

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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ABSTRACT

Simultaneous abatement of 2,4-dichlorophenoxyacetic acid (2,4-D at 70 $\mu\text{g L}^{-1}$), *Escherichia coli* and *Klebsiella pneumoniae* (laboratory and wild strains) cells from real groundwater samples containing natural iron concentrations ($\sim 0.3 \text{ mg L}^{-1}$) 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 $t_{30\text{W}}$ 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 $t_{30\text{W}}$ 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 ($t_{30\text{W}}$) 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

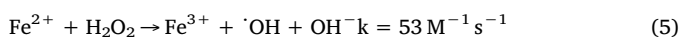
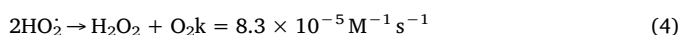
et al., 2012; Oturan and Aaron, 2014; Robertson et al., 2012; Salgado-Transito et al., 2015; Vilar et al., 2012). However, AOTs likely use complicated and expensive systems (such as ozone) or large amounts of chemicals such as nanoparticles (photocatalysis) or iron, hydrogen peroxide at acid pH (photo-Fenton).

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; Rodríguez-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

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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.



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). Sciaccia 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 ($70 \text{ } \mu\text{g L}^{-1}$) 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 photo-induced 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 $6 \text{ } \mu\text{g L}^{-1}$ and $5 \text{ } \mu\text{g L}^{-1}$ 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.

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