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Solar Advanced Oxidation Processes as disinfection tertiary treatments for real wastewater: Implications for water reclamation

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ABSTRACT

The aim of this study was to assess the disinfection of a real secondary effluent from a municipal wastewater treatment plant using added H_2O_2 (20 and 50 mg L^{-1}), TiO_2 (100 mg L^{-1}) and photo-Fenton under natural solar radiation in compound parabolic collector photo-reactors. For this purpose, the naturally occurring *Escherichia coli*, spores of sulphite-reducing clostridia (SRC), somatic coliphages (SOMCPH) and F-specific RNA bacteriophages (FRNA) were tested before and along the different solar treatments. Results for *E. coli* showed the different treatments efficiency rank: photo-Fenton pH 3 > H_2O_2 (20 mg L^{-1})/solar > TiO_2/solar > solar photo-inactivation. On the other hand, for viral indicators the ranking was: photo-Fenton pH 3 > TiO_2/solar > H_2O_2 (20 mg L^{-1})/solar > solar photo-inactivation. SRC was the most resistant indicator microorganism in all the evaluated processes. For the first time these solar processes have been evaluated for naturally occurring conventional indicators such as *E. coli* and alternative indicators such as SOMCPH and FRNA as viral indicators or spores of SRC as protozoan indicators. Some of the tested solar photo-oxidation treatments have shown their capability to reduce *E. coli* concentrations to a suitable level for water reuse (according to different reclaimed water guidelines) within affordable treatment times.

1. Introduction

Pathogens causing waterborne infectious diseases constitute one of the health risks associated with urban wastewater, and they have been identified as the main cause of contamination in streams, rivers and estuaries in the United States [1]. Diarrhoea is the most widespread waterborne infectious disease worldwide, mostly among children, and it is one of the leading causes of mortality in developing countries, where circa 88% of cases are associated with poor water quality and sanitation [2]. Among other measures, proper treatment of wastewater and drinking water disinfection are required in order to tackle this problem [3].

Other situations related to the scarcity of fresh water also render it necessary to conduct further research on new water disinfection technologies. First, the availability of safe fresh water is diminishing at an alarming rate both in high and low income countries, and sunny areas worldwide are particularly affected [4]. This situation will inexorably lead to the use of non-conventional water resources such as reclaimed water. Second, there is an increasing presence of disinfection by-products (DBPs) in drinking water due to the widespread use of chlorination and ozonation [5,6]. Third, many countries and communities are unable to assume the economic and energy costs of some of the current disinfection treatments. Moreover, the efficacy of some widely used disinfection treatments is limited as regards removal of resistant waterborne pathogens such as *Cryptosporidium sp* and *Giardia sp* [7].

Advanced Oxidation Processes (AOPs) have been widely demonstrated to be reliable for wastewater treatment since they have a high capacity to oxidise nearly all organic pollutants. This capacity derives from the generation of hydroxyl radicals ($OH^{\bullet}$), the second most oxidant species after fluorine which acts unselectively [8]. Moreover, the use of solar light means that these treatments are environmentally friendly. Among solar AOPs, heterogeneous and homogeneous photocatalysis (i.e., TiO_2 and photo-Fenton) are the processes which have received most research attention in recent decades for wastewater treatment purposes. Recently, these AOPs have also begun to be studied for water disinfection purposes [9].

This study analysed the disinfection capacity of four water disinfection methods based on the use of natural solar radiation. These were: solar photo-inactivation (with no additives or catalysts), H_2O_2 (20 and 50 mg L^{-1}) with solar light, solar heterogeneous photocatalysis (TiO_2), and solar photo-Fenton. Recently, these treatments have been proven to have a good capacity to inactivate microorganisms in water. Solar photo-inactivation has been

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applied for inactivation of a wide range of waterborne pathogens [10]. The synergistic effect of H_2O_2 and sunlight has been demonstrated to be effective for water disinfection against bacterial cells and fungal spores [11,12]. Since the 90's, the use of TiO_2/solar UVA for inactivation of *Escherichia coli*, *Enterococcus faecalis*, total coliforms and several fungal spores has been studied in depth by several groups [13–16]. Moreover, the almost neutral pH photo-Fenton method has been studied as a novel mild water disinfection treatment. This treatment was successfully tested with *E. coli*, *E. faecalis*, *Salmonella* spp. and fungal spores in different contaminated waters [17–19].

The concentration of pathogenic microorganisms in highly polluted waters, i.e., wastewater, may be low and furthermore, difficult to quantify. Consequently, indicators are used to represent both the potential occurrence and the response of pathogens to water disinfection, and faecal bacteria (faecal coliforms, *E. coli*, enterococci) are commonly used for this purpose. However, these indicators do not provide information on the occurrence and behaviour of viruses and protozoa. Hence, alternative indicators are used to evaluate water treatments: somatic coliphages (SOMCPH), F-specific RNA phages (FRNA) and bacteriophages infecting *Bacteroides* are used as viral indicators [20], and spores of sulphite-reducing clostridia (SRC) are used as indicators of oocysts of *Cryptosporidium sp* [21,22]. These indicators are present in wastewater, and their concentrations in a secondary effluent are sufficiently high to monitor 4 log reductions of *E. coli*, 3.5–4 log of SOMCPH, 2.5–3 log of FRNA and 2–2.5 log of SRC without requiring costly and complicated concentration procedures [23].

The aim of this study was to assess the disinfection of a real secondary effluent from a Municipal Wastewater Treatment Plant (MWWTP) using added H_2O_2, TiO_2 and photo-Fenton under natural solar irradiation in Compound Parabolic Collector (CPC) photo-reactors. For this purpose, the naturally occurring *E. coli*, SRC, SOMCPH and FRNA were tested before and throughout the different solar treatments in order to: (i) determine the inactivation of these microbial indicators in real wastewater effluents, (ii) determine the indicator removal efficiency of the technologies studied, and (iii) evaluate the feasibility of these disinfection technologies for water reclamation.

2. Materials and methods

2.1. Collection of Municipal Wastewater Treatment Plant samples

Samples of real MWWTP effluent (MWWTP) were collected from the municipal wastewater treatment plant in the city of Almería (Southeast of Spain) and used to carry out all the disinfection experiments. This treatment plant uses activated sludge plus decantation as secondary treatment, and produces 11,594,704 m^3 of secondary effluent per year. Fresh secondary effluent was collected every day in batches of 60 L. The samples were used for solar tests within 2 h of collection. Several parameters were evaluated for the chemical and microbiological characterisation of the effluent. Table 1 shows pH, inorganic ion concentrations, turbidity, conductivity, Total Organic Carbon (TOC) and *E. coli* concentrations, averaged for all MWWTP effluent samples used in all the experiments. Ion determination was performed by ion chromatography (IC) using a DX-600 model (Dionex Corporation, Sunnyvale, CA) for anions and a DX-120 model for cations. TOC and Total Carbon (TC) were analysed using a TOC-5050 (Shimadzu Corporation, Kyoto, Japan). Turbidity was measured with a turbidimeter (Model 2100N, Hach, USA). The natural presence of iron in MWWTPE was analysed using a spectrophotometric technique with phenanthroline/acetic acid (UV–vis measurements, limit of detection 0.05 mg L^{-1}). No iron was detected in any of the samples used.

2.2. Indicator detection and quantification

E. coli was detected using the plate counting method in Chromocult® Coliform agar (Merck, Germany) with different sample volumes: (a) 25, 50, 250, and 500 μL of sample was spread over 90 mm diameter Petri dishes, and (b) 5 mL of sample was poured and cultured twice (total of 10 mL) in 140 mm diameter Petri dishes. In both cases, plates were incubated at 44 °C for 20 ± 4 h and enumerated. For detection of SRC, water samples were cultured on sulphite polymyxin sulphadiazine (SPS, Cultimed, Panreac) agar medium at 44 °C under anaerobic conditions for 24 h. Plaque forming units (PFUs) of SOMCPH in the WG5 *E. coli* strain were counted by the double agar layer technique following ISO standard 10705-2 [24]. FRNA were determined in *Salmonella* strain WG49 according to ISO 10705-1 [25]. The limit of detection for bacteria and bacteriophages was 10 CFU and 1 PFU/100 mL, respectively.

2.3. Solar CPC photo-reactors

The efficiency of solar photo-activated treatments may be enhanced by the use of solar Compound Parabolic Collector (CPC) reactors. Their high efficiency in solar radiation (both direct and diffuse) collection accelerates the inactivation rates of the different solar treatments due to optimal collection of solar UVA photons [26,27].

All experiments were performed under natural solar radiation at Plataforma Solar de Almería, located at 37°84′N and 2°34′W. The solar CPC reactors used for this study have been described elsewhere [28]. They consist on a CPC photo-reactor tube module placed on a tilted platform connected to a recirculation tank and a centrifugal pump. Total volume of the photo-reactor was 10 L, illuminated volume was 4.5 L and the irradiated collector surface was 0.4 m^2. Based on our previous experience, the selected water flow rate was 10 L/min. The experimental setup allowed two experiments to be carried out simultaneously in two identical solar CPC reactors.

2.4. Solar experiments

All experiments were conducted on sunny days in September and October 2011. The weather conditions (ambient temperature and solar irradiance) were those typical for this time of the year at this location. The average solar UVA irradiance for all tests was 38 W m^{-2} within the period 11:00–15:00 local time, with maximum values of 50 W m^{-2} registered between 13:00 pm and 15:00 pm. The temperature of the water samples during the solar tests (5 h) ranged from 28 °C ± 1.6 °C to 39.8 °C ± 3.8 °C.

Inactivation results are presented as the average of at least two replicates for each treatment. Water and reagents were added to the reactor tank and re-circulated for 15 min to ensure adaptation and homogenisation, with the CPC mirror covered by an opaque sheet. After that, the first sample was taken and the reactor was exposed to solar radiation. Samples were collected at regular intervals to determine indicator concentrations. Frequency of sampling varied depending on the treatment. Duration of all experiments was 5 h, starting at 11:00–11:30 a.m. local time. Water temperature (Checktemp, Hanna instruments, Spain), dissolved oxygen (DO) and pH (multi720, WTW, Germany) were measured in the reactor during the experiments. For each treatment, dark control tests were carried out using the same conditions (reactor, reagents, etc.) in a covered reactor. *E. coli* re-growth was evaluated 24 and 48 h after the treatment. This was performed with samples that reached the limit of detection. The samples were stored in the dark at 25 °C and enumerated again as described above. These results showed no re-growth in any of the treatments.

Table 1
Chemical and microbiological characterisation of the municipal wastewater treatment plant secondary effluent used (El Bobar, Almería, Spain).

Secondary effluent characterisation			
pH	7.31 ± 0.30	Turbidity (NTU)	8 ± 4
PO_4^{3-} (mg L^{-1})	6 ± 3	Conductivity (μS/cm)	1530 ± 152
NO_2^- (mg L^{-1})	3.4 ± 0.5	NO_3^- (mg L^{-1})	12 ± 15
Cl^- (mg L^{-1})	289.7 ± 64	SO_4^{2-} (mg L^{-1})	114.7 ± 32
NH_4^+ (mg L^{-1})	35 ± 21	Mg^{2+} (mg L^{-1})	27 ± 3
HCO_3^- (mg L^{-1})	85 ± 3	K^+ (mg L^{-1})	24.3 ± 3
Na^+ (mg L^{-1})	182 ± 11	Ca^{2+} (mg L^{-1})	78 ± 3
DOC (mg L^{-1})	16.3 ± 3	$Fe^{2+/3+}$ (mg L^{-1})	0 ± 0
E. coli (CFU/100 mL)	$1 \times 10^5 \pm 9 \times 10^4$		

2.5. Reagents

Aeroxide P25 (Evonik Corporation, Germany) TiO_2 catalyst was used as received from the manufacturer as slurry to conduct heterogeneous photocatalytic experiment. The catalyst concentration used was 100 mg L^{-1} according to previous findings for the same CPC reactor configuration [28].

Ferrous sulphate heptahydrate ($FeSO_4 \cdot 7H_2O$, PANREAC, Spain) was used as source of Fe^{2+} at concentrations of 5 mg L^{-1} and 10 mg L^{-1} for homogeneous photo-Fenton reaction. Fe^{2+} and total iron (Fe^{tot}) concentrations were measured according to ISO 6332. Fe^{3+} concentration was determined subtracting Fe^{2+} from Fe^{tot}. The concentration ratio of iron:H_2O_2 used was 1:2. Sulphuric acid (Merk, Germany, analytical grade) was used when acidic conditions were required for photo-Fenton experiments.

Hydrogen peroxide 30% (w/v) (Riedel-de Haën, Germany) solution was added directly into the samples. H_2O_2 concentration was determined with spectrometric methods as described elsewhere [29] with a range of 0.1–100 mg L^{-1}. The experiments of H_2O_2/solar UVA were performed with 20 and 50 mg L^{-1}. For photo-Fenton tests, a freshly prepared solution of bovine liver catalase (0.1 g L^{-1}, Sigma–Aldrich, USA) was added to samples in a ratio 0.1/5 (v/v) to eliminate residual hydrogen peroxide and avoid Fenton reactions after samples collection. H_2O_2 and catalase at these concentrations have been demonstrated to have no detrimental effect on *E. coli* viability [18].

2.6. Solar radiation

UVA radiation was measured with a global UVA pyranometer (300–400 nm, Model CUV4, Kipp & Zonen, Netherlands) tilted 37°, the same angle as the local latitude. The pyranometer provides data in terms of incident UVA-W m^{-2}, which is the solar radiant UVA energy rate incident on a surface per unit area. In this study, the inactivation rate is plotted against both experimental time (t), and cumulative energy per unit of volume (Q_{UV}) received in the photoreactor. Q_{UV} (Eq. (1)) is commonly used to compare results under different conditions [9].

$$Q_{UV,n} = Q_{UV,n-1} + \frac{\Delta t_n \overline{UV}_{G,n} A_r}{V_t} \qquad \Delta t_n = t_n - t_{n-1} \tag{1}$$

where $Q_{UV,n}$, $Q_{UV,n-1}$ is the UV energy accumulated per litre (kJ L^{-1}) at times n and $n-1$, $UV_{G,n}$ is the average incident radiation on the irradiated area, Δt_n is the experimental time of sample, A_r is the illuminated area of collector (m^2), and V_t is the total volume of water treated (L).

2.7. Kinetics evaluation

The inactivation results are usually evaluated using the Chick–Watson's law (Eq. (2)).

$$\log\left(\frac{N}{N_0}\right) = -k_t \cdot t \tag{2}$$

where N/N_0 is the reduction in the concentration of microorganism, k_t is the disinfection kinetic constant, and t is the experimental time. However the kinetics of the photo-induced disinfection process depends also on the photon flux received inside the photoreactor. For this reason, to properly assess the inactivation rates of processes carried out under natural solar radiation, the modified Chick–Watson's law was proposed (Eq. (3) [28]):

$$\log\left(\frac{N}{N_0}\right) = -k_{Q_{UV}} \cdot Q_{UV} \tag{3}$$

where $k_{Q_{UV}}$ is the new disinfection kinetic constant and Q_{UV} accounts for the solar UVA energy per unit of volume accumulated in the system.

3. Results and discussion

3.1. Inactivation of microbial indicators by solar photo-inactivation

The effect of solar radiation on pathogen viability is shown in Fig. 1(a), while Tables 2 and 3 show the first order decay rate of microbial indicators (k_t and $k_{Q_{UV}}$, respectively) for all the solar treatments evaluated in this study. According to the inactivation rates, the order of sensitivity to solar photo-inactivation was: *E. coli* > FRNA ≥ SOMCPH > SRC.

Dark control experiments were performed under the same conditions in a covered reactor, so that the water presented the same thermal behaviour as under solar UVA exposure, thus enabling the effect of mild solar heating on these indicators to be evaluated (Fig. 1(b)). This effect was either low or null for all indicators except FRNA, which showed a significant reduction (1.8-log). The sequence of sensitivity to this increase in temperature (29–38 °C) was FRNA > *E. coli* ≥ SOMCPH ≥ SRC.

Different responses to mild heating have been reported for the indicators studied here [30–33]. Temperature has a clear influence on the inactivation rate of microorganisms in solar water disinfection [34]. The synergistic effect of UVA radiation and temperature has also been evaluated in a number of Solar Water Disinfection (SODIS) articles [10], which have demonstrated that inactivation of bacteria by solar disinfection is substantially faster at temperatures above 45 °C [35]. In the present study, the range of temperatures was too low (<40 °C) to observe such strong synergy. As can be seen in our results (Fig. 1), the inactivation of *E. coli* and SOMCPH seemed to be mainly due to solar UVA irradiation. FRNA bacteriophage concentrations decreased 2 logs under solar exposure, although the thermal effect accounts for most of the inactivation (1.8 log). No significant reduction in SRC spores was observed in either case. The bactericidal effect of solar UVA radiation has been well described elsewhere [10,30] for a wide range of pathogens. The effect is based on the oxidative action of several reactive oxygen species (ROS) generated in water under UVA radiation. The main variables affecting the inactivation rate are photon flux and the way that solar radiation is delivered into the system [36], the dissolved oxygen

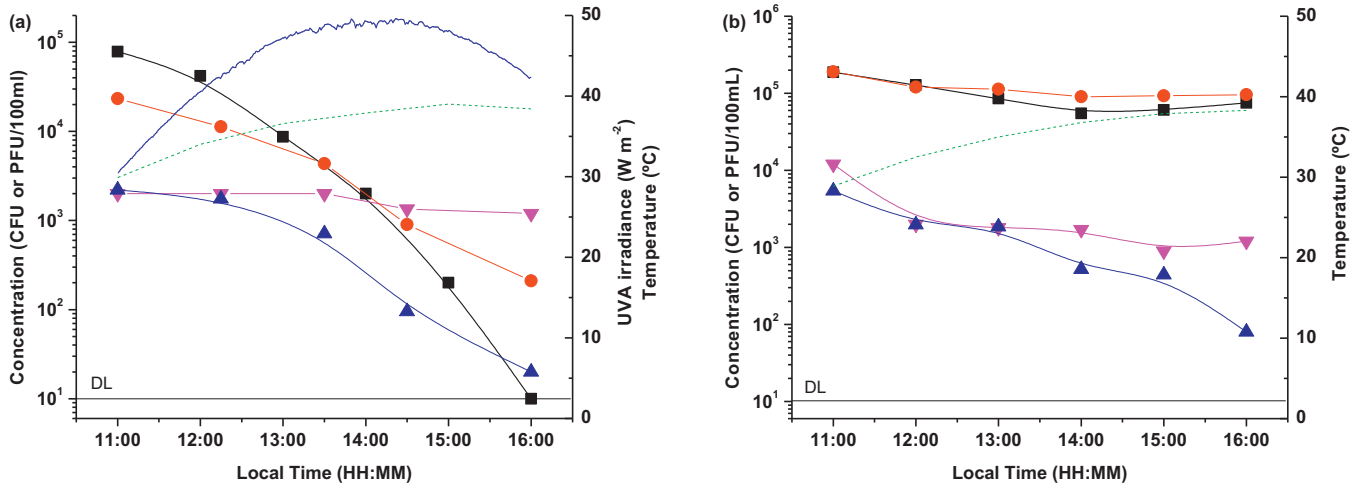

Fig. 1. Inactivation of all the microbial indicators tested in a representative single experiment of solar photo-inactivation (a) and corresponding experiment performed in the dark (b). *E. coli* (–■–); SRC, sulphite-reducing clostridia (—▼—); SOMCPH, somatic coliphages (—●—); FRNA, F-specific RNA bacteriophages (—▲—); DL, detection limit; CFU, colony forming units; PFU: plaque forming units. UVA irradiance (▬); temperature (▬ ▬).

Table 2
Decay rates ± standard errors in min^{-1} of the microbiological parameters in the different treatments. SRC, spores of sulphite reducing clostridia; SOMCPH, somatic coliphages; FRNA, F specific bacteriophages infecting *Salmonella* strain WG49.

Treatment	Decay rate – k_t (min^{-1})			
	E. coli	SRC	SOMCPH	FRNA
Solar photo-inactivation	0.0133 ± 0.0004	0.0019 ± 0.0012	0.0074 ± 0.0006	0.0085 ± 0.0017
H_2O_2/solar (20 mg L^{-1})	0.0342 ± 0.0094	0.0065 ± 0.0000	0.0077 ± 0.0002	0.0118 ± 0.0018
H_2O_2/solar (50 mg L^{-1})	0.0201 ± 0.0032	0.0066 ± 0.0006	0.0065 ± 0.0004	0.0107 ± 0.0014
TiO_2/solar (100 mg L^{-1})	0.0135 ± 0.0013	0.0022 ± 0.0004	0.0141 ± 0.0010	0.0627 ± 0.0186
Photo-Fenton pH 3	0.5377 ± 0.1355	0.0051 ± 0.0005	0.0390 ± 0.0010	0.4525 ± 0.0921
Photo-Fenton natural pH	0.0263 ± 0.0000	0.0039 ± 0.0000	0.0126 ± 0.0000	0.0137 ± 0.0000

[37], chemical composition and turbidity of the water [38]. Much research has been reported on the photo-inactivation of microorganisms, and the results have varied widely, being influenced not only by the above-mentioned parameters but also by other factors such as the origin of the microbial contamination (naturally occurring or lab seeded), water temperature, the source of light (sun, solar light simulators or UV lamps) and the design of the photo-reactor. Most studies have been carried out using low volume static batch systems, such as 2 L PET bottles, following the so-called SODIS method. However, this paper presents results for a re-circulated batch system. This permitted treatment of higher volumes but also required longer treatment times to reach the detection limit because the UVA radiation dose was not delivered continuously into the system [36]. Therefore, the total volume of treated water should be taken into account when comparing the treatment times (or UVA dose in terms of kJ m^{-2}) required by both types of systems to disinfect water.

E. coli is the most frequently studied bacterium. Using the SODIS method, this waterborne pathogen has been found to be inactivated within 6 h under full sunlight [10]. However, naturally occurring faecal coliforms have shown a higher resistance to sunlight [39,40], with a substantial difference being observed between the inactivation of *E. coli* and that of bacterial endospores (belonging to *Bacillus*) in experiments performed in pure water seeded with laboratory-grown microorganisms, which is in agreement with the results obtained in this study. Furthermore, in experiments using UV irradiation, the same sequence of sensitivity, *E. coli* > human viruses > spores of *Bacillus subtilis*, has been reported [41]. Wegelin et al. [30] also observed that animal viruses and bacteriophages f2 (F-specific RNA bacteriophages) were more resistant than *E. coli* to UV irradiation. In contrast, the inactivation kinetics of *E. coli* and coliforms reported by Caslake et al. [42] were markedly faster than those described here. Nevertheless, it should be borne in mind that the coliforms studied by these authors consisted of a mix of pure laboratory-grown enteric bacteria cultures.

The variability observed in microbial inactivation in the experiments carried out in this study may have been due to numerous factors which changed daily between the different experiments,

Table 3
Decay rates in L kJ^{-1} of the microbiological parameters in the different treatments. SRC, spores of sulphite reducing clostridia; SOMCPH, somatic coliphages; FRNA, F specific RNA bacteriophages infecting *Salmonella* strain WG49.

Treatment	Decay rate – $k_{Q_{UV}}$ (L kJ^{-1})			
	E. coli	SRC	SOMCPH	FRNA
Solar photo-inactivation	0.110 ± 0.010	0.012 ± 0.004	0.061 ± 0.004	0.075 ± 0.007
H_2O_2/solar (20 mg L^{-1})	0.410 ± 0.050	0.055 ± 0.005	0.068 ± 0.004	0.132 ± 0.007
H_2O_2/solar (50 mg L^{-1})	0.290 ± 0.020	0.064 ± 0.008	0.068 ± 0.003	0.125 ± 0.012
TiO_2/solar (100 mg L^{-1})	0.150 ± 0.010	0.029 ± 0.003	0.149 ± 0.006	0.500 ± 0.300
Photo-Fenton pH 3	0.800 ± 0.030	0.064 ± 0.004	0.500 ± 0.090	0.490 ± 0.050
Photo-Fenton natural pH	0.300 ± 0.040	0.043 ± 0.004	0.133 ± 0.006	0.135 ± 0.010

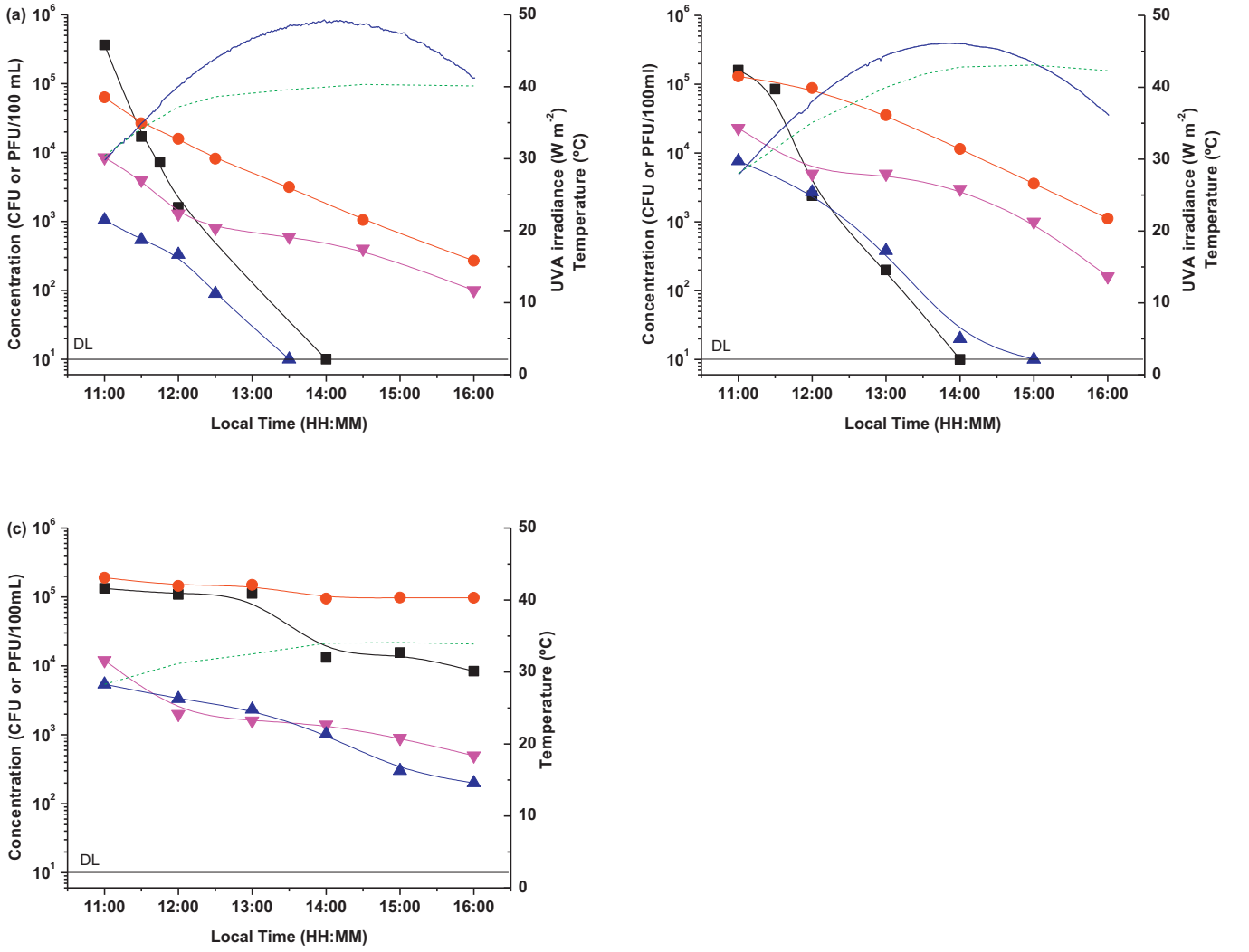

Fig. 2. Inactivation of all the microbial indicators tested in a representative single experiment of H_2O_2 (20 mg L^{-1} and 50 mg L^{-1})/solar UVA (a) and (b) respectively; experiment performed with H_2O_2 (20 mg L^{-1}) in the dark (c). *E. coli* (–■–); SRC, sulphite-reducing clostridia (—▼—); SOMCPH, somatic coliphages (—●—); FRNA, F-specific RNA bacteriophages (—▲—); DL, detection limit; CFU, colony forming units; PFU, plaque forming units. UVA irradiance (▬); temperature (▬).

such as the level of solar irradiance, water temperature, and the load and nature of microbial and chemical contamination of the secondary effluent. However, although replicates were performed on different days and with the secondary effluent samples that varied slightly in turbidity and microbial indicator concentrations, they gave similar results, as shown in standard errors of k_t (Table 2) and $k_{Q_{UV}}$ (Table 3). This low variability of the results was observed for all treatments.

3.2. Inactivation of microbial indicators by H_2O_2/Solar

Fig. 2(a) and (b) shows representative observations of microbial indicator inactivation with 20 and 50 mg L^{-1} of H_2O_2 respectively, under natural solar irradiation. The drop in concentration of each indicator during solar exposure with added 20 mg L^{-1} of H_2O_2 was 5.3-log for *E. coli*, 3-log for FRNA, 2.3-log for SOMCPH and 1.9-log for SRC. The addition of 50 mg L^{-1} of H_2O_2 resulted in similar inactivation levels, i.e., *E. coli* (5.2-log), FRNA (2.8-log), SOMCPH (2-log) and SRC (2.1-log). For comparison purposes, the inactivation rate constants should be taken into account (Tables 2 and 3) in order to establish the order of resistance of each indicator to this solar treatment. As expected, both H_2O_2 concentrations led to similar *k*-values, and the sequence of inactivation rate observed was: *E. coli* > FRNA > SOMCPH > SRC. During solar treatment, H_2O_2 consumption was 12 mg L^{-1} and 30 mg L^{-1}, respectively. The main factor responsible for this consumption was the chemical oxygen demand (COD) of the water samples, although temperature and pH may also have played a role. As demonstrated in previous contributions [12], there was no direct relationship between the amount of H_2O_2 consumed and the inactivation rate.

To determine the influence of the low H_2O_2 concentrations, we performed dark control tests in the same reactor under the same conditions except that the reactor was covered. The viability of each indicator in the presence of H_2O_2 (20 mg L^{-1}) was evaluated over 5 h (Fig. 2(c)). As reported in the literature for other microorganisms, we observed a non-significant decrease in the concentration of all of the indicators. The poor effect of H_2O_2 in the dark on survival of seeded *E. coli* has also been described by Rincón and Pulgarín [43], of naturally occurring *E. coli* by Fisher et al. [44], and of wild fungi by Sichel et al. [11]. The harmful effect of H_2O_2 itself depends on the microorganism and on the dosage applied; bacterial growth is inhibited in the range of 10–1000 mg L^{-1}, while at higher concentrations the organisms are destroyed [45]. The low H_2O_2 concentrations used in this study only exerted a damaging effect on the indicators evaluated when combined with sunlight.

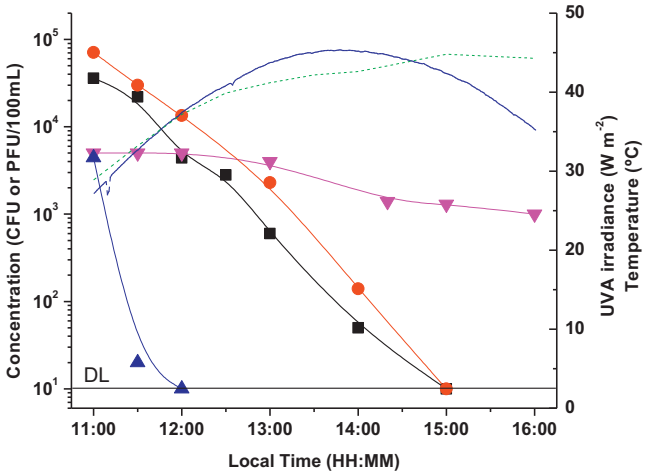

Fig. 3. Inactivation of all the microbial indicators tested in a representative single experiment of TiO_2 (100 mg L^{-1})/solar UVA. *E. coli* (–■–); SRC, sulphite-reducing clostridia (—▼—); SOMCPH, somatic coliphages (—●—); FRNA, F-specific RNA bacteriophages (—▲—). UVA irradiance (▬); temperature (▬).

The synergistic effect of low amounts of H_2O_2 and solar irradiation to inactivate different microorganisms has been reported for *E. coli*, bacteriophage T7, *Bacillus* spores and *Fusarium* spp. spores [12,44,46]. The different levels of sensitivity to this treatment shown by the indicators is in agreement with other reported results for inoculated *E. coli* [47], *Bacillus* spores [48], MS2 (F-specific RNA bacteriophage) and somatic coliphages T4 and T7 [49,50]. There are some similarities between the values of T90 (time required to achieve a 90% reduction) for naturally occurring *E. coli* reported by Fisher et al. [44] from contaminated ditch water and our results. In both cases, T90 ranged between 2 and 3 h.

These authors attributed cell inhibition or death to the following mechanisms occurring simultaneously: (i) direct photo-inactivation (Fig. 1(a)), (ii) internal Fenton and Haber–Weiss reactions leading to internal cell injures, this occurs when small, uncharged H_2O_2 molecules diffuse through membranes into the cell, increasing intracellular H_2O_2 levels. These molecules then react with free or loosely bound iron from cellular sources of iron (sulphur cluster, enterobactin, ferritin, and siderophores) leading to $OH^{\bullet}$ generation via a Haber–Weiss reaction), and (iii) inactivation of catalase and superoxide dismutase (SOD), which occurs under UV-A radiation stress, favouring an increase in H_2O_2 inside the cells. Although the complete mechanism still remains unclear, since there is no experimental evidence of what is happening inside the cells, our results may be explained by the above mechanisms.

Moreover, no enhancement of inactivation was observed when 50 mg L^{-1} of H_2O_2 was used (Fig. 2(b)). The same effect has been observed before for *Fusarium* spp. [11,12,18]. This finding could be also explained by the internal reaction mechanisms, as they are limited by the iron available inside cells and not by the concentration of added H_2O_2. In this case, as with solar photo-inactivation [36], the amount of oxidative species needed to induce the destruction or inhibition of a certain microorganism are generated under given conditions (i.e., 20 mg L^{-1} of H_2O_2), but additional generation of ROS does not necessarily lead to better inactivation results.

3.3. Inactivation of microbial indicators by heterogeneous photocatalysis with TiO_2

Fig. 3 shows the inactivation of the four microbial indicators under solar heterogeneous photocatalysis using 100 mg L^{-1} of suspended TiO_2. The improvement in disinfection achieved by the addition of TiO_2 was very different for the microorganisms tested (see inactivation kinetics in Fig. 3 and decay rates in Tables 2 and 3). The viral indicators (SOMCPH and FRNA) were the most sensitive, particularly FRNA, the inactivation rates of which were much higher than with solar light alone. *E. coli* inactivation was similar under both treatments. In contrast, SRC were hardly affected by the addition of titanium dioxide. According to inactivation rate constants (Tables 2 and 3), the sequence of microbial indicator sensitivity to the TiO_2/solar treatment was: FRNA > SOMCPH ≥ *E. coli* > SRC.

A similar sequence of sensitivity has been reported elsewhere with seeded microorganisms. The greater sensitivity of MS2 (F-specific RNA bacteriophage) compared to *E. coli* [51] and phages ϕX174 and PR772 (both somatic coliphages) has also been reported [52]. In addition, the greater sensitivity of *E. coli* when compared to SRC [53] and the higher effect of TiO_2 photo-oxidation on bacteriophage MS2 has been reported [54]. Regarding animal viruses, it has been described that enteroviruses are more sensitive to TiO_2 photo-oxidation than *E. coli* [55]. On the other hand, some papers reports contradictory results with seeded *E. coli* [56–59].

Under the experimental conditions applied in this study, it was not possible to obtain the best photocatalytic disinfection results due to the existence of certain detrimental factors, such as the presence of a high amount of carbonates/bicarbonates in the water, which decreases photocatalytic efficiency [28]. Similarly, organic matter competed for hydroxyl radicals generated under solar irradiation. Nevertheless, the reduction in Dissolved Organic Carbon (DOC) was measured throughout the experiment and this parameter did not suffer any significant change. The high water pH (7.31 ± 0.30) did not favour the interaction between photo-catalyst particles and microorganism cells [60].

3.4. Inactivation of microbial indicators by solar photo-Fenton

The inactivation of microbial indicators with photo-Fenton was tested in the real effluent at pH 3 and at natural pH (7.31 ± 0.30) using 10 mg L^{-1} of Fe^{2+} and several doses of H_2O_2 (20 mg L^{-1} per dose), added during the solar experiments when the hydrogen peroxide fell below 10 mg L^{-1}. The addition time of H_2O_2 doses varied depending on each experiment. The inactivation patterns obtained are shown in Fig. 4(a) and (b) and decay rate constants are given in Tables 2 and 3. As expected, the results differed depending on the pH and also on the microorganisms, and very short inactivation times were needed at pH 3 compared with natural pH, for all tested indicators. The sensitivity of the indicators against the photo-Fenton treatment at both pH values was: *E. coli* > FRNA > SOMCPH > SRC.

Dark control tests to evaluate the viability of the indicators at pH 3 were also performed in a covered reactor for 5 h. The indicator most affected by pH 3 was *E. coli*, which showed a > 2-log reduction within 60 min. FRNA and SOMCPH showed a 1-log and 0.2-log drop in concentration, respectively, in 60 min. On the other hand, pH 3 did not affect SRC, as its concentrations remained constant throughout the 5 h.

Dissolved iron in the neutral pH photo-Fenton tests was zero or below the detection limit of the quantification method; consequently k_t and $k_{Q_{UV}}$ values of microbial indicators were similar to those obtained for the H_2O_2/solar treatment. In contrast, the effect on all indicators except for SRC of photo-Fenton at pH 3 was dramatic: for *E. coli*, FRNA, and SOMCPH, the concentration reductions were 5-log (10 min), 3.8-log (10 min), and 5-log (1.5 h), respectively. The SRC concentration did not show a significant enhancement in inactivation, demonstrating the very strong resistance of this pathogen even to photo-Fenton at pH 3.

The high efficiency of the process at pH 3 was due to the photo-Fenton reaction which occurred between the added H_2O_2 and the dissolved iron in the effluent, and the stress that the very acidic conditions represented for the microorganisms may also have

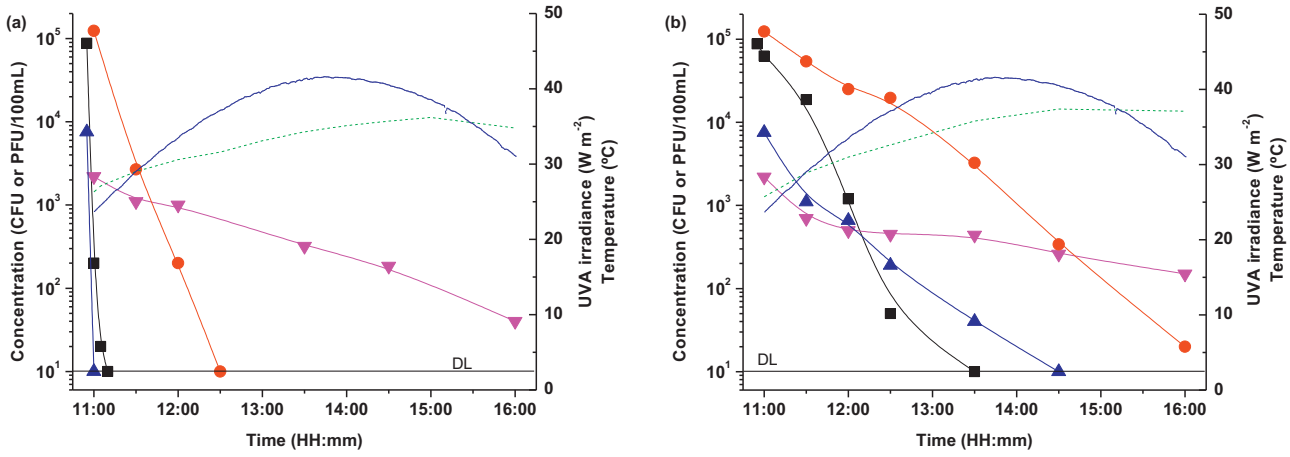

Fig. 4. Inactivation of all the microbial indicators tested in a representative single experiment of photo-Fenton pH3 (a) and natural pH (b): 10 mg L^{-1} of Fe^{+2} and 20 mg L^{-1} of H_2O_2 at pH3. *E. coli* (–■–); SRC, sulphite reducing clostridia (—▼—); SOMCPH, somatic coliphages (—●—); FRNA, F-specific RNA bacteriophages (—▲—). UVA irradiance (▬); temperature (▬).

favoured inactivation. Although 10 mg L^{-1} of iron was added, only low amounts of dissolved iron were present due to the chemical composition of the water (Table 4). The dissolved iron at pH 3 was between 1 and 3 mg L^{-1}, generating sufficient hydroxyl radicals to produce lethal damage to microbial cells, as has been reported for fungal spores, virus and bacteria [12,18,61]. It was the photo-Fenton reaction which was primarily responsible for this dramatic drop in indicator concentrations, because similar experiments at neutral pH or with only added H_2O_2 showed slower inactivation profiles (Tables 2 and 3). Due to the photo-Fenton reactions, the reduction in DOC at pH 3 was around 10 mg L^{-1} in all experiments (from 15 to 5 mg L^{-1}), while in the near neutral pH photo-Fenton reaction, the decrease in DOC was not appreciable. Therefore, to minimise the supplementary cost of acidification and neutralisation, it would seem advisable to conduct further research into the effect of pH values between 3 and neutral.

3.5. Photo oxidation effects on microbial inactivation

These treatments have typically been studied using laboratory strain microorganisms seeded in water (i.e., *E. coli* K12). However, very little information is available about disinfection of naturally occurring *E. coli* [44], and nothing has been reported about viral indicators or bacterial spores until now. Moreover, seeded microorganisms are more sensitive to disinfection than naturally occurring ones [62–66].

A global comparison of all the inactivation rates achieved for each naturally occurring indicator (Fig. 5) shows that SRC was the indicator with the lowest inactivation rates for all treatments. In contrast, *E. coli* was always the most easily inactivated indicator except in the case of the treatment with TiO_2/solar, where FRNA phages were the most susceptible microorganisms. Regarding the elimination of *E. coli*, which is currently the recommended indicator worldwide [67], in the USA [68] in Spain [69], etc., the different treatments ranked as follows: photo-Fenton pH 3 > H_2O_2 (20 mg L^{-1})/solar > TiO_2/solar > solar photo-inactivation. On the other hand, for viral indicators the ranking was: photo-Fenton pH 3 > TiO_2/solar > H_2O_2 (20 mg L^{-1})/solar > solar photo-inactivation. These different rankings should be taken into account when intending to combine photo-oxidation processes.

The sensitivity of the viral indicators to the disinfection procedures tested was moderate when compared with *E. coli* and SRC (Fig. 5), as suggested in reported studies of seeded viruses [41]. SOMCPH, which are a mixture of different DNA viruses, showed a higher resistance than FRNA phages to the tested photo-oxidative processes (Fig. 5), in agreement with other studies performed with seeded bacteriophages [52]. The few reports on seeded human viruses presented very variable results: rotaviruses and FRNA phages f2 and MS2 showed similar levels of resistance; nevertheless the encephalomyocarditis virus presented levels of resistance similar to those of SOMCPH [30]. Thus, it can be deduced that naturally occurring SOMCPH are good surrogate indicators for human viruses in photo-oxidative systems, since they cover viruses that are not covered by either *E. coli* or F-specific RNA phages.

It is difficult to compare inactivation of SRC and *Cryptosporidium* oocysts, since data on inactivation of naturally occurring oocysts with these treatments are not available. Nevertheless, seeded *Cryptosporidium* oocysts [70–72] and *Achantamoeba* cysts [41] have been described to be quite resistant to photo-oxidation treatments. Additionally, it has been reported that with tertiary treatments such as UV irradiation and chlorination, SRC inactivate similarly to *Cryptosporidium* infectious oocysts [73–75]. Therefore, among the currently used model microorganisms, SRC appears to be the best indicator of the fate of protozoan oocysts when photo-oxidation treatments are used.

3.6. Applicability of photo-oxidation treatments in water reclamation

The treatment time required to fulfil the microbiological criteria stated in water reclamation policies and guidelines has been estimated for four solar-promoted photo-oxidation treatments. Table 5 presents a summary of the required time (solar exposure) for each treatment to eliminate 3-, 4- and 6-log of naturally occurring *E. coli* under the experimental conditions used in this study. In the worst case scenario of a highly contaminated secondary effluent (i.e., 10^6 CFU-*E. coli* per 100 mL), these reductions would provide reclaimed water suitable for unrestricted irrigation which fulfilled the criteria established by: (i) the WHO (2006), (ii) Spanish regulations [69] and (iii) the USEPA [68]. These three different

Table 4
Iron measurement during the photo-Fenton experiment.

pH	Added Fe[a]	Dissolved Fe[a]	Total H_2O_2[a]
3	10	1.1	80
3	10	3.2	120
3	10	2.9	140
8	10	0	100

[a] Expressed in mg L^{-1}.

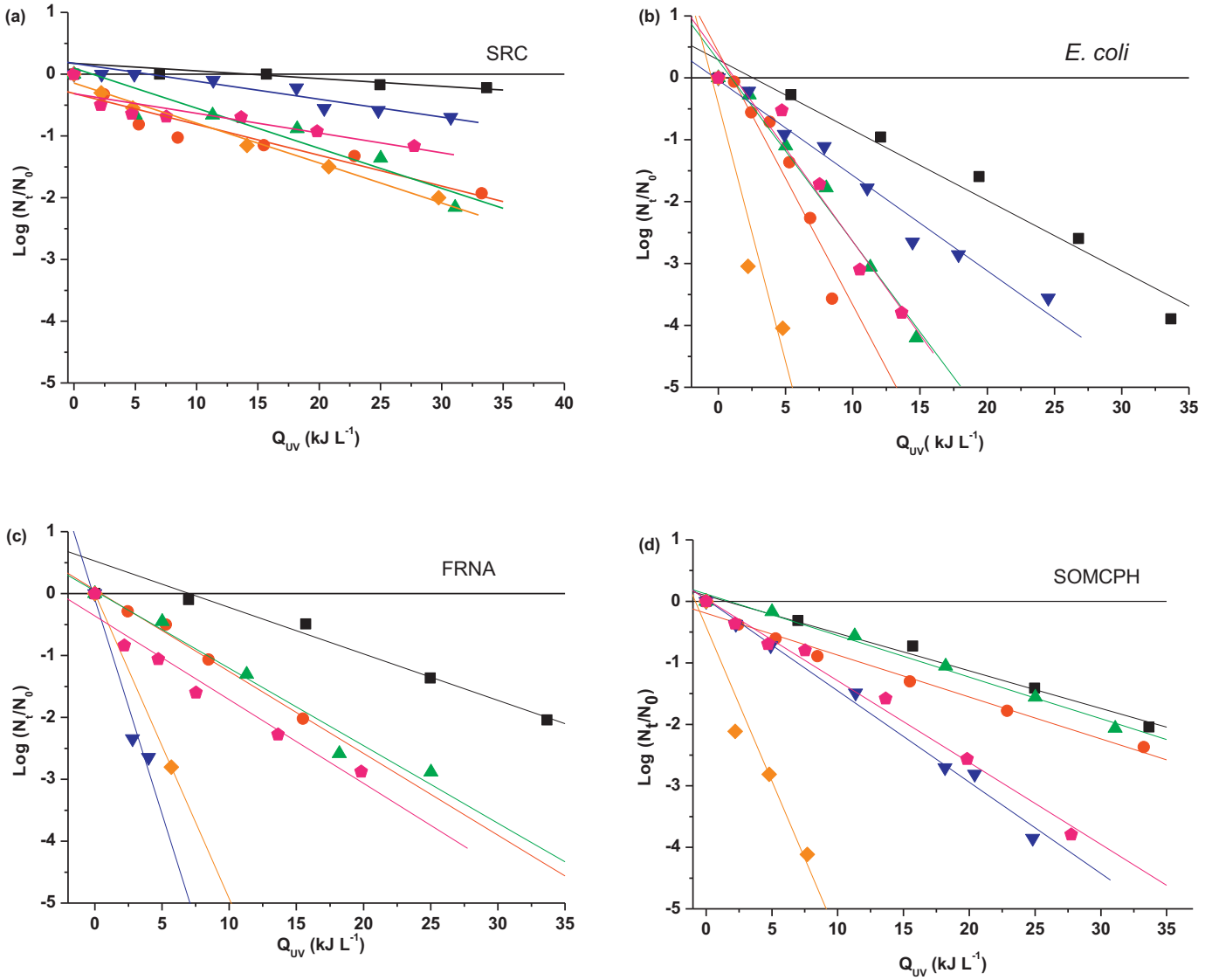

Fig. 5. Log inactivation of SRC (a), *E. coli* (b), FRNA (c), and SOMCPH (d) in each photo oxidation treatment: solar disinfection (–■–), H_2O_2-20 mg L^{-1} (—●—), photo-Fenton pH 3 (–◆–), TiO_2 (—▼—), H_2O_2-50 mg L^{-1} (—▲—), photo-Fenton natural pH (–⬟–). SRC, sulphite-reducing clostridia; SOMCPH, somatic coliphages; FRNA, F-specific RNA phages. Q_{UV}, accumulated energy.

regulations on reclaimed water quality stipulate different requirements: <1000, <100, and <1 *E. coli* (CFU per 100 mL), respectively. The design of a solar treatment plant which fulfilled these criteria would need a residence time of at least 6 min for photo-Fenton at pH 3, or 2 h for H_2O_2 (20 mg L^{-1})/solar (Table 5). These treatment times are still lengthy for a continuous flow reactor.

Previous experiences with solar pilot reactors are based on batch flow reactors which can treat variable volumes (tens of litres) of water, as they are modular. Only a few studies have been reported on continuous flow systems, for example, a sequential batch photo-reactor based on solar photo-inactivation for drinking water purification in isolated communities with lack of access to drinking water. In this case, the reactor was tested using *E. coli* in natural well water [29].

The capability of several solar technologies for real MWWTP effluents disinfection (for further reclamation) using a solar CPC photoreactor prototype has been shown here. According to our results, in the case of a continuous flow reactor for the treatment of large amounts of water (several m^3 per day), the CPC surface area required should be enormous (hundreds of m^2), as the treatment times range from few minutes to 2 h (4-log decrease). The design of this system should be enhanced for the real applications; this will be achieved by reducing the residence time. For example, from the solar collection point of view, the best choice of the optical path length (it depends on the water quality) would improve the income of solar photons in the photoreactor. Therefore, a proper design of a CPC plant for real MWWTP effluents based on the real load of contaminated water is needed prior to report on surface areas of solar collectors and capacity of the treated volumes per day. Additionally, more research should be conducted on these solar photo-oxidative technologies with the aim of finding out the limitations of these

Table 5
Time needed (h) to achieve 3, 4 and 6 log reductions using photo oxidation processes assayed.

Treatment	Time needed (h)		
	3-log reduction	4-log reduction	6-log reduction
Solar photo-inactivation	3.76	5.01	7.52
H_2O_2/solar (20 mg L^{-1})	1.46	1.95	2.93
TiO_2/solar (100 mg L^{-1})	3.70	4.94	7.41
Photo-Fenton pH 3	0.09	0.12	0.19

processes from the photo-chemical and photo-biological point of view.

4. Conclusions

The novelty of the study reported here is that it was performed with naturally occurring microorganisms, and to the best of our knowledge, this is the first time that a description has been given of the effects of photo-oxidation processes on naturally occurring viral indicators and spores of sulphite-reducing clostridia, which are considered indicators of protozoan (oo)cysts. In addition, the results confirm previously described studies suggesting that a single microbial indicator may not be enough to guarantee a low risk of infection. Nevertheless, it would also be possible to estimate the effects of these treatments on viruses and protozoa using the indicators, and to determine the treatment required to bring pathogens down to acceptable levels. In addition, the photo-oxidation treatments tested were capable of achieving the disinfection level necessary to reduce microbial health risks for users and they seemed to be functional as regards the treatment time required to achieve the regulatory limits. Consequently, from a practical point of view, this is highly valuable information when deciding which of these treatments is the most feasible to obtain safe reclaimed water.

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