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Comparative effect of simulated solar light, UV, UV/H_2O_2 and photo-Fenton treatment (UV–Vis/H_2O_2/$Fe^{2+,3+}$) in the *Escherichia coli* inactivation in artificial seawater

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ABSTRACT

Innovative disinfection technologies are being studied for seawater, seeking a viable alternative to chlorination. This study proposes the use of H_2O_2/UV_{254} and photo-Fenton as disinfection treatment in seawater. The irradiations were carried out using a sunlight simulator (Suntest) and a cylindrical UV reactor. The efficiency of the treatment was compared for Milli-Q water, Leman Lake water and artificial seawater. The presence of bicarbonates and organic matter was investigated in order to evaluate possible effects on the photo-Fenton disinfection treatment. The photo-Fenton treatment, employing 1 mg L^{-1} Fe^{2+} and 10 mg L^{-1} of H_2O_2, led to the fastest bacterial inactivation kinetics. Using H_2O_2/UV_{254} high disinfection rates were obtained similar to those obtained with photo-Fenton under UV_{254} light. In Milli-Q water, the rate of inactivation for *Escherichia coli* was higher than in Leman Lake water and seawater due to the lack of inorganic ions affecting negatively bacteria inactivation. The presence of bicarbonate showed scavenging of the $OH^{\bullet}$ radicals generated in the treatment of photo-Fenton and H_2O_2/UV_{254}. Despite the negative effect of inorganic ions, especially HCO_3^-, the disinfection treatments with AOPs in lake water and seawater improved significantly the disinfection compared to light alone (simulated sunlight and UV_{254}). In the treatment of photo-Fenton with simulated sunlight, dissolved organic matter had a beneficial effect by increasing the rate of inactivation. This is associated with the formation of Fe^{3+}-organo photosensitive complexes leading to the formation of ROS able to inactivate bacteria. This effect was not observed in the photo-Fenton with UV_{254}. Growth of *E. coli* surviving in seawater was observed 24 and 48 h after treatment with UV light. However, growth of surviving bacteria was not detected after photo-Fenton with UV_{254} and H_2O_2/UV_{254} treatments.

This study suggests H_2O_2/UV_{254} and photo-Fenton treatments for the disinfection of seawater, in spite its high concentration of salts.

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1. Introduction

During the last years several disinfection technologies have been applied for drinking waters and wastewaters, but only in recent years have been tested for marine water.

Due to the development of new legal requirements, several seawater activities as the treatment of ballast water, aquaculture and industrial refrigeration systems have developed an interest for disinfection technologies. Ballast water is an important vector in the transport of invasive species, assuming a serious risk to ecosystems. In 2004, the International Marine Organization (IMO) adopted the Ballast Water Management Convention to prevent the spread of harmful aquatic organisms from one region to another, by establishing standards and procedures for the management and control of ships' ballast water and sediment (IMO, 2004). In an industrial context, the accumulation of fouling on heat exchangers in coastal power stations using seawater for cooling purposes, can be a cause of significant economic losses (Nebot et al., 2006; Bott, 1995). The first stage of the fouling process is an uncontrolled growth of microbial organisms on surfaces (Petrucci and Rosellini, 2005). In aquaculture, the water quality is an important factor in the production process and expansion of the intensive aquaculture system (Jorquera et al., 2002). The Directive, 2008/56/CE establishes principles on which develop strategies to achieve good environmental status of marine waters.

Sodium hypochlorite (NaClO) is a very common disinfection agent due to its low price and high effectiveness. However, there are increasing environmental concerns regarding the use of chlorination for the disinfection of natural water related to the formation of potentially harmful chloro-organic by products trough reactions with natural organic matter (NOM). For this reason, in accordance with Best Available Technology (BAT) (Directive, 2008/1/EC), it is important to find friendly technologies with the environment and that do not produce toxic waste.

Some treatments as ultraviolet light (UV) (Hess-Erga et al., 2010), exposure to ozone (Hess-Erga et al., 2010; Grguric et al., 1994), thermal treatment (Jacobsen and Liltved, 1988) and sonication (Holm et al., 2008) are being studied as an alternative to chlorination. The advanced oxidation processes (AOPs) are presented as treatment of future in disinfection and removal of contaminants from water. There are seldom studies of these processes in marine waters.

Advanced oxidation processes represent a group of techniques used for the treatment of water characterized by the generation of radicals, such as the hydroxyl radical ($OH^{\bullet}$) and may be an alternative to chlorine disinfection (De la Cruz et al., 2012; Marugan et al., 2008; Rincon et al., 2001; Pulgarin and Kiwi, 1996). UV/H_2O_2 is an AOP based on the combination of UV light and hydrogen peroxide, generating $OH^{\bullet}$ radicals. UV/H_2O_2 has been recently reported for the disinfection of seawater (Penru et al., 2012). Others as Fenton and photo-Fenton have been reported in the degradation of contaminants and in water disinfection (Spuhler et al., 2010; Moncayo-Lasso et al., 2009; Lipczynska-Kochany and Kochany, 2008; Herrera et al., 1998; Ribordy et al., 1997).

Fenton (Eq. (1)) and photo-Fenton processes (Eqs. (1) and (2)) in water produce $OH^{\bullet}$ radicals and peroxy radicals ($OH_2^{\bullet}/O_2^{\bullet-}$) (Eq. (3)).

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\bullet} \quad (1)$$

$$Fe(OH)^{2+} + h\upsilon \rightarrow Fe^{2+} + OH^{\bullet} \quad (2)$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HO_2^{\bullet} + H^+ \quad (3)$$

The effect of light irradiation in Eq. (2) shows Fe^{2+} formation by photo-reduction of the aqua-Fe^{3+} complexes leading to additional production of $OH^{\bullet}$ radicals (Moncayo-Lasso et al., 2009; Pulgarin et al., 1995). The formation of different iron complexes depends on the pH, perceived as the limiting factor for the photo-Fenton system. The most photoactive iron complex, $Fe(OH)^{2+}$, is predominant at low pH ($\approx$2.8). At high pH, the predominant species, $Fe(OH)_2^+$, is apparently less photoactive. In natural waters with NOM, iron can form Fe^{3+}-organo complexes that are stable at neutral pH. These complexes typically have higher molar absorption coefficients in the near-UV and visible regions. Their excitation produces Fe^{2+} and a ligand radical (Eq (4)).

$$R - COO - Fe^{3+} + h\upsilon \rightarrow R - COO^{\bullet} + Fe^{2+} \rightarrow R^{\bullet} + CO_2 \quad (4)$$

Some of these complexes with iron species in high oxidation state may be involved in the formation of radicals within the photo-Fenton system (Pignatello et al., 2006; Hug and Leupin, 2003; Feng and Nansheng, 2000).

Escherichia coli is the most employed bacterium in disinfection studies because it is a faecal indicator. But this indicator – and other bacterial ones – has shown to have a reactivation capacity after UV irradiation, and so the efficiency of disinfection is reduced. This reactivation ability must be checked for other photo-treatment technologies as AOPs.

The aim of this study was to evaluate the disinfection efficiency of *E. coli* by some alternative disinfection techniques: simulated solar light, ultraviolet radiation, H_2O_2/UV_{254} and photo-Fenton system. A special effort has been done in studying the effect of the water matrix on the efficiency of the diverse technologies. In this paper, we carried out a systematic study on the photo-inactivation and reactivation of *E. coli* in Milli-Q water, Leman Lake water and artificial seawater. Finally, we studied the effect of dissolved bicarbonates and NOM since these compounds affect the effectiveness of the treatments used.

2. Materials and methods

2.1. Chemicals

The chemicals used for experiments were reagent grade, supplied by Sigma–Aldrich or Fluka. Photo-Fenton experiments were carried out employing ferrous sulfate heptahydrate (Fluka Chemika), iron (III) oxide (Fe_2O_3, Sigma–Aldrich), hydrogen peroxide (35% by weight, Sigma–Aldrich) and

catalase (Catalase, from bovine liver; Sigma–Aldrich) to neutralize the hydrogen peroxide (in H_2O_2/UV and photo-Fenton experiments) prior to plating the samples. Hydrogen peroxide analyses were carried out with Titanium (IV) oxysulfate ($TiOSO_4$, Fluka). All solutions were prepared with Milli-Q water (18.2 MΩ cm) immediately prior to irradiation. Three different types of water were used for the experiments: a) Milli-Q water (18.2 MΩ cm), b) natural water from the Leman Lake and c) artificial seawater prepared by adding 35 g L^{-1} of sea salt (natural sea salt from Unión Salinera Española, Grupo Salins). Some physicochemical characteristics of these waters are shown in Table 1

2.2. Analytical methods

The evolution of H_2O_2 concentration was measured by a colorimetric method based in the absorbance of the yellow complex formed between Titanium (IV) oxysulfate and H_2O_2 by using a spectrophotometer (Perkin–Elmer UV–Vis lambda 20 spectrophotometer) at 410 nm. The yellow color produced in the reaction is due to the formation of pertitanic acid (Eisenberg, 1943). The absorbance vs. concentration relationship was linear in the range 0.1–100 mg L^{-1} (Sichel et al., 2009). To relate the absorbance vs. concentration a calibration curve of six points was used ($R^2 > 0.99$) from 0 to 20 mg H_2O_2 L^{-1}. Also, residual H_2O_2 was measured right after each experiment with peroxide test (colorimetric test strips methods, 1–100 mg L^{-1}, Merck).

Total and dissolved iron concentrations were measured with atomic absorption employing an ICP-OES spectrometer SHIMADZU ICPE-9000. For dissolved iron, the samples were filtered (0.45 μm) to eliminate the precipitated iron.

The pH (pH/Ion S220, Seven Compact, Mettler Toledo) of the samples was measured before and after each treatment.

2.3. Bacterial strain and growth media

The bacterial strain used was *E. coli* K12 (MG1655) and was supplied by DSM, German Collection of Microorganisms and Cell Cultures. *E. coli* K12 is non-pathogenic and approximates the wild-type *E. coli*, a typical indicator bacteria for enteric pathogens. Strain samples were stored in cryo-vials containing 20% glycerol at −20 °C.

Bacterial pre-cultures were prepared for each series of experiments by streaking out a loopfull from the strain sample onto Plate Count Agar (PCA, Merck). Subsequently the plates were incubated for 24 h at 37 °C (Incubator Heraeus instrument). From the growing colonies, one was re-plated on a separate PCA and incubated again for 24 h at 37 °C. To prepare the bacterial pellet for the experiments, one colony was picked from the pre-cultures and loop-inoculated into a 50 mL sterile PE Eppendorf flask containing 5 mL of Luria Bertani (LB) medium. The flask was then incubated at 37 °C and 180 rpm in a shaker incubator. After 8 h, cells were diluted (1% v/v) in a 500 mL Erlenmeyer flask containing 150 mL of pre-heated LB broth and incubated aerobically at 37 °C for 16 h until stationary physiological phase was reached. Bacterial growth and stationary phase was monitored by optical density at 600 nm. Component of LB medium included sodium chloride (10 g), tryptone (10 g) and yeast extract (5 g) in 1 L of deionized water; this solution was then sterilized by autoclaving for 20 min at 121 °C.

Cells were harvested by centrifugation (15 min at 5000× *g* RCF and 4 °C) in a universal centrifuge (Hermle Z323K). The bacterial pellet was re-suspended and washed three times with a saline solution (NaCl/KCl). The final pellet was re-suspended in saline solution to the initial volume. This procedure resulted in a cell density of approximately 10^9 Colony Forming Units (CFU) per milliliter. The bacterial solution was diluted in experimental water to the required cell density corresponding to 10^6 CFU mL^{-1}. The saline solution included sodium chloride (8 g) and potassium chloride (0.8 g) in 1 L of deionized water. The pH of the solution was adjusted to 7–7.5 and the solution was then sterilized by autoclaving for 20 min at 121 °C.

CFU were monitored by plating on PCA. 1 mL of the samples was withdrawn. Exceeding H_2O_2 was neutralized with catalase, samples were diluted in 10% steps and pour plated on PCA. Plates were incubated for 24 h at 37 °C and the CFU were counted manually.

2.4. Experimental

Two groups of experiments were carried out in this study concerning to the light source. The light sources used were a sunlight simulator (Suntest) and a UV lamp emitting at 254 nm wavelength. In each case, different experimental conditions were evaluated in three types of water at neutral pH. We studied the effects of iron concentration, bicarbonate ion content and the presence of organic matter on the proposed disinfection treatments. In those experiments with NOM and bicarbonate, concentrations were 0.8–1 mg C L^{-1} and 100 mg HCO_3^- L^{-1}, respectively. The NOM was in natural water of Lake Leman and was not added by other means.

For all experiments, the initial populations of bacteria in the water were 10^6–10^7 CFU mL^{-1}. Bacterial cells were suspended in the water and the solution was kept under stirring for 1 h in order to provide a time for bacteria acclimatization before starting the experiment. The addition of reagents (ferrous sulfate and/or hydrogen peroxide) was conducted in a single dosage and the light source was initiated immediately

Table 1 – Some physicochemical characteristics of the types of water used in the experimentation.

Parameter	Milli-Q water	Leman lake water	Artificial seawater
Conductivity at 20 °C (μS/cm)	<0.055	250	5×10^4
Transmittance at 254 nm (%)[a]	100	96	84
pH	7.83	7.79	7.81
Total Organic Carbon (TOC) (mg C/L)	<0.005	0.8–1	0.8–1
Hydrogen carbonates (mg HCO_3^-/L)	–	100	100
Chloride (mg Cl^-/L)	–	8	19,491
Sodium (mg Na^+/L)	–	8.5	8740
Sulfate (mg SO_4^-/L)	–	48	2743

[a] Measurements compared with Milli-Q water.

to start the test. Dark control experiments were carried out and for reproducibility reasons, each experiment was carried out in triplicate.

2.4.1. *Suntest experiment*

Six circular pyrex reactors of 50 mL (3.5 cm in diameter and 7 cm high) were placed on a magnetic stirrer in a solar simulator (CPC Suntest System Heraeus Noblelinght, Hanau, Germany). The lamp had a wavelength spectral distribution with about 0.5% of the emitted photons at wavelengths shorter than 300 nm (UV-C range) and about 7% between 300 and 400 nm. The emission spectrum in the Suntest between 400 and 800 nm follows the solar spectrum and cut off with a filter wavelengths shorter than 290 nm. Temperature in the reactor never exceeded 33 °C. The radiation intensity was 500 Wm^{-2} and was monitored by a combination of a UV radiometer and a pyranometer connected to a data-logger (CUV and CM6b respectively, Kipp & Zonen, Defft, Holland). All the experiments were carried out in equilibrium with air at 650 rpm of agitation.

In photo-Fenton experiments the Fe^{2+} and H_2O_2 concentrations used were 1 mg L^{-1} and 10 mg L^{-1} respectively. A similar concentration of H_2O_2 was used for the H_2O_2/simulated solar light system. The artificial seawater was prepared by the addition of sea salt on the natural lake water to keep the same amount of NOM. In experiments without NOM, seawater was prepared using Milli-Q water. For several treatments it was necessary to remove the bicarbonates found in the Lake Leman water. This was conducted by pH modification process that can be summarized in three steps: (1) water acidification to pH 4 by addition of hydrochloric acid solution 0.1 M to transform all bicarbonates into CO_2, (2) removal of CO_2 from the water by applying aeration for 1 h and (3) adjust pH to 7.5–8 by addition of a sodium hydroxide solution 0.1 M. Experimental conditions were evaluated for each type of water:

A) Milli-Q water: (I) simulated solar light only; (II) H_2O_2/simulated solar light; (III) Fe^{2+}/H_2O_2/simulated solar light; (IV) $Fe^{2+}/H_2O_2/HCO_3^-$/simulated solar light; (V) dark control
B) Leman Lake water: (I) simulated solar light only; (II) H_2O_2/simulated solar light; (III) Fe^{2+}/H_2O_2/NOM/simulated solar light; (IV) Fe^{2+}/H_2O_2/NOM/HCO_3^-/simulated solar light; (V) dark control
C) Artificial seawater: (I) simulated solar light only; (II) H_2O_2/simulated solar light; (III) Fe^{2+}/H_2O_2/NOM/simulated solar light; (IV) Fe^{2+}/H_2O_2/NOM/HCO_3^-/simulated solar light; (V) $Fe^{2+}/H_2O_2/HCO_3^-$/simulated solar light; (VI) dark control

2.4.2. *UV (254 nm) reactor experiment*

UV light treatments were carried out in a cylindrical water-jacketed glass reactor (6 cm in diameter and 25 cm high), in batch mode. A low-pressure mercury lamp (Pen-Ray 90-0012-01, 4.9 W) immersed and isolated by a cylindrical quartz tube was used, emitting the primary energy at 254 nm. A volume of 450 mL of water was treated in each experiment. The water in the reactor was maintained well mixed by a magnetic stirrer and the temperature never exceeded 23 °C.

For experiments with Milli-Q water and Leman Lake water, Fe^{2+} concentration was 1 mg L^{-1}. In artificial seawater several concentrations of Fe^{2+} (1, 3 and 5 mg L^{-1}) were studied. Also, was tested with Fe_2O_3 as a natural source of iron for the photo-Fenton system. In waters with Fe_2O_3, in presence of oxygen and hydrogen peroxide, photo-leaching processes may exist. The photoexcitation followed by a charge transfer reduces the Fe^{3+} to Fe^{2+}. The dissolution process of Fe_2O_3 is completed by the detachment of Fe^{2+} from the mineral surface (Rodriguez et al., 2009; Pulgarin and Kiwi, 1995). In all cases the concentration of H_2O_2 used was 10 mg L^{-1}. The seawater, with and without natural organic matter (NOM), was prepared similarly as in the experiments with Suntest. Also, the removal of bicarbonates in Lake Leman water was performed as described in the above section. In another experiment, humic acid was added on the seawater at a concentration of 1 mg L^{-1} as a different source of organic matter. Humic acids can form Fe^{3+}-organo complexes that favor the photo-Fenton treatment in water at neutral pH. Several experimental conditions were carried out:

A) Milli-Q water: (I) UV_{254} only; (II) H_2O_2/UV_{254}; (III) $Fe^{2+}/H_2O_2/UV_{254}$; (IV) $Fe^{2+}/H_2O_2/HCO_3^-/UV_{254}$; (IV) dark control
B) Leman Lake water: (I) UV_{254} only; (II) H_2O_2/NOM/UV_{254}; (III) Fe^{2+}/H_2O_2/NOM/UV_{254}; (IV) Fe^{2+}/H_2O_2/NOM/HCO_3^-/UV_{254}; (IV) dark control
C) Artificial seawater: (I) UV_{254} only; (II) H_2O_2/NOM/UV_{254}; (III) Fe^{2+}(1 mg L^{-1})/H_2O_2/NOM/UV_{254}; (IV) Fe^{2+}(1 mg L^{-1})/H_2O_2/NOM/HCO_3^-/UV_{254}; (V) Fe^{2+}(3 mg L^{-1})/H_2O_2/NOM/HCO_3^-/UV_{254}; (VI) Fe^{2+}(5 mg L^{-1})/H_2O_2/NOM/$HCO_3^-/h\upsilon$, (VII) Fe_2O_3 (1 mg L^{-1})/H_2O_2/NOM/HCO_3^-/UV_{254}, (VIII) Fe^{2+}(1 mg L^{-1})/$H_2O_2/HCO_3^-/UV_{254}$; (IX) Fe^{2+}(1 mg L^{-1})/H_2O_2/HCO_3^-/Humic acids/UV_{254} (X) dark control

2.4.2.1. *Post-irradiation event*. During the storage of treated water, growth of bacteria surviving can take place. The organic matter in the water or the byproducts generated from lysis of the bacteria may promote bacterial growth making useless the disinfection treatment.

In this study, growth of surviving bacteria in artificial seawater after 24 h and 48 h in the dark was measured for three different treatments (UV_{254}, UV_{254}/H_2O_2 and photo-Fenton). Samples were withdrawn after achieving total inactivation into the UV reactor for each treatment and were then placed for either 24 or 48 h in the dark at room temperature before measurement of CFU by pour-plating on PCA.

2.5. *Data treatment*

The bacterial inactivation kinetics can be described by the first order kinetic model proposed by Chicks–Watson (Eq. (5)) (Brahmi et al., 2010; McGuigan et al., 1998):

$$N_t = N_0 e^{-kt} \tag{5}$$

where N_0 is the concentration of viable organisms before radiation exposure; N_t is the concentration of organisms surviving after irradiation time t; t is the irradiation time (at constant light flux) and k is the first order inactivation rate. To compare inactivation between the different treatments tested, the constants of inactivation rate were obtained, k_{obs}, from fitting of plots of log(CFU mL^{-1}) vs. time. The fitting was carried out by GInaFiT, a tool of Microsoft © Excel for testing

different types of microbial survival models on experimental data (Geeraerd et al., 2005). The root mean square error of the fit to the experimental data was evaluated. The R^2 of the model was in most cases superior to 0.9. The standard deviation was calculated using a minimum of three measures. The deviation did not exceed 15% and confidence interval for the fitting was 95%.

3. Results and discussion

3.1. *Experiments carried out with simulated solar light (Suntest)*

Experiments with *E. coli* were carried out in a Suntest simulator with Milli-Q water (pH 7.5–8), Leman Lake water (pH 7.5–8) and artificial seawater (pH 7.5–8). The rates k_{obs} obtained by fitting the raw data using the first order kinetic model ($N_t = N_0 e^{-kobs*t}$) are presented in Table 2.

3.1.1. E. coli *inactivation in Milli-Q water*

Raw data obtained from the experiments in Milli-Q water are presented in Fig. 1A. The lines show the one-order exponential fitting used to calculate k_{obs}. Dark control experiments show no variation of bacteria up to 240 min. The k_{obs} followed the order: Fe^{2+}/H_2O_2/simulated solar light > $Fe^{2+}/H_2O_2/HCO_3^-$/simulated solar light > H_2O_2/simulated solar light > simulated solar light only. Under only artificial solar light irradiation the kinetic parameter k_{obs}^{solar} was 0.047 min^{-1} (Table 2). Simulated solar irradiation alone is not sufficient for the total elimination of bacteria after 4 h treatment, however it is able to remove 5 logs of CFU/ml. Previous studies refer to UV-A/B as cause of excited states of oxygen via intracellular chromophores acting as photosensitizers. UV-A light (320–400 nm) kill bacteria by inactivation by depletion of ATP. Furthermore, the wavelength between 320 and 400 nm may catalyze the formation of intracellular ROS ($O_2^{\bullet-}$, H_2O_2 and $OH^{\bullet}$) causing damage to cellular DNA. UV-B (290–320 nm) radiation causes direct DNA damage by inducing the formation of DNA photoproducts. The accumulation of DNA photoproducts can be lethal to cells through the blockage of DNA replication and RNA transcription (Spuhler et al., 2010; Bosshard et al., 2009; Rincon and Pulgarin, 2007; Hoerter et al., 1996). The H_2O_2/simulated solar light system showed an inactivation rate of 0.058 min^{-1}, approximately 25% greater than with simulated solar light only. This enhanced inactivation could be due to several different processes: (1) the diffusion of H_2O_2 into the cell and (2) direct attack from H_2O_2 to the cell membrane. The photolysis process of H_2O_2 does not play a role in bacterial inactivation because photon absorption of H_2O_2 is not significant at the wavelength used ($\lambda > 290$ nm) and the quantum yield is low (Spuhler et al., 2010; Malato et al., 2009). On the other hand, when H_2O_2 is present in the extracellular medium, its diffusion into the cell is possible since it is a stable and uncharged molecule. H_2O_2 diffusion into the cell increases the possibility of $OH^{\bullet}$ generation via Fenton reactions with intracellular iron. If the free iron is not available, the H_2O_2 can cause the oxidation of iron-sulfur clusters ([4Fe–4S]) and the release of iron which may contribute to the Fenton reaction (Spuhler et al., 2010; Imlay, 2008, 2003). Furthermore, H_2O_2 can directly attack the cell membrane by lipid peroxidation, affecting viability of the cell and increasing the membrane permeability (Halliwell and Aruoma, 1991).

Photo-Fenton treatment showed higher inactivation rates compared with light alone applied to *E. coli* suspended in Milli-Q water. In the photo-Fenton system without bicarbonates, k_{obs} was increased to 503% over the k_{obs}^{solar}. This is about five times faster than the only light inactivation. In the case of photo-Fenton with bicarbonates, k_{obs} increased up to 247%. The presence of bicarbonates during photo-Fenton treatment appears thus to be responsible for a lowering of the inactivation rate by a factor of 2 (Table 2, Milli-Q water). This system generates oxidative species ($OH^{\bullet}$ and ROS) simultaneously inside and outside the cell. The reactive species in contact with the bacteria induce cell damage leading to death. Photo-

Table 2 – Inactivation rates (K_{obs}[min^{-1}] obtained from suntest experiment. $K_{obs}^{h\upsilon}$ was set to 100% and in order to facilitate comparing, the variation of K_{obs} compared $K_{obs}^{h\upsilon}$ was displayed in parentheses. *hυ* correspond to simulated solar radiation (>290 nm). (*) Data fitting that presented a low goodness of fit ($R^2 < 0.9$).

Milli-Q	Simulated solar light	$H_2O_2/h\upsilon$	$Fe^{2+}/H_2O_2/h\upsilon$	$Fe^{2+}/H_2O_2/HCO_3^-$ /hυ
	0.0470 ± 0.0011	0.0576 ± 0.0016	0.2365 ± 0.0018	0.1161 ± 0.0129
r^2	0.9245	0.9835	0.9214	0.9399
(% compared to solar light only)	(100%)	(123%)	(503%)	(247%)

Lake Leman water	Simulated solar light	$H_2O_2/h\upsilon$	$Fe^{2+}/H_2O_2/NOM/h\upsilon$	$Fe^{2+}/H_2O_2/NOM/HCO_3^-$ /hυ
	0.0521 ± 0.0003	0.0539 ± 0.0065	0.1145 ± 0.0170	0.0924 ± 0.008
r^2	0.9754	0.9690	0.9490	0.8480*
(% compared to solar light only)	(100%)	(103%)	(220%)	(177%)

Seawater	Simulated solar light	$H_2O_2/h\upsilon$	$Fe^{2+}/H_2O_2/NOM/h\upsilon$	$Fe^{2+}/H_2O_2/NOM/HCO_3^-$ /hυ	$Fe^{2+}/H_2O_2/HCO_3^-$ /hυ
	0.0341 ± 0.0028	0.0315 ± 0.0054	0.1064 ± 0.0096	0.0858 ± 0.0043	0.0542 ± 0.0052
r^2	0.8210*	0.9233	0.9549	0.9676	0.9715
(% compared to solar light only)	(100%)	(92%)	(312%)	(252%)	(159%)

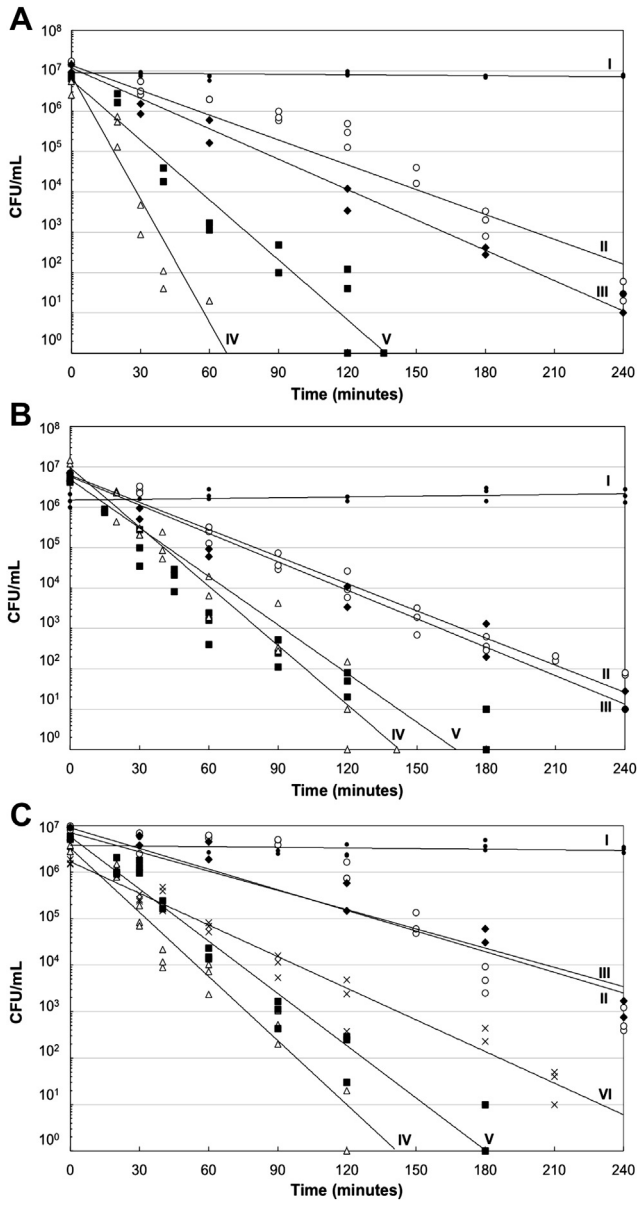

Fig. 1 – *E. coli* inactivation following different phototreatments with simulated solar light in (A) Milli-Q water, (B) Leman Lake water, (C) Artificial seawater. Bacterial cells were suspended in the reactor and after acclimation, Fe^{2+} and H_2O_2 were added to the corresponding systems. During the experimental period, samples were taken to measure the evolution of the concentration of bacteria. Remaining H_2O_2 was eliminated by catalase before plating. Markers represent raw data and lines show a one-term exponential fitting; (I) (●) dark control; (II) (○) *hν* only, *hν* > 290 nm; (III) (◆) 10 mg H_2O_2 L^{-1}/*hν*; (IV) (△) 1 mg Fe^{2+} L^{-1}/10 mg H_2O_2 L^{-1}/*hν*; (V) (■) 1 mg Fe^{2+} L^{-1}/10 mg H_2O_2 L^{-1}/100 mg HCO_3^- L^{-1}/*hν*; (VI) (×) 1 mg Fe^{2+} L^{-1}/10 mg H_2O_2 L^{-1}/100 mg HCO_3^- L^{-1}/*hν*/without NOM.

Fenton reaction can lead to the collapse of normal cellular metabolism via lipid peroxidation, cross-linking of protein and DNA mutations. The $OH^•$ radical with an oxidation potential (2.70 V), causes damage on the bacterial membrane. Moreover, the presence of H_2O_2 in the extracellular medium may lead to an increase in membrane permeability, facilitating the diffusion of Fe^{2+} (Spuhler et al., 2010; Moncayo-Lasso et al., 2009; Rincon and Pulgarin, 2007; Cho et al., 2004). There are some processes that can explain the inhibition of photo-Fenton by HCO_3^-. An important reason for the significant decrease of $OH^•$ is that bicarbonate ions react with hydroxyl radicals to produce less reactive radicals, $^•CO_3^-$ (Eqs. (6) and (7)). The radical $^•CO_3^-$ is an electrophilic species reacting slower compared to $OH^•$.

$$OH^• + HCO_3^- \rightarrow {}^•CO_3^- + H_2O \quad (6)$$

$$OH^• + CO_3^{2-} \rightarrow {}^•CO_3^- + OH^- \quad (7)$$

Furthermore, HCO_3^- absorbs light hindering its penetration in the water, thus protecting the bacteria (Grebel et al., 2010; Rincon and Pulgarin, 2004; Liao et al., 2001; Chen et al., 1997).

3.1.2. *E. coli inactivation in Leman Lake water*

Disinfection results of *E. coli* in Leman Lake water are presented in Fig. 1B. As shown in Table 2, k_{obs} decreases in the order: Fe^{2+}/H_2O_2/NOM/simulated solar light > Fe^{2+}/H_2O_2/NOM/HCO_3^-/simulated solar light > H_2O_2/simulated solar light ≈ hv only. k_{obs} for simulated solar light was 0.052 min^{-1}, similar to k_{obs}^{solar} in Milli-Q water. The presence of NOM in the lake water can act as a filter of radiation and affect the inactivation of *E. coli*. However, UV light absorption by dissolved organic matter (DOM) promote the triplet state ($^3DOM^*$) (Eq. (8)). The deactivation of this excited state of the DOM occurs in several ways, including reaction with oxygen to form singlet oxygen ($^1O_2$) (Eq. (9)). Singlet oxygen can react forming peroxidation products that interact with water contaminants (Canonica, 2007; Georgi et al., 2007; Buschmann et al, 2005).

$$DOM + hv \rightarrow {}^1DOM^* \rightarrow {}^3DOM^* \quad (8)$$

$$^3DOM^* + O_2 \rightarrow DOM + {}^1O_2 \quad (9)$$

In lake water, the combination of H_2O_2 and simulated solar light did not increase the bacterial inactivation rate compared to using simulated solar light only. The absence of osmotic stress due to the presence of inorganic ions in the water prevent excessive diffusion of H_2O_2 inside the cell. In addition, inorganic ions may compete with H_2O_2 for the contact sites of the cell membrane, protecting it from the direct attack of H_2O_2 and other ROS (Spuhler et al., 2010). In the photo-Fenton system *E. coli* photo-inactivation in neutral pH water increases up to 177% (with HCO_3^-) and 220% (without HCO_3^-). Same as Milli-Q water, in Leman Lake water the presence of bicarbonates affects the photo-Fenton system, slowing the inactivation of *E. coli*. Probably due to the scavenging of $OH^•$ by bicarbonate in lake water being similar to Milli-Q water.

Comparing k_{obs} of photo-Fenton in lake water and Milli-Q water, we observe that the inactivation rate is higher in Milli-Q water (Table 2). In the system Fe^{2+}/H_2O_2/NOM/simulated solar light, the difference between the two types of water is due to the presence of ions such as phosphate, sulfate, organosulfonate, fluoride, bromide, and chloride (Pignatello et al., 2006). Besides the ion content of the water of Lake Leman, this may be due to the evolution of the pH during the

experiment. The lake water has a strong buffer effect and the pH does not change during the treatment (final pH 7.5–8). Instead, the pH in Milli-Q water for the system Fe^{2+}/H_2O_2/simulated solar light decreases to a final pH of 5. It is generally accepted that the photo-Fenton system is limited by pH. The most photoactive iron complex, $Fe(OH)^{2+}$, is predominant at low pH. At high pH, Fe^{3+} tends to precipitate and the predominant species, $Fe(OH)_2^+$, is considerably less photoactive. The pH decrease in Milli-Q water could increase the $Fe(OH)^{2+}$ complex concentration leading to a higher bacterial rate of inactivation (Pignatello et al., 2006; Hug and Leupin, 2003; Feng and Nansheng, 2000).

3.1.3. E. coli *inactivation in artificial seawater*

The results on artificial seawater are displayed in Fig. 1C. The ranking for k_{obs} was: Fe^{2+}/H_2O_2/NOM/simulated solar light > Fe^{2+}/H_2O_2/NOM/HCO_3^-/simulated solar light > $Fe^{2+}/H_2O_2/HCO_3^-$/simulated solar light > H_2O_2/simulated solar light ≈ simulated solar light only (Table 2). As in the previous experiments, the concentration of *E. coli* did not change for 4 h in the dark control. During the course of the experiment there is no significant decrease in the number of bacteria by osmotic stress effect. The system studied using only *hν* presented a k_{obs} of 0.034 m^{-1}, lower than in Milli-Q water and Leman Lake water. This was due to the decrease in the transmittance of light in water with high salt concentration. In the first phase of this treatment a delay in the inactivation of *E. coli* was observed. This kinetic phase is known as shoulder and can be attributed to the need of bacteria to absorb a threshold UV dose to begin producing severe damages that cause its inactivation (Severin et al., 1984). The shoulder and the lower inactivation rate may be due to the decrease in the transmittance of light in water with high salt concentration. Ions as Cl^- and SO_4^{2-} in high concentrations can absorb light and have a protective effect on the bacteria (Rincon and Pulgarin, 2004). In order to compare the inactivation rate of this treatment with those obtained in the other systems studied, despite the presence of a shoulder, the data were fitted using the one-term exponential model. As in the lake water, we would expect a positive effect of the NOM (Eq (8)) and the possible formation of carbonate radicals (Eqs. (5) and (6)). However, the negative effect of a high concentration of salt in artificial seawater and the corresponding limitations in the attenuation of light is stronger so an actual decrease in the disinfection rate is observed.

The H_2O_2/simulated solar light system showed an inactivation rate similar to that obtained with light alone. As discussed in previous sections, inorganic ions could have a protective effect on bacteria against H_2O_2. Furthermore, due to the high concentrations of inorganic ions in seawater, there is a strong osmotic stress but with opposite effect that in Milli-Q water, hindering the diffusion of H_2O_2 into the cell.

The effect of the bicarbonate was also detected in artificial seawater. In the presence of bicarbonate, the disinfection rate in the photo-Fenton system was 252% of k_{obs}^{solar}. Without bicarbonates in the water, k_{obs} increased to 312% of k_{obs}^{solar}. Similar to the other types of water, there is a scavenger effect of $OH^\bullet$ by bicarbonate ions in artificial seawater. Hydroxyl radicals are removed, decreasing the rate of *E. coli* inactivation. The presence of NOM also contributes to the rate of inactivation by photo-Fenton. Dissolved organic matter in the concentrations tested (0.8–1 mg L^{-1}) had a positive effect on k_{obs}. Photo-Fenton systems (Fe^{2+}/H_2O_2/NOM/HCO_3^-/simulated solar light and $Fe^{2+}/H_2O_2/HCO_3^-$/simulated solar light) presented inactivation rates of 252% and 159% of k_{obs}^{solar}, respectively. The content of organic matter could be seen as a problem, consuming the $OH^\bullet$ or attenuating the active light. However, the results showed an increase in the rate of inactivation compared to the system without NOM. The positive effect of organic matter may be due to two mechanisms: (i) The formation of the triplet state of the DOM as a result of the absorption of light (UV/visible), discussed above. (II) Complex formation between iron and NOM that could have a significant effect on the photo-Fenton treatment.

In the application of photo-Fenton, the formation of Fe^{3+}-NOM complexes may be very beneficial for the treatment. These complexes typically have higher molar absorption coefficients in the near-UV and visible regions. The excitation by light absorption of Fe^{3+} complexes undergoing metal-to-ligand charge transfer (LMCT), dissociating to give Fe^{2+} and an oxidized ligand (L_{ox}) (Eq. (10)) (Georgi et al., 2007; Pignatello et al., 2006).

$$Fe^{3+}(L)n + h\nu \rightarrow Fe^{2+}(L)n-1 + L_{ox}^{\bullet} \quad (10)$$

This process could be positive for recycling Fe^{3+}/Fe^{2+} as well as the rate of $OH^\bullet$ formation.

Comparing k_{obs} of photo-Fenton obtained for the three types of water, the order is Milli-Q > Leman Lake water > Artificial seawater (Table 2). The possible reason for this is the concentration of ions such as chloride, sulfate, phosphate and bromide, as mentioned in the previous section. The rate of inactivation would decrease with increasing concentration of these ions. Despite the high concentration of inorganic ions in seawater, the photo-Fenton treatment significantly improved disinfection with simulated solar light.

3.2. *UV (UV_{254}) reactor*

The results of the experiments with *E. coli* carried out in the UV_{254} reactor with Milli-Q water (pH 7.83), Leman Lake water (pH 7.79) and artificial seawater (pH 7.81) are presented in Fig. 2 and will be discussed in detail below. No changes in pH were observed, possibly due to the short duration of treatments. The kinetic data, until the point where 99.9% of disinfection was reached, were approximated by a one-term exponential model ($N_t = N_0 e^{-kobst}$). The observed inactivation rates k_{obs} are presented in Table 3.

3.2.1. E. coli *inactivation in Milli-Q water*

The inactivation rates obtained in experiments with milli-Q water are shown in Table 3. Milli-Q. The bacteria concentration was unchanged during the experimental period in the dark control (results not shown). The order of the systems studied according to k_{obs} was: $Fe^{2+}/H_2O_2/UV_{254}$ > $Fe^{2+}/H_2O_2/HCO_3^-/UV_{254}$ ≈ $H_2O_2/HCO_3^-/UV_{254}$ > UV_{254}. k_{obs}^{UV} was 0.1167 s^{-1}. UV treatment was efficient and fast, with maximum treatment time of 5 min. The water temperature never exceeded 23 °C, not taking into account thermal disinfection. Disinfection is due to photons at 254 nm wavelength. Ultraviolet light

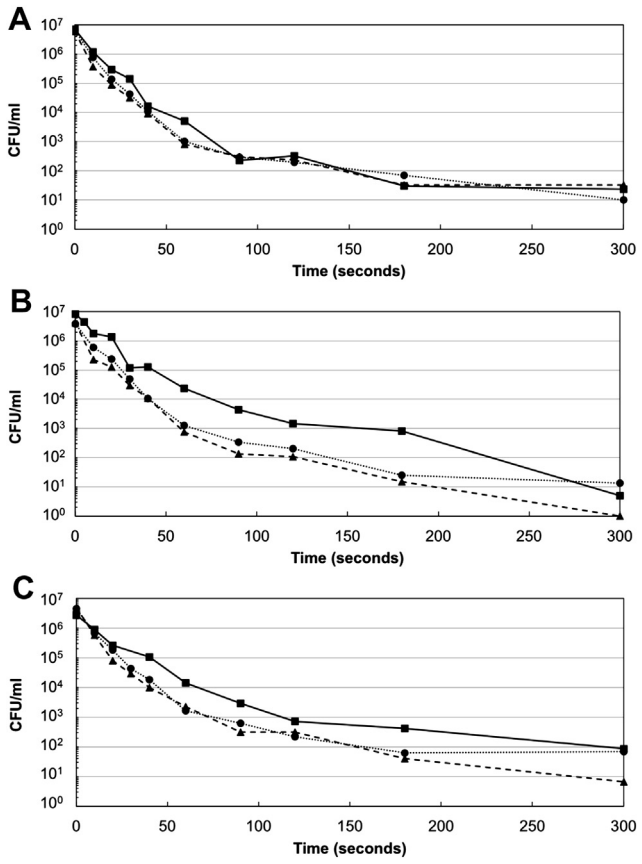

Fig. 2 – *E. coli* inactivation following three different treatments in (A) Milli-Q water, (B) Leman Lake water, (C) Artificial seawater. Bacterial cells were suspended in the reactor and after acclimation, Fe^{2+} and H_2O_2 were added to the corresponding systems. Remaining H_2O_2 was eliminated by catalase before plating. Markers represent the average of raw data during treatment; (■) UV_{254}; (●) 10 mg H_2O_2 L^{-1}/UV_{254}; (▲) 1 mg Fe^{2+} L^{-1}/10 mg H_2O_2 L^{-1}/ UV_{254}.

causes damage in essential components of the bacteria (proteins, lipids, membrane and DNA), but the most significant damage caused by UV light is the result of direct photochemical damage on intracellular DNA. The typical UV damage induces the formation of thymine–thymine cyclobutane cys-syn thymine–thymine photodimers and pyrimidine (6-4) pyrimidine photoproducts (TT (6-4) photoproducts) (Cho et al., 2010; Belov et al., 2009; Taghipour, 2004).

The H_2O_2/UV_{254} treatment improved the *E. coli* disinfection compared to UV_{254} treatment, k_{obs} increased to 130% over the k_{obs}^{UV}. The inactivation process improvement is due to the formation of $OH^{\bullet}$ radicals that cause significant damage to the bacteria. H_2O_2/UV_{254} is an advanced oxidation process that forms hydroxyl radicals by photolysis reaction of H_2O_2 (Eq. (11)).

$$H_2O_2 + h\upsilon \rightarrow 2OH^{\bullet} \quad (11)$$

The quantum yield of this reaction with UV light at a wavelength of 254 nm is 0.5, two hydroxyl radicals formed per quantum of radiation absorbed. (Penru et al., 2012; Crittenden et al., 1999). Probably, the sum of the effects from UV radiation and $OH^{\bullet}$ radicals (highly reactive) is the major route of inactivation of *E. coli* in this system. However, there are other possible ways of inactivation with lesser roles. Representative one can be H_2O_2 direct attack on the lipid membrane of the bacterium, increasing the permeability of the cell, or the penetration of H_2O_2 into the cell increasing the probability of radical generation via Fenton with intracellular iron (Imlay, 2003).

Photo-Fenton treatment $Fe_2^{+}/H_2O_2/HCO_3^{-}/UV_{254}$ showed an inactivation rate higher than when UV-light is applied as the light activation source. Photo-Fenton system did not show significant improvement in disinfection compared with $H_2O_2/HCO_3^{-}/UV_{254}$ treatment. Photo-Fenton effects on bacteria are similar to those explained above for the experiment in the Suntest cavity. $OH^{\bullet}$ radicals generated by photo-Fenton can attack the bacteria and cause significant damages on the cell membrane. Moreover, the diffusion of Fe^{2+} and H_2O_2 within the cell may generate radicals via Fenton reactions inside bacteria that attack the DNA and other cellular components (Spuhler et al., 2010; Rincon and Pulgarin, 2007). Milli-Q water did not contain inorganic ions and organic material that could have an effect on the photo-Fenton treatment.

Control experiments to assess the effect of bicarbonates were carried out by photo-Fenton treatment without HCO_3^{-} in Milli-Q water. The system studied was $Fe^{2+}/H_2O_2/UV_{254}$. It was observed that in Milli-Q water with HCO_3^{-} k_{obs} was 0.1540 s^{-1}, while in water without HCO_3^{-} k_{obs} was 0.1986 s^{-1}. These data confirm the fact that the bicarbonate ions react with hydroxyl radicals to produce less reactive radicals, $^{\bullet}CO_3^{-}$ (Eqs. (5) and (6)).

3.2.2. E. coli *inactivation in Leman Lake water*

The rates of inactivation obtained in experiments with Leman Lake water are present in Table 3. In dark control, the bacterial concentration remained constant. According to the results presented in Table 3, the kinetic order of the inactivation of *E coli* due to the following treatments is: $Fe^{2+}/H_2O_2/NOM/UV_{254}$ > $Fe^{2+}/H_2O_2/NOM/HCO_3^{-}/UV_{254}$ ≈ $H_2O_2/NOM/HCO_3^{-}/UV_{254}$ > UV_{254}. The photo-Fenton system without HCO_3^{-} is the most effective treatment for the removal of *E. coli* in the Leman Lake water.

The presence of inorganic ions and organic matter are responsible for the differences between lake and Milli-Q water. In all systems studied, k_{obs} is lower for lake water compared to Milli-Q water. Cl^{-}, HCO_3^{-} and SO_4^{2-} have a protective effect on the bacteria by absorbing some of the UV light (Rincon and Pulgarin, 2004). It is also known that the natural organic material absorbs ultraviolet light, decreasing its bactericidal effect. However, organic matter and inorganic ions exposed to UV light can form radicals that interact with bacteria (Canonica, 2007; Buschmann et al, 2005). Probably the UV light attenuation is greater than the advantages due to radical formation, therefore seeing an overall decrease in the rate of inactivation compared with Milli-Q water.

The $Fe^{2+}/H_2O_2/NOM/HCO_3^{-}/UV_{254}$ and $H_2O_2/NOM/HCO_3^{-}/UV_{254}$ systems increase *E. coli* photo-inactivation compared with irradiation applying UV light. The inactivation

Table 3 – Inactivation rates (K_{obs}[s^{-1}]) obtained from the experiments with UV reactor. The process kinetics up until the point where 99.9% of disinfection was reached were approximated by a one-term exponential model ($N_t = N_0 e^{-kobst}$). K_{obs}^{UV} was set to 100% and in order to facilitate comparing, the variation of K_{obs} compared K_{obs}^{UV} was displayed in parentheses. UV corresponds to radiation of low-pressure mercury lamp (254 nm).

Milli-Q	UV_{254}	H_2O_2/UV_{254}	$Fe^{2+}/H_2O_2/UV_{254}$	Fe^{2+} (1 mg L^{-1})/H_2O_2/ HCO_3^- /UV_{254}
	0.1167 ± 0.0047	0.1517 ± 0.0054	0.1986 ± 0.0259	0.1540 ± 0.0102
r^2	0.9699	0.9634	0.9459	0.9471
(% compared to UV only)	(100%)	(130%)	(170%)	(132%)
Lake Leman water	UV_{254}	$H_2O_2/NOM/HCO_3^-$ /UV_{254}	$Fe^{2+}/H_2O_2/NOM/UV_{254}$	Fe^{2+} (1 mg L^{-1})/ H_2O_2/NOM/ HCO_3^- /UV_{254}
	0.0949 ± 0.0102	0.1416 ± 0.0101	0.1828 ± 0.0210	0.1466 ± 0.0076
r^2	0.9355	0.9609	0.9305	0.9340
(% compared to UV only)	(100%)	(149%)	(193%)	(154%)
Seawater	UV_{254}	$H_2O_2/NOM/HCO_3^-$ /UV_{254}	$Fe^{2+}/H_2O_2/NOM/UV_{254}$	Fe^{2+} (1 mg L^{-1})/ H_2O_2/NOM/ HCO_3^- / UV_{254}	Fe^{2+} (3 mg L^{-1})/H_2O_2/ NOM/ HCO_3^- /UV_{254}
	0.0795 ± 0.0078	0.1322 ± 0.0091	0.1823 ± 0.0221	0.1376 ± 0.0030	0.1365 ± 0.0232
r^2	0.9507	0.9627	0.9002	0.9348	0.9697
(% compared to UV only)	(100%)	(166%)	(229%)	(173%)	(172%)
	Fe^{2+} (5 mg L^{-1})/H_2O_2/NOM/HCO_3^- /UV_{254}	$Fe_2O_3/H_2O_2/NOM/HCO_3^-$ /UV_{254}	Fe^{2+} (1 mg L^{-1})/H_2O_2/ HCO_3^- /UV_{254}	Fe^{2+} (1 mg L^{-1})/H_2O_2/HCO_3^- / UV_{254}/Humic acid
	0.1348 ± 0.0226	0.1411 ± 0.0176	0.1348 ± 0.0095	0.1309 ± 0.0096
r^2	0.9195	0.9493	0.9161	0.9254
(% compared to UV only)	(170%)	(178%)	(170%)	(165%)

rate of photo-Fenton and UV/H_2O_2 are 0.1466 and 0.1416 s^{-1}, respectively. The small differences between inactivation rates may indicate that the dominant process in both treatments is the generation of hydroxyl radicals by photolysis of hydrogen peroxide. However, there are some processes such as the formation of Fe^{3+}-NOM complexes that promote the Fe^{3+}/Fe^{2+} recycling and enhance $OH^{\bullet}$ formation by the photo-Fenton. These processes may not have a significant effect in short treatments (on the order of seconds) as observed on previous experiences with the Suntest (on the order of hours). Furthermore, it is possible that the Fe^{3+}-NOM complexes are not photoactive at the wavelength emitted by the lamp used (254 nm). In the photo-Fenton treatment, the presence of bicarbonate in the water had a negative effect on the rate of inactivation of *E. coli*. Bicarbonate/carbonate species are the most important scavengers in our water. These ions are able to remove a large amount of the $OH^{\bullet}$ generated in the process (Liu et al., 2012).

Comparing k_{obs} of photo-Fenton in lake water and Milli-Q water (Table 3), we observed that the process has a greater inactivation rate in Milli-Q water. The difference may be due to two factors: the first, inhibition by the presence of inorganic ions that may cause the precipitation of iron, light absorption, scavenging of $OH^{\bullet}$, or coordination to dissolved Fe^{3+} to form a less reactive complex and, second, the scavenging of hydroxyl radicals and UV-light absorption by the organic matter present in lake water. However, the advantage of Milli-Q water treatment is not very important because with short treatment times, the pH of the water does not decrease. The photoactive iron complexes are formed at low pH as reported in the last decade (Pignatello et al., 2006; Hug and Leupin, 2003; Feng and Nansheng, 2000).

3.2.3. E. coli *inactivation in artificial seawater*

Treatments with UV_{254} as light source have greater applicability in the disinfection of large volumes of water (e.g. ballast water of ships) due to its higher bacterial inactivation rate compared with the simulated sunlight. Furthermore, UV systems are much more compact and require relatively little space for installation, which is essential for their industrial application. For these reasons and trying to optimize several parameters of photo-Fenton in seawater, it was studied the effect of iron concentration, type of iron source or content of humic acids as organic matter source.

Several authors affirm that in waters with high concentrations of ions, such as seawater, photocatalytic treatments lose effectiveness due to scavenging processes of $OH^{\bullet}$ radicals (Grebel et al., 2010; Rincon and Pulgarin, 2004). So, to confirm this statement ten different systems were investigated. Table 3 shows the rates of inactivation that were obtained. In darkness, the concentration of *E. coli* did not change during the course of the experiment.

According to k_{obs}, the ranking for the different treatments in artificial seawater was: $Fe^{2+}/H_2O_2/NOM/UV_{254} > Fe^{2+}/H_2O_2/NOM/HCO_3^-/UV_{254} \approx H_2O_2/NOM/HCO_3^-/UV_{254} > UV_{254}$. As in Milli-Q water and Leman Lake water, H_2O_2/UV_{254} and photo-Fenton improved the UV light disinfection process. As in the previous results, bicarbonate ions had a negative role in the disinfection of *E. coli*.

Ultraviolet light k_{obs} was 0.0795 s^{-1}. Comparing the different types of water, the UV inactivation rate was found to decrease when increasing the solution salt concentration. Ions absorb light causing a protective effect on the bacteria (Spuhler et al., 2010; Rincon and Pulgarin, 2004). This was observed with the measurements of water absorbance for UV radiation at 254 nm.

The H_2O_2/UV_{254} and photo-Fenton treatments in artificial seawater showed lower inactivation rate than in Milli-Q water. However, the bacteria inactivation rates were similar to those obtained in lake water. Apparently, salinity does not have a major negative impact on the inactivation process.

In photo-Fenton experiments performed in the UV reactor with seawater the presence of NOM had no effect on the rate of inactivation. Tests were performed with and without natural organic matter, and the effect of humic acid addition as a source of organic matter was also studied. The rates of inactivation (k_{obs}) for the three cases were similar (Table 3). The complex formation between iron and NOM could have a positive effect on the system photo-Fenton (Pignatello et al., 2006). This effect was not observed when using a low pressure UV lamp as a light source (treatment duration of seconds).

Iron concentrations of 1, 3 and 5 mg Fe^{2+} L^{-1} were tested. Also, tests using Fe_2O_3 as iron source (1 mg L^{-1}) were conducted. With increasing iron concentration would be expected an improvement in the disinfection of *E. coli*. However, the rate of inactivation is similar to the three concentrations tested, even decreases slightly with increasing iron. At neutral pH, the iron species precipitated, increasing turbidity and turning the color of the water yellow-orange. Furthermore, the precipitated species are considerably less Fenton-reactive and do not re-dissolve readily (Pignatello et al., 2006). Adding iron can increase the absorption of UV light by non-photoactive iron complexes as well as the observed turbidity. Excess iron can have a protective role for bacteria. The results of photo-Fenton with Fe_2O_3 were similar to those obtained with Fe^{2+}.

The results of iron concentrations showed that in water at neutral pH, immediately after starting the experiment, the iron precipitates, being the dissolved iron fraction practically non-existent (Fig. 3). Iron evolution was similar in all photo-Fenton experiments performed. Under the simultaneous action of oxygen and H_2O_2, the Fe^{2+} added is quickly oxidized to Fe^{3+} and subsequently transformed in insoluble iron species (Morgan and Lahav, 2007; Jolivet at al., 2006). Therefore, from

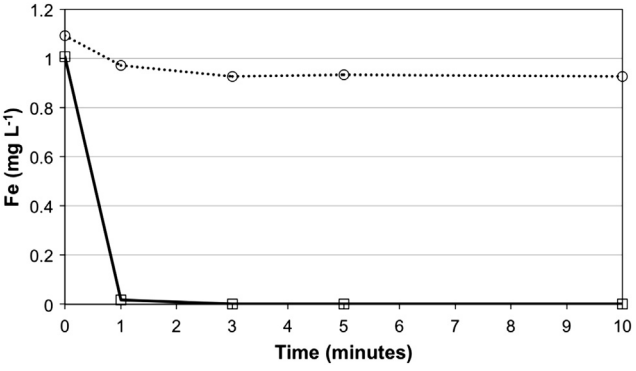

Fig. 3 – Variation of total iron (dashed lines) and dissolved iron (solid line) in the experiments.

the first step of the treatment the iron was not present in solution and the process was governed by a heterogenous photo-Fenton process. Recent studies have found clear improvements in the oxidation of organic compounds by heterogeneous photo-Fenton treatment at neutral pH. These studies raise the hypothesis that, at the surface of iron oxides, in a similar way to the homogenous photo-Fenton process, light accelerates the production of reactive species ($OH^{\bullet}$ or ferryl) from H_2O_2 by enhancing the recycling of Fe^{3+} to Fe^{2+} (González-Olmos et al., 2012).

3.2.4. *Post-irradiation effects in artificial seawater*

By the end of UV irradiation treatments, inactivated (dead), damaged (sub-lethal) and undamaged bacteria may be found in the water. These damaged cells can recover when kept in the dark. Even a small amount of repaired and survived cells can be sufficient for growth in the presence of easily assimilable organic carbon (Spuhler et al., 2010; Reed, 2004). The growth results of *E. coli* surviving after treatments in seawater are presented in Fig. 4.

With UV light alone was needed 9 min treatment to achieve complete inactivation of *E. coli* in seawater. 24 and 48 h after the end of treatment was observed growth of *E. coli* (Fig. 4I). The bacteria that have received sub-lethal damage are able to utilize repair processes. These may occur with light through enzymes like photolyase (Hallmich and Gehr, 2010), but this is possible also in the dark (Rincon and Pulgarin, 2007; Salcedo et al., 2007). In addition, the nutritional sources for bacteria growth as the NOM present in seawater and the organic material from dead bacteria, favor the growth processes.

In the H_2O_2/UV_{254} system, total inactivation was achieved with 7 min treatment. Bacterial reactivation was studied at 7 min and 9 min of treatment and in neither case was observed growth of *E. coli* after 24 and 48 h (Fig. 4. II). *E. coli* uses two cytoplasmic SOD isozymes to neutralize $O_2^{\cdot-}$ and peroxidase/catalase (Imlay, 2008) to neutralize external/internal H_2O_2. Equation (12) shows the decomposition H_2O_2 by catalase

$$2H_2O_2 + \text{cat} \rightarrow 2\,H_2O + O_2 \quad (12)$$

Despite the bacteria's repair mechanism, *E. coli* did not growth. The absence of bacterial growth may be explained by severe damage caused on bacteria with this treatment. Radicals produced in the process may cause greater damage than the UV light only. Furthermore, the presence of residual hydrogen peroxide after treatments (6.62 ± 0.78 mg L^{-1}) may prevent regrowth through some mechanisms: a) high levels of H_2O_2 inactivate the enzymes responsible for the defense of bacteria against oxidative stress, b) damage on the bacteria preventing the repair mechanism to function normally. Hydrogen peroxide deteriorate cell lipids, c) damage in the wall-cell allow peroxide to enter into the bacterial cell (Rincon and Pulgarin, 2007).

When photo-Fenton treatment was used, growth of surviving bacteria was not observed. As with H_2O_2/UV_{254} treatment, damage caused by radicals and the residual H_2O_2 prevent the growth. Moreover, residual H_2O_2 in the presence of Fe^{3+} ions preclude bacterial regrowth (Fig. 3. III). In the dark, the combination of Fe^{3+} ions and H_2O_2 produce $OH^{\bullet}$ via Fenton processes.

The bacterial growth results confirmed the photo-Fenton and H_2O_2/UV_{254} as potential treatments for disinfection of seawater. Disinfection by these methods was effective a long time after treatment and was not observed growth of surviving bacteria. This point is very important for possible industrial applications, such as treatment of ballast water.

Photo-Fenton and H_2O_2/UV_{254} treatments outperformed the UV treatment in two fundamental aspects, the greatest inactivation rate of *E. coli* and the absence of regrowth.

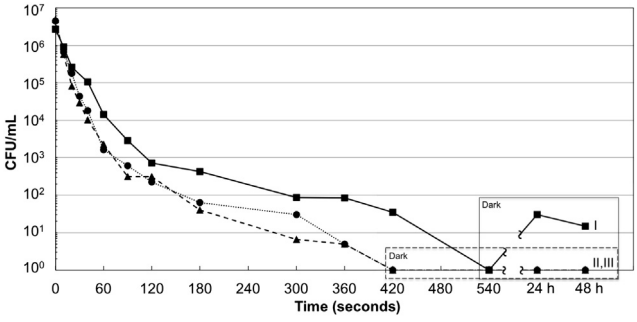

Fig. 4 – *E. coli* inactivation following three different treatments in artificial seawater (pH 7.5–8) and post-irradiation events. For bacterial growth of surviving bacteria, samples were taken after total inactivation and were placed in the dark 24 and 48 h. For each sample, remaining H_2O_2 was removed by catalase before plating. Markers represent the average of raw data during treatment and after 24 and 48 h when treatment was stopped. (■) UV_{254}; (●) 10 mg H_2O_2 L^{-1}/UV_{254}; (▲) 1 mg Fe^{2+} L^{-1}/10 mg H_2O_2 L^{-1}/UV_{254}. (I) Solid line box contains results on dark growth of surviving bacteria after UV_{254} treatment; (II, III) Box with dashed line shows the results of growth of surviving bacteria in darkness after H_2O_2/UV_{254} and photo-Fenton treatments.

4. Conclusions

The treatments showed some differences depending on the water used. In general, the bacterial inactivation rate decreased in the following order: Milli-Q > Leman Lake water ≈ Seawater. Highest disinfection rates were obtained in Milli-Q water due to the absence of inorganic ions. Inactivation rates obtained in Leman Lake water and seawater were similar despite the significant differences of salts concentration. Photo-Fenton and H_2O_2/UV_{254} treatments were effective in a wide range of salinity, showing a disinfection potential applicability in seawater.

The combination of light with Fe^{2+} and/or H_2O_2 (photo-Fenton system and H_2O_2/UV_{254}) in water at neutral pH was effective for bacterial inactivation, improving treatments using light alone. The treatments studied required low concentrations of reactives (1 mg L^{-1} Fe^{2+} and/or 10 mg L^{-1} H_2O_2) to achieve high levels of bacteria inactivation. Adding more amount of Fe^{2+} did not increase the effectiveness of photo-Fenton. This is important for the economic viability of the treatments.

In seawater, photo-Fenton treatment was affected by natural organic matter (NOM) depending on the light source used.

In the experiments with suntest, the organic matter had a beneficial effect on the disinfection, increasing the inactivation rate more than 30% in comparison with the water without NOM. However, the positive effect of NOM was not observed when using UV_{254} light.

In this work, the most influential inorganic ion on treatments has been the bicarbonate. Bicarbonate ions competing for hydroxyl radicals that are formed in H_2O_2/UV_{254} and photo-Fenton treatments. The scavenging effect of HCO_3^- at a concentration of 100 mg L^{-1}, decreased the *E. coli* inactivation rate about 20% compared to water without bicarbonate. Despite the presence of bicarbonate, photo-Fenton and H_2O_2/UV were effective in *E. coli* disinfection, improving the inactivation using only light.

Experimental results showed growth of surviving *E. coli* 24 and 48 h after treatment with UV light alone in seawater. However, growth of surviving bacteria was not observed after photo-Fenton and H_2O_2/UV_{254} treatments. The AOPs can cause more severe damage that ultraviolet light preventing the bacterial growth. Moreover, the presence of residual peroxide and/or iron after treatments can help to limit the growth of bacteria. In the dark, the presence of iron ions and residual H_2O_2 may produce $OH^{\bullet}$ via Fenton processes.

This work showed the potential for the application of H_2O_2/UV_{254} and photo-Fenton as disinfection treatment of marine water. Moreover, at industrial scale, the costs of these treatments might not be high, because only low concentrations of the reagent would be needed. It could be tested at pilot scale level to check if those treatments are a real alternative to standard water disinfection technologies.

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