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Fe^{3+} and TiO_2 solar-light-assisted inactivation of *E. coli* at field scale Implications in solar disinfection at low temperature of large quantities of water

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Abstract

Field disinfection of water in a large solar photoreactor (35–70 l) was conducted at ‘‘natural’’ temperature of 35 °C by different photocatalytic processes: sunlight/TiO_2, sunlight/TiO_2/Fe^{3+}, sunlight/Fe^{3+}/H_2O_2 and referenced by the control experiment of direct sunlight alone. Experiments under direct solar radiation were carried out using a compound parabolic collector (CPC) placed at the EPFL in Lausanne, Switzerland. Leman Lake water contaminated with *E. coli* K 12 was exposed to sunlight in sunny days on 2003. In these conditions, under solar irradiation, total disinfection was not reached after 5 h of treatment; and bacterial recovery was observed during the subsequent 24 h in the dark. The addition of TiO_2, TiO_2/Fe^{3+} or Fe^{3+}/H_2O_2 to the water accelerates the bactericidal action of sunlight, leading to total disinfection by helio-photocatalysis.
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Keywords: Solar water disinfection; TiO_2; Iron photo-assisted reaction; Photocatalysis; Water disinfection; *E. coli* K 12; Photo-Fenton

1. Introduction

In recent years, there has been a growing interest in the development of new processes for water disinfection, since the traditional processes such as chlorination lead to the production of toxic disinfection by-products [1]. In order to minimize the risk to humans, modifications of conventional disinfection including, the removal of chloro-organic compounds have been proposed [2,3]. Standard water disinfection techniques are often too expensive regarding capital investment, operation, and maintenance to be used in many regions of developing countries. In this respect, the use of solar energy as an alternative to chlorination has recently been explored. Moreover, this technology could also be economically viable in countries with a high degree of sunlight radiation.

The bactericidal effect of sunlight has been known for many years [4,5]. Recently Reed presented a complete review of solar disinfection used as a water treatment process [6].

The disinfecting effect of solar light can be enhanced by addition of a catalyst and such generated system belongs to the group of advanced oxidation processes (AOP). AOP could be an attractive alternative for the treatment of contaminated ground, surface and wastewaters containing hardly biodegradable anthropogenic substances, as well as for the purification and disinfection of drinking water [7]. AOP is characterized by the production of $OH^\bullet$ radicals and other highly oxidative radicals [8]. Photoinduced AOP can be roughly divided into heterogeneous and homogeneous processes.

In the case of heterogeneous photocatalytic oxidation via TiO_2, when the semi-conductor is suspended in water and irradiated with near UV ($\lambda < 395$ nm), $OH^\bullet$ radicals are generated by the reaction of holes and electrons respectively with donors and acceptors of electrons, Eqs. (1)–(7). The electron acceptors commonly used are the O_2 from air and the H_2O_2. The generated $OH^\bullet$ radical is highly toxic towards microorganisms and very reactive in the oxidation of organic substances. Therefore, a solar photocatalytic treatment could become an efficient method to simultaneously inactivate bacteria and degrade organic matter. Reduction by

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photocatalysis of the amount of organic matter present in drinking water sources could limit the generation of harmful disinfection byproducts (DBPs) during an optional subsequent chlorination process.

$$TiO_2 + h\nu \rightarrow TiO_2\,(e^- + h^+) \tag{1}$$

$$TiO_2\,(h^+) + RX_{ad} \rightarrow TiO_2 + RX_{ad}^{\bullet +} \tag{2}$$

$$TiO_2\,(h^+) + H_2O_{ad} \rightarrow TiO_2 + OH_{ad}^{\bullet} + H^+ \tag{3}$$

$$TiO_2\,(h^+) + {}^-OH_{ad} \rightarrow TiO_2 + OH_{ad}^{\bullet} \tag{4}$$

$$TiO_2\,(e^-) + O_2 \rightarrow TiO_2 + O_2^{\bullet -} \tag{5}$$

$$O_2^{\bullet -} + H^+ \rightarrow HO_2^{\bullet} \tag{6}$$

$$HO_2^{\bullet} + HO_2^{\bullet} \rightarrow O_2 + H_2O_2 \tag{7}$$

$$H_2O_2 + TiO_2\,(e^-) \rightarrow OH^{\bullet} + HO^- + TiO_2 \tag{8}$$

The photocatalytic degradation of various organic compounds by illuminated TiO_2 has already been reported in the literature [7]. Photocatalytic inactivation of bacteria and viruses has also been investigated, but to a lesser extend [9]. In our previous papers, bacterial suspensions in deionized water, tap water and wastewater have been used to test the helio-photocatalytic disinfection via TiO_2 catalyst illuminated with sunlight. It was found that the effectiveness of the process depends on the physico-chemical [10,11] and biological [12,13] characteristics of treated water.

On the other hand, in homogeneous processes, the use of a catalytic cation (i.e. Fe^{2+} or Cu^{2+}) and H_2O_2 leads under UV–vis light to the photo-oxidation of organic compounds principally via the generation of hydroxyl radicals ($OH^{\bullet}$) which will subsequently attack the organic pollutants.

In absence of light, we are in presence of the Fenton process that can be outlined as follows:

$$M^{n+} + H_2O_2 \rightarrow M^{(n+1)+} + OH^- + OH^{\bullet} \tag{9}$$

where M is a transition metal such as Fe or Cu. In the absence of light and complexing ligands other than water, the most accepted mechanism of H_2O_2 decomposition in acidic homogeneous solution, involves the formation of hydroperoxyle/superoxide ($HO_2^{\bullet}/O_2^-$) and hydroxyl radicals ($OH^{\bullet}$) [14–16]. The $OH^{\bullet}$ radical, once in solution attacks almost every organic compound. For Fe^{2+}, the most accepted scheme of the overall process is described in the following equations:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\bullet}$$
$$K = 63\ \text{l/mol s} \tag{10}$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HO_2^{\bullet} + H^+$$
$$K = 3.1 \times 10^{-3}\ \text{l/mol s} \tag{11}$$

$$Fe^{2+} + OH^{\bullet} \rightarrow Fe^{3+} + OH^-$$
$$K = 3.0 \times 10^{8}\ \text{l/mol s} \tag{12}$$

$$OH^{\bullet} + H_2O_2 \rightarrow HO_2^{\bullet} + H_2O$$
$$K = 3.3 \times 10^{7}\ \text{l/mol s} \tag{13}$$

$$Fe^{3+} + HO_2^{\bullet} \rightarrow Fe^{2+} + H^+ + O_2$$
$$K = 2.0 \times 10^{3}\ \text{l/mol s} \tag{14}$$

$$Fe^{3+} + O_2^{\bullet -} \rightarrow Fe^{2+} + O_2$$
$$K = 5.0 \times 10^{7}\ \text{l/mol s} \tag{15}$$

$$Fe^{2+} + HO_2^{\bullet} \rightarrow Fe^{3+} + HO_2^-$$
$$K = 1.2 \times 10^{6}\ \text{l/mol s} \tag{16}$$

Fenton reaction (Fe^{3+}/H_2O_2) rates are strongly increased by irradiation with UV–vis light [17,18], this type of photo-assisted reaction is referred to as the photo-Fenton reaction Eq. (17) [19]. The positive effect of irradiation on the degradation rate is due to the photochemical mediated regeneration of ferrous (Fe^{2+}) by photoreduction of aqua-complexes of ferric ions ($Fe(OH)^{2+}$) which concomitantly leads to the additional generation of $OH^{\bullet}$.

$$Fe(OH)^{2+} + H_2O + h\nu \rightarrow OH^{\bullet} + H^+ + Fe^{2+} + OH^- \tag{17}$$

The new generated ferrous ion reacts with H_2O_2 generating a second $OH^{\bullet}$ radical and ferric ion Eq. (10), and the cycle continues. The main advantage of the photo-Fenton process is the light sensitivity up to a wavelength of 600 nm (35% of the solar irradiation). The depth of light penetration is higher when compared with TiO_2 photo-assisted processes and the contact between the pollutant and the oxidizing agent seems to be favourable, since homogeneous solution is used [20]. Fenton and photo-Fenton processes have been used with great success to treat a wide variety of contaminants [21–24].

In contrast, to our knowledge, there are no reported studies concerning the use of photo-Fenton system to disinfect water at pilot scale. However, Fenton reactions can naturally occur in biological systems, especially where hydrogen peroxide is formed during the course of normal cell functioning. In fact, there are numerous studies [25] indicating the toxic nature of iron and copper and their role in aging of biological systems. Both copper and iron combined with H_2O_2 have been investigated as a substitute for conventional disinfectants [26]. Fenton-based disinfection using hydrogen peroxide is considered more efficient, with essentially no side effects, when compared to glutaraldehyde [27].

Fenton reaction has been found to be the key reaction in the oxidation of membrane lipids, aminoacids and in the reactions where biological reduction agents are present, such as ascorbic acid or thiols [28]. Its occurrence is also supposed in heart diseases, such as ischemia and reperfusion. The investigations of Fenton reaction in cell biology are in progress [28].

In a recent work, different advanced oxidation processes, i.e. the systems UV–vis/TiO_2, UV–vis/TiO_2/H_2O_2, UV–vis/Fe^{3+}/H_2O_2, Fe^{3+}/H_2O_2 and UV–vis/H_2O_2, have been studied and heir performances for the bacterial inactivation were compared at laboratory scale [29].

In the present study, field experiments under solar radiation are reported. Natural water contaminated with *Escherichia coli* K12 was exposed to sunlight and the bacterial culturability was monitored for photocatalytic systems such as sunlight/TiO_2, sunlight/TiO_2/H_2O_2, sunlight/Fe^{3+}/H_2O_2. The following topics are addressed in this study: (a) bacterial inactivation by sunlight in the presence and absence of TiO_2, (b) the influence of iron addition on the TiO_2 photo-assisted disinfection process, (c) bacterial inactivation by photo-Fenton system, (d) the durability of the different disinfection systems.

2. Experimental

2.1. Solar photoreactor

The configuration of the CPC is the same as that at the Plataforma Solar de Almeria, PSA [30]. CPC is a static collector with a reflective surface describing an in-volute around a cylindrical reactor tube (Fig. 1). Due to the reflector design, almost all the UV radiation arriving at the CPC aperture area (not only direct, but also diffuse) can be collected and so be available for the process in the reactor. The light reflected by the CPC is distributed around the back of the tubular photoreactor and as a result, most of the reactor tube circumference is illuminated. The CPC has three modules (collector surface 3.08 m^2, photoreactor volume 24 l, and total reactor volume was 37 l whereas one module consists of eight tubes and mounted on a fixed platform 40° tilted (local latitude). The three modules are connected in series with water directly flowing through them at 20.5 l/min, leading finally to a recirculation tank connected to a centrifugal pump. Water temperature reached in the described CPC reactor was in our case of 30–35 °C.

Solar ultraviolet radiation is determined during the experiments by means of a global UV radiometer (KIPP&ZONEN, model CUV3), also mounted on a 40° fixed-angle platform (the same angle as the CPC). Solar-UV power varies during experiments, especially when clouds are passing by. Data combination from several days and their comparison with other photocatalytic experiments is done by the application of Eq. (18).

$$Q_{\mathrm{UV},n} = Q_{\mathrm{UV},n-1} + \Delta t_n \overline{\mathrm{UV}}_{\mathrm{G},n} \frac{A_{\mathrm{CPC}}}{V_{\mathrm{TOT}}} \tag{18}$$

where $\Delta t_n = t_n - t_{n-1}$, t_n is the experimental time for each sample, $\mathrm{UV}_{\mathrm{G},n}$ the average UV_{G} (global UV radiation) during t_n, A_{CPC} = 3.08 m^2, V_{TOT} = 35–70 l and $Q_{\mathrm{UV},n}$ the accumulated energy incident on the photoreactor for each sample during the experiment per unit of volume (kJ/l). The total solar radiation was measured by means of a radiometer KIPP&ZONEN, model CUV4.

Fig. 1. View of the photoreactor used at the EPFL, Lausanne, Switzerland, compound parabolic collector (CPC).

2.2. Materials

The photocatalyst used was TiO_2 Degussa P-25 (Frankfurt, Germany), which contains mainly anatase, and a specific surface area of 50 m^2/g. TiO_2 in suspension at 40 mg/l was used in disinfection experiments at large scale. Iron chloride $FeCl_3 \cdot 6H_2O$ was supplied by Fluka (Buchs, Switzerland) and was used without further purification. Hydrogen peroxide (30 M) was supplied by Merck AG (Darmstadt, Germany). All solutions were prepared with Millipore water (Ω 18.2 M cm) and were prepared immediately prior to irradiation.

2.3. Bacterial strain and growth media

A complete description was presented previously [12]. The bacterial strain used was *Escherichia coli* K12 (ATCC 23716) and was supplied by DSM, German Collection of Microorganisms and Cell Cultures. *E. coli* K12 was inoculated into Luria Bertani (LB) medium and grown overnight at 37 °C by constant agitation under aerobic conditions. Components of LB medium included sodium chloride (10 g), tryptone (10 g) and yeast extract (5 g) in 1 l of deionized water; this solution was then sterilized by autoclaving for 20 min at 121 °C. Aliquots of the overnight culture were inoculated into sterilized LB medium and incubated aerobically at 37 °C. Bacterial growth was monitored by optical density at 600 nm. At an exponential growth phase, bacterial cells were collected by centrifugation at 500 g for 10 min at 4 °C and the bacterial pellet was washed three times with a tryptone solution (1%, W/V). Finally, the bacterial pellet was resuspended in tryptone solution and diluted in natural water to the required cell density corresponding to $10^4 - 10^6$ colony forming units per milliliter (CFU/ml). Thereafter, bacterial suspension was exposed to the sunlight irradiation in the presence or absence of TiO_2, Fe^{3+}/TiO_2, or Fe^{3+}/H_2O_2. Illuminated samples were taken during illumination period and after 24 h in the subsequent dark period. Samples were plated on agar Plate-Count-Agar (PCA, Merck, Germany) plates. In the curves of the experiments performed under sunlight irradiation, the reported results are the mean value of four samples collected at the same time interval. The error bars (Figs. 3–5) indicate standard deviation (S.D.) of the results.

Natural water coming from the Leman Lake was used to suspend bacteria. Table 1 shows some physicochemical characteristics of this water source. The initial pH of water was not modified from 7.55.

Table 1
Some physicochemical characteristics of water coming from the Leman Lake

Parameter	Unit	Value
Turbidity	FTU	0.18
Conductivity at 20 °C	μS/cm	252
Absorbance	254 nm	1.079
pH	–	7.55
Chloride	mg Cl^-/l	8.0
Nitrate	mg $NO_3{}^-$/l	2.7
Sulfate	mg $SO_4{}^{-2}$/l	48
Hydrogencarbonates	mg $CO_3{}^-$/l	107
Total organic carbon (TOC)	mg C/l	0.8
Orthophosphates	mg $PO_4{}^{-3}$/l	0.010
Aluminium	mg Al^{3+}/l	14
Nitrites	mg $NO_2{}^-$/l	0.002
Ammonium	mg NH_4/l	0.001

Water is filtrated on sand before distribution without significant modification of its chemical characteristics. The values correspond to the average of three samples taken on February and October, 2002 at the water distribution station of Haute-Pierre, near from Lausanne.

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. 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.

3. Results and discussion

3.1. Phototreatment with a solar CPC reactor in absence of TiO_2

In the absence of TiO_2: bacteria were not completely eliminated after 40 kJ/l of incident energy per unit of volume (Fig. 2a).

It is well known that sunlight is able to inactivate microorganisms due to the synergistic effect of the UV and heating of water by infrared radiation [31]. In our experiments, very little modification of the water temperature (30–35 °C) was detected throughout. Therefore, inactivation was predominantly due to optical, rather than thermal effects. The UV-A (or far-UV) wavelengths typically cause indirect damage to cellular DNA through catalyzing the formation of reactive oxygen species (ROS) such as $O_2{}^{\bullet -}$, H_2O_2 and $OH^{\bullet}$. In contrast, UV-B (or near-UV) radiation can cause direct DNA damage by inducing the formation of DNA photoproducts. The accumulation of DNA photoproducts can be lethal to cells through the blockage of DNA replication and RNA transcription [32].

In Fig. 2a the concentration of active (culturable) bacteria decreased as the energy increased up to a solar energy equivalent to 10 kJ/l and reaching a plateau even if the illumination was prolonged. The same behavior was observed for an other period of the year as shown in Fig. 2b and c. Similar tendency has been reported in one of our previous paper [33]. That could be due to:

(a) Some bacteria which are not culturable at the beginning of the process recover their culturability under the phototreatment. Recently, it has been suggested that some readily culturable species of bacteria, when subjected to prolonged starvation or other stress, may enter a survival state in which they are not detectable by culturability tests [34]. Authors [35] suggest that the non-culturable cells are in a ‘‘viable but non-culturable’’ (VBNC) state in which they remain viable but cannot be cultured. But these non-culturable cells of *E. coli* could not be dead, they are damaged and their cellular damage is reversed probably favored by the decrease of light intensity to enable the injured cell to resume growth. Bacteria can recover their culturability under phototreatment also by the photoreactivation processes [32].
(b) A possible replication of the remaining culturable cells.
(c) The damaged bacteria are repaired. Bacteria have evolved four main mechanisms in the repair or damage tolerance of UV radiation-damaged DNA, including nucleotide excision repair (NER), mutagenic DNA repair (MDR), and recombinational DNA repair and even photoreactivation [36].
(d) Diminishing of the bactericide action of the sunlight by both the decrease in UV intensity and the modification of the visible part of the spectrum along the day [11]. Indeed, light intensity (inset in Fig. 2a and b) diminishes approximately to 50% at the end of each experiment.

The combined action of factors (a) to (d), which are opposed to the solar disinfection, results in an equilibrium between bacterial inactivation, reactivation and growth, that leads in Fig. 2a and b to a quasi-stationary state where undetectable level of bacteria (<1 CFU/ml) was not reached.

Fig. 2c shows the lost of bacteria culturability during phototreatment; there is a clear difference in the responses of *E. coli* for selective and non-selective media. At 4 kJ/l of accumulated energy, no bacteria is detected in selective medium (ECC) whereas 10^3 CFU/ml are detected in non-selective one (PCA). Great differences were observed (data not shown) in other experiments carried out with different initial bacterial concentration when using the photolytic and photocatalytic systems. If the culture medium chosen to evaluate the photodisinfection efficiency (in the presence or absence of TiO_2), is not appropriated, the treatment could be stopped from a false ‘‘negative’’ test indicating erroneously the absence of bacteria colonies. Even if in this paper, we worked with pure *E. coli* culture, the detection was always performed using a non-selective medium (PCA) except for the comparison presented in Fig. 2c.

3.2. Sunlight disinfection in the presence of TiO_2

The bacterial inactivation by sunlight is strongly enhanced by the presence of TiO_2 (Fig. 3a and b). In this case, the number of active (culturable) bacteria decreases to non-detectable values (<1 CFU/ml) when an accumulated solar energy incident on the photoreactor per unit of volume of 21.0 kJ/l

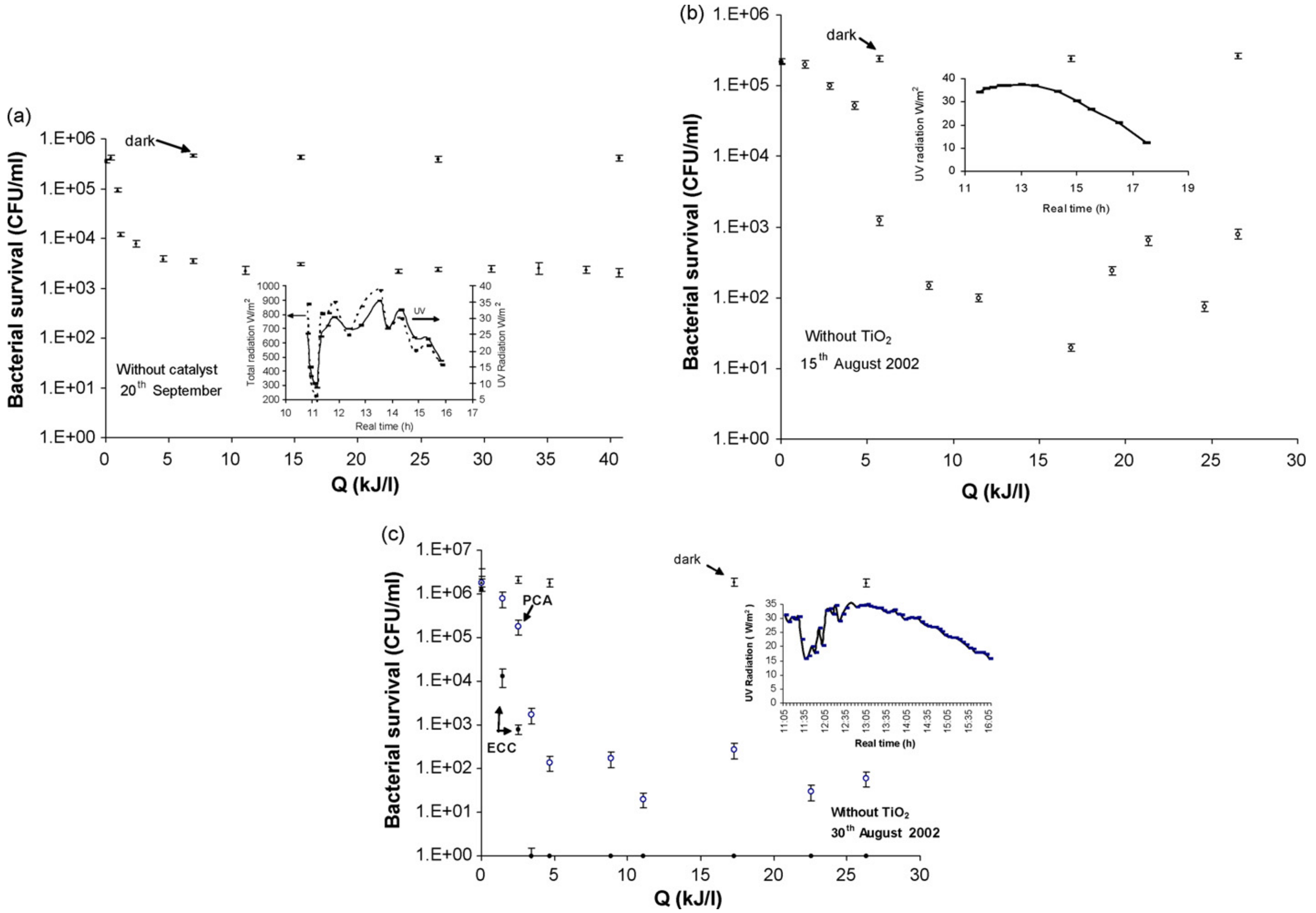

Fig. 2. (a) Inactivation of *E. coli* by direct sunlight in a CPC photoreactor. The inset contains the UV (–) and total (. . .) solar irradiance measured during the period of the experiment. 20th September 2003, in Lausanne, Switzerland. Dark control for samples that corresponds to 0, 1, 2, 3 and 5 h of the illumination exposure. Conditions: V_{TOT} = 35 l, recirculation rate = 20.5 l/min, illumination time = 5 h, temperature = 30–35 °C. The small bars show the standard deviation (S.D.) of four samples taken at the same moment. Initial concentration 10^5 CFU/ml. (b) Inactivation of *E. coli* by sunlight. On 15th August 2002. Summer in Lausanne, Switzerland (○). Dark control (●). Conditions: V_{TOT} = 70 l, recirculation rate = 20.5 l/min, illumination time = 5 h, temperature = 30–35 °C. Inset contains a UV solar irradiance vs. time from 11:30 to 17:30. Each point represents an average value of four samples taken at the same time. The bars show the S.D. (c) Inactivation of *E. coli* by sunlight. On 30th August 2002. Summer in Lausanne, Switzerland. Dark control (●). Conditions: V_{TOT} = 57 l, recirculation rate = 20.5 l/min, illumination time = 5 h. Temperature = 30–35 °C. Inset contains a UV solar irradiance vs. time from 11:05 to 16:05. Each point represents an average value of four samples taken at the same time. The bars show the S.D. Bacteria culturability was monitored by a selective, ECC (●) and non-selective, PCA (○) media.

(Fig. 3a) or 13.5 kJ/l (Fig. 3b) is applied, contrary to that observed when TiO_2 is absent. Solar disinfection with TiO_2 is a consequence of both direct action of the light on the microorganisms and the photocatalytic action of the excited TiO_2 particles. Control experiments of Fig. 3a and b show that after 5 h of stirring, in the dark, all *E. coli* survive in the presence of TiO_2. This indicates that disinfection with TiO_2 in the dark does not occur. These controls also showed that the transfer of *E. coli* from the culture media to the lake water does not cause a detrimental impact on their culturability.

Some reports have described photokilling of bacteria [37–39], viruses [40] and tumor cells [41] by photocatalytic treatment of water. Different mechanisms involved in the bactericidal action of TiO_2 photocatalysis have been proposed [38,42,43], some of which were reviewed by Blake et al. [9]. Results from the above studies suggest that the cell membrane is the primary site of reactive photogenerated oxygen species attack. Oxidative attack of the cell membrane, especially by $OH^{\bullet}$, leads to lipid peroxidation. Furthermore, some of the H_2O_2 generated under illuminated TiO_2 Eqs. (5)–(7) might subsequently lead to additional production $OH^{\bullet}$ radicals in the bulk of the suspension Eq. (8) or into the cells via an intracellular photo-Fenton reaction Eqs. (10)–(17). The required Fe^{3+}/Fe^{2+} results from the light-assisted dissociation of proteinic iron complexes such as enterobactin. The combination of cell membrane damage, and further oxidative attack of internal cellular components, ultimately results in cell death.

In the majority of the experiments the TiO_2 is used in suspension; but in this case an additional step of recovering is required. It is why, some authors intended to use fixed TiO_2 but the disinfection rate in this case is dramatically decreased as compared with those observed in illuminated TiO_2 suspensions. Most of the reported experiments have been carried out in small

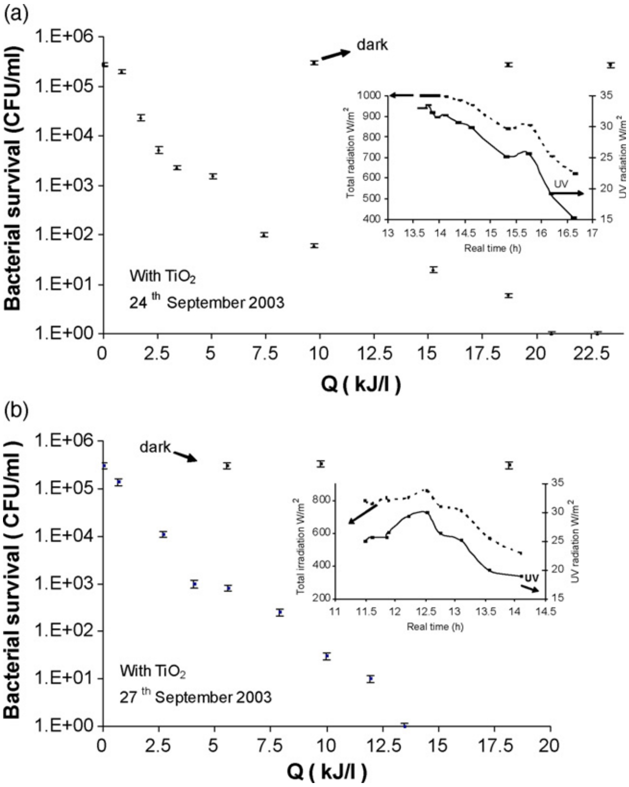

Fig. 3. Inactivation of *E. coli* by sunlight in the presence of TiO_2 in a CPC photoreactor. The inset contains the UV (–) and total (. . .) solar irradiance measured during the period of the experiment. 24th September 2003 (a) and 27th September 2003 (b), in Lausanne, Switzerland. Dark control for samples that corresponds to 0, 1, 2 and 3 h of the illumination exposure. Conditions: $TiO_2 = 40$ mg/l, $V_{TOT} = 35$ l, recirculation rate = 20.5 l/min, illumination time = 3 h, temperature = 30–35 °C. The bars show the S.D. of four samples taken at the same moment. Initial concentration 10^5 CFU/ml.

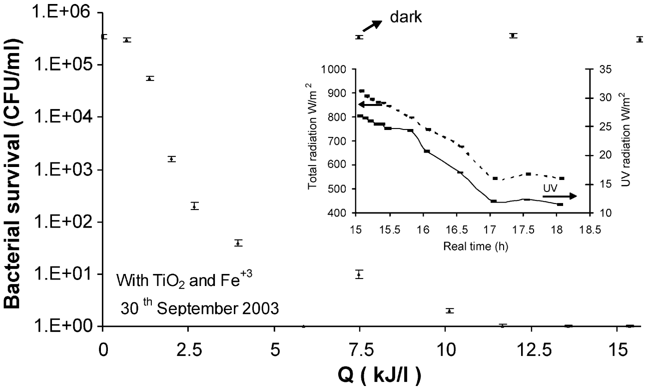

Fig. 4. Inactivation of *E. coli* by sunlight in the presence of TiO_2 and Fe^{3+} in a CPC photoreactor. The inset contains the UV (–) and total (. . .) solar irradiance measured during the period of the experiment. 30th September 2003, in Lausanne, Switzerland. Dark control for samples that corresponds to 0, 1, 2 and 3 h of the illumination exposure. Conditions: $TiO_2 = 40$ mg/l, $Fe^{3+} = 0.3$ mg/l $V_{TOT} = 35$ l, recirculation rate = 20.5 l/min, illumination time = 3 h, temperature = 30–35 °C. The bars show the S.D. of four samples taken at the same moment. Initial concentration 10^5 CFU/ml.

reactors at laboratory scale. A few reports with natural water at field scale using photoreactors illuminated by solar radiation have been published. Among the reactors used we can mention: (a) CPC photoreactors [33,44,45], (b) borosilicate glass and PET plastic SODIS reactors fitted with flexible plastic insets coated with TiO_2 powder [46]; (c) continuous flow recirculating sytems with different reflector profile V-groove, parabolic and compound parabolic [47]. None of the reported works used the Fe^{3+} as photocatalyst for solar disinfection purposes.

3.3. Sunlight disinfection in the presence of Fe^{3+} and TiO_2

The Fig. 4 shows that the presence of Fe^{3+} increases the effectiveness of the photocatalytic system under the direct solar irradiation in a CPC reactor. The number of active (culturable) bacteria decreases to non-detectable values (<1 CFU/ml) when an accumulated solar energy incident on the photoreactor per unit of volume of 11.0 kJ/l is applied, contrary to that observed when Fe^{3+} is absent (20 kJ/l in Fig. 3a and 14 kJ/l in Fig. 3b, respectively).

The beneficial effect of Fe^{3+} on the photocatalytic disinfection of water is attributed to electron trapping at the semiconductor surface. Fe^{3+} behaves as an electron scavenger, thus preventing the recombination of electron-hole pairs, Eq. (19). In addition, the ferric cations are easily adsorbed on the TiO_2 surface.

The trapping of photoelectrons Eq. (19) leaves photogenerated holes available for reaction with hydroxyl ions present in water to form $OH^{\bullet}$ radicals. However, a decreased activity above the optimum metal ions concentration is possibly due to the oxidation of Fe^{2+} by $OH^{\bullet}$ radicals or holes. The competition for holes between Fe^{2+} and OH^{-} ions, means that less $OH^{\bullet}$ radicals would be generated for the bacteria inactivation or the oxidation of organic molecules.

The reactivity of the iron aquocomplexes with e^{-} photogenerated at the conduction band of TiO_2 could be beneficial or detrimental for the oxidative processes depending on the iron species present in the medium. A positive effect was observed with Fe(III) species with low photochemical activity, while this effect is negative for the photoactives monomeric complexes $Fe(OH)^{2+}$ [48].

The presence of Fe^{3+}/Fe^{2+} in TiO_2 suspension strongly influences the reaction of generated hydrogen peroxide and surface peroxidic groups. In the combined system $Fe^{3+}/TiO_2/$UV–vis, the re-oxidation reactions of Fe(II) to Fe(III) on the illuminated TiO_2 surface is due to oxidative species such as $HO^{\bullet}$, $O_2^{\bullet-}$, $HO_2^{\bullet}$, H_2O_2 produced by the processes represented in Eqs. (1)–(8). So, the reaction of Fe^{2+} with H_2O_2 photogenerated by the system TiO_2/UV–vis Eq. (20) represents a supplementary source of $OH^{\bullet}$ radicals resulting in the increase of organic substances decomposition and bacterial inactivation rate in the Fe^{3+}/TiO_2/UV–vis system.

$$Fe^{3+} + e^{-} \rightarrow Fe^{2+} \tag{19}$$

$$M^{(n-1)+} + H_2O_2 + H^{+} \rightarrow M^{n+} + OH^{\bullet} + H_2O \tag{20}$$

In addition, H_2O_2 produced during the TiO_2 mediated photocatalysis may also diffuse across the cell membrane and produce intracellular $OH^{\bullet}$ radicals according to the Fenton mechanism Eq. (19).

In previous work at laboratory scale, we observed that the inactivation rates were lower for the UV–vis/TiO_2 photocatalysis in the presence of Fe^{3+} (0.3 mg/l) at TiO_2 concentrations higher that 0.1 g/l. For TiO_2 concentrations between 0.02 and 0.1 g/l a significant positive effect of Fe^{3+} was observed. The oxidative activity of two systems is very dependent on the relative concentration of Fe^{3+} and TiO_2 at the applied experimental conditions [29].

On the other hand, there have been a few studies on the TiO_2-assisted photoreaction of biomolecules. A recent study reported the photocatalytic degradation via TiO_2 of certain pyrimidine bases such as uracil, thymine, a-metiluracil and cytosine in an aqueous suspension by the TiO_2 Fe^{3+} dopped [49]. It has been reported that the photooxidation rate of these organic substances increases with the increase in the level of Fe^{3+} in TiO_2 Fe^{3+} doped up to approximately 0.75% of Fe^{3+} followed by a decrease in rate with further increase in Fe^{3+} level. A similar effect was reported for $CHCl_3$ degradation [49]. The increase in the photodegradation rate with the increase in the amount of Fe^{3+} was explained by the ability of the doping metal ion to act as a charge carrier trap for e^-. In addition, authors explain this fact, by the close proximity of the energy levels of Fe^{2+}/Fe^{3+} and Ti^{3+}/Ti^{4+}. As a result of this proximity, it is expected that the trapped electron in the Fe^{2+} could easily be transferred to a neighboring surfacial Ti^{4+}, which then leads to interfacial electron transfer, thus resulting in decreased e^-/h^+ recombination. The existence of an optimal doping concentration was interpretable in terms of a charge in the space-charge layer thickness. The decrease in photodegradation rate was explained because after the optimal Fe^{3+} concentrations the recombination rate increases exponentially with the dopant concentration due to the decrease of the average distance between the trap sites with the number of dopants within a particle [49,50]. Some of the latter arguments could also explain our findings concerning the increase in the lost of culturability of *E. coli* when Fe^{3+} is added to TiO_2 photocatalytic system (Fig. 4).

3.4. Sunlight disinfection in the presence of Fe^{3+} and H_2O_2

The bacterial inactivation by sunlight is increased by Fe^{3+} and H_2O_2. Fig. 5a and b show that the photo-Fenton reaction was more efficient than TiO_2 and TiO_2/Fe^{3+} photo-assisted systems (Figs. 3a,b and Fig. 4). However, the UV irradiance range was higher for the photo-Fenton (34–37 W/m^2) and (35–10 W/m^2) compared to that of TiO_2/Fe^{3+} system (12–25 W/m^2) and that of TiO_2 (15–33 W/m^2). Any modification of chemical

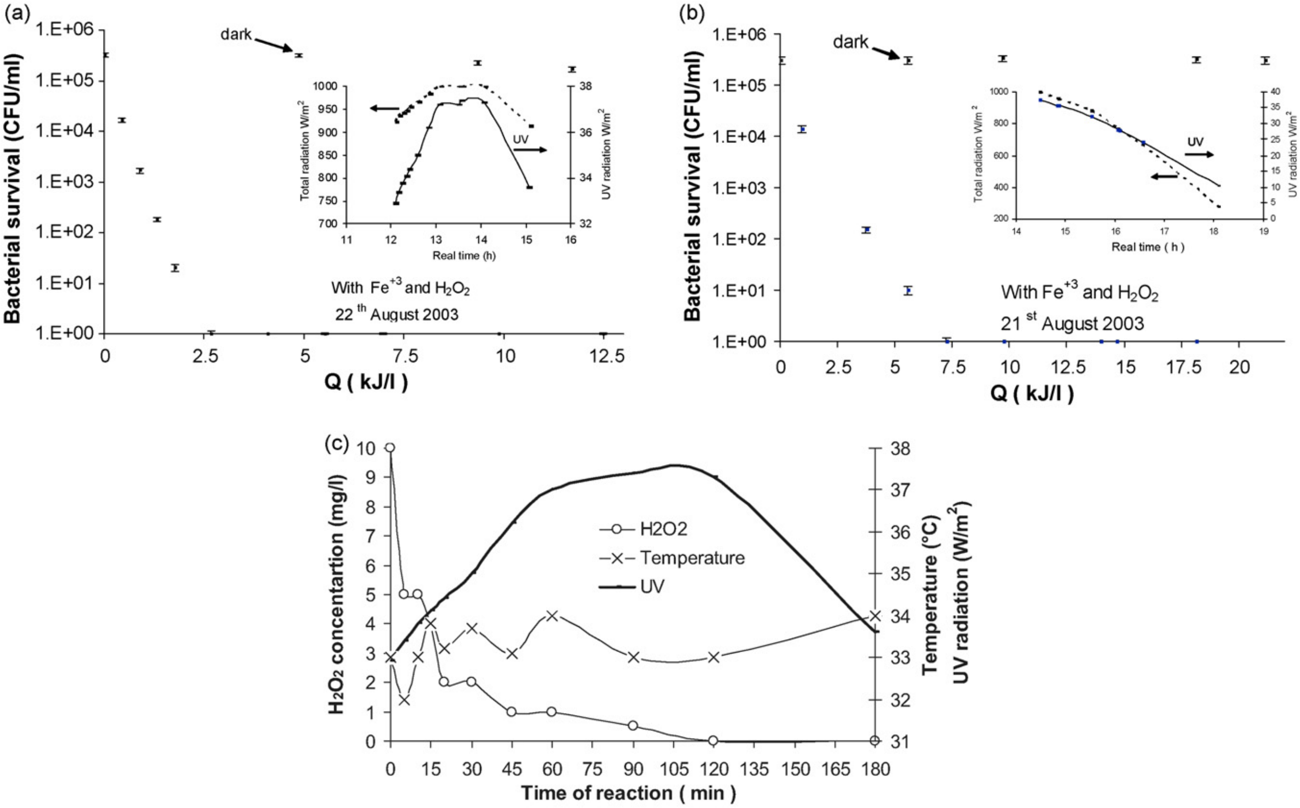

Fig. 5. (a) Inactivation of *E. coli* by sunlight in the presence of Fe^{3+}/H_2O_2 in CPC photoreactor. The inset contains the UV (–) and total (. . .) solar irradiance measured during the period of the experiment. 22nd August 2003, in Lausanne, Switzerland. Dark control for samples that corresponds to 0, 1, 2 and 3 h of the illumination exposure. Conditions: Fe^{3+} = 0.3 mg/l, H_2O_2 = 10 mg/l, V_{TOT} = 35 l, recirculation rate = 20.5 l/min, illumination time = 3 h, natural pH 7.5, temperature = 30–35 °C. The bars show the S.D. of four samples. (b) Inactivation of *E. coli* by sunlight in the presence of Fe^{3+}/H_2O_2 in a CPC photoreactor. The inset contains the UV (–) and total (. . .) solar irradiance measured during the period of the experiment. 21st August 2003, in Lausanne, Switzerland. Dark control for samples that corresponds to 0, 1, 2 and 3 h of the illumination exposure. Conditions: Fe^{3+} = 0.3 mg/l, V_{TOT} = 35 l, recirculation rate = 20.5 l/min, illumination time = 3 h, natural pH of 7.5, temperature = 30–35 °C. The bars show the S.D. of four samples. (c) Evolution of temperature, H_2O_2 concentration and UV irradiation during the inactivation of *E. coli* by sunlight in the presence of Fe^{3+} and H_2O_2. 22nd August 2003 in Lausanne, Switzerland.

composition of natural water was introduced before the treatment.

Normal aerobic metabolism gives rise to active oxygen species such as superoxide radical ($HO_2^{\bullet}$) and H_2O_2. In the presence of iron complexes these species react to produce $OH^{\bullet}$ radicals. Cellular defenses against $HO_2^{\bullet}$ and H_2O_2 include the enzymes Superoxide dismutase (SOD) and catalasa as well as a network of genome controlled responses. The oxidative radicals lead to oxidative stress in bacteria consisting in: lipid peroxidation of the membrane cell, crosslinking of protein, mutation in DNA, inactivation of protein and disintegration of membranes cell wall. It is possible that in these experiments there is a photo-Fenton reaction also inside the cell which generates enough concentration of ROS which cause lethal effect on bacteria.

Comparison of the different systems (photo-Fenton system, TiO_2/Fe^{3+}/light and TiO_2/light) at field scale is risky because the experiments were made in different days, with different spectral characteristics of applied solar intensity. In addition, it was reported [33] that the solar UV dose is not a suitable parameter to predict and standardize the impact of the solar photocatalytic process on bacteria. That was demonstrated by numerous repetitions of the experiments in the presence and absence of TiO_2 using the same type of water and reactor that in the present experiments. Indeed, the relative UV and visible wavelengths intensities, characteristics of each season and taking different times of the day significantly affect the solar photoinactivation, and photoreactivation as well as the bacterial behavior in the subsequent dark period. This is illustrated in Figs. 2a–c, 3a,b, 4 and 5a,b; where at 2.5 kJ/l of accumulated energy per unit of volume; the extent of bacterial inactivation is different for each system.

As shown (Fig. 5c), H_2O_2 is consumed as a result of photo-Fenton reaction Eqs. (10)–(17) where oxidative species participate in bacterial inactivation. According to Eqs. (10), (11) and (13), H_2O_2 was consumed and at 75 min of irradiation, less than 0.5 mg/l was detected (Test Peroxides, Merck Merckoquant).

In the other hand, solar photocatalytic process with TiO_2 has a heterogeneous nature. Interaction between the suspended catalyst nanoparticles and the bacteria is a requisite [43], as very short-lived $OH^{\bullet}$ radicals are produced from H_2O or OH^- (Eqs. (3) and (4)). Thus, the attack of the strongly oxidizing $HO^{\bullet}$ radicals is possible only over a few sites of the bacterial membrane (where there is a cell–catalyst surface interaction), not always producing a lethal damage. In contrast, the photocatalytic treatment with photo-Fenton reaction, being a homogeneous process, is not subject to such limitations and the generated ROS can simultaneously attack the bacterial membrane in several points, producing probably a more widespread damage.

3.5. Durability of solar disinfection after a field scale phototreatment

The final sample taken during the experiment carried out on September 20th, 24th, and 30th as well as August 22nd (showed in Figs. 2–5) were incubated in dark conditions at 37 °C for 24 h (Section 2.4).

Bacterial recovery was observed during the subsequent dark period following the inactivation experiment without catalyst addition (not showed here). This takes place possibly because the photoinactivation was not complete, and hence some still active (culturable) *E. coli* continue to be active and replicate in the dark. A similar behavior of bacteria population during the post irradiation steps has been reported elsewhere [33]. In the presence of TiO_2 as well as in the photo-Fenton reaction no bacterial recovery was observed after 24 h of incubation in the dark.

To standardize the field disinfection experiments under direct solar irradiation, it is necessary to determine the effective disinfection time, EDT*x* (*x* means the time for dark incubation), which avoids bacterial growth after stopping of the photocatalytic treatment. This EDT is dependent not only on the classical parameters such as chemical characteristics of water, type and initial concentration of bacteria, reactor geometry, light intensity, etc., but also on the UV and visible composition of sunlight spectra in different geographic locations, seasons and period of day. EDT_{24} (after 24 h incubation) was reached for the three photocatalytic systems (Figs. 3–5) but not for the experiment in the absence of catalyst (Fig. 2).

4. Conclusions

Field experiments under direct sunlight using a CPC reactor demonstrate that at 30–35 °C the photocatalytic treatment via TiO_2 is more effective for the water disinfection than solar disinfection without catalyst. Moreover, the addition of Fe^{3+} to TiO_2 suspensions was beneficial for the photocatalytic inactivation of *E. coli*. However, photo-Fenton reaction (Fe^{3+}/H_2O_2/sunlight) showed a more effective disinfecting action with regard to TiO_2/sulight and TiO_2/Fe^{3+}/sunlight systems.

The effective disinfection time (EDT_{24}) was reached under solar light in the presence but not in the absence of TiO_2. EDT_{24} was also attained in the Fe^{3+}/H_2O_2/sunlight system.

However, the comparison carried out in this paper between different photocatalytic systems has to be considered with caution because of the following reasons:

(a) The UV solar dose (KJ/l) required to reach a target disinfection level is not a suitable parameter to compare the influence of different experimental conditions on the solar disinfection rate.
(b) Comparative runs were not carried out simultaneously and the comparison under different experimental conditions was carried out from the accumulated UV or global dose (KJ/l). This procedure did not take in account the fact that light spectra and intensities making up the accumulated doses were not necessarily similar for all the compared runs.
(c) For the experiments in the presence of iron, the optimal iron concentration was not defined and the optimal pH (near to 3) was not used.
(d) The bacterial response, under TiO_2/sunlight and Fe^{3+}/H_2O_2/sunlight systems, could be modified depending on the

relative concentrations of Fe^{3+}, H_2O_2 and TiO_2 and these concentrations were not optimized for each system.

In the $TiO_2/Fe^{3+}/H_2O_2$/sunlight system, only 40 mg/l of TiO_2 and 0.3 mg/l of Fe^{3+} were enough to reach a relatively short EDT_{24}. The fact that such a low Fe^{3+} concentration enhances the solar photocatalytic disinfection is promising in view of disinfecting water sources containing Fe^{3+} as is often the case in tropical countries. The mixing, before photocatalytic treatment, of different water sources, of which one of them contains iron, is other possible strategy.

For a specific type of water, a compromise has to be done between the amount of used TiO_2, the set up and time required for TiO_2 decantation and the aimed water production. Once the TiO_2 concentration is fixed, the x (in hours) obtained from measured EDTx will give the necessary information for manage the water production capability of the system as well as the limits for water storage and consumption. When water storage time is higher than x, the use of a chemical disinfectant as chlorine could be considered after the solar photocatalytic treatment in order to ensure a residual disinfection effect. In this case the formation of disinfection by-products (DBPs) such as trihalomethanes is limited as the precedent photocatalytic treatment degrades an important part of the organic substances naturally present in water called disinfection by products precursors (DBPPs) and from which the DBPs are generated during chlorination [10].

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