b that during the first 15 min the photocatalytic bacterial inactivation rate was the same, irrespective of the presence of high or low DO concentrations, while at the further stages of the reaction, the inactivation rate decreased when initial DO concentration is lower. That confirms that in the absence of an electron acceptor such as oxygen to favor Eq. (1), there is a higher recombination of the electron–hole pairs (Eq. (8)) and consequently there is a reduction in the production of ROS, which are responsible for the photocatalytic disinfection.

$$TiO_2(e^-) + TiO_2(h^+) \rightarrow TiO_2 \quad (8)$$

The relatively high disinfection rate observed during the first step in water containing low DO concentration (Line a in Fig. 5b) could be indicative of participation in the reaction of lattice oxygen as suggested by Dhananjeyan et al. [20]. The surface bound OH^- participating in the reaction in Eq. (9) may be available from the following reaction involving lattice oxygen (oxide ion) as in Eq. (10), where *I* is an adsorption site on the catalyst.

$$TiO_2(h^+) + OH^-_{ad} \rightarrow TiO_2 + {}^{\bullet}OH_{ad} \quad (9)$$

$$O^{2-}_L(h^+) + I + H_2O \rightarrow O_LH^- + I - OH^- \quad (10)$$

Moreover, the initial low DO concentration could generate some changes in the mass transfer of all chemical species involved in the photocatalytic process. At a low DO concentration the inactivation rate (calculated from the points between 0 and 60 min in Fig. 5b (Line Δ) was 0.1031 min^{-1} ($R^2 = 0.9566$), while in the system containing a higher DO concentration (Line ◇) it was 0.2001 min^{-1} ($R^2 = 0.967$).

Other authors have discussed the role of DO in water on bacterial inactivation by photocatalysis. Wei et al. [21] used suspended TiO_2 under solar irradiation. The bactericidal activity increased with the concentration of DO. The composition of the gas flowing in the solutions exerted a dramatic effect on the bacterial activity of irradiated TiO_2 when switched from 100% O_2 to 100% N_2. Reed and co-workers [22,23] reported that even in the absence of TiO_2, solar disinfection depends on the DO concentration.

At 60 min of illumination (Fig. 5a, Line Δ and Curve a in the inset) DO concentration was increased, but no rise in the disinfection rate was observed at this stage.

3.2.4. Influence of organic matter and minerals on the photocatalytic disinfection

We studied the photocatalytic inactivation of a mixture of *E. coli* and *Bacillus* sp. in the presence of broth media (Nutrient Broth N2, Oxoid Switzerland), labeled NB. This solution contains, among other compounds, sodium chloride and peptone. Therefore, the chemical composition of the solution was different in the presence and absence of NB. For instance, in the first case, an initial chemical oxygen demand, COD see above, of 8 mg O_2/l was measured while this value was only 0.1 mg O_2/l in the second case.

Organic and inorganic matter present in NB diminish the *E. coli* disinfection rate as observed in Fig. 6a and b. In the absence of the NB medium (Fig. 6a), *E. coli* were inactivated after 30 min of photocatalytic treatment while active *Bacillus* sp. remain almost constant at 10^4 CFU/ml after 1 h of treatment. NB medium is a very rich medium that protects bacteria against photocatalysis and provides bacteria with optimal growth conditions. Thus, bacterial growth probably takes place and counter-balances the photocatalytic inactivation of bacteria in Fig. 6b. On the other hand, organic substances present in NB medium

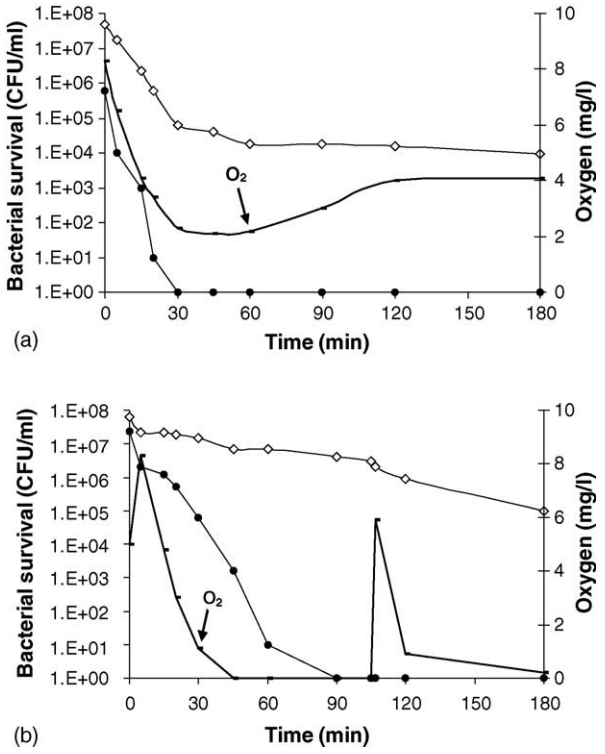

Fig. 6. Effect of nutrient broth (NB) media on the photocatalytic inactivation of *E. coli* (●) and *Bacillus* sp. (◇) mixed culture, using the CAPHORE system. Evolution of DO in the system (–). Bacteria were detected by PCA (non-selective medium), and ECD (specific to identified coliforms). (a) Experiment without NB; (b) experiment with NB. TiO_2 1 g/l.

are in competition with bacteria for oxidative species, such as ${}^{\bullet}OH$. Moreover, certain anions present in NB could also inhibit the photocatalytic inactivation of bacteria [24]. In addition, adsorbed organic matter (RX_{ad}) reacts with the photogenerated h^+ (Eq. (11)) competing for the oxidative reactions as the production of ${}^{\bullet}OH$ (Eqs. (9) and (12)).

$$TiO_2(h^+) + RX_{ad} \rightarrow TiO_2 + RX^{\bullet+}_{ad} \quad (11)$$

$$TiO_2(h^+) + H_2O_{ad} \rightarrow TiO_2 + {}^{\bullet}OH_{ad} + H^+ \quad (12)$$

Fig. 6a and b also show that during photocatalytic disinfection, the dissolved oxygen evolves differently depending on the presence or absence of NB. In the presence of NB the dissolved oxygen concentration decreases to non-detectable values in 45 min. At 100 min of photocatalytic treatment, O_2 was added for a second time (Fig. 6b), but was quickly consumed without significantly influencing the inactivation rate.

The DO decrease in the presence of NB could be explained by the presence of organic matter contained in the NB, which is attacked by reactive oxidative species (ROS) generated during the photocatalytic treatment. High ROS consumption by organic matter implies concomitant high O_2 consumption due to the reaction in Eq. (1). Moreover, ${}^{\bullet}OH$

Table 2
Photocatalytic *E. coli* and *Bacillus* sp. inactivation rates in the presence and absence of nutrient broth (NB) in the CAPHORE system

System	k (min^{-1})	R^2
With NB		
E. coli	0.2290	0.9680
Bacillus sp.	0.0660	0.8950
Without NB		
E. coli	0.4860	0.9340
Bacillus sp.	0.2230	0.9980

The disinfection constants were calculated from Fig. 6, according to the Chick's Law. The period for calculating the k's was 30 and 60 min for *E. coli* and *Bacillus* sp., respectively.

diffusion and reactivity in the system is probably modified by the presence of organic and inorganic matter in NB media. Thus, in presence of NB, the photocatalytic bacteria inactivation process was limited by O_2 availability.

Decrease in DO concentration is also due to the endogenous respiration of the bacteria, as well as to the aerobic consumption as energy source of the organic compounds contained in NB substrate. In the present work, the effect of the metabolic role of each organism on the evolution of DO concentration was not systematically studied. However, this factor may also contribute to the difference observed between Fig. 6a and b concerning the relative viabilities of *E. coli* and *Bacillus* sp.

Table 2 shows that in the presence of NB, the *E. coli* and *Bacillus* sp. inactivation rates decreased 2 and 3 times, respectively, over their inactivation rate in the absence of NB. As mentioned above, *Bacillus* sp. were less sensitive to photocatalytic treatment than *E. coli* and probably the growth of *Bacillus* was also more positively affected by the presence of NB, than that of *E. coli*. This fact is probably related to the metabolism of each strain.

The effect of oxygen on bacterial photocatalytic inactivation is crucial in water containing organic matter. For practical reasons, oxygenation of this kind of water seems to be essential to optimize the process.

For evaluation of the effect of the CAPHORE system on a real water; the response to the photocatalytic treatment of a bacterial community present in wastewater is studied in the next section.

3.3. Effect of the photocatalytic process on a microbial community present in partially treated wastewater

Domestic wastewater is known to harbor high levels of pathogenic agents that are still present in the effluents of biological treatment plants. The presence of such pathogenic agents and other harmful chemical compounds after biological treatment makes direct use of these effluents for agricultural purposes impossible. It is therefore of great interest to control their elimination by applying adequate disinfection treatment. The common water disinfection processes (chlorination, ozonation) often lead to the formation of toxic disinfection by-products (DBPs). The most important DBPs are the trihalomethanes (THM), which are cause of concern due to their carcinogenic and mutagenic potential [25,26]. DBPs result mainly from the organic compounds present in water that combine with chemical oxidants during the classical chemical disinfection.

Since wastewater contains high levels of organic matter, a correspondingly high level of DBPs is consequently generated during their chemical disinfection. The use of new technologies for disinfection of domestic wastewater has therefore recently gained in importance. Among them, photocatalytic disinfection with TiO_2 to limit DBP formation has recently been explored, since this process simultaneously attacks bacterial populations and organic substances [27].

Photocatalytic processing of pretreated municipal wastewater is therefore reported in this section. Samples were taken at the outlet of a biological wastewater treatment plant (Vidy, Lausanne, Switzerland in June, 2002) and were irradiated in the CAPHORE for 5 h in the presence of TiO_2. The evolution during phototreatment of non-selective bacteria, Gram-negative, *E. coli*, other coliforms and *Enterococcus* sp. was monitored using different selective and non-selective media. After 1, 2 and 5 h of phototreatment, aqueous suspensions of bacteria were also left in the dark under continuous agitation and sampled after 24 h for plating. The TiO_2 concentration was chosen based on information obtained in previous work during processing of similar partially treated wastewater [5]. The turbidity of

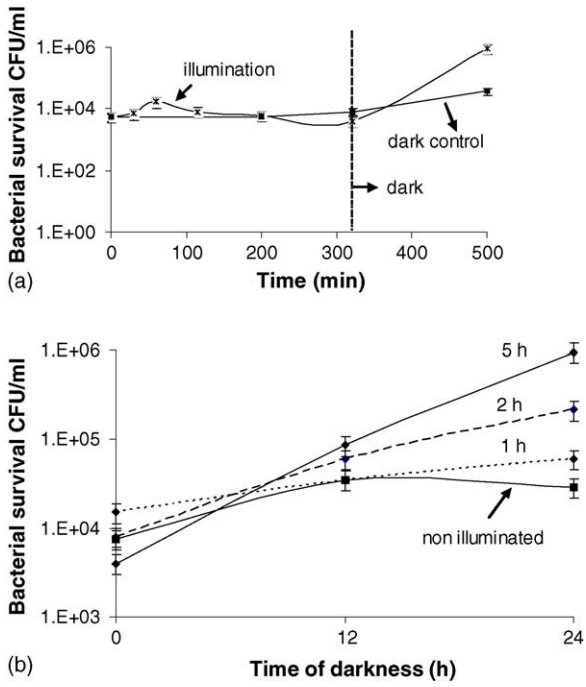

Fig. 7. Photocatalytic disinfection of wastewater in the CAPHORE. (a) Illumination off at 5 h. (b) durability of disinfection, samples in the dark after previous illumination of 1, 2 and 5 h. Bacteria detected by non-selective media (PCA). TiO_2 0.5 g/l.

wastewater (see Section 2.5) was 19 nephelometric turbidity units (NTU). In a previous work, it was presented that water turbidity higher than 30 NTU affect negatively the rate of photocatalytic disinfection [5].

The photocatalytic treatment illustrated in Fig. 7a shows a slow decrease in bacterial counts during 5 h of irradiation. A non-selective medium was used for bacterial detection. Thereafter, in the dark, bacterial population increased reaching levels up to two log higher than those observed when counting was performed at the start of irradiation. This rise in the dark is higher for the samples previously illuminated for 5 h than for those illuminated for 1 or 2 h (Fig. 7a and b). The control experiment without TiO_2 but under illumination showed that bacteria were not inactivated by UV-A (not shown here).

These observations suggest the presence of one, or several, of the following situations:

(a) There is light-induced adaptation of some bacteria to both UV and oxidants photogenerated on TiO_2.
(b) Some of the bacteria strains are less negatively affected by photocatalytic conditions and developed better in the plate, without competition from photo-inactivated strains.
(c) Some bacteria that were not culturable before illumination became culturable under light.
(d) Damage to some bacteria (i.e. to DNA) caused by $^{\bullet}OH$ and UV light could be repaired in the dark. Dark repair is a mode of reactivation consisting of excision-resynthesis and post-replication repair processes. This involves various enzymes which reverse the detrimental UV photodimerization of pyrimidines by reforming the monomeric pyrimidine [14,28]. It is possible that after photocatalysis, a self-mechanism similar to that observed after UV exposure appears.
(e) Some organic substances present during and after photocatalytic treatment become a source of nutrients for microorganisms. Photocatalysis can partially degrade organic compounds present in the wastewater, providing a carbon source that could facilitate the growth of some microorganisms after the end of illumination.

Effective disinfection time (EDT) is defined as the time required for the total inactivation of bacteria without regrowth during a subsequent dark period referenced at 24 or 48 h after ending phototreatment [6]. By this definition, EDT_{24} was not reached for the whole population.

The following sections describe the decay of some specific bacterial groups present in the same wastewater during photocatalytic treatment. Plating and counting was carried out in selective media.

3.3.1. Sensitivity of different specific groups of bacteria to photocatalytic treatment

The sensitivity of bacteria to photocatalytic treatment was different for each specific group of the microbial community contained in partially treated wastewater. Fig. 8 shows that the concentration of active bacteria decreases with irradiation time, thereafter, some bacterial populations continue to decrease in the dark. Samples were taken after different periods of exposure to the photocatalytic process and then incubated in the dark. Much less repair was observed for the specific bacteria populations (monitored by specific media) (Fig. 8), than those observed by counting the whole population in a non-selective medium (Figs. 7a and b). For *Enterococcus* sp. and *E. coli* no bacterial recovery was observed after the following 24 h in the dark. The sequence of the photocatalytic inactivation of bacteria detected using selective media, from higher to lower was:

$$E.\,coli > Enterococcus\ \text{sp.} > \text{all coliforms excluding } E.\,coli > \text{total Gram-negative.}$$

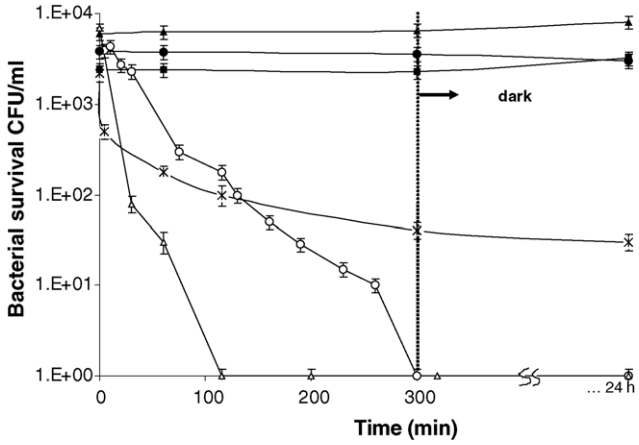

Fig. 8. Effect of the photocatalytic (0.5 g/l TiO_2) process on wastewater containing a microbial community. All Gram-negative except *E. coli* (∗). *Enterococcus* sp. (○), *E. coli* (△). The vertical line shows the beginning of dark incubation. Control without illumination, all Gram-negative except *E. coli* (■). *Enterococcus* sp. (●), *E. coli* (▲).

Note that in this work we used selective media for the detection of very few bacteria groups. These monitored bacteria represent only a small part of the total population. Moreover, differences in bacterial culturability after photocatalytic treatment have been reported [6], depending on the using of selective or non-selective media. Culturability also varies from one selective medium to another (Section 3.3.4). Therefore, these factors must be taken into account in the interpretation of results obtained with selective media. These two statements partially explain the differences in photocatalytic bacterial inactivation observed in Figs. 7a, b and 8, as well as the reported post-irradiation events. Indeed, after illumination, no growth was observed for specific selected groups (Fig. 7a and b), whereas, it was significant for whole population (Fig. 8).

In the next section, the response of each bacteria group to photocatalytic treatment is discussed.

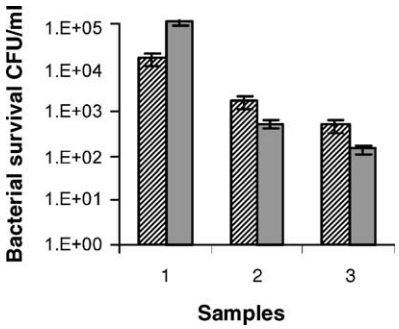

Fig. 9. Gram-negative bacteria monitored for different periods of photocatalytic treatment in the CAPHORE, and after 24 h in the dark. Samples were taken: (1) before illumination, after (2) 1 h and (3) 5 h of phototreatment. (▨) After illumination, (▩) after 24 h in the dark. Bacteria were detected by ECD media. TiO_2 0.5 g/l.

3.3.2. Evolution of Gram-negative bacteria detected by ECD media

Fig. 9 shows that the viable Gram-negative bacteria present in wastewater decreased from 10^4 to 10^2 CFU/ml after 5 h of photocatalytic treatment. Samples illuminated for a period of 1 and 5 h were placed in the dark and we observed that during the following 24 h, bacteria continued to decrease (Fig. 9). Bacterial regrowth due to dark repair does not occur in this case. Note that the length of phototreatment was not enough to assure total Gram-negative bacteria death. Thus, EDT_{24} was not reached for the whole Gram-negative bacteria population. The method used to detect bacteria indicates an average response to the photocatalytic system of all of the Gram-negative bacteria strains present in the sample. All bacteria, which are resistant to the action of the specific substances present in ECD media (biliar salts), grow in this media; but most of the Gram-positive are inhibited. Within the Gram-negative bacteria families, there are differences in their capacities to repair UV-damaged cells by liquid holding (LH) [14]. During counting after photocatalytic exposure in the present work, the bacteria observed in the selective medium reveal this diversity. LH means enhanced survival of UV-irradiated (A and/or B) cells after keeping them in a nutrient-free buffer and in the dark for several hours before cultivation on media. LH has been observed for *E. coli* B by some authors [29,30]. Other authors have observed LH recovery of bacteria when the samples are immersed in different types of chemical solutions. For instance, the repair of single-strand breaks induced in *E. coli* DNA by aerobic UV-A radiation has been observed after holding the irradiated cells in phosphate buffer [16,31]. Enzymes are involved in dark repair, such as LH recovery pathways [14].

3.3.3. Coliform evolution detected with ECC medium

For all of the coliform group strains, active bacteria decrease with irradiation time (Fig. 10), but only *E. coli* (Line △) present in the consortium were totally inactivated

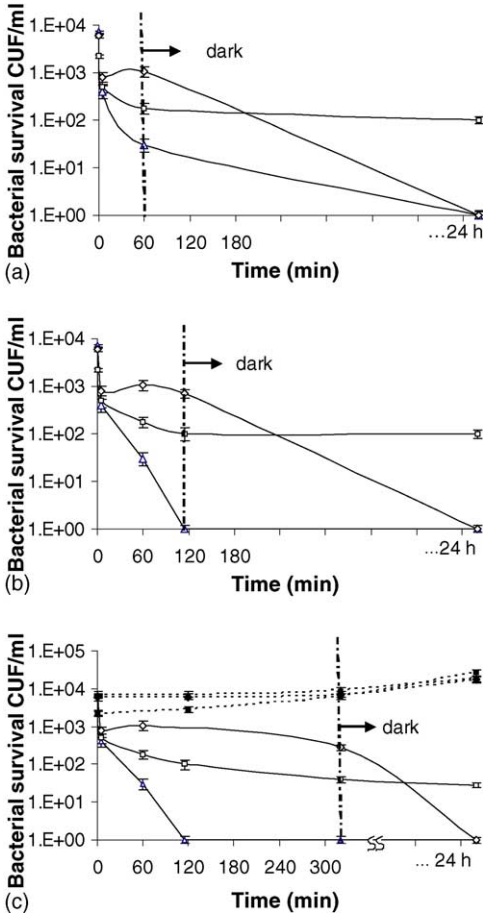

Fig. 10. Evolution of coliforms and other Gram-negatives from wastewater taken from the outlet of the Vidy aerobic biological treatment plant, Lausanne, during photocatalytic disinfection (TiO_2 0.5 g/l) in the CAPHORE. Samples irradiated for 1 h (a), 2 h (b) and 5 h (c). Dark behavior of the samples after 24 h is also shown. *E. coli* (△), all coliforms except *E. coli* (◇), Gram-negative other than coliforms (□). The vertical line shows the beginning of dark incubation. Control experiments without illumination are shown in Fig. c (▲,◆ and ■).

after 2 h of illumination. When illumination is turned off after 1, 2 and 5 h, bacterial population continues to decrease in the dark for other coliforms and other Gram-negative groups. No dark repair was observed after the following 24 h, for all groups, indicating irreversible damage to cells. Fig. 10c shows also the results of the control experiments. No significant changes were observed in the presence of non illuminated TiO_2 during the period of experimentation (Lines ◆, ■ and ▲). The control experiments without TiO_2 but UV-A showed low bacterial inactivation. In ECC medium, Gram-negative bacteria other than coliforms can also be detected.

According to these results, EDT_{24} was reached for *E. coli* and for all coliforms excluding *E. coli,* but not for Gram-negative other than coliforms. Similar *E. coli* sensitivity had previously been observed in our laboratory [5] for photocatalytic treatment of a pure culture of K12 *E. coli* suspended in deionized water.

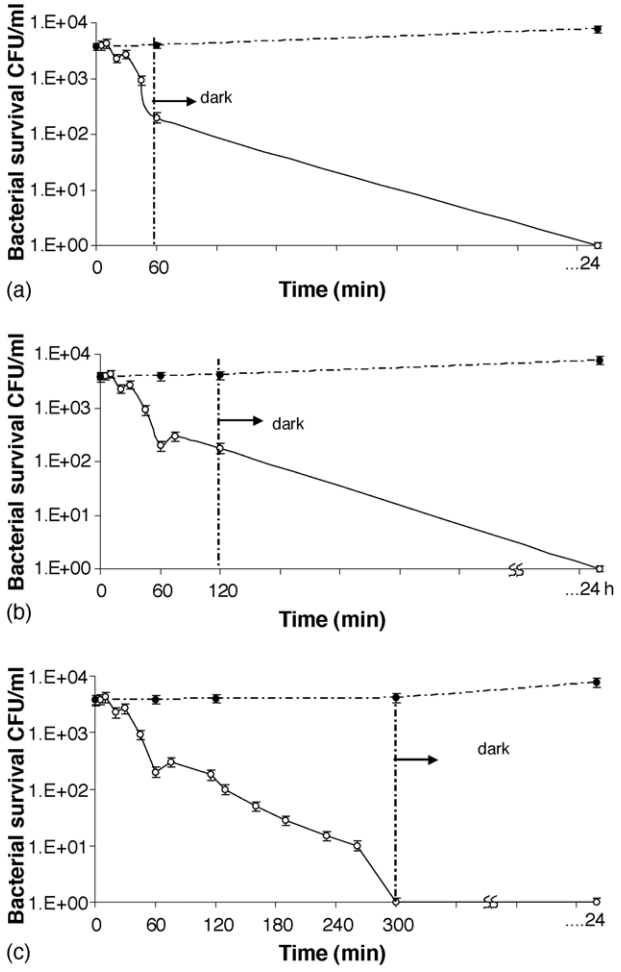

Fig. 11. Evolution of *Enterococcus* sp. during photocatalytic (TiO_2 0.5 g/l) disinfection using CAPHORE. Wastewater coming from the outlet of a biological treatment plant, Vidy Lausanne. Samples irradiated for 1 h (a), 2 h (b) and 5 h (c). Behavior of the photo-treated samples after 24 h in the dark. The vertical line shows the beginning of the dark incubation.

The sequence of the detrimental effect of photocatalysis on the different bacteria groups, in ECC medium from less to more resistant was:

$$E.\,coli > \text{all coliforms except } E.\,coli > \text{other Gram-negative}.$$

So, we have confirmed in this type of reactor, the results obtained in previous work where *E. coli* was found to be more sensitive to the photocatalytic treatment than all coliforms except *E. coli* (other coliforms) and Gram-negative other than coliforms.

3.3.4. *Enterococcus sp. evolution during the photocatalytic treatment*

Enterococcus sp. was more resistant than *E. coli* to TiO_2-light attack (Fig. 8) reaching non-detectable values (<1 CFU/ml) only after 5 h of illumination, while *E. coli* reached non-detectable values after 2 h. However it was less resistant than other coliforms (other than *E. coli*) and other Gram-negative bacteria (Fig. 8). The separate samples taken during illumination periods show that the concentration of active *Enterococcus* sp. also decreased in the dark (Fig. 11), therefore no detectable dark repair of photo-treated bacteria was observed during the following 24 h after turning off illumination. Figs. 8 and 11 show that EDT_{24} was reached for *Enterococcus* sp.

It is known that *Enterococcus* sp. are more resistant than coliforms to antimicrobial agents. Saito et al. [32] reported that illuminated TiO_2 had a bactericidal effect on strains of mutant streptococci, but no differences were observed between the strains of mutant streptococci covering all serotypes. This was probably because killing took place too quickly under their experimental conditions. The wavelength range of light used in the referenced study was from 300 to 400 nm (peak 352). Another paper reports differences in the photocatalytic bactericidal effects of TiO_2 on different strains of mutant streptococci [33]. To our knowledge little has been published concerning the resistance of *Streptococcus* sp. to photocatalysis in water containing other bacteria strains and chemicals. Herrera Melian et al. [8] explored photocatalytic disinfection of urban wastewater containing these bacteria. They reported that solar and UV-lamp disinfection of streptococci is slower than that observed for *E. coli* plus other coliforms, and no major differences were observed in the presence or absence of TiO_2.

In contrast to this, Sinton et al. [34] compared the sunlight (without TiO_2) inactivation of *Enterococcus* sp., fecal coliforms and *E. coli* from waste stabilization pond (WSP) effluent. The inactivation of *Enterococcus* sp. was more rapid than that of fecal coliforms and *E. coli*. They observed, using different UV-B and visible light filters, that *Enterococcus* sp. tended to be inactivated by a wide range of wavelengths, whereas fecal coliforms were mainly susceptible to wavelengths of <318 nm. It is not clear to the authors why *Enterococcus* suffer more UV-generated photooxidative damage than fecal coliforms in a WSP, but they would appear to possess a narrower range of biochemical defense mechanisms.

In this work, our findings concerning stronger resistance of *Enterococcus* sp. than other Gram-negative or other coliforms could be explained by the fact that *Enterococuss* sp. are much more affected than other bacteria by wavelengths longer than those of the CAPHORE lamp (340–380 nm). Nevertheless, the combined effect of oxidative species generated during photocatalysis, combined with the sensitivity of *Enterococcus* sp. to UV-B light, was sufficient to reach undetectable levels (<1 CFU/ml) during illumination and to limit their repair and recovery in the dark.

The contribution of light (at the different wavelengths) on bacterial inactivation is not negligible because in the photocatalytic treatment a simultaneous (synergistic?) effect of light and TiO_2 is present. Note that TiO_2 is active below 380 nm and not all wavelengths emitted by the lamp are equally effective in the germicidal action. The contribution

Table 3
Percentage of bacteria inactivated after a 5-h illumination period and after a subsequent dark period of 24 h

Bacteria (selective media)	Initial concentration of bacteria (CFU/ml)	Bacterial inactivation after 5 h of illumination (%)	Bacterial inactivation during the subsequent 24 h in the dark (%)	Total inactivation (%)
E. coli (ECC)	7.0×10^3	100.00	0	100.00
All coliforms except *E. coli* (ECC)	6.0×10^3	95.33	4.67	100.00
Other Gram-negative different to coliforms	2.2×10^3	98.18	0.55	98.73
Total coliforms (ECC)	1.3×10^4	97.85	2.15	100.00
Enterococcus sp.	3.8×10^3	100.00	0	100.00
Total Gram-negative (ECD)	1.6×10^4	96.88	3.00	99.88

No regrowth of bacteria was observed. For this calculation, the detection limit was zero.

of each wavelength to overall disinfection was not determined in this study.

Table 3 shows the percentages of bacteria removed during both the phototreatment and the following 24-h dark period. Note that the inactivation during phototreatment fluctuated between 95.33 and 100% while inactivation in the subsequent dark period varies between 30 and 100%, showing that the magnitude of the ''residual disinfecting effect'' in the dark probably depends on the type of bacteria treated, and not necessarily on the reduction in active bacteria during the preceding photocatalytic treatment. In other words, the bacterial injury generated by photocatalytic treatment continues to be enhanced in the dark. This delayed process termed the ''residual effect'' [6], but is probably not induced by the residual presence of any active oxidating species.

Fig. 12 shows the response of *E. coli* in wastewater to the photocatalytic treatment. Bacterial monitoring was carried out in different ECC and ECD culture media which are selective to detect *E. coli* and total Gram-negative. The majority of Gram-negative bacteria present in the sample belong to the coliform group. The difference observed between the two media is probably due to the different inhibitory substances present in them.

In real water with a mixed population, the results obtained by a standard plate count should be corroborated with direct methods. Direct microscope count of the living bacteria using fluorescent labeling [35,36] or cellular

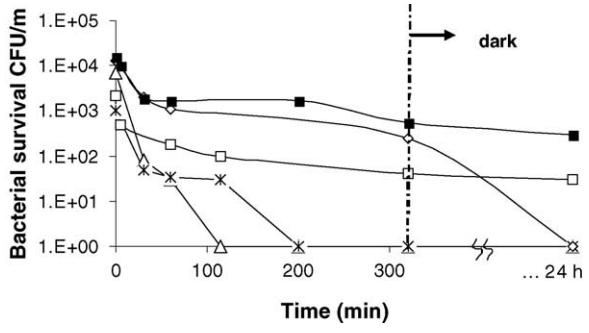

Fig. 12. Evolution of *E. coli* detected with ECC (△) and ECD (✱) media, Gram-negative detected with ECD medium (■) and total coliform detected with ECC medium (◇). Other Gram-negatives detected with ECC medium (□). The vertical line shows the beginning of dark incubation. TiO_2 0.5 g/l.

revivification [37,38] seem to be more appropriate ways to study the non-culturability phenomena observed under oxidative stress. When the use of direct methods is not possible, comparison of different media is necessary to find the most adequate. Some authors have increased culturability by adding specific compounds, like catalase [39].

Note that, during the following 24 h in the dark, the growth curve is different for each group of damaged bacteria. Probably the photocatalytic treatment induces modifications in the cell division rate depending on the type of bacterial group. Moreover, most of the critical synthesis of bio-molecules necessary for bacterial recovery (after UV-A and B radiation) takes place during the first hours of post-irradiation [40]. To our knowledge, no systematic studies on the photocatalytic response and dark reactivation of different bacterial strains have been made while there are several studies concerning dark repair after UV (A, B and C) treatment of bacteria in water [16,28,41]. Thus, in a mixture of bacteria, resistance to the phototreatment and consequently their survival capabilities during and after irradiation may be different for each type of bacteria.

It is well known that microorganisms vary significantly in their abilities to tolerate various sterilization and disinfection procedures. The basis for this difference is largely attributable to their morphology (structure) and biochemical characteristics. A thick rigid layer composed of an overlapping lattice of two sugars that are cross-linked by amino acid bridges is found in both Gram-positive and Gram-negative cells. However, most of the Gram-positive cell wall is composed of a thick peptidoglycan layer, while Gram-negative cell walls have a more complicated structure. The Gram-negative cell wall structure is thinner with distinct layers. The outer layer is enclosed by a cytoplasmic membrane, with a typical trilaminar structure. The main component of the Gram-negative cell wall is a lipopolysaccharide. Moreover, phospholipids, protein, lipoprotein and a small amount of peptidoglycan are present [11]. These characteristics influence the sensitivity of Gram-positive bacteria to illuminated TiO_2 compared, to Gram-negative bacteria because in any case, regardless of the subsequent photokilling mechanism, the first contact between bacteria and TiO_2 (or its photogenerated species) takes place on the surface of the cell envelope.

The extent of damage induced on each bacteria type (individually treated) by photocatalysis has not been estimated nor compared to the inactivating effect of UV-A light only. In a bacterial community, the extent of damage suffered by a specific type of bacteria is probably different from that observed for the same bacteria in a pure culture. Furthermore, the self-defense mechanism and the response of bacteria to UV-A or B light are different from the response to photocatalysis. As the adsorption of TiO_2 on bacterial cells is necessary to obtain a bactericidal effect, (the photocatalytic reaction is induced on the surface of TiO_2), the affinity of each bacterial strain to TiO_2 probably influences its bactericidal effects. In addition, differences observed in resistance to photocatalytic treatment of different bacteria groups could also be due to the growth phase of each microorganism when the treatment is applied, as it is known that bacteria are more resistant in stationary than in exponential growth [6].

In the CAPHORE, *E. coli* was more sensitive than other specific bacteria to photocatalytic attack, as observed in previous experiments carried out using a solar lamp [6]. All this information makes the use of *E. coli* as the sole microbial indicator for monitoring the effectiveness of disinfection in the photocatalytic system questionable.

Illuminated TiO_2 in the CAPHORE not only inactivates bacteria, but also degrades organic compounds present in wastewater or released by bacterial lysis during photocatalytic treatment. This simultaneous action has also been reported elsewhere [42] and was especially noted during the treatment of solution artificially contaminated with both *E. coli* and dihydroxybenzenes [27]. Other papers report the photocatalytic treatment of *E. coli* and formaldehyde [43], as well as wastewater taken at the outlet of a biological treatment plant [6].

4. Conclusions

The effectiveness of the CAPHORE was tested using water contaminated with a mixed *E. coli* and *Bacillus* sp. population. *E. coli* was found to be more sensitive to photocatalytic treatment than *Bacillus* sp. Bacterial inactivation was dependent on the oxygen concentration and the chemical composition of water. It was observed that the presence of oxygen clearly enhanced the inactivation process of the mixed culture.

A sharp drop in dissolved oxygen was observed during photocatalytic disinfection in the presence of a mixture of organic and inorganic matter. In this situation, photogenerated ROS simultaneously attack the bacteria and the organic compounds, leading to heavy consumption of O_2, which is involved in the photocatalytic production of ROS.

The effect of oxygen on bacterial photocatalytic inactivation is crucial in water containing a large amount of organic matter. For practical reasons, good oxygenation of this kind of water seems to be essential to prevent the inhibition of bacterial inactivation.

A low photocatalytic inactivation rate was observed for the whole bacterial population present in domestic wastewater detected by non-selective media. Among the microbial community typical of such wastewater, *E. coli* was the most sensitive to photocatalytic treatment compared to other bacteria groups (*Enterococcus* sp., total Gram-negative and other coliforms), detected by selective media. No bacterial recovery of these photo-treated bacteria was observed during the subsequent 24 h in the dark.

EDT_{24} of *E. coli* was attained after 2 h of photocatalytic treatment. In contrast, it was reached only after 5 h for *Enterococcus* sp. and all coliforms except *E. coli*. EDT_{24} was not attained for the whole population. Therefore, the use of the photocatalytic system under these experimental conditions is not recommended for disinfection of this type of partially treated wastewater.

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