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Absence of *E. coli* regrowth after Fe^{3+} and TiO_2 solar photoassisted disinfection of water in CPC solar photoreactor

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Abstract

Field disinfection of water in a large solar compound parabolic collector (CPC) photoreactor (35–70 l) was conducted at 35 °C by different photocatalytic processes: sunlight/TiO_2, sunlight/TiO_2/Fe^{3+}, sunlight/Fe^{3+}/H_2O_2 and compared to the control experiment of direct sunlight alone. Experiments were carried out using a CPC and natural water spiked with *E. coli* K 12. Under these conditions, total disinfection by bare sunlight irradiation 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 solar-photocatalysis. No bacterial regrowth was observed during 24 h after stopping sunlight exposure. For some samples, the decrease of bacteria continues in the dark. A ''residual disinfection effect'' was observed for these samples before reaching the total inactivation. The effective disinfection time (EDT_{24}), defined as the treatment time required to prevent any bacterial regrowth during the subsequent 24 h in the dark, after stopping the phototreatment, was reached in the presence but not in the absence of different photocatalytic systems. EDT_{24} was 2 h 30 min, 2 h and 1 h 30 min for sunlight/TiO_2, sunlight/TiO_2/Fe^{3+} and sunlight/Fe^{3+}/H_2O_2 systems, respectively. The post irradiation events observed when the phototreated water is poured into an optimal growth medium are also discussed.

Keywords: Solar water disinfection; Photocatalysis; TiO_2; Iron photo-assisted reaction; *E. coli K 12*; Photo-Fenton; Efficient disinfection time (EDT); Residual disinfection effect

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. In order to minimize the risk to humans, modifications of conventional disinfection, including, the removal of chloro-organic compounds, have been proposed [1]. 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 by several groups since this technology could be economically viable in countries with a high degree of sunlight radiation. The bactericidal effect of sunlight has been known for many years [2]. The practical use of solar disinfection of drinking water was first studied by Acra et al., who filled polyethylene bags with water before exposing them to full sunlight [3]. Recently, Reed presented a complete review of solar disinfection used as a water treatment process [4].

The disinfecting effect of solar light can be enhanced by addition of a catalyst in the presence of O_2 (from air) or other electron acceptors such as H_2O_2. The generated oxidative conditions could become 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 [5]. Photoinduced catalytic process, which belongs to the family of advanced oxidation processes (AOPs), can be roughly divided into heterogeneous and homogeneous and both are characterized by the production of hydroxyl radicals ($OH^{\bullet}$) and other highly oxidative species [6]. In the case of heterogeneous

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photocatalysis, the degradation of various organic compounds by illuminated TiO_2 has already been reported in the literature [5]. Photocatalytic inactivation of bacteria and viruses has also been investigated, but to a lesser extent [7]. In our previous papers, bacterial suspensions in deionized water, tap water and wastewater have been used to test the solar-photocatalytic disinfection via TiO_2 catalyst illuminated with sunlight. It was found that the effectiveness of the process depends on the physico-chemical [8,9] and biological [10,11] characteristics of treated water, as well as the physico-chemical properties of TiO_2 [12]. However, most studies evaluating TiO_2 photo-inactivation in water disinfection have examined the levels of inactivation but have not taken into account the levels of repair that may follow.

The most studied homogeneous photocatalytic process is the photo-Fenton system which consists in the improvement by action of light of the Fenton process. Latter system can be outlined as follows:

$$M^{n+} + H_2O_2 \xrightarrow{\text{dark}} M^{(n+1)+} + OH^- + OH\cdot \quad (1)$$

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 hydroxyperoxyl ($HO_2^{\bullet}/O_2^{\bullet -}$) and hydroxyl radicals ($OH^{\bullet}$) [5]. The $OH^{\bullet}$ radical, once in solution attacks almost every organic compound.

Fenton reaction (Fe^{3+}/H_2O_2) rates are strongly increased by irradiation with UV/visible light [13,14], this type of photoassisted reaction is referred to as the photo-Fenton reaction Eq. (2). 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 $OH^{\bullet}$.

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

The new generated ferrous ion reacts with H_2O_2 generating a second $^{\bullet}OH$ radical and ferric ion Eq. (3), and the cycle continues Eq. (4).

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + {}^{\bullet}OH \qquad K = 63\,l/mol\,s \quad (3)$$

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

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 favorable, since homogeneous solution is used. Fenton and photo-Fenton processes have been used with great success to treat a wide variety of contaminants [14].

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 [15] indicating the toxic nature of iron and copper and their role in aging of biological systems. Fenton reaction has been found to be the key reaction in the oxidation of membrane lipids, amino acids and in the reactions where biological reduction agents are present, such as ascorbic acid or thiols [16]. Both copper and iron combined with H_2O_2 have been investigated as a substitute for conventional disinfectants [17].

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 their performances for bacterial inactivation were compared at laboratory scale [18]. In the present study, field experiments with a CPC reactor 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/Fe^{3+}, sunlight/Fe^{3+}/H_2O_2 (photo-Fenton system). The study focuses on the durability of the different disinfection systems during 24 h after stopping of the field scale phototreatment. Thus, samples containing phototreated water have been incubated, with and without supplemented nutrient medium, and the active bacteria have been monitored during 24 h in the dark.

2. Experimental details

2.1. Materials

The photocatalyst was TiO_2 Degussa P-25 (Frankfurt, Germany), which contains mainly anatase and specific surface area of 50 $m^2\,g^{-1}$. The catalyst was used as received without previous photo-activation or washing. TiO_2 in suspension at 40 mg/l was used in disinfection experiments at large scale. Iron chloride $FeCl_36H_2O$ was supplied by Fluka (Buchs, Switzerland) and was used without further purification. Hydrogen peroxide was supplied by Merck AG (Darmstadt, Germany). All solutions were prepared with Millipore water (18.2 MΩ cm) and were prepared immediately prior to irradiation. Sodium chloride, tryptone and yeast extract were also supplied by Merck AG (Darmstadt, Germany). H_2O_2 was detected by a Test Peroxides, Merckoquant, Merck (Darmstadt, Germany).

Natural water coming from the Leman Lake was used to suspend bacteria. Water was filtered on sand before distribution without significant modification of its chemical characteristics at the water distribution station of Haute-Pierre, near Lausanne [19]. The total organic carbon was 0.8 mg C/l. The initial pH of water was not modified from 7.55.

2.2. Solar photoreactor

A complete description was presented previously [19]. The CPC has three modules (collector surface 3.08 m^2), photo-reactor 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 35 °C in the CPC reactor. Solar UV 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 accumulated energy incident on the photoreactor for each sample during the experiment per unit of volume ($Q_{UV,n}$) is done by the application of Eq. (5). Where $\Delta t_n = t_n - t_{n-1}$, t_n is the experimental time for each sample, $UV_{G,n}$ is the average UV_G (global UV radiation) during t_n, $A_{CPC} = 3.08\ m^2$, $V_{TOT} = 37$ l and $Q_{UV,n}$ is expressed in (kJ/l).

$$Q_{UV,n} = Q_{UV,n\text{-}1} + \Delta t_n \overline{UV}_{G,n} \frac{A_{CPC}}{V_{TOT}} \quad (5)$$

2.3. Bacterial strain and growth media

A complete description was presented previously [19]. The bacterial strain used was *E. coli* K12 (ATCC 23716) and was supplied by DSM, German Collection of Microorganisms and Cell Cultures. *E. coli* K12 was inoculated into sterilized 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. 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 the exponential growth phase, bacterial cells were collected by centrifugation and the bacterial pellet was washed with a tryptone solution. 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 sunlight irradiation. The reported results are the mean value of four samples collected at the same time interval. 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, as well as from results obtained in previous work [20]. One sample was immediately plated on agar Plate-Count-Agar (PCA, Merck, Germany) plates as an experimental non-repair control; colonies were enumerated after 24 h incubation at 37 °C.

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. In some experiments, the irradiated samples were diluted 10 times in LB and transferred to an incubator under mechanical agitation at 37 °C for 24 h; thereafter, samples were plated on agar for counting after 24 h incubation at 37 °C.

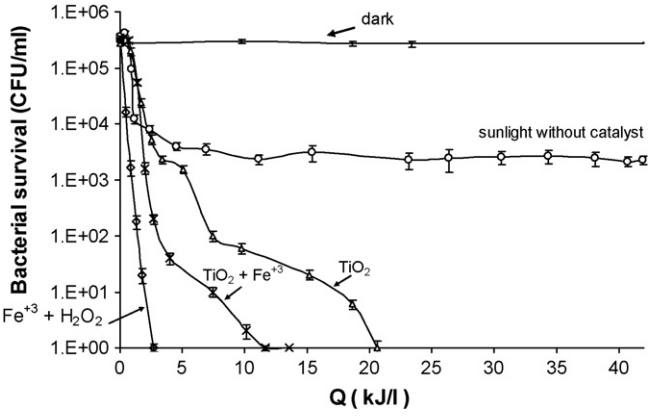


Fig. 1. Inactivation of *E. coli* by sunlight in a CPC photoreactor during the summer of 2003, 22nd of August (◇), and 20th (○) 24th (△), 30th of September (*), in Lausanne-Switzerland. Dark control for samples that corresponds to 0, 1, 2, 3 and 5 h of the illumination exposure (◆). In the presence of TiO_2 (△), TiO_2 and Fe^{3+} (*), Fe^{3+} and H_2O_2 /sunlight (◇), in the absence of catalyst (○). Conditions: $TiO_2 = 40$ mg/l, $Fe^{3+} = 0.3$ mg/l, initial H_2O_2 concentration: 10 mg/l, $V_{TOT} = 35$ l, recirculation rate = 20.5 l/min, initial pH = 7.55, final pH = 7.70 (△), 7.4 (*), 6.8 (◇) and 7.5 (○). Illumination time = 3 h for photocatalytic experiments and 5 h for system without catalyst. Mean solar intensity in W/m^2: 29.07 (△), 22.00 (*), 35.03 (◇), 24.28 (○). Temperature = 30–35 °C. The bars show the standard deviation (S.D.) of four samples taken at the same time.

3. Results and discussion

3.1. Sunlight disinfection in the absence of TiO_2

Fig. 1 (trace ○) shows that bacteria were not completely eliminated after 40 kJ/l of incident energy per unit of volume. In these experiments, very little modification of the water temperature (30–35 °C) was detected throughout. Therefore, inactivation was predominantly due to photonic rather than thermal effects. The concentration of active (culturable) bacteria decreased as the energy increased up to a solar energy equivalent to 10 kJ/l and reached a plateau even if the illumination was prolonged. Similar tendency has been reported in one of our previous paper [19]. That could be due to: (a) some bacteria which are not culturable at the beginning of the process recover their culturability under the phototreatment; (b) a possible replication of the remaining culturable cells; (c) the damaged bacteria are repaired; (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 [9]. The combined action of factors (a)–(d), which are opposed to the solar disinfection, results in an equilibrium between bacterial inactivation, reactivation and growth, that leads to a quasi-stationary state where undetectable level of bacteria (<1 CFU/ml) was not reached.

The partial lost of bacterial culturability by UV is due to direct photolysis. UV-A 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 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 [21].

3.2. Sunlight disinfection in the presence of TiO_2

The bacterial inactivation by sunlight in a CPC photoreactor is strongly enhanced by the presence of TiO_2 (Fig. 1 trace △). 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 is applied, contrary to that observed when TiO_2 is absent (trace ○). This result is in accordance with previous findings [7]. Control experiments (trace ■) 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.

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. Some reports have described photokilling of bacteria [22], viruses and tumor cells by photocatalytic treatment. Different mechanisms involved in the bactericidal action of TiO_2 photocatalysis have been proposed some of which were reviewed by Blake et al. [7]. Results from the above studies suggest that the cell membrane is the primary site of reactive photogenerated oxygen species attack [23]. Oxidative attack of the cell membrane, especially by $OH^{\bullet}$, leads to lipid peroxidation and the cell death. Recently, the formation of the peroxidation products due to the photocatalysis of *E. coli* K12 cells has been measured by attenuated total reflection Fourier infrared spectroscopy (ATR-FTIR) [24]. Furthermore, some of the H_2O_2 generated under illuminated TiO_2 might subsequently lead to additional production $OH^{\bullet}$ radicals at the interface TiO_2–cells or inside the cells via an intracellular photo-Fenton reaction. The combination of cell membrane damage, and further oxidative attack of internal cellular components, ultimately results in cell death.

In the majority of the cases, the TiO_2 is used in suspension. In a previous work, we 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 [25]. A few reports with natural water at field scale using photoreactors illuminated by direct solar radiation have been published. Among the reactors used we can mention: (a) CPC photoreactors [19,26,27]; (b) borosilicate glass and PET plastic SODIS reactors fitted with flexible plastic inserts coated with TiO_2 powder [28]; (c) continuous flow recirculating systems with different reflector profile V-groove, parabolic and compound parabolic [29]. However, most studies evaluating TiO_2 photo-inactivation in water disinfection have examined the levels of inactivation but have not taken into account the levels of repair that may follow. None of the reported studies used Fe^{3+} as photocatalyst for solar disinfection purposes.

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

Fig. 1 shows that the presence of 0.3 mg/l of Fe^{3+} increases the effectiveness of the TiO_2 (40 mg/l) 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) as shown in Fig. 1 (trace △.). The beneficial effect of Fe^{3+} on the photocatalytic disinfection of water is attributed to electron trapping at the semiconductor surface. Fe^{3+} forms aquacomplexes (Fe^{3+}aq) that behave as electron scavengers, thus preventing the recombination of electron-hole pairs, Eq. (6). This reaction is thermodynamically favorable with regard to the oxide-reductive potential of Fe(III)/Fe(II) (E versus NHE = +0.77 V) and that of the conduction band of TiO_2 (−0.1 to 0.2 V).

The trapping of photoelectrons Eq. (6) leaves photogenerated holes available for reaction with hydroxyl ions present in water to form hydroxyl radicals. However, a decreased activity observed above the optimum metal ions concentration (not shown here) is possibly due to the oxidation of Fe^{2+} by $HO^{\bullet}$ radicals or holes. The competition for holes between Fe^{2+} and OH^{-} ions means that less $HO^{\bullet}$ radicals would be generated for the bacteria inactivation or the oxidation of organic molecules.

$$Fe^{3+}aq + e^{-}(TiO_2) \rightarrow Fe^{2+} \quad (6)$$

The presence of Fe^{3+}/Fe^{2+} in TiO_2 suspension strongly influences the reaction of generated H_2O_2 and surface peroxidic groups. In the combined system Fe^{3+}/TiO_2/sunlight, 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 and h^+ produced by the photocatatytic process via TiO_2. So, the reaction of Fe^{2+} with H_2O_2 photogenerated by the system TiO_2/sunlight 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/sunlight system.

In previous work at laboratory scale, we observed that the inactivation rate for the UV-Vis/TiO_2 photocatalysis was increased in the presence of 0.3 mg/l of Fe^{3+} for TiO_2 concentrations between 0.02 and 0.1 g/l. At higher TiO_2 concentration beneficial effect of Fe^{3+} additions was not observed. The oxidative activity of this complex system is then very dependent on the relative concentration of Fe^{3+} and TiO_2 at the applied experimental conditions [18].

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

The bacterial inactivation by sunlight is increased by the simultaneous addition of Fe^{3+} and H_2O_2. Fig. 1 shows that the photo Fenton reaction (trace ◇) was more efficient than TiO_2 and TiO_2/Fe^{3+} photoassisted systems (traces △ and *).

However, it should be noted that the UV light intensity range, which could affect the bacterial response [19], was higher for the photo-Fenton (34–37 W/m^2) than for the TiO_2/Fe^{3+} (1–25 W/m^2) and TiO_2 (15–33 W/m^2) systems. Normal aerobic bacterial metabolism gives rise to active oxygen species such as hydroperoxide radical ($HO_2^{\bullet}$) and H_2O_2. Cellular defenses against $HO_2^{\bullet}$ and H_2O_2 include the enzymes Superoxide dismutase (SOD) and catalase as well as a network of genome controlled responses. This self-regulated endo-bacterial Fenton process is probably disrupted by the external addition of iron species and H_2O_2. In this case, the detrimental effect of $OH^{\bullet}$ generated in the bulk of the solution could be increased by the action of additional $OH^{\bullet}$ produced inside the cell. These concomitant actions lead to crosslinking of proteins, mutation in DNA, inactivation of proteins, lipid peroxidation of the membrane cell, and disintegration of membranes cell wall. Thus, possible photo-Fenton reactions inside the cell, which contribute to the lethal effect on bacteria have to be considered.

In the experiment of 22nd of August, (trace ◇ in Fig. 1), H_2O_2 is consumed (not shown here) as a result of photo-Fenton reaction, according to Eqs. (3)–(4), and at 75 min of irradiation, less than 0.5 mg/l of H_2O_2 was detected.

In our conditions, the order of water disinfection rate for the different photoinactivation systems at field scale with a CPC reactor under solar irradiation was as follow: Sunlight ≪ sun-sunlight–TiO_2 < sunlight/TiO_2/Fe^{3+} < sunlight/Fe^{3+}/H_2O_2.

However, this order has to be taken with caution because experiments at field scale under direct solar exposition were performed at different days, with different intensities and spectral characteristics of applied solar radiation. According to our previous results, at laboratory and field scale, the disinfection rate increases proportionally with the UV intensity but not during all the stages of the process. Indeed, once the minimum efficient solar dose (UV intensity × time of irradiation) has been received, the disinfection rate is not necessarily enhanced by any further increase of dose. Therefore, it has already been reported [19] that, under these conditions, the solar UV dose (kJ/l) is not always a suitable parameter for monitoring the impact of the solar photocatalytic process on bacteria. Indeed, the relative UV and visible wavelength intensities, characteristics of each day period and each season significantly affect the solar photoinactivation, and photoreactivation as well as the bacterial behavior in the subsequent dark period.

Nevertheless, the lesser *E. coli* inactivation observed with TiO_2 could be due to the heterogeneous nature of this photo assisted process. Indeed, interaction between the suspended catalyst nanoparticles and the bacteria is a pre-requisite [30], as very short-lived hydroxyl radicals are produced at the surface of TiO_2 from H_2O or OH^-. Moreover, the attack of the strongly oxidizing $HO^{\bullet}$ radicals at the TiO_2 surface 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 a more widespread damage.

To evaluate the durability of the solar photocatalytic disinfection on 35 l of natural water spiked with *E. coli*, the post irradiation events occurring in water stored during the subsequent 24 h in the dark are studied in the Section 3.5.

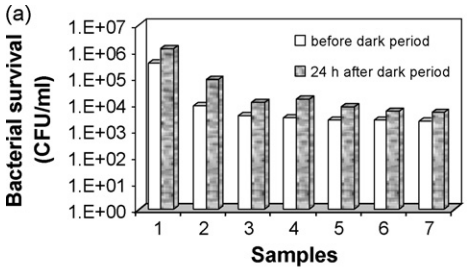
3.5. Durability of solar disinfection in water after tested photo-assisted processes

Some samples, including the final sample, were taken during the experiment carried out on September 20th, 24th, and 30th as well as August 22nd, (showed in Fig. 1) 2003, and incubated in dark conditions at 37 °C for 24 h.

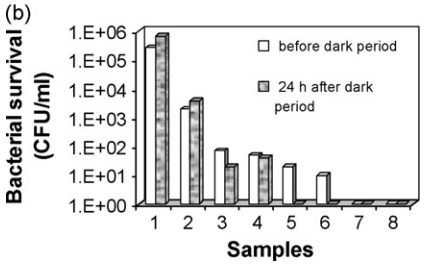
For monitoring the post irradiation effects and consequently the disinfection durability after different field disinfection experiments, it is necessary to determine the effective disinfection time (EDT_x) for different tested inactivating systems. In EDT_x, x is the number of hours that the water can be stored in the dark, without bacterial regrowth after stopping of the photo-treatment during a time T [19,20]. This EDT depends on the used photo-assisted system, the 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 at different geographic locations, seasons and period of day.

3.5.1. Post irradiation events after a solar treatment in the absence of TiO_2

In Fig. 2a bacterial recovery (black column) was observed during the subsequent 24 h dark period for the samples (white column) selected from the experiment under solar illumination carried out at 30–35 °C without catalyst addition. This recovery probably takes place due to the fact that the photoinactivation was not complete, and hence some still active (culturable) *E. coli* cells continue to be active and replicate in the dark. Conditions are favorable for *E. coli* replication since even the non-illuminated sample undergoes a growth (sample 1 in Fig. 2a) although the low concentration of dissolved organic carbon (0.8 mg/l) of water limits the bacterial growth. Furthermore, the DNA damage caused by sunlight can be repaired by some bacteria in the dark. A similar behavior of *E. coli* population has been previously reported elsewhere [19]. In another of our experiments (not shown here), carried out on the 26th of September 2003, water in the absence of TiO_2 was irradiated during 6 h using a CPC photoreactor and in this case the active *E. coli* concentration reached gradually an undetectable value of culturable bacteria (1 CFU/ml) at the end of irradiation. However, bacterial recovery of 5-log was observed in the final irradiated sample after 24 h of dark incubation, whereas for the not illuminated control 1-log increase the same way observed during the same time. This fact reveals that, even if bacteria were not cultivable at the end of illumination, some of them were actually not killed and recovered their culturability after a period of time in the dark. In addition, a conversion of non-culturable cells into culturable ones by the previous action of light (photoactivation) and



Sample	Illumination time (h)
1	0.00
2	0.50
3	1.00
4	2.00
5	3.00
6	4.50
7	5.00



Sample	Illumination time (h)
1	0.00
2	0.50
3	1.00
4	1.50
5	1.75
6	2.00
7	2.5
8	3.00

Fig. 2. Evolution of *E. coli* in the dark after solar disinfection: (a) without TiO_2, (b) with TiO_2, date from Fig. 1, on September 20th and 24th, 2003. *E. coli* concentration of illuminated samples (□) and the same samples after 24 h in the dark (■). TiO_2 = 0.04 g/l. UV range of previous illumination: 12–35 W/m^2 on September 20th and 15–33 W/m^2 on September 24th. Each point represents an average value of four samples taken at the same time. The average S.D. was 3%, between four samples taken at the same time.

duplication in the dark period is also probable. No enough UV intensity and dose was applied to prevent enzymatic DNA repair, which result in bacterial reactivation. Thus, EDT_{24} was not reached either in the experiment discussed here in September 24th, 2003 or in the reported experiment of September, 2003. Consequently, under these conditions a much longer time of illumination should be applied to reach the EDT_{24} under bare solar treatment.

3.5.2. Post irradiation events after a solar treatment in the presence of TiO_2

In Fig. 2b, bacterial recovery after 24 h in the dark (black column) is observed for samples 1 and 2, whereas in samples 3 and 4 no regrowth of bacteria was observed during dark incubation. In samples 5 and 6, the bacterial concentration attained during photocatalytic treatment (white column) continues to decrease in the dark and reaches undetectable level (<1 CFU/ml) after 24 h. Samples 7 and 8 presented already non-detectable value at the moment of sampling, this value was maintained during the subsequent 24 h in the dark. The behavior in the dark of phototreated samples 5 and 6 suggests that during photocatalytic disinfection, radicals and other oxidative species induce damage that continues to affect bacteria in the dark generating a ''residual effect''. The ''residual effect'' is certainly not induced by $^{\bullet}OH$ radicals which have an extremely short lifetime (10^{-6}–10^{-9} s), and must be produced close to the bacterial membrane for being active in the oxidation of some of its components. In a previous article we reported that this residual effect in the dark is dependent on the light intensity previously applied [9,11], as well as on the initial concentration of bacteria, and the chemical composition of water [20].

Some hypotheses which could explain the appearance of a ''residual'', or delayed effect are:

(a) The inactivation of some enzymes responsible for the defense of bacteria towards oxidative stress continues in the dark. It has been reported that exposure to light in the presence of a photo sensitizer of some enzymes (such as catalase), which play an important role in the bacterial defense against oxidative stress, resulted in loss of enzymatic or functional activity due to direct photooxidative damage [31]. A further decline in activity after cessation of illumination was also observed by other authors suggesting that subsequent dark reactions can also contribute to the loss of enzyme activity [32]. Our observations concerning the post-irradiation events in the dark after solar-photocatalytic treatment could then be related with these findings. Nevertheless this fact has not been strictly proved for photocatalytic systems.

(b) Alteration in membrane permeability may facilitate entrance into the cells of chemical compounds such as H_2O_2 and cations that increase the perturbation of the redox reactions regulated in the cells. This perturbation could continue in the dark. Alterations in membrane architecture caused by lipid peroxidation and the conformational changes in many membrane-bound proteins and electron mediators lead to changes in how these compounds are orientated across the cell membrane. Consequently, functional changes are expected [33]. Illuminated TiO_2 has an adverse effect on the permeability of *E. coli* cell membranes. This was demonstrated when the treatment of *E. coli* with TiO_2 and near UV light resulted in an immediate increase in permeability to small molecules such as *o*-nitrophenol β-D-galactopyranoside (ONPG), and the leakage of large molecules such as β-D-galactosidase after 20 min. In the latter work, kinetic data showed that cell wall damage took place in less than 20 min, followed by a progressive damage of cytoplasmic membrane and intra-

cellular components. The results from the ONPG assay correlated well with the loss of cell viability. The alteration on the permeability of *E. coli* cell membranes explain also the rapid leakage of K^+ observed in *Streptococcus sobrinus* [22]. In addition, the oxidative species generated during photocatalysis could be able to disrupt the cell wall sufficiently to allow the access of the photocatalyst into the bacterium. In this case, additional lethal damage to the cell would be produced under illumination via intracellular photocatalysis. However, evidence of TiO_2 penetration into the bacteria has not been reported. Once the outer and inner membranes are damaged, it is possible that some biomolecules such as proteins are destroyed and, consequently, the repair is unattainable in the dark, after the illumination period.

(c) The damage on the cell is so extensive that the mechanisms of repair in the dark probably do not take place. Lipid peroxidation reaction was the first evidence proposed as the underlying mechanism of death of *E. coli* [33]. Unsaturated fatty acids (polyunsaturated phospholipids) are the primary target of $OH^\bullet$ radicals and the other reactive oxygen species such as O_2^- or H_2O_2 [33,34]. Oxidative deterioration of lipids leads the formation of malondialdehyde during the last stage of the breakdown of endoperoxidases which are formed during intramolecular rearrangements in the structure of unsaturated fatty acids.

On the other hand, the oxidation of proteins implicated in the process of repair and the blockage of their synthesis probably blocks the repair mechanisms. This is associated with the reported slow release of intracellular protein and RNA by bacterial cells during the photocatalytic inactivation of *Streptococcus sobrinus* [22]. However, the nature and function of these proteins were not reported. According to these authors, the expulsion of these cellular contents and the deleterious effects of TiO_2 photocatalysis on cellular respiratory activity probably make impossible the bacterial recovery.

(d) The damage inside the cell may continue even in the dark via Fenton reaction Eqs. (3)–(4). Once lethal oxidation reactions are initiated by the TiO_2 photocatalytic reaction in the complex cellular systems, the damage may continue in the dark via the Fenton reaction or the free radical chain reactions of lipid peroxidation.

On the other hand, the hypothesis presented in the point (c) suggests that TiO_2/UV-Vis system causes a DNA damage, which has not been demonstrated *in vivo* until now. Of the experimental evidence in this regard [35,36], it is important to mention the work of Hidaka et al. [37], who performed *in vitro* experiments monitoring the fate of DNA, RNA and their pyrimidine and purine bases once irradiated by UVA and UVB in the presence of TiO_2 particles. They verified the detrimental effect on DNA and RNA by scanning electron microscopy and gel permeation. Recent results from our laboratory (not yet published) suggest that the action of illuminated TiO_2 on bacteria perturbs also their iron captage or leashing leading to the disruption of the intracellular Fenton system which in normal situation regulates the production of oxidative species in the cells. These oxidative species especially $OH^\bullet$, will subsequently attack DNA and other internal cellular components.

(e) Some TiO_2 particles remaining in slurry may still retain their bactericidal activity in the dark. Pham et al. [38] have reported that intermittent illumination reduced viable *Bacillus pumilus* spores more effectively than continuous exposure to UV (λ 365 nm) in photocatalytic system. In contrast, in one of our precedent work we observed that at the beginning of the treatment, numerous interruptions of the *E. coli* illumination cause a retardation in the disinfection rate [9]. The rate of recombination of h^+/e^- could change in the different stage of the photocatalytic reaction in complex system TiO_2/sunlight/bacteria.

In another paper it was reported that TiO_2-treated cells continue to lose their viability even after UV light is turned off [30]. This situation has been observed at advanced states of treatment while at the early states of treatment bacteria recover their culturability [9]. Other authors have also observed a negligible *E. coli* regrowth during 72 h in the dark after a photocatalytic treatment using a CPC photoreactor [26]. Probably the half-life time of h^+ and e^-, the ratio between irradiation and dark exposure times, as well as the extent of each period itself determine not only the $OH^\bullet$ production efficiency but also the extent of the bacterial recovery. Therefore, this subject must be studied in more detail.

(f) The possibility that the degradation products coming from organic substances present in natural water as well as from those secreted by bacteria, affect the sensitivity of bacteria to photocatalysis cannot be excluded, specially if generated by-products are toxic for bacteria. Regarding this point, it has been reported that when a suspension of *E. coli*, TiO_2 and dihydroxybenzenes (catechol, resorcinol, hydroquinone) was illuminated for 2 h, the bacterial inactivation was higher when dihydroxybenzenes were not present, because they protect bacteria toward the OH• attack. However, the dihydroxybenzene that experiences the highest degradation (resorcinol) during the first 20 min of illumination induces the lowest bacterial protection. That means that *E. coli* inactivation is related not only to the photoreactivity of dihydroxybenzenes but also to the nature of intermediates formed that could play a synergistic (toxic?) role in the *E. coli* inactivation process [39].

To our knowledge, no systematic studies on the dark reactivation of different bacterial strains after photocatalytic treatment have been made while there are several studies concerning dark repair after UV (A, B and C) exposition of bacteria in water [2]. In our photocatalytic experiments, similar mechanisms among others are probably implied in the process of repair.

3.5.3. Post irradiation events after a solar treatment in the presence of $TiO_2 + Fe^{3+}$

Fig. 3 illustrates the absence of bacterial recovery of final sample subjected to solar irradiation, in the presence of

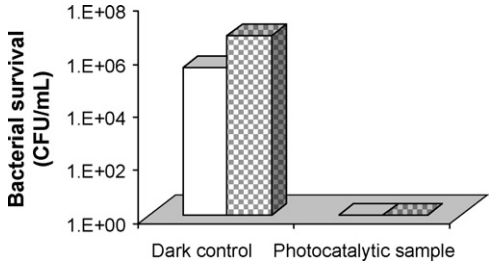


Fig. 3. Evolution of *E. coli* in the dark after solar disinfection under illuminated Fe^{3+} + TiO_2 system, date from Fig. 1 (final sample), on September 30th, 2003. *E. coli* concentration of illuminated sample (□) and the same samples after 24 h in the dark (▩). TiO_2 = 0.04 g/l, Fe^{3+} = 0.3 g/l. UV range of previous illumination: 12–25 W/m^2. Each point represents an average value of four samples taken at the same time. The average S.D. was 3 %, between four samples taken at the same time.

$TiO_2 + Fe^{3+}$. In this work, the photocatalytic disinfection of water in the presence of TiO_2 and Fe^{3+} on September 30th, 2003 exhibits a similar behavior to the samples presented in Fig. 2b after treatment with the TiO_2/sunlight system. Fig. 3 illustrates the behavior, after 24 h in the dark, of the final sample which corresponds to 2.3 h of irradiation.

3.5.4. Post irradiation events after a solar treatment in the simultaneous presence of Fe^{3+} and H_2O_2

Each sample taken during the solar exposure carried out on August 22nd (shown in Fig. 1, trace △) was incubated under dark conditions at 37 °C for 24 h. Fig. 4 shows that no bacterial recovery was observed for any incubated sample. This residual effect is probably due to: (a) a prolongation of the inactivation by the action of the subsequent Fenton reaction in the dark, when there is remaining amount of H_2O_2; and (b) even in samples where the H_2O_2 has been completely consumed, *E. coli* damage produced during the photo-Fenton process is so strong that ''injured'' bacteria continue to die in the dark.

Thus, EDT_{24} (no bacterial regrowth after 24 h of dark incubation) was reached for the three photocatalytic systems (Fig. 1 traces △, * and ◇) but not for the experiment in the absence of catalyst (trace ○).

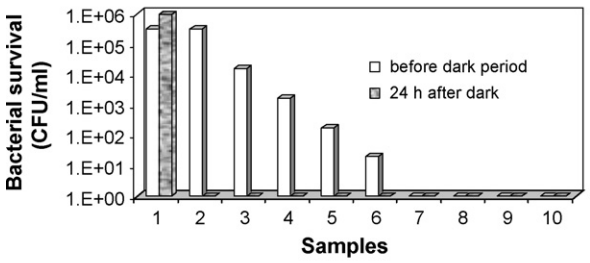
3.6. Post irradiation events in samples after addition of nutrient broth (LB medium)

At the end of illumination period in experiments presented in Fig. 1, a duplicate of the final samples (end of illumination period) were diluted 20 times in LB medium and incubated in dark conditions at 37 °C for 24 h. Final samples correspond to 3 h of irradiation for photocatalytic samples traces (△, *, ◇) Fig. 1 and 5 h of irradiation for samples without catalyst, (trace ○).

3.6.1. Evolution in LB medium of samples previously illuminated without catalyst addition

The incubation in the LB medium of initial (no illuminated) samples showed an increase in bacterial concentration between 3 and 4 orders of magnitude in all experiments (Fig. 5a–f). Fig. 5a shows also that, as expected, bacterial recovery in the dark was observed (bacteria increase from 10^3 to 10^7 CFU/ml) in the final sample of the experiment without TiO_2 addition presented in Fig. 1 trace (○). LB, which is a rich culture medium, supplies the nutrients necessary for bacterial survival and growth of remaining *E. coli*. A second experiment carried out with a sample taken from another photoinactivation run in the absence of TiO_2 (no shown in Fig. 1) illustrates in Fig. 5b that the concentration of active bacteria increases from undetectable values (<1 CFU/ml) to 10^8 CFU/ml in the subsequent dark period. This is due to the reasons discussed above when LB was not added in addition to the supply of carbon source and nutrients contained in LB, which accelerates resuscitation and growth of remaining bacteria.

It is known that under various stressful conditions, bacterial cells may become ''viable but not-culturable'' (VBNC) as a protective mechanism [40]. There have been numerous reports on the appearance of culturable cells from a population of non-culturable ones after addition of nutrients in a process termed ''resuscitation''. Nevertheless, the authors have attributed these observations to the presence of a low (not detectable) level of residual culturable cells that simply grow and divide in response to the added nutrients [41]. Indeed, cells could be defined operationally as alive or dead depending on the method used to determine cell viability. The capacity of cells to respond to the addition of nutrients after UV exposure has been studied



Sample	Illumination time (h)
1	0.00
2	0.00
3	0.17
4	0.25
5	0.33
6	0.50
7	0.75
8	1.00
9	1.50
10	2.5
11	3.00

Fig. 4. Evolution of *E. coli* in the dark after solar disinfection by photo-Fenton reaction, date from Fig. 1, on August 22nd, 2003. *E. coli* concentration of illuminated samples (□) and the same samples after 24 h in the dark (▩). Fe^{3+} = 0.3 mg/l. Initial H_2O_2 concentration: 10 mg/l. UV range of previous illumination: 33–37 W/m^2. Each point represents an average value of four samples taken at the same time. The average S.D. was 3%, between four samples taken at the same time. Sample 1: Fe^{3+} without H_2O_2. Sample 2 is non-illuminated (but contains H_2O_2).

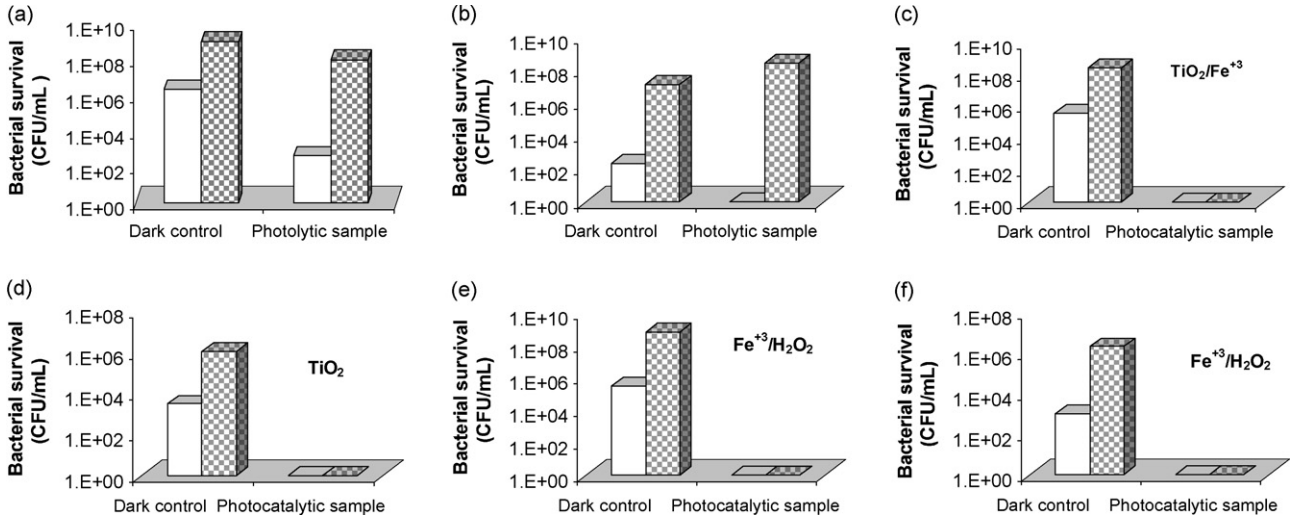


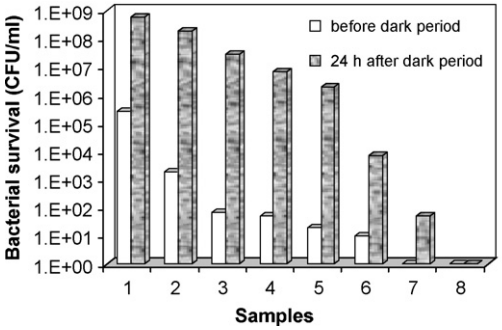
Fig. 5. Evolution of *E. coli* in the dark after solar disinfection: without catalyst, experiment of September 20th (a), September 26th (b), TiO_2/Fe^{3+}/sunlight system experiment of September 30th (c), TiO_2/sunlight system experiment of September 15th (d), Fe^{3+}/H_2O_2/sunlight experiment of August 22nd, and 23rd, 2003 (e) and (f). In the latter two runs, dark controls are carried out without H_2O_2 addition. TiO_2 = 0.04 g/l, Fe^{3+} = 0.3 mg/l. Initial H_2O_2 concentration: 10 mg/l. Initial (non illuminated) and final illuminated samples were incubated in LB media during 24 h. Active *E. coli* population is represented by columns before (□) and after (▩) 24 h of dark incubation. UV range of previous illumination: 11–31 W/m^2 on September 26th, 10–30 W/m^2 on September 15th, 13–27 W/m^2 on August 23rd.

[42]. It was determined by cell elongation, protein synthesis capability and glucose metabolism measurements, as well as by the study of the effect of aeration conditions on the regulation of bacterial metabolic fluxes through the Embden–Meyerhof and pentose phosphate pathways. Thus, as a function of the increase of UV dose, the metabolic imbalance increase and the capacity of bacteria to respond to nutrient addition by protein synthesis and cell wall synthesis declines. Fig. 5a and b demonstrate that in the studied photolytic (without TiO_2) conditions, a bacteriostatic but not bactericidal effect of solar disinfection of 35 l of water at 35 °C occurred.

3.6.2. Evolution in a LB medium of samples previously illuminated under: TiO_2, TiO_2/Fe^{3+} and Fe^{3+}/H_2O_2

In our studies, *E. coli* cells lost their culturability after all the photocatalytic treatments (Fig. 1, traces △, ◇, *), thereafter, during the subsequent dark periods neither *E. coli* recovery (without LB incubation, Figs. 2b, 3 and 4) nor resuscitation (with LB incubation, Fig. 5c–f) was observed for the final samples. Consequently, a bactericidal effect was obtained with all tested processes.

Fig. 6 shows the bacterial concentration after a 24 h dark incubation in LB of TiO_2/sunlight treated samples taken from the experiment of September 24th presented in Figs. 1 and 6. These values can be compared to those of Fig. 2b which shows the bacterial dark recovery in the absence of LB for the same samples. The bacterial concentration of the non phototreated sample (sample 1) increased 2.5 and 2500 times, respectively, in the absence (Fig. 2b) and in the presence (Fig. 6) of the rich LB medium. In this latter case, the bacterial concentration values observed after dark incubation decreased as the previous photocatalytic treatment time increased. Sample number 8, in Fig. 6, does not show a bacterial growth. In the photocatalytically treated samples 2–6 there are probably three types of population: (a) dead bacteria, (b) other bacteria which recover their culturability under light and grow in the dark, and (c) bacteria that were not yet affected by photocatalysis and still culturable after different periods of photocatalytic treatment.



Sample	Illumination time (h)
1	0.00
2	0.50
3	1.00
4	1.50
5	1.75
6	2.00
7	2.5
8	3.00

Fig. 6. Evolution of *E. coli* in the dark after solar photocatalytic treatment, TiO_2/sunlight; date from Fig. 1, 24th of September, 2003. *E. coli* concentration of illuminated samples (□) and the same samples after 24 h in the dark (▩). TiO_2 = 0.04 g/l. All samples were diluted on LB before dark incubation. Each point represents an average value of 4 samples taken at the same time. The average standard deviation was 3%, between four samples taken at the same time.

This latter population is the main responsible for the bacterial growth in the presence of nutrients contained in LB medium. In sample 7 of Fig. 6, the number of bacteria increased from a non-detectable level (<1 CFU/ml) to 10^2 CFU/ml during 24 h in the dark. This illustrates the fact that a few viable cells remaining in the sample are enough to undergo growth. In sample 8 all bacteria were really killed since not cultivable in the very rich LB medium.

4. Conclusions

Field experiments under direct sunlight using a CPC reactor demonstrate that at 30–35 °C the photocatalytic disinfection via TiO_2 of 35 l of *E. coli* contaminated water is more effective for the water disinfection than solar disinfection without catalyst. Moreover, the addition of 0.3 mg/l of Fe^{3+} to 40 mg/l of TiO_2 suspensions was beneficial for the photocatalytic inactivation rate 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. An effective disinfection time (EDT_{24}) lower than 3 h was found for all the photocatalytic systems and for samples containing < 1CFU/ml of culturable bacteria after photocatalytic treatment. Bacterial recovery was not observed even when final samples were incubated in LB medium. This finding means that the disinfection was complete at this moment. In these conditions, UV intensity and doses generated a combined photolytic and photocatalytic conditions that were sufficient to kill all bacteria.

Monitoring of bacteria population during storage of water after phototreatment is crucial because by-products photogenerated from organic matter naturally present in water or from bacterial lysis could be favorable for the subsequent bacterial regrowth during water storing in the dark. In this work, field scale experiments demonstrated evidence of absence of bacterial regrowth after Fe^{3+} and TiO_2 solar photoassisted disinfection of *E. coli* contaminated water. 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, at natural (not acidic) pH, 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 another possible strategy.

Thus, photocatalytic inactivation is a simple process and could be affordable for use in semi-urban and rural areas within developing countries and warrants further study. Indeed, further research should be carried out on the efficiency of solar photocatalytic disinfection methods for the specific water sources and locations, in order to explore the potential use of these technologies.

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