Simultaneous abatement of organics (2,4-dichlorophenoxyacetic acid) and inactivation of resistant wild and laboratory bacteria strains by photo-induced processes in natural groundwater samples

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ARTICLE INFO

ABSTRACT

Simultaneous abatement of 2,4-dichlorophenoxyacetic acid (2,4-D at 70 $\mu g\,L^{-1}$), *Escherichia coli* and *Klebsiella pneumoniae* (laboratory and wild strains) cells from real groundwater samples containing natural iron concentrations ($\sim 0.3\,mg\,L^{-1}$) was studied by addition of H_2O_2 (10 mg L^{-1}) using a 18-L compound parabolic collector-CPC solar reactor (under natural sunlight irradiation 150,000 $J\,m^{-2}$) equipped with a UV-(A + B + Visible) lamp (15 W: UV-B and UV-A intensities were 0.74 and 6.47 $W\,m^{-2}$ respectively) powered by a photovoltaic panel.

Viability of *E. coli* K12 and *K. pneumoniae* laboratory strains at high initial concentrations of 10^6–10^7 cells mL^{-1} (followed by DVC-FISH) dropped 4.69 and 2.18 Logs, respectively after 30 min t_{30W} of combined UV-A + B-Visible lamps and sunlight irradiation (SL + UV + H_2O_2). Moreover, the initial 2,4-D concentration underwent a strong reduction reaching concentrations below the detection limit after 5 min t_{30W} of SL + UV + H_2O_2 treatment. Regarding real wild bacteria strains often present in natural well waters, which were at low initial concentrations, total culturability (initial concentration 10^1 CFU mL^{-1}) and viability (initial concentration 10^2–10^3 cells mL^{-1}) reductions were reached after 30 min (t_{30W}) of combined treatment. Participation of several photochemical and dark events such as photocatalysis (by iron hydro-oxides and other metal oxides naturally present), Fenton and photo-Fenton (by natural dissolved iron), UV-A + B/NO_3^-, Dissolved organic matter (DOM)/UV + Vis and UV-B photolysis of H_2O_2 are suggested as responsible of simultaneous 2,4-D abatement and microbial inactivation in natural groundwater samples.

1. Introduction

In Latin America, one third of people in rural communities (31%) uses groundwater as its main drinking water source (UNEP-GEO, 2010). However, these sources are threatened by the lack of primary sewage and drinking water systems, the former polluting the water with bacteria and the latter avoiding water disinfection. On the other hand, agricultural practices, which are often the main economic activity of these communities, use large amounts of pesticides (most of them toxic and non-biodegradable) and their bad handling can make also possible chemical groundwater pollution.

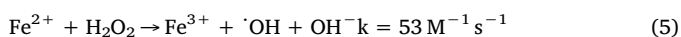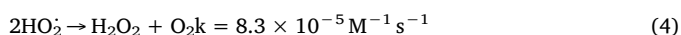Advanced oxidation technologies (AOTs) seem to be promising to removal of chemical and microbiological pollution in waters (Lanao et al., 2012; Oturan and Aaron, 2014; Robertson et al., 2012; Salgado-Transito et al., 2015; Vilar et al., 2012). However, AOTs likely use complicated and expensive systems (such as ozone) or large amounts of chemicals such as nanoparticles (photocatalysis) or iron, hydrogen peroxide at acid pH (photo-Fenton).

Regarding photo-Fenton processes which has demonstrated an excellent performance removing either chemicals or bacteria from water (García-Fernández et al., 2012; Giannakis et al., 2016a,b; Ortega-Gómez et al., 2012; Pignatello et al., 2006; Rodriguez-Chueca et al., 2014; Santos-Juanes et al., 2017; Sciacca et al., 2011; Tsydenova et al., 2015), several strategies have been explored in order to increase its pH range, especially toward circumneutral or neutral values. One of the most popular is the adding of different iron complexing agents such as

EDTA and citrate among others which forms ferric complexes highly soluble at neutral or circumneutral pH and photochemically active (Clarizia et al., 2017; Georgi et al., 2007; Miralles-Cuevas et al., 2014; Nogueira et al., 2017; Pignatello et al., 2006; Romero et al., 2016; Schenone et al., 2015). Light absorption by these ferric complexes yields ferrous ions and oxidized ligand through ligand to metal charge transfer process (LMCT) from complex excited states. Oxidized ligand reacts with molecular oxygen yielding superoxide radicals ($O_2^{-\cdot}$). This latter generates hydrogen peroxide (H_2O_2) by disproportion reactions, thus, finally, ferrous ions and H_2O_2 can produce Fenton or photo-Fenton reaction leading to the formation of hydroxyl radicals highly oxidants.

$$Fe^{3+}(L) + h\nu \rightarrow Fe^{2+} + L_{ox}^{\cdot} \tag{1}$$

$$L_{ox}^{\cdot} + O_2 \rightarrow CO_2 + O_2^{-\cdot} \tag{2}$$

$$HO_2^{\cdot} \leftrightarrow O_2^{-\cdot} + H^+ \; pk_a = 4.8 \tag{3}$$

$$2HO_2^{\cdot} \rightarrow H_2O_2 + O_2 \; k = 8.3 \times 10^{-5} M^{-1} s^{-1} \tag{4}$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\cdot}OH + OH^- \; k = 53 M^{-1} s^{-1} \tag{5}$$

Although that this approach seems to be promising to extend the pH range of photo-Fenton process to achieve waterborne organics abatement and bacteria inactivation, it shows different drawbacks such as cost of chelator addition, total organic carbon (TOC) increasing (by addition of chelators) and the most important, the ecotoxicity and biodegradability of chelators and byproducts formed after the photochemical event (Clarizia et al., 2017).

Another approach has been also explored in order to remove either waterborne organic pollutants or microorganisms in natural water sources without adding iron salts to drinking water production, since iron concentration in these kind of samples is restricted to concentrations below $0.3 \, mg \, L^{-1}$ because at higher concentrations, iron can produce bad taste to the water and clothes staining (WHO, 2011). Sciacca et al. (2010) found that the simple adding of hydrogen peroxide ($10 \, mg \, L^{-1}$) and sunlight irradiation may enhance solar disinfection of surface natural waters since natural dissolved or colloidal iron present on these samples should induce Fenton and photo-Fenton processes yielding $^{\cdot}OH$ radicals responsible of cell inactivation. Ndounla et al. (2013) reported for the first time, bacteria inactivation using the same approach in natural well waters.

It is well known that photochemical events such as UV/NO_3^-, UV/NO_2^- and triplet excited states of dissolved organic matter ($^3DOM^*$) may play an important role in organic pollutants removal from natural surface waters (Canonica et al., 1996;; De Laurentiis et al., 2014; Gligorovski et al., 2015; McNeill and Canonica, 2016).

Further, Gutierrez-Zapata et al. (2017a) suggested at laboratory scale that the simple addition of H_2O_2 ($10 \, mg \, L^{-1}$) upon simulated solar light could enhance natural abiotic processes such as $UV/NO_3^- - NO_2^-$ dissolved organic matter (DOM)/light, photo-Fenton and photocatalysis leading to the generation of reactive oxygen species (ROS) able to remove the most part of 2,4-dichlorophenoxyacetic acid (2,4-D) ($24.3 \, mg \, L^{-1}$), a herbicide highly used from natural groundwater samples. Moreover, it was also found that the chemical matrix of groundwater (especially the presence of carbonate and fluoride) could exert a positive effect on the photocatalytic and/or photo-Fenton processes photoinduced by the presence of added H_2O_2.

Then, the same authors demonstrated that 2,4-D herbicide at very low concentrations ($70 \, \mu g \, L^{-1}$) and *E. coli* cells could be simultaneously removed and inactivated with success from simulated groundwater by the addition of $10 \, mg \, L^{-1}$ of H_2O_2 in CPC reactors (30 L) irradiated by natural sunlight (Gutierrez-Zapata et al., 2017b). In contrast, *Klebsiella pneumoniae* cells which are highly resistant strains to the oxidative stress were not inactivated. This study highlighted the necessity to evaluate other bacterial strains different to *E. coli* in this kind of disinfection processes. Moreover, these results demonstrated the necessity

to enhance the different photochemical processes in order to obtain *K. pneumoniae* inactivation without the addition of foreign chemical substances. For this reason, an UV-B + A lamp was coupled to the system with the aim to enhance the photochemical events which may be photoinduced by these wavelengths.

Since this enhanced photo-induced abiotic process seems to have promising features to degrade emergent pollutants (such as pesticides) (Klamerth et al., 2010; Miralles-Cuevas et al., 2015; Navarro et al., 2011) and inactivate some bacteria strains in artificial and natural waters (Giannakis et al., 2014, 2016a,b,c, 2017; Gutierrez-Zapata et al., 2017c; Ndounla et al., 2013; Rodriguez-Chueca et al., 2012, 2014; Spuhler et al., 2010), herein this study was addressed to evaluate and enhance its performance by using a modified CPC solar reactor with UV-B + A + visible lamps powered by a photovoltaic panel in order to reach the simultaneous total inactivation of microorganisms including those highly resistant to the disinfection such as *K. pneumoniae* (which in previous studies reported for us seems to be resistant to the photochemical treatments), removal of 2,4-D in real groundwater samples. Furthermore, it was also evaluated the performance of this modified reactor on the inactivation of real wild bacteria strains present in natural groundwater samples.

2. Materials and methods

2.1. Reagents

Hydrogen peroxide (H_2O_2) (Carlo Erba), 2,4-D (Sigma-Aldrich), 2,4-Dichlorophenol, formaldehyde (Carlo Erba), formamide (Amresco), ethanol (Merck), sodium chloride (Sigma-Aldrich), EDTA (Merck), SDS (Fisher), Tris/HCl (Amresco), FISH probe ES-445 (Microsynth GmbH), nalidixic acid (Acros Organics), nutrient broth (Difco), yeast extract (Oxoid), peptone (Difco), plate count agar (Oxoid), EMB agar (Merck), Chromocult (Merck), humic acid (Alfa Aesar), Potassium Nitrate (Merck), Sodium phosphate (Scharlau), Sodium bicarbonate (Merck), Sodium fluoride (Merck), Sodium chloride (Sigma), Sodium sulfate (Merck), Chloride of manganese tetra hydrated (Fisher) and Milli-Q water. FISH probes Kpn (5′-CCT ACA CAC CAG CGT GCC-3′) (Microsynth GmbH) and ES-445 (5′-CTT TAC TCC CTT CCT CCC-3′) (Microsynth GmbH). All the reagents were used without further purification.

2.2. Determination of 2,4-D and hydrogen peroxide

Concentrations of 2,4-D and 2,4-Dichlorophenol (2,4-DCP) were followed by HPLC (LC20AT- Shimadzu). Solid-phase extraction clean-up with C-18 was performed previously (activation and elution with ethyl acetate). Limits of quantification (LOQ) of the chromatographic methods were $6 \, \mu g \, L^{-1}$ and $5 \, \mu g \, L^{-1}$ for 2,4-D and 2,4-Dichlorophenol, respectively. Each sample was injected three times and its average was reported.

Hydrogen peroxide was quantified by the method titanium (IV) oxysulfate DIN 38402H15. Total iron was measured by the method 3500-Fe D (AWWA, APHA, and WEF, 2012). Each sample was injected three times and its average was reported.

2.3. Bacterial cell viability

1 mL of sample was centrifuged at 13,000 RPM and discarding 900 μL to obtain a final volume of 100 μL which was used to evaluate viability of *E. coli* K12 (ATCC 23716) and *K. pneumoniae* (ATCC BAA-1705) by using the method described by Gutierrez-Zapata et al., 2017b,c. The limit of detection was 30 Cell mL^{-1}. Each sample was measured by triplicate and its average was reported.
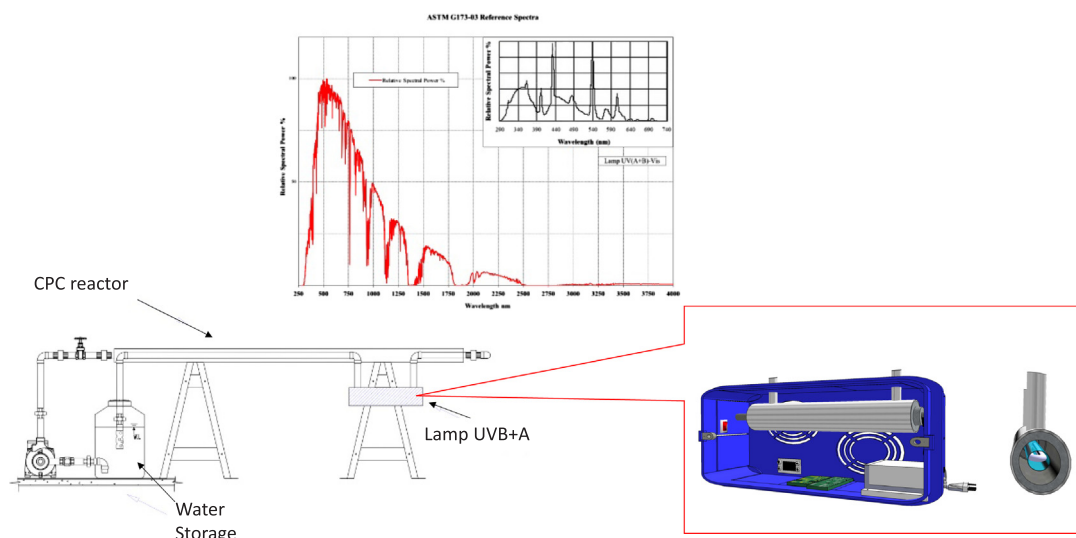
Fig. 1. Emission spectrum of UV-B + A + Visible light lamps, sunlight emission spectrum and CPC + UVB + A + Visible lamps reactor design.

2.4. Experiments with addition of 2,4-D and laboratory microbial strains on real groundwater

Experiments were performed using a compound parabolic collector-CPC solar reactor under natural sunlight irradiation. CPC reactor was made of Pyrex® glass tubes (32 mm o.d.) placed over the reflective surface of anodized aluminum (length 1.92 m, width 0.61 m; irradiated surface: 0.708 m^2) inclined to 3°, corresponding to the latitude of the location site (Cali, Colombia). The operating conditions were a flow rate of 18 L min^{-1}, a total volume of 18 L and an irradiated volume of 6.36 L. Assays were conducted in the Universidad del Valle (co-ordinates: 3.22′38.27″N, 76.31′56.97″W) in Cali Colombia.

CPC reactor was equipped with a 14 W UV-B + A + Visible lamp (ReptiGlo 10.0 PT2169 Exo-Terra Lighting Germany) (Fig. 1) with an emission spectrum between 280 nm and 700 nm, (emission maxima at 310, 365, 430 and 540 nm). UV-B and UV-A intensities were measured by using an UV photo-radiometer A Delta OHM using a UV-B (280–315) and UV-A (315–400 nm) probe at 0.6 cm of distance which corresponds to the reactor radius (Fig. 1). The UV-B and UV-A intensities were 0.74 and 6.47 W m^{-2} respectively. Furthermore, the illuminated volume by lamp was 1.36 L. The power source for the operation of the pump and lamp was a solar panel (AMPA ASM-250P24 solar pv module with dimensions of 1485 × 668 × 35 mm) with a maximum power of 250 W and maximum system open circuit voltage of 2000 V DC. An inverter INTI IIP 24,600 (maximum power 1200 W; input and output voltage 24 V-DC and 110-AC respectively) was also used.

The herbicide 2,4-D was added to the reservoir of CPC reactor to achieve a concentration of 70 µg L^{-1} and microorganisms *E. coli* and *K. pneumoniae* were added at initial concentrations of 10^6–10^7 cells mL^{-1}.

Afterward, hydrogen peroxide was added, and the collector was uncovered. Samples were taken every 25,000 J m^{-2}, by extracting water samples with sterile syringes. Total accumulated radiation was 150,000 J m^{-2} (UV-A spectral range (315–400 nm), with a UV-A t_{30W} of 30 min. This parameter (t_{30W}) was used to standardize the solar irradiation, assuming the average solar UV flux on a perfect sunny day to be around 30 W m^{-2} as it was described by Hincapié Pérez et al. (2006) and it was calculated following Eq. (6), where V_t is total volume, V_i is irradiated volume, UV_g is average solar UV irradiation and Δt_n is irradiated average time.

$$t_{30W,n} = t_{30W,n-1} + \Delta t_n \frac{UV_{G,n}}{3} \frac{Vi}{Vt} \qquad (6)$$

The three experiments evaluated were: (i) groundwater sample in the presence of 10 mg L^{-1} of H$_2$O$_2$/solar light, (ii) groundwater

containing 10 mg L^{-1} of H$_2$O$_2$/dark and (iii) groundwater irradiated with solar light in the absence of H$_2$O$_2$. Remaining H$_2$O$_2$ after experiments was neutralized with 0.1 mL of sodium thiosulfate solution (10% w/v), in 100 mL of samples.

Control parameters were temperature, pH, hydrogen peroxide concentration and instantaneous radiation. The radiant flux was monitored with a Photo-radiometer (HD 2102.2 – Delta Ohm) with a radiometric probe UV-A (315–400 nm).

The washing procedure of the CPC reactor was done by recirculating a detergent-aqueous solution. Then, water at pH 2.0 was recirculated for 2 h. Subsequently, the system was disinfected by passing through the CPC reactor a Ca(OCl)$_2$ solution (free chlorine concentration around 5 mg L^{-1}) at pH 5 during 2 h. Previously to the treatment, the system was purged with natural groundwater samples.

Experiments were not done by triplicate since physical-chemical features of groundwater samples and sunlight irradiation were not constants (see Table 1).

2.5. Experiments with wild microbial strains on real groundwater

Inactivation of wild bacterial strains present in groundwater samples was evaluated by culturability (using plate count) and viability (DVC-FISH). The treatment was performed by addition of hydrogen peroxide (10 mg L^{-1}), using a CPC reactor (18 L) under natural sunlight irradiation (Total UV-A 77,060 J m^{-2}) and coupled to UVA + B-Vis Lamp (14 W). Samples were taken at the beginning and end of treatment. Culturability was evaluated in the solid media EMB agar and Chromocult using concentrated sample 10^2 and dilutions between 10^0

Table 1

Physicochemical characteristics of groundwater sample in assays with added 2,4-D and *E.coli/K. pneumoniae* laboratory and wild strains.

Parameter	Value (Laboratory strains)	Value (wild strains)
pH	7.56	7.01
Total iron (mg L^{-1})	0.39	0.22
Bicarbonate (mg mL^{-1})	235	264
Phosphate (mg L^{-1})	0.24	0.17
Fluoride (mg mL^{-1})	1.23	1.0
Chloride (30 mg L^{-1})	25	30
Nitrate (mg L^{-1})	1.77	0.97
Conductivity (µS cm^{-1})	245	231
Dissolved organic carbon (DOC) (mg L^{-1})	1.13	4.54

and 10^{-6}. In addition, biochemical tests were performed to confirm the identification of isolated pathogens (Brenner, 2005). Each sample was injected three times. Reported values were the average. Viability was assessed for *E. coli* and *K. pneumoniae* DVC – FISH as it was described in Section 2.3.

2.6. Scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM-EDX)

The secondary electron micrographs of the samples were obtained by scanning electron microscopy (SEM) using Philips 505 equipment. The energy dispersive X-ray analysis (EDS) of the samples was performed using an EDAX 9100 analyzer at a working potential of 15 kV and graphite-supported samples metalized with gold.

3. Results and discussion

3.1. Experiments with addition of 2,4-D and laboratory microbial strains on real groundwater

Physical-chemical characterization of raw groundwater samples revealed the presence of chemical substances as dissolved organic matter (DOM), NO_3^-, fluoride, carbonates, phosphates, and iron among others (Table 1).

2,4-D underwent a partial degradation of 51 and 63,29% when well water was only irradiated with UV-B + A + visible lamp (UV) and sunlight (SL) respectively without addition of H_2O_2 after 30 min (t_{30w}). Fig. 2 shows that the same experiments reached 2 and 3 logs of viability reduction respectively after 30 min (t_{30w}).

Experiments using only CPC (SL) reactor and CPC reactor coupled with UV-B + A-Vis lamp (UV) by adding of 10 mg L^{-1} of H_2O_2 (SL + H_2O_2) and (SL + UV + H_2O_2) respectively (Fig. 2) exhibited a complete 2,4-D abatement contained in real groundwater samples after t_{30W} of 20 min (240 min of solar irradiation). However, the system

SL + UV + H_2O_2 showed a better performance, especially at the beginning of the experiment than SL + H_2O_2. Meanwhile, the using of UV-B + A + Visible lamps alone (without real sunlight irradiation, UV + H_2O_2) and the presence of 10 mg L^{-1} of H_2O_2, led to the degradation of 80% of 2,4-D after the same irradiation time (t_{30W} of 20 min). In all samples, the concentration of metabolite 2,4-dichlorophenol (2,4-DCP) was always below the detection limit (5 μg L^{-1}).

Regarding bacteria inactivation (Fig. 2), all treatments did not reach total inactivation of 10^{-6} cells mL^{-1}. However, for *E. coli* K12 strain, treatment SL + UV + H_2O_2 was the most efficient achieving the removal of 4.69 Logs of viable cells after 30 min (t30W) of irradiation, whereas SL + H_2O_2 and UV + H_2O_2 treatments reached the elimination of 4.06 Logs and 3.68 Logs respectively. Furthermore, *K. pneumoniae* inactivation assays showed that the treatment SL + UV + H_2O_2 reached a viability decreasing of 2.18 Logs, while SL + H_2O_2 and UV + H_2O_2 treatments exhibited a viability decreasing of 1.04 Logs and 1.40 logs respectively. These results described a different behavior than those reported previously to *E. coli* abatement, where it was reached its total inactivation (Gutierrez-Zapata et al., 2017b). The difference could be linked that in results reported herein, UV-A accumulated radiation from sunlight was lower than in experiments reported previously (In the current study Total UV-A was 150,000 J m^{-2} and the previous was 300,000 J m^{-2}).

However, *E. coli* concentration detected in monitored natural groundwater samples showed maximum values around 2.98 logs (in CFU mL^{-1}). In addition, in these samples the mean value of fecal coliforms was around of 3.93 logs. Thus, the treatment SL + UV + H_2O_2 (4.69 Log) may exceed the removal of *E. coli* required to achieve the total elimination of this strain in natural groundwater samples.

Regarding the inactivation of *K. pneumoniae,* the highest viability reduction was obtained after 30 min (t_{30W}) by using the system SL + UV + H_2O_2 (2.18 log). This result was higher than those obtained
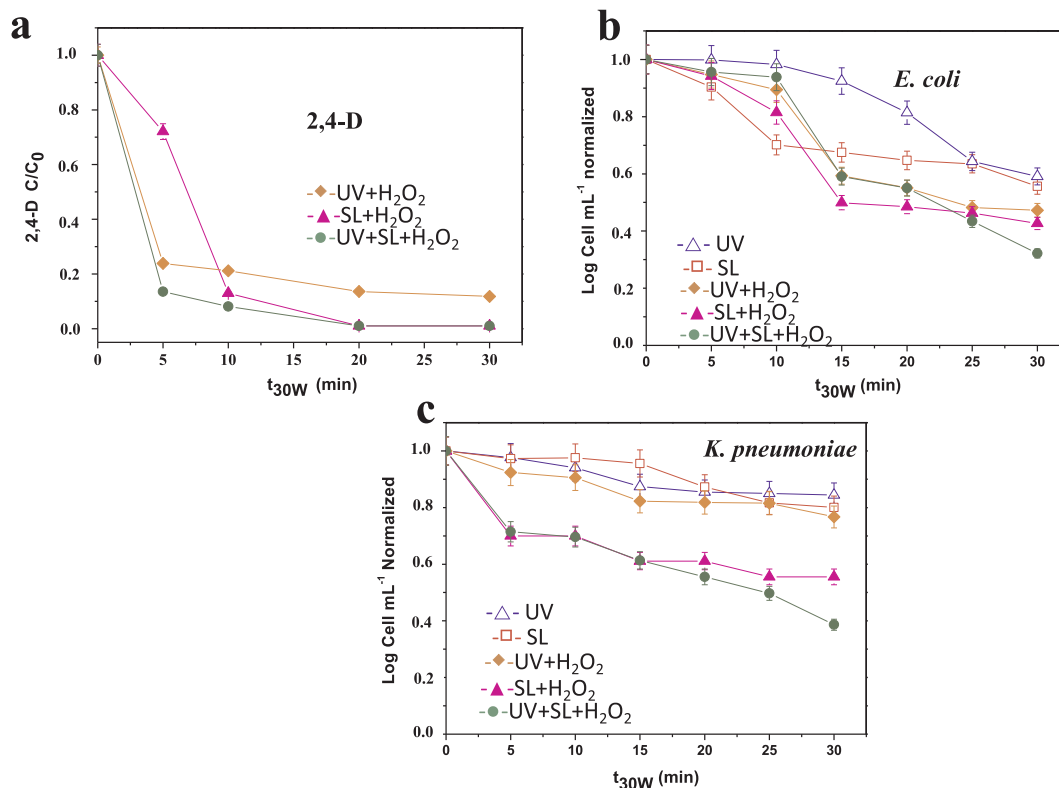

Fig. 2. Degradation of (a) 70 μg L^{-1} of 2,4-D and viability decreasing of laboratory strains of (b) *Escherichia coli* K-12 and (c) *Klebsiella pneumoniae* cells upon different experimental conditions. Cumulated irradiation of 150,000 J m^{-2}. The UV-B and UV-A intensities were 0.74 and 6.47 W m^{-2} respectively.
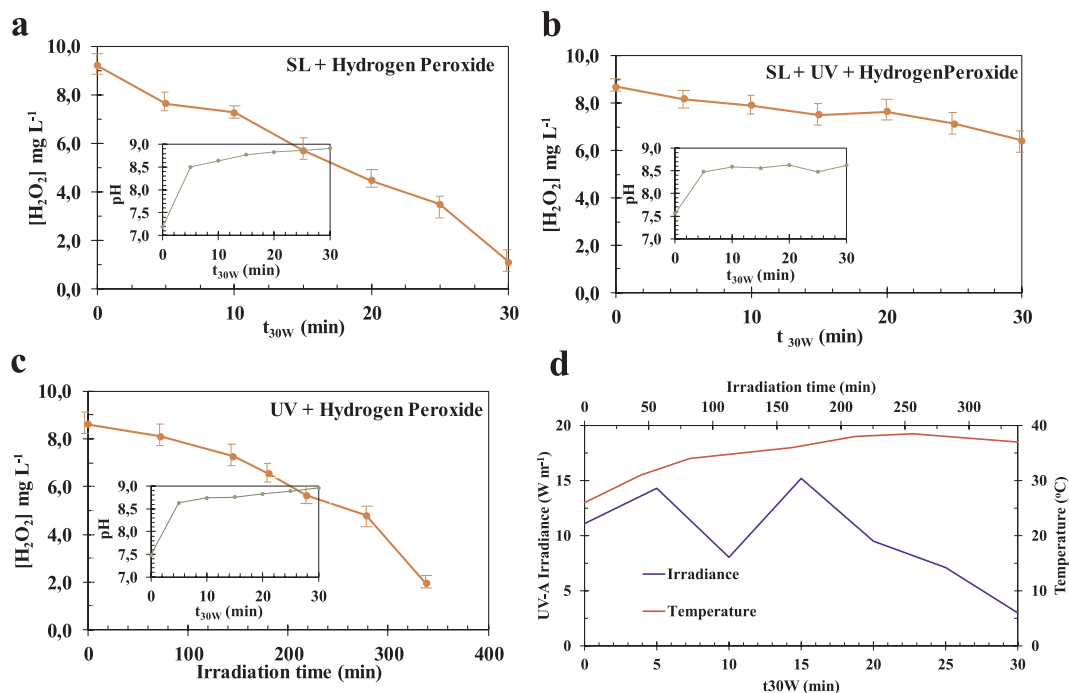
Fig. 3. Evolution of pH and H_2O_2 concentration (a) 10 mg L^{-1} of H_2O_2; (b) combined sunlight and UV-B + A light in presence of 10 mg L^{-1} of H_2O_2; (c) UV-B + A lamp with 10 mg L^{-1} of H_2O_2; and (d) evolution of UV irradiance and temperature during the solar experiments.

previously by using only sunlight irradiation in presence of H_2O_2 10 mg L^{-1} in simulated groundwater (1.71 logs) after 35 min (t_{30w}). Therefore, the coupling with UV-B + A + Visible lamps showed a remarkable improvement on the *K. pneumoniae* inactivation. Natural samples showed that concentration of *K. pneumoniae* ranged from 5 to 18 cells 100 mL^{-1}; hence, the treatment may allow the removal of this microorganism under the studied conditions.

On the other hand, the excessive resistance of *K. pneumoniae* observed should be linked to morphological and physiological reasons as it was already mentioned (Gutierrez-Zapata et al., 2017b). However, it is necessary to point out that *K. pneumoniae* seems to be a microorganism with a high antioxidant capacity (Hochman and Goldberg, 1991), inactivating H_2O_2, which could explain its resistance to the treatment and its high H_2O_2 consumption in experiments where its viability was not strongly reduced (SL + H_2O_2 and UV-H_2O_2) (Gutierrez-Zapata et al., 2017b).

Physicochemical parameters such pH, temperature and H_2O_2 concentration were monitored during the treatments (Fig. 3). Initial pH in all treatments ranged from 7.19 to 7.56, while final pH rose to 8.62–8.96, showing the same pH behavior as the previous studies (Gutierrez-Zapata et al., 2017b). The final concentration of hydrogen peroxide was 5.81 mg L^{-1} for SL + UV + H_2O_2, 1.12 mg L^{-1}, for SL + H_2O_2, and 1.92 mg L^{-1} for UV + H_2O_2. The maximum temperature reached during the experiment was 38.5 °C, which granted that the hydrogen peroxide did not undergo thermal decomposition and that bacteria inactivation was not achieved by temperature rising.

UV irradiance and temperature were also followed (Fig. 3d) showing that it was not constant, probably due to the presence of clouds. The average UV irradiance during the whole experiment was around 9.74 W m^{-2} with SL + H_2O_2 and 11,1 W m^{-2} with SL + UV + H_2O_2.

3.2. Experiments with wild microbial strains on real groundwater

Wild bacteria strains were followed by culturability (plate count) and viability (DVC-FISH) techniques. It was found by culturability initial concentrations of 1.9×10^1, 9×10^0, and 1.0×10° CFU mL^{-1} of

total coliforms, fecal coliforms and *E. coli* respectively. After 237 min of SL + UV + H_2O_2 treatment, culturability of all wild bacteria strain was completely reduced, (Table 1, water characterization).

Regarding viability followed by DVC-FISH method, the initial counts for *E. coli* and *K. pneumoniae* (1.5×10^2 and 1.6×10^3 cells mL^{-1} respectively) were higher than those obtained by culturability techniques. Previous studies reported that DVC-FISH method can evidence higher concentrations of bacteria than culturability techniques, such as most probable number (MPN), and plate count in natural surface waters (Servais et al., 2009).

However, after 30 min (t_{30W} or 237 min of sunlight irradiation) of SL + UV + H_2O_2 treatment, there was a strong viability decreasing of both bacteria strains (< 30 cells mL^{-1}). Residual H_2O_2 concentration at the end of the experiment was around 3.8 mg L^{-1} and the maximum temperature reached was 27 °C. pH value underwent the same behavior observed previously, since it increased from 7.01 to 8.67. The accumulated UV-A radiation was 77,060 J m^{-2} and the maximum instantaneous UV-A radiation was 5.5 W m^{-2} with a t_{30W} of 15.4 min.
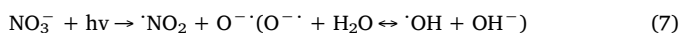
3.3. Photochemical induced mechanisms responsible of 2,4-D abatement and bacteria inactivation in real groundwater

Since natural sunlight irradiation can induce ˙OH production by natural photo-induced abiotic processes in natural waters (Rojas et al., 2011; Vione et al., 2014; Gligorovski et al., 2015), the coupling with UV-B + A + Visible lamps could strongly enhance these photochemical events on natural groundwater samples. Results showed that 2,4-D concentration and bacteria viability underwent a slight decreasing when experiments with natural well waters without H_2O_2 were performed.

Well water characterization revealed the presence of nitrates at concentrations ranging between 1.77 and 0.97 mg L^{-1} when experiments with laboratory and wild strains were performed respectively. It is well known that NO_3^- and NO_2^- participate in photochemical reactions by UV-B + A irradiation in natural waters leading to the formation of ˙OH radicals. Nitrates (NO_3^-) shows two absorption bands: one weak at 302 nm ($\varepsilon = 7.2$ M^{-1} cm^{-1}) due to n → π* transitions and

other strong at 200 nm (ε = 9900 M^{-1} cm^{-1}) due to $\pi \to \pi^*$ (Goldstein and Rabani, 2007; Herrmann, 2007; Vione et al., 2014; Gligorovski et al., 2015).

Thus, excitation with wavelengths beyond 280 nm (UV-B) produces $n \to \pi^*$ transitions yielding the $\cdot OH$ and NO_2 production through reactions (7) and (8).

$$NO_3^- + hv \to \cdot NO_2 + O^{-\cdot}(O^{-\cdot} + H_2O \leftrightarrow \cdot OH + OH^-) \qquad (7)$$

$$NO_3^- + hv \to NO_2 + O^\cdot \qquad (8)$$

On the other hand, nitrite ions (NO_2^-) and undissociated nitrous acid (HNO_2) can undergo in aqueous solutions photochemical reactions induced by UV-A light absorption (300–400 nm) leading to the production of $\cdot OH$ radicals at neutral pH (Fischer and Warneck, 1996; Vione et al., 2014; Gligorovski et al., 2015).

$$NO_2^- + hv \to \cdot NO + O^{-\cdot} \qquad (9)$$

$$O^{-\cdot} + H^+ \leftrightarrow \cdot OH \qquad (10)$$

Moreover, although hydrogen peroxide absorbs light mainly in the UV-C region (100–280 nm), this molecule shows an absorption tail in the UV-B and UV-A (Rojas et al., 2011); thus, it should be possible that hydrogen peroxide undergoes photolysis upon UV-A + B-Vis lamps irradiation, especially UV-B light, which may induce the production of $\cdot OH$ radicals (Rojas et al., 2011; De Laurentiis et al., 2014; Vione et al., 2014; Gligorovski et al., 2015).

$$H_2O_2 + hv \to 2\cdot OH \qquad (11)$$
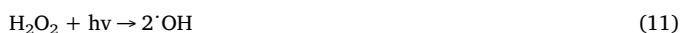
Besides, dissolved organic matter (DOM), which it could be present in groundwater samples (DOC: 1.13 and 4.54 mg L^{-1} in laboratory and wild strain experiments respectively), can participate in photo-induced processes in natural waters (Vione et al., 2014). Often these substances such humic and fulvic acids (HA and FA respectively), absorb light in the range of 300 and 500 nm (also emitted by UV-B + A + Visible lamp) (Canonica et al., 1996; Dong and Rosario-Ortiz, 2012; De Laurentiis et al., 2014; Vione et al., 2014; Gligorovski et al., 2015). This light absorption produces excited states which can be quenched through electronic or energy transfer reactions leading to the production of ROS such as hydroxyl radical and singlet oxygen ($^1O_2$).

$$DOM + hv \to DOM^* + O_2 \to 1O_2 + \cdot OH \qquad (12)$$
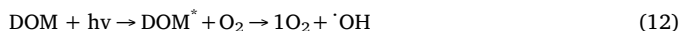
UV-B + A + Visible lamp could also enhance photocatalytic and photo-Fenton processes produced by colloidal and dissolved iron (Total iron concentration 0.39 mg L^{-1}) (Ruales Lonfat et al., 2015; Gutierrez-Zapata et al., 2017a). SEM micrographs of solids recovered by filtration from natural groundwater samples (Fig. 4) revealed the presence of particles with heterogeneous sizes and shapes. Their composition was evaluated by energy dispersive X-ray spectroscopy (EDS) and it was found signals linked to Ca ($K\alpha1$ line 3.690 KeV), Ti ($K\alpha1$ line 4.508 KeV), Mn ($K\alpha1$ 5.894 KeV) and Fe ($K\alpha1$ 6.398 KeV).

Taking into account these results, it seems possible to suggest the presence of manganese, iron, Ti and calcium oxides. Thus, the participation of iron (Hydr)oxides in photo-induced processes leading to the generation of $\cdot OH$ radicals in groundwater upon UV-A irradiation should be also possible. It is well known that iron (hydr)oxides such as goethite and lepidocrocite could be present in natural groundwater (Appelo and Postma, 2005). On the other hand, since Ti is present in solution, it might regard that TiO_2, which is a very active photocatalyst, could also participate in bacteria inactivation and 2,4-D oxidation (Augugliaro et al., 2012; Rengifo-Herrera et al., 2013) while manganese oxides could lead to the H_2O_2 decomposition producing as well $\cdot OH$ radicals (Watts et al., 2005; Bokare and Choi, 2014).

Dissolved iron can be also present in natural surface and well waters as ferric-humic acid complexes (Georgi et al., 2007). It is well known that these complexes absorb UV-A light producing LMCT transitions responsible of $\cdot OH$ radical formation (see Eqs. (1)–(5)).

The coupling of UV-B + A + Visible light lamp could enhance these natural abiotic photochemical processes mentioned above since these light sources emit at wavelengths where nitrites, nitrates, hydrogen peroxide, metal oxides (iron (hydr)oxides and TiO_2) and DOM absorb light. Photo-induced $\cdot OH$ radicals production could be enhanced increasing the oxidant capacity and being able to degrade efficiently 2,4-D herbicide and inactivate bacteria (even those highly resistant as *K. pneumoniae*) in groundwater samples.

On the other hand, physical-chemical features of groundwater samples could enhance photocatalytic and photochemical processes, as it was already reported. Gutierrez-Zapata et al. (2017a,b) claimed that presence of certain ions could exert a key role either removing 2,4-D or inactivating bacteria. Fluoride (1.23 mg L^{-1}) which is present on natural groundwater samples could induce the replacement of surface hydroxyl groups in colloidal iron oxides or oxyhydroxides and TiO_2 inducing $\cdot OH$ production as result of an accelerated water oxidation by photo-induced valence band holes (Du et al., 2008).

Observed pH rising could be due to the fact that carbonates can scavenge $\cdot OH$ radicals leading to the carbonate radical ($CO_3^{-\cdot}$) production, which shows an important oxidative potential (E° ($CO_3^{-\cdot}$/$CO_3^=$) = 1.78 V vs NHE) being able to degrade organics (Dell'Arciprete et al., 2013; De Laurentiis et al., 2014) and to inactivate bacteria as well (Wolcott et al., 1994). Reactions (13) and (14) reveal that during this reaction, the pH must increase as it was confirmed during the experiments.

$$CO_3^{2-} + \cdot OH \to OH^- CO_3^{-\cdot} \qquad (13)$$

$$2CO_3^{2-} + 2H^+ \to 2CO_2 + H_2O_2 \qquad (14)$$

$$CO_3^\cdot + OM \to OM_{ox} + CO_3^{2-} \qquad (15)$$

Bacteria are sensitive to UV-B + A light irradiation. These wavelengths can produce damages in DNA and some enzymes and proteins weakening cells. However, specifically UV-B light is more efficient inactivating bacteria cells than UV-A. Furthermore, H_2O_2 can also help to inactivate bacteria cells since it may produce detrimental effects on the cell membrane by increasing its permeability (Santos et al., 2013; Giannakis et al., 2016a,b).

The coupling of the UV-B + A + Vis lamp and the CPC reactor could be a promising alternative to pesticides and bacteria (including bacteria strains highly resistant toward AOPs such as *K. pneumoniae*) removal from real natural groundwater. Furthermore, the solar panel increases the sustainability of treatment. However, the performance of this technology must be studied evaluating the inactivating effect on different microorganisms since their response to the photo-treatment could be different depending of strains, species and cell states (Santos et al., 2013).

4. Conclusions

Herein it was shown that coupling of UV-B + A + visible lamp electrically powered by solar photovoltaic panel to a CPC reactor enhanced both 2,4-D removal and decreasing of *E. coli* and *K. pneumoniae* viability improving natural abiotic photochemical events in well waters by addition of H_2O_2.

Despite solar CPC reactor was equipped with a UV-B + A + Visible lamp, combined treatment showed low reduction of bacteria viability when laboratory strains of *E. coli* and *K. pneumoniae* at high initial concentrations ranging 10^6–10^7 cells mL^{-1} were used. However, culturability (initial concentrations around 10^1 CFU mL^{-1}) and viability (initial concentration ranging from 10^2 to 10^3 cells mL^{-1}) of wild bacteria strains naturally present in well waters were completely reduced during the combined treatment demonstrating that this process should be a promising alternative to be applied as drinking water production system.
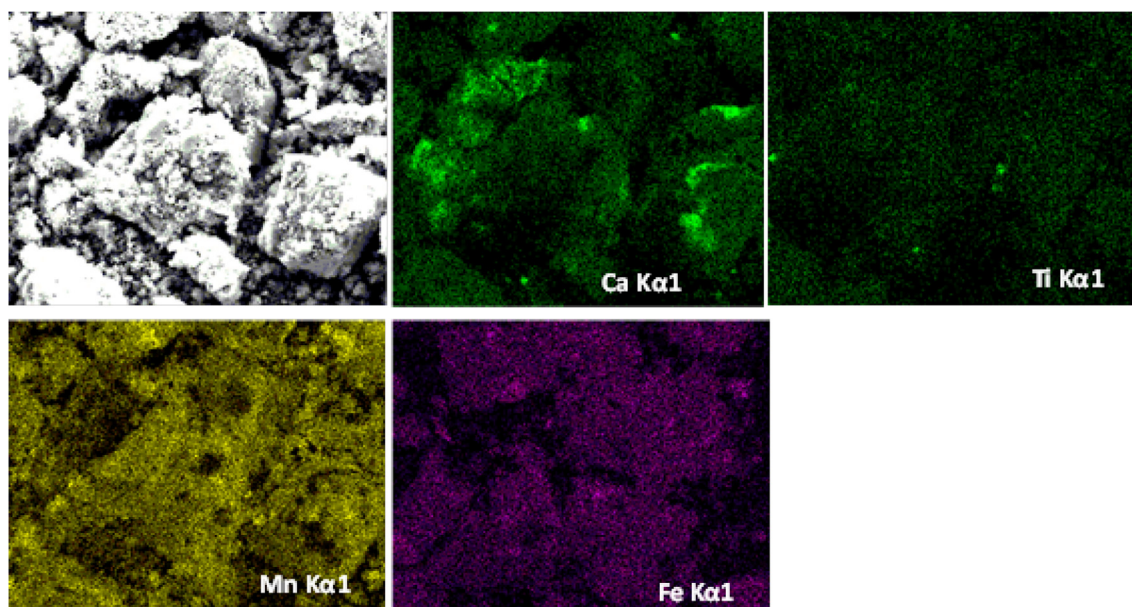
Fig. 4. SEM micrographs and EDX measurements of solids recovered by filtration from natural groundwater.

Acknowledgements

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