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Addition of hydrogen peroxide enhances abiotic sunlight-induced processes to simultaneous emerging pollutants and bacteria abatement in simulated groundwater using CPC solar reactors

Hector Mario Gutiérrez-Zapata [a], Janeth Sanabria [a,*], Julián Andrés Rengifo-Herrera [b,*]

[a] Environmental Microbiology and Biotechnology Laboratory, Engineering School of Environmental & Natural Resources, Engineering Faculty, Universidad del Valle - Sede Meléndez, A.A. 25360 Santiago de Cali, Colombia

[b] Centro de Investigación y Desarrollo en Ciencias Aplicadas "Dr. J.J. Ronco" (CINDECA), Departamento de Química, Facultad de Ciencias Exactas, UNLP-CCT La Plata, CONICET, 47 No. 257, 1900 La Plata, Buenos Aires, Argentina

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ABSTRACT

Results revealed that almost 90% of 2,4-D (70 μg L^{-1}), present in simulated groundwater containing 0.3 mg L^{-1} of iron at pH 7.0, was degraded after 320 min (60 min t_{30w}) of natural sunlight irradiation while the viability of *Escherichia coli* cells (followed by DVC-FISH) was completely reduced after 220 min (40 min t_{30w}) by simple addition of 10 mg L^{-1} of hydrogen peroxide. *Klebsiella pneumoniae* exhibited an especial behavior since its viability was only reduced in 3.5 logs after 320 min of sunlight irradiation (60 min t_{30w}). Dark experiment (in presence 10 mg L^{-1} of H_2O_2) showed that Fenton processes may also play an important role reducing the 40% of 2,4-D after 320 min (60 min t_{30w}) while viability of *E. coli* and *K. pneumoniae* underwent a reduction of 2.5 and 2 logs respectively. Photolysis experiments were not able to degrade 2,4-D and *E. coli* and *K. pneumoniae* viability was partially reduced (2 logs).

Results showed that high 2,4-D abatement could be due to photo-induced and/or dark processes such as photo-Fenton and Fenton (dissolved and colloidal iron), photocatalysis (colloidal iron) and UV-B photolysis of H_2O_2. Viability reduction of microorganisms should be related to combined effects of UV-A + B irradiation, rising of temperature (44 °C), photo-Fenton, Fenton and photocatalytic processes.

1. Introduction

In Latin America and especially in rural communities, the 31% of people uses groundwater as the main source of drinking water (UNEP-GEO, 2010), however, the lack of suitable sanitation and the economic activities (agriculture and the uncontrolled use of agrochemicals) in most of these communities makes possible for these water sources to be susceptible to microbiological and chemical pollution. Thus, finding new technologies to the abatement of microbiological and chemical pollution in groundwater has risen as an important issue.

Natural waters have the ability to degrade itself small quantities of organic pollutants via biotic (*e.g.* microorganisms) and abiotic processes (*e.g.* hydrolysis and photochemical events) (De Laurentiis et al., 2014; Gligorovski et al., 2015). It is well known that the presence of nitrites/nitrates and dissolved organic matter in natural waters can stimulate photochemical abiotic processes induced by sunlight which can lead to the production of reactive oxygen species (ROS) such as hydroxyl radical (·OH), anion superoxide radical ($\cdot O_2^-$), hydrogen peroxide (H_2O_2), anion carbonate radical ($CO_3^{-\cdot}$), and singlet oxygen ($^1O_2$) able to degrade several organic pollutants and inactivate microorganisms (Canonica, 2007; Dell'Arciprete et al., 2012; De Laurentiis et al., 2014). A specific especial case, is the presence of natural iron species (dissolved or colloidal) which in the presence of natural amounts of H_2O_2 (10^{-7}–10^{-11} M (Burns et al., 2012) could induce Fenton, photo-Fenton or photocatalytic reactions also responsible of the organic pollutants abatement (Nakatani et al., 2007). However, these natural photochemical-induced abiotic processes do not produce high amounts of ROS in natural waters (Burns et al., 2012) (in general, they ranged from 10^{-10} to 10^{-12} M) and this fact may reduce its ability to remove microbiological and chemical pollution.

Recently, Gutiérrez-Zapata et al. (2016) found that the addition of 10 mg L^{-1} of hydrogen peroxide on natural groundwater containing already small amounts of iron (0.3 mg L^{-1}) was enough to remove the most part of the herbicide 2,4.dichlorophenoxyacetic

* Corresponding authors.
E-mail addresses: janeth.sanabria@correounivalle.edu.co (J. Sanabria), julianregifo@quimica.unlp.edu.ar (J.A. Rengifo-Herrera).

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acid (2,4-D). On the other hand, several studies have already reported that the addition of hydrogen peroxide to natural surface waters or groundwater containing natural iron concentrations (0.1–0.3 mg L^{-1}) leads to the complete inactivation of several bacteria (Sciacca et al., 2010, 2011; Ndounla et al., 2013; Ndounla and Pulgarin, 2014, 2015). All these findings allow suggesting that it should be possible to enhance the natural abiotic photo-induced processes responsible of the formation of ROS by the simple addition of hydrogen peroxide, increasing the ability of natural waters to remove either chemical or microbiological pollution.

Bacterial inactivation by photo-Fenton processes in surface waters, groundwater, wastewaters and Milli-Q water has often been studied by culturability techniques, mainly by plate count (PC) (Sciacca et al., 2010; Spuhler et al., 2010; Sciacca et al., 2011; García-Fernández et al., 2012; Moncayo-Lasso et al., 2012; Rodriguez-Chueca et al., 2012; Ndounla et al., 2013; Ndounla and Pulgarin, 2014; Ruales-Lonfat et al., 2014; Ndounla and Pulgarin, 2015; Ruales-Lonfat et al., 2016; Ortega-Gómez et al., 2016). However, one of the main drawbacks of culturability techniques is their inability to detect viable but nonculturable cells (VBNC) which could maintain their virulence (Li et al., 2014; Ramamurthy et al., 2014; Kong et al., 2015). In 2004, Garcia-Armisen and Servais (2004) used a methodology based in Direct Viable Count (DVC) coupled with fluorescence in situ hybridization (FISH) to enumerate viable *E. coli* cells in freshwaters. Further, in 2009, Servais et al. (2009) compared a culturability method (most probable number-MPN) with DVC-FISH in freshwaters, finding a high number of *E. coli* cells in VBNC state which was not detected by MPN technique. Moreover, in photocatalytic reactions with TiO_2 nanoparticles, there were differences between viability and plate count method (PC) results due to photocatalytic processes may induce to a part of the bacteria population to enter into a viable but non-culturable state (VBNC). Therefore, it has been established that PC method alone cannot be used as a tool to measure the disinfection in such studies (Swetha et al., 2012).

On the other hand, bacteria inactivation by photo-Fenton reaction in natural waters disinfection have only been focused in two microorganisms: *E. coli* (Sciacca et al., 2010, 2011; Ndounla et al., 2013; Ndounla and Pulgarin, 2014; Ndounla and Pulgarin, 2015; and *Salmonella* (Sciacca et al., 2010, 2011; Ndounla et al., 2013; Ndounla and Pulgarin, 2015). *Klebsiella pneumoniae* is a vegetative bacterium which has shown resistance to antibiotics, H_2O_2 and water disinfection procedures such as the chlorination and it is considered as highly virulent and pathogenic microorganism (McKeon et al., 1995; Podschun et al., 2001; Szabo and Minamyer, 2014). A prominent polysaccharide capsule could be responsible of its high virulence and resistance (Podschun and Ullmann, 1998). Culturability loss of *K. pneumoniae* cells produced by photocatalytic reactions over TiO_2 have been reported on the literature (Venieri et al., 2014; Korösi et al., 2016). *K. pneumoniae* was resistant to photocatalytic reactions using TiO_2 (even using Degussa P-25) (Venieri et al., 2014), however culturability was strongly decreased when irradiated TiO_2 nanoparticles and H_2O_2 were simultaneously present. On the other hand, irradiated metal doped TiO_2 (Co and Mn) led an important culturability reduction. To our knowledge, there are not studies about the culturability or viability reduction of waterborne *Klebsiella pneumoniae* by photo-Fenton.

This study shows for the first time, the simultaneous removal of a chemical pollutant at very low concentrations (70 μg L^{-1}), the 2,4-dichlorophenoxyacetic acid (2,4-D), and the viability followed by DVC-FISH of two microorganisms: *Escherichia coli* and *Klebsiella pneumoniae* (at initial concentrations of 10^6 cells mL^{-1}) by the addition of hydrogen peroxide (10 mg L^{-1}) to simulated groundwater containing natural iron concentrations at neutral pH in CPC reactors under natural sunlight irradiation.

2. Experimental methods

2.1. Reagents

Ferric sulfate ($Fe_2(SO_4)_3{\cdot}nH_2O$) (Merck), 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), humic acid (Alpha Aesar), Potassium Nitrate (Merck), Sodium phosphate (Sharlau), Sodium bicarbonate (Merck), Sodium fluoride (Merck), Sodium chloride (sigma), Sodium sulfate (Merck), Chloride of manganese tetrahydrated (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. Analysis of 2,4-dichlorophenoxyacetic acid (2,4-D) and hydrogen peroxide

Concentrations of 2,4-D and 2,4-Dichlorophenol (2,4-DCP) were followed by HPLC (LC20AT- Shimadzu) using as mobile phase acetonitrile (55%), aqueous solution of acetic acid at pH 3.0 (30%) and Milli-Q water (15%) and a HPLC column C-18 Nucleosil 100-5. An isocratic flux of 0.8 mL min^{-1} and a UV detector at 280 nm were used. 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 μg L^{-1} and 5 μg L^{-1} for 2,4-D and 2,4-DCP, correspondingly.

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). Dissolved oxygen was measured using an oximeter (YSI 550a). UV 254 was measured by method 5910B (AWWA, APHA and WEF, 2012).

2.3. Direct viable count by fluorescence in situ hybridization (DVC-FISH) viability

Viability of *E. coli* K12 (ATCC 23716) and *K. pneumoniae* (ATCC BAA-1705) were evaluated using direct viable count by fluorescent in situ hybridization (DVC-FISH). Samples were incubated for 18 h at 35 °C ±2 °C in a solution of nutrient broth with yeast extract and 40 μg mL^{-1} of nalidixic acid. After incubation, 1 mL of sample was centrifuged at 13000 RPM. Then, samples were re-suspended in 1 mL of 1 × phosphate buffered saline (PBS), this procedure was repeated two times and fixated with 4% (v/v) *para*-formaldehyde for 2 h at 4 °C. Samples were concentrated by centrifugation and the pellets were washed with 1 mL of PBS. Procedure was repeated twice to ensure *para*-formaldehyde removal; the final pellet was re-suspended in 500 μL of 1 × PBS and 500 μL of ethanol. Then, 10 μL of cell suspension were transferred to each well of a 8-well microscope slide. The loaded wells with sample were dried and subsequently dehydrated with ethanol. Subsequently, they were incubated with the hybridization solution. In the case of hybridization for *E. coli* and *K. pneumoniae* the probe ES-445 (Kenzaka et al., 2001) and KPN (Kempf et al., 2000) was used respectively. For *E. coli* hybridization, the samples were incubated with the hybridization solution containing 25 ng μL^{-1} of the probe at 48 °C for 2 h in the dark. The *E. coli* post-hybridization washing was performed at 45 °C for 20 min using 30% formamide washing solution. In the case of *K. pneumoniae*, the samples were incubated in the hybridization solution at 55 °C for 90 min in the dark and post-hybridization washing was done at 40 °C for 5 min using

the washing solution containing 30% formamide. After hybridization, the microscope slides were washed with sterile distilled water, air dried and measured with epifluorescence microscopy. Detection limits were calculated based on the volume of the sample analysed, the replicates performed and the effective field of view of the microscope examined. In samples with viable count cell less than 3 logs were added 40 μL of fixed sample per well. It was also counted 1254 visual fields contained in an area of 0.04 mm^2. The DVC-FISH detection limit was 250 *E. coli* or *K. pneumoniae* cells mL^{-1}. Quantification of cells was performed with the epifluorescence microscope Nikon-90i (Cy3 filter), using the software NIS element AR.

2.4. Microbial disinfection and 2,4-D degradation experiments under sunlight irradiation and peroxide at pilot scale using a CPC reactor

The experiments were conducted using a compound parabolic collector (CPC) under sunlight irradiation. The CPC was made of Pyrex® glass tubes (32 mm o.d.) placed over reflective surface of anodized aluminum inclined to 3°, corresponding to the latitude of the location site (Cali, Colombia), with a volume irradiated of 10.77 L. The operating conditions were: flow rate of 30 L min^{-1} and total volume of 30 L. Experiments were conducted on a platform situated at Universidad del Valle (coordinates: 3.22′38.27″N, 76.31′56.97″W).

The composition of the test water was pH 7.0, Fe^{+3} (0.3 mg/L), Mn^{+2} (0.27 mg/L), bicarbonate (353 mg/L), phosphate (0.48 mg/L), fluoride (1.0 mg/L), sulfate (58.7 mg/L), chloride (20.0 mg/L), nitrate (4.7 mg /L) and humic acid (3.2 mg/L).

The herbicide 2,4-D was added to reservoir of CPC reactor to achieve a concentration of 70 μg L^{-1} and microorganisms *E. coli* and *K. pneumoniae* were added at an initial concentration of 10^6–10^7 cells mL^{-1}. Afterwards, 10 mg L^{-1} of hydrogen peroxide was added and then the collector was uncovered and experiment began under solar light irradiation (H_2O_2/SL). Samples were taken every 25,000 J m^{-2}, by extracting water samples with sterile syringes. The accumulated radiation was 300,000 J m^{-2} with the UV-A spectral range (315–400 nm), with a UVA t_{30W} of 60 min. This parameter (t_{30W}) was defined to standardize the solar irradiation (Hincapié-Pérez et al., 2006). Exceeding H_2O_2 was neutralized with 0.1 mL of sodium thiosulfate solution (10% W/V), in 100 mL of samples before the DVC-FISH and HPLC analyzes. The control experiments were: dark/H_2O_2 (Dark/H_2O_2), and solar light in the absence of H_2O_2 (photolysis).

It was evaluated as control parameters temperature, pH, 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).

3. Results and discussion

3.1. Simultaneous removal of 2,4-D and viability decreasing of bacteria cells at simulated groundwater in a CPC reactor under natural sunlight irradiation

Fig. 1 exhibits the degradation of 70 μg L^{-1} of 2,4-D and the viability reduction of *E. coli* and *K. pneumoniae* cells (10^6 cells mL^{-1}) present simultaneously in simulated groundwater under different experimental conditions in a CPC solar reactor. Furthermore, 2,4-DCP was not detected because its concentration was always under the LOQ (5 μg L^{-1}) of the HPLC technique; this fact is important since for USEPA, the limit suggested of 2,4-DCP in water is 10 μg L^{-1} (USEPA, 2015) and experimental results showed that always the 2,4-DCP generated during the process was below of this limit. Simulated groundwater upon natural sunlight (photolysis) in absence of hydrogen peroxide did not show 2.4-D degradation (Fig. 1a). Bacteria viability for both strains revealed a reduction of 2 logs after 320 min (60 min t_{30w}) (Fig. 1b and c). In contrast, dark experiment containing 10 mg L^{-1} of hydrogen peroxide revealed a partial 2,4-D degradation of 40% and a slight decreasing of cell viability to both strains (2–2.5 logs). However, the simultaneous presence of the same H_2O_2 concentration and natural sunlight led more than the 90% of 2,4-D removal after 320 min of sunlight irradiation (60 min t_{30w}) (The final concentration of 2,4-D was under the limits suggested by the US-EPA of 70 μg L^{-1} (USEPA, 2007)) while *E. coli* viability was completely reduced after 220 min of irradiation (45 min t_{30w}). Viability of *K. pneumoniae* was only reduced in 3.5 logs after 320 min (60 min t_{30w}) of sunlight irradiation exhibiting an important resistance.

Fig. 2 shows the H_2O_2 consumption during the dark and irradiated experiments. Irradiated experiment exhibited a H_2O_2 consumption of 5 mg L^{-1} after 320 min (60 min t_{30w}) of sunlight irradiation while under dark conditions, the H_2O_2 consumption was 6 mg L^{-1} after the same time.

Insert in Fig. 2 shows also the pH changes during the experiments. In both cases, irradiated or dark experiments, pH increased from 7.0 to almost 9.0 after 320 min (60 min t_{30w}).

Since temperature and UV-A irradiance can exert an important role in waterborne bacteria abatement (Wegelin et al., 1994; Sommer et al., 1996; Keogh et al., 2015), both parameters were also followed during the solar experiments (Fig. 3). Experiments upon the simultaneous presence of hydrogen peroxide and sunlight revealed that the temperature reached almost 44 °C after 320 min while the UV-A irradiance was more or less constant during the first 260 min and then it underwent a strong dropping caused by the presence of clouds. Dark experiments revealed that the temperature reached the same value (data not shown).

3.2. Suggested mechanisms of 2,4-D and bacteria cells abatement achieved in groundwater in CPC reactor under natural sunlight irradiation

Dark and photo-induced processes should be responsible of bacteria and 2,4-D abatement observed.

Dark experiments in presence of H_2O_2 (10 mg L^{-1}) exhibited a partial 2,4-D degradation and cell viability reduction caused probably by homogeneous or heterogeneous Fenton reactions (presence of 0.3 mg L^{-1} of iron) which also lead to the formation of hydroxyl radicals (Pignatello et al., 2006). Moreover, Uhl et al. (2015) have suggested that the simple presence of H_2O_2 at low concentrations (near to 1 mM) could be toxic for *E. coli* by inducing intracellular Fenton reactions.

At pH values of 7.0, soluble complexed iron species (especially with humic acids) together with some iron (hydr)oxides could lead like-Fenton reactions yielding ·OH radicals (Ruales-Lonfat et al., 2014; Jack et al., 2015; Giannakis et al., 2016). Humic acids (HA) can form soluble complexes at neutral pH with ferric ions which are very active in Fenton (Lipczynska-Kochany and Kochany, 2008) (Fig. 4).

Photochemical events should be also involved in the 2,4-D and bacteria removal in simulated groundwater samples (Fig. 4). These samples contain inorganic anions such as nitrates, phosphates, fluoride and carbonates and dissolved organic matter in form of humic acids, which could induce some photochemical events by sunlight irradiation leading to the production of reactive oxygen species (ROS) such as hydroxyl radical (·OH) and singlet oxygen ($^1O_2$) among others (De Laurentiis et al., 2014; Gligorovski et al., 2015).

Humic acids make part in natural waters of the dissolved organic matter (DOM) and it is well known that DOM can absorb sunlight yielding singlet excited states which are transformed by

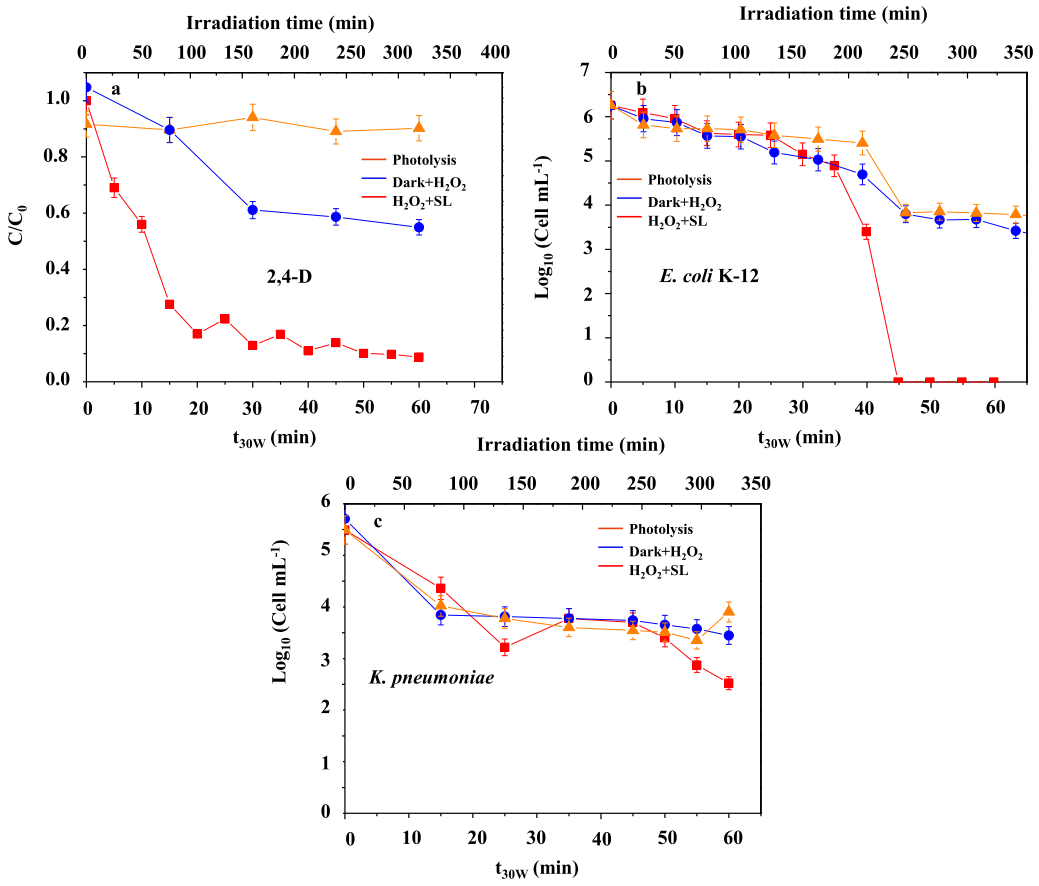

Fig. 1. Experiments photolysis, dark (Dark + H_2O_2) and irradiated (H_2O_2 + SL). (a) Degradation of 2,4-D (70 μg L^{-1}), (b) viability *E. coli* cell (10^6 cells mL^{-1}) and (c) viability *K. pneumoniae* cell (10^6 cells mL^{-1}).

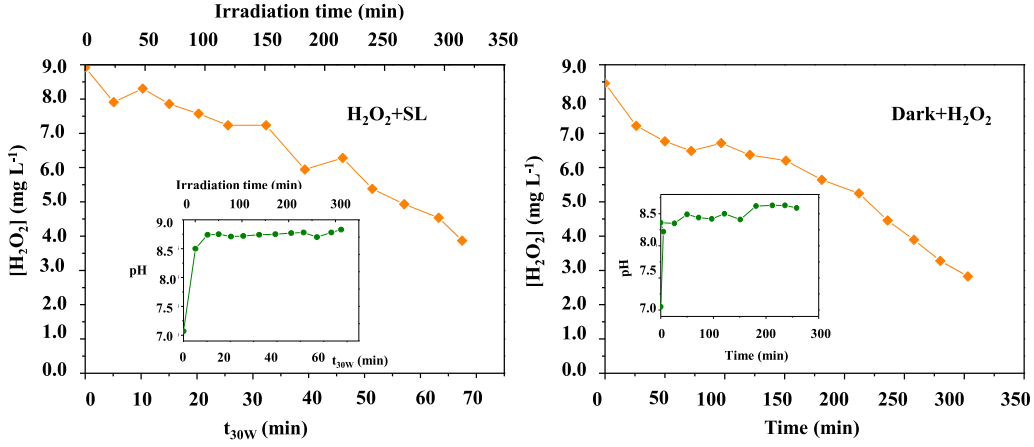

Fig. 2. H_2O_2 consumption and pH changes. Irradiated experiment (H_2O_2 + SL) and dark experiment (Dark + H_2O_2).

inter-system crossing (ICS) into excited triple states. These excited states can be quenched through energy transfer mechanisms by molecular oxygen leading to the generation of singlet oxygen ($^1O_2$) (Eq. (1)). Moreover, triplet excited DOM can also react with carbonates producing $CO_3^{-\cdot}$ radicals (Eq. (2)).

$$DOM + h\nu \rightarrow {}^1DOM^* \rightarrow {}^3DOM + O_2 \rightarrow {}^1O_2 \quad (1)$$

$${}^3DOM + CO_3^{=} \rightarrow DOM^{-\cdot} + CO_3^{-\cdot} \quad (2)$$

Nitrates can absorb UV light at acid and neutral pH producing ·OH radicals (Eq. (3)).

$$NO_3^- + h\nu + H^+ \rightarrow {}^{\cdot}OH + {}^{\cdot}NO_2 \quad (3)$$

This photochemical events could show a minor role in the degradation of 2,4-D and bacteria abatement since it is well known that in natural waters, the amounts of photo-induced ROS by solar light are very low (in general, they range from 10^{-10} to 10^{-12} M (Burns et al., 2012)).

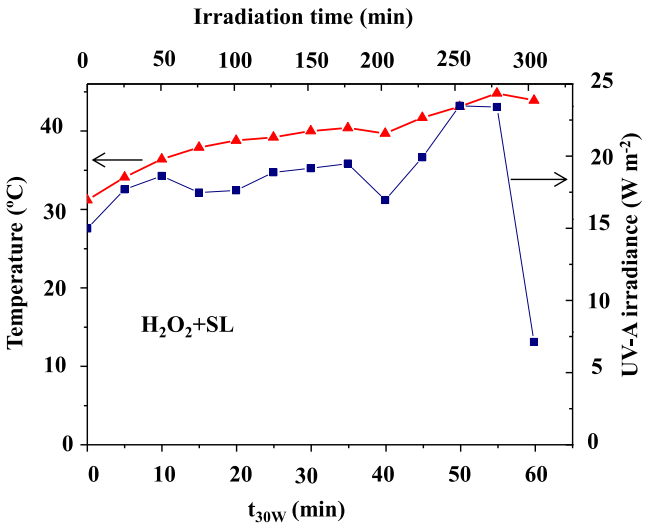

Fig. 3. Temperature (°C) and UV-A irradiance (W m^{-2}) of experiment irradiated (H_2O_2 + SL).

On the other hand, UV-A+B irradiation and temperature near to 50 °C can cause detrimental effects on the microorganisms. It is well known that combined effect of UV light and temperature (50 °C) is efficient to inactivate waterborne pathogen (Wegelin et al., 1994; Sommer et al., 1996; Keogh et al., 2015). UV-A+B light can affect negatively the activity of antioxidant enzymes present on bacteria such as superoxide dismutase (SOD), catalase (CAT), and could also produce partial degradation of intracellular iron-containing proteins, releasing Fe^{2+} ions into the cell and allowing the presence of intracellular Fenton reactions (Kapuscinski and Mitchell, 1981; Imlay, 2008; Giannakis et al., 2016). Thus, the only presence of UV-A+B light in combination with high temperatures could partially affect the viability of the microorganisms being responsible of the low viability reduction observed during the photolysis experiments.

When natural sunlight and 10 mg L^{-1} of hydrogen peroxide were simultaneously present in simulated groundwater effluents, 2,4-D was almost completely removed and *E. coli* viability was totally reduced. The presence of hydrogen peroxide and sunlight can induces photo-Fenton (dissolved and colloidal iron in form of iron hydr(oxides)) and photocatalytic processes (colloidal iron) yielding ·OH radicals (Bernabeu et al., 2012; Klamerth et al., 2013; Ruales-Lonfat et al., 2014; Giannakis et al., 2016).

Iron oxides can participate in photocatalytic processes by sunlight irradiation where electron-hole separation is achieved. These charge carriers can participate in photo-oxidative and photo-reductive reactions leading to the production of ·OH radicals among others reactive oxygen species (Eqs. (4)(8)) (Ruales-Lonfat et al., 2014; Giannakis et al., 2016).

$$Fe_2O_3 + h\nu \rightarrow Fe_2O_3(e^- + h^+) \quad (4)$$

$$e^-_{(cb)} + O_2 \rightarrow O_2^{-\cdot} \quad (5)$$

$$h^+_{(vb)} + O_2^{-\cdot} \rightarrow {}^1O_2 \quad (6)$$

$$e^-_{(cb)} + > Fe^{3+} \rightarrow > Fe^{2+} \quad (7)$$

$$h^+_{(vb)} + P_{(ads)} \rightarrow P^{\cdot+}_{(ads)} \quad (8)$$

Furthermore, irradiated iron hydr(oxides) can photo-promote iron reduction of $>Fe^{3+}$-OH to $>Fe^{2+}$; this latter can subsequently react with H_2O_2 leading to the production of ·OH radicals (Eqs. (9) and (10)) (Ruales-Lonfat et al., 2015).

$$> Fe^{3+} - OH + h\nu \rightarrow > Fe^{2+} + {\cdot}OH \quad (9)$$

$$> Fe^{2+} + H_2O_2 \rightarrow > Fe^{3+} + OH^- + {\cdot}OH \quad (10)$$

Photolysis of H_2O_2 caused by UV-B light (which is partially filtered by the Pyrex glass of CPC reactors) should also participate in the production of ·OH radicals (Gligorovski et al., 2015).

$$H_2O_2 + h\nu(UV - A + B) \rightarrow 2{\cdot}OH \quad (11)$$

It is well known that phosphate and carbonates, both present in simulated groundwater samples, exhibit a detrimental effect on photo-Fenton reactions (Lipczynska-Kochany et al., 1995; Pignatello et al., 2006; Soler et al., 2009), however, Gutiérrez-Zapata et al. (2016) found that carbonate could have an ambivalent behavior. Carbonates can scavenge hydroxyl radicals decreasing the degradation of organics by photo-Fenton reactions (Reaction (2)), but however, this effect should depend of its concentration. At very high concentrations, such as those reported herein, the effect may be slightly positive. This effect should take place since carbonate anions can scavenge ·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) able to degrade organics (Dell'Arciprete et al., 2012; De Laurentiis et al., 2014) and also to inactivate bacteria (Wolcott et al., 1994).

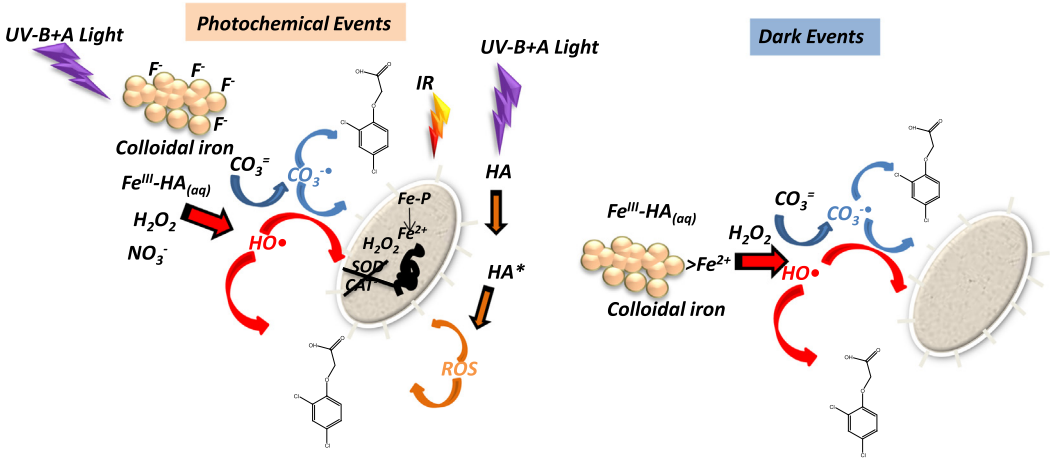

Fig. 4. Suggested mechanism of simultaneous 2,4-D degradation and *E. coli* – *K. pneumoniae* disinfection in simulated groundwater samples.

$$CO_3^{2-} + {}^{\cdot}OH \rightarrow OH^- + CO_3^{-\cdot} \quad (12)$$

$$2CO_3^{-\cdot} + 2H^+ \rightarrow 2CO_2 + H_2O_2 \quad (13)$$

$$CO_3^{-\cdot} + OM \rightarrow OM_{ox} + CO_3^{2-} \quad (14)$$

Reactions (12) and (13) reveal that during this reaction, pH may increase as it was confirmed during the experiments.

Moreover, Gutiérrez-Zapata et al. (2016) reported also the positive effect of fluoride (at concentrations of 1.2 mg L^{-1}) on the degradation of 2,4-D in Milli-Q water by photo-induced reactions involving iron and H_2O_2. Fluoride could induce the replacement of surface hydroxyl groups on iron (hydr)oxides (α- and γ-Fe_2O_3, Fe_3O_4, and α-δ-γ-FeOOH), inducing ·OH radicals production and accelerating the oxidation of water by the valence band holes (h^+_{VB}) (Du et al., 2008). On the other hand, Hu et al. (2009) claimed that the replacement of surface hydroxyl groups by fluoride could lead to the negative shifting of the flat band potential, allowing to the hematite (α-Fe_2O_3) and TiO_2 semiconductors reduce efficiently the molecular oxygen reduction and decreasing the h^+_{VB}/e^-_{CB} recombination (Fig. 4).

Regarding the viability reduction of *K. pneumoniae*, its lower viability reduction in comparison with *E. coli* cells could be due to morphological and physiological factors. *K. pneumoniae* exhibits a prominent polysaccharide capsule, which could protect the microorganism from the oxidative attack of photo-induced ·OH radicals and other ROS (Podschun et al., 2001). Furthermore, *K. pneumoniae* shows three different types of catalase (KpA, KpT and KpCP) while *E. coli* cells exhibits only two (Hochman and Goldberg, 1991). Thus, *K. pneumoniae* seems to be a microorganism with a high antioxidant capacity, especially inactivating H_2O_2, which could explain its resistance to the treatment.

This point highlights the importance of evaluate the waterborne bacteria inactivation by photochemical reactions such as photo-Fenton with microorganisms different to *E. coli* which can be more pathogenic. In addition, the complete viability reduction of *E. coli* cells is also very important from the sanitary point of view since this fact should guarantee the elimination of the potential regrowth and maintenance of virulence of this microorganism and in consequence the reduction of its pathogenic risk.

4. Conclusions

This study reports for the first time, the simultaneous removal of an organic pollutant (2,4-D) and bacteria inactivation (followed by viability procedures) of *E. coli* and *K. pneumoniae* (this latter in a lesser extent) in simulated groundwater samples containing natural iron concentrations using natural sunlight and CPC reactors at neutral pH.

Several dark (Fenton reactions) and photo-induced processes such as photo-Fenton, photocatalysis (with dissolved and colloidal iron), UV-B H_2O_2, nitrites/UV-B and photochemical reactions of dissolved organic matter (as humic acids) should be responsible of ·OH radicals formation and the pollutant and microbial removal.

However, *K. pneumoniae* was not efficiently removed. This fact should be related to morphological and physiological features, since *K. pneumoniae* shows a prominent polysaccharide capsule and a high capacity to inactivate H_2O_2 which might help to the microorganism to protect it from the oxidative stress caused by H_2O_2 photo-induced reactions.

This latter highlights the importance of evaluate the waterborne bacteria inactivation by photochemical reactions such as photo-Fenton with microorganisms different to *E. coli* which can be more pathogenic. Moreover, the complete viability reduction of *E. coli* cells is also very important from the sanitary point of view since this fact should guarantee its potential regrowth and maintenance of virulence of this microorganism and in consequence the removal of its pathogenic risk.

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