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Technical Note

Dramatic enhancement of solar disinfection (SODIS) of wild *Salmonella* sp. in PET bottles by H_2O_2 addition on natural water of Burkina Faso containing dissolved iron

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ABSTRACT

Disinfection of surface water containing dissolved iron (0.3 mg L^{-1}) at natural neutral pH (~7.5) was carried out via solar disinfection (SODIS) treatment in PET bottles with H_2O_2 (10 mg L^{-1}). Wild coliforms and *Salmonella* sp. were monitored for 6 h of sunlight irradiation and 72 h of dark post-treatment period. In our conditions, SODIS treatment could not avoid *Salmonella* sp. re-growth during dark storage, meanwhile the addition of 10 mg L^{-1} of H_2O_2 showed a strong enhancement of the inactivation rate without any re-growth of both bacteria. Finally, total coliforms (*Escherichia coli* included) demonstrated to be an inappropriate indicator for monitoring bacterial contamination in water during solar disinfection processes.

1. Introduction

Solar water disinfection in bottles, known as solar disinfection (SODIS) process, is a widely known, simple and low-cost treatment to improve water quality for drinking purposes (Wegelin et al., 1994). Nevertheless, a complete SODIS treatment requires long-time of solar exposure (around 6 h), which can increase during cloudy days (up to 48 h), followed by an eventual storage to cool the water (Oates et al., 2003). Bacterial inactivation by SODIS treatment is the consequence of two synergic factors: (i) the effect of UV-A between 320 and 400 nm and the visible irradiation between 400 and 490 nm, and (ii) the increasing temperature which must reach at least 45 °C (Wegelin et al., 1994; Sommer et al., 1996; McGuigan et al., 1998).

SODIS treatment for water disinfection has often been evaluated through the monitoring of *Escherichia coli* inactivation as the bacterial indicator. However, Berney et al. (2006) have found that this microorganism is one of the most sensitive to the effect of solar irradiation and the increase of temperature. For this reason, it would not represent a suitable indicator of SODIS performance for drinking water bacterial inactivation. On the other hand, *Salmonella typhimurium* an extremely pathogen microorganism, seems to be more resistant to SODIS treatment (Smith et al., 2000; Winfield and Groisman, 2003; Berney et al., 2006).

Recent studies have addressed the potential of additives to the enhancement of SODIS treatment using reactive oxygen species (ROS) production such as ·OH radical, H_2O_2, superoxide radical anion ($\cdot O_2^-$) and singlet oxygen ($^1O_2$) (Fisher et al., 2008). The ROS production for bacterial inactivation in natural waters through helio-photocatalytic process has also been explored in compound parabolic collector (CPC) solar photoreactors with TiO_2 as photo-catalyst (Rincòn and Pulgarin, 2004, 2006, 2007a,b; Fernández-Ibáñez et al., 2009; Van Grieken et al., 2009). However, solar photo-Fenton process seems to be the most promising process for ROS production and bacteria inactivation at neutral pH (Rincòn and Pulgarin, 2007a,b; Moncayo-Lasso et al., 2008, 2009).

Fenton process (dark) uses Fe^{II}/Fe^{III} and hydrogen peroxide. In the presence of dissolved iron ions the H_2O_2 is rapidly and efficiently decomposed producing ·OH radicals (Eqs. (1) and (2)). Under solar irradiation, photons up to 580 nm activate Fe^{III} organo-complexes (and also aqua-complexes) by photo-reduction and increase the ·OH production (Eq. (3)) (Sun and Huang, 1993; Bandara et al., 1996; Pignatello et al., 2006; Rincòn and Pulgarin, 2007a).

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^- + HO^\cdot \quad (k = 63\ M^{-1}\ s^{-1}) \tag{1}$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HO_2^\cdot + H^+ \quad (k = 3.1 \times 10^{-3}\ M^{-1}\ s^{-1}) \tag{2}$$

$$Fe^{3+} + H_2O + h\nu \rightarrow Fe^{2+} + HO^\cdot + H^+ \tag{3}$$

Natural surface water in the Sahelian areas contains large quantities of iron as it flows on ferruginous substrates (Ben Yahmed, 2005). This characteristic could consecutively lead to homoge-

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Table 1
Physico-chemical and bacteriological parameters of the urban dam No. 3 in Ouagadougou. A composite of 20 L with 5 L in upper waters, 5 L in middle water and 10 L downstream in the deeper part, was sampled.

Parameter	Value
T (°C)	31.1
pH	7.54
Turbidity (NTU)	26
Bicarbonate (mg L^{-1})	159
Iron total (mg L^{-1})	0.3
Copper total (mg L^{-1})	0
Conductivity (μS cm^{-1})	385
TOC (mg L^{-1})	7.4
Absorption at 254 nm	0.225
Total coliforms (CFU mL^{-1})	3.4×10^2
Salmonella sp. (CFU mL^{-1})	6.8×10^2

neous photo-Fenton processes under solar light when adding H_2O_2. This study aims to evaluate in field conditions the comparative effect at natural neutral pH of (i) the addition of H_2O_2 in the dark, (ii) SODIS process as it is known, and (iii) SODIS adding H_2O_2 on wild coliforms and *Salmonella* sp. contained in water of the urban dams in Ouagadougou, Burkina Faso.

2. Experimental

2.1. Reagents and materials

Hydrogen peroxide at 30% AnalaR Normapur from VWR was added into plastic bottles at the beginning of the solar bacterial inactivation. Common Polyethylene Terephtalate (PET) bottles, which initially contained mineral water were bought and used the same day. Surface water was sampled 1 h before experimentations at the third urban dam of Ouagadougou. Water characteristics are summarized in Table 1. As prescribed by SODIS code of use, water turbidity does not exceed 30 NTU (EAWAG/SANDEC, 2002).

2.2. Analytical methods

Hydrogen peroxide evolution was followed by strip Merckoquant (Merk) test with a detection limit around 0.5 mg L^{-1}. Temperature and pH were measured with a Universal pocket meter WTW 340i equipped with a WTW SenTix 41-3 probe. The water natural pH was 7.5 and was not modified during the experiments. Turbidity was determined by the nephelometric method described in APHA standards methods (APHA, 2001). Total iron was evaluated with the HACH FerroVer method using a HACH 2010 spectrophotometer and UV absorbance at 254 nm with a Biomate 3 model. Total organic compounds (TOC) were monitored using a Shimadzu 500. Before measuring, each sample was stored at 4 °C after addition of sodium bisulfite to eliminate the H_2O_2 and stop any Fenton and photo-Fenton reaction. Experimental configuration permitted to monitor bacterial inactivation twice (in two different bottles) without H_2O_2 addition (classic SODIS) and with 10 mg L^{-1} of H_2O_2 treatments. Average values of bacterial concentration are the media of three determinations.

2.3. Photo-catalytic procedures

Thirteen new PET bottles of 1.5 L were used in parallel and filled with natural surface water from urban dam No. 3, Ouagadougou. The experimental configuration is summarized in Fig. 1. As proposed by SODIS designers, the exposed bottles were laid down perpendicularly to the incident solar irradiation on a corrugated iron sheet for 6 h (EAWAG/SANDEC, 2002). UV-A solar radiation intensity during the experiments was monitored with an ACADUS 85 UV radiometer. The average and maximum UV-A intensity were 28.6 and 36 W m^{-2} respectively. Temperature in SODIS bottles reached 48.2 °C; it reached 41.6 °C in bottles covered with aluminium sheets (dark controls under heating), and 38.1 °C in reference solution in the dark.

Dam water was sampled 1 h before the irradiation experiment, which started at 10:00 a.m. During the experiment, each bottle was agitated and sampled at the start and after 0.5, 1, 1.5, 2, 3, 4, 5 and 6 h. Then, the bottles were stored in the dark at 37 °C and sampled 24, 48 h and 72 h after the end of the experiment to monitor bacterial re-growth during dark cooling.

Wild bacterial strains naturally present in surface water were followed during experiments. Without filtering, samples were plated on Chromocult Coliform agar (Merck). This selective agar allows the identification of different kinds of bacteria colonies through different colours: total coliforms salmon to red colour and *Salmonella* colourless. Colonies were counted after 24 h at 37 °C in the dark. Bacterial trends were worked out with the two main populations: total coliforms and *Salmonella* sp.

3. Results and discussion

3.1. Effect of H_2O_2 addition under dark conditions on total coliforms and Salmonella sp. (Fig. 1, PET bottle Nos. 5, 6, 7, 8, 12 and 13)

Fig. 2a and b shows that by addition of 10 mg L^{-1} H_2O_2, both total coliforms and *Salmonella* sp. were inactivated without regrowth if a final temperature of 41.6 °C was reached (bottle Nos.

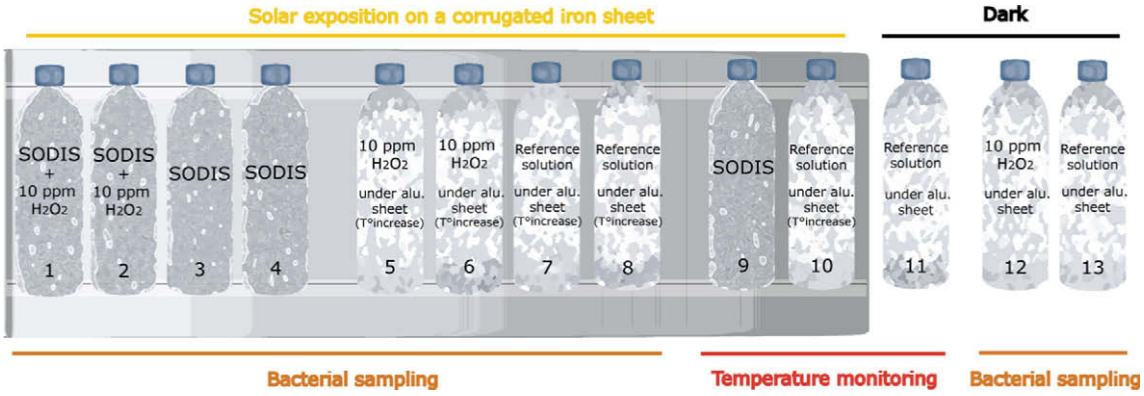

Fig. 1. Experimental configuration to follow bacterial trends and temperature for 6 h of solar irradiation. Bacterial number and temperature were monitored in different bottles in order to avoid contamination.

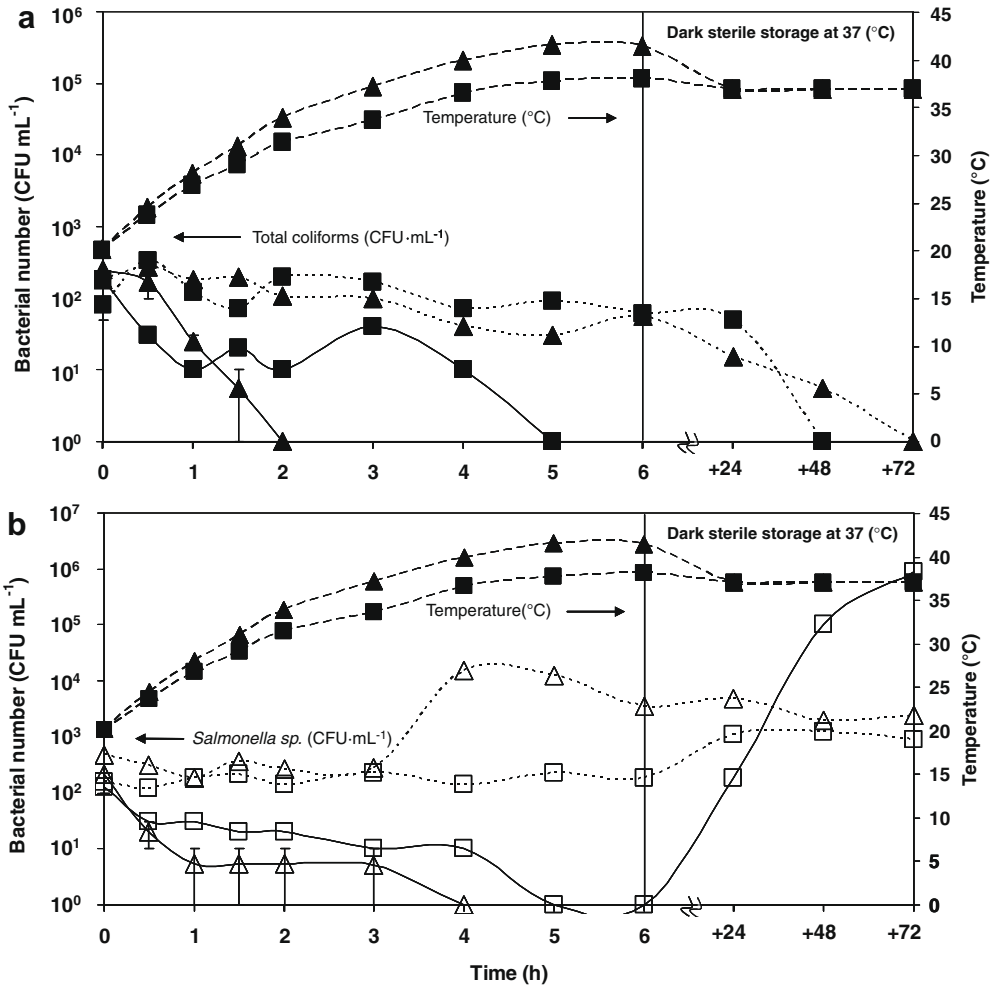

Fig. 2. Bacterial inactivation under dark conditions in presence (10 mg L^{-1}) and absence of H_2O_2. (a) Total coliforms; (··▲··) without H_2O_2 under aluminium sheet and (··■··) at ambient temperature, (-▲-) with H_2O_2 under aluminium sheet and (-■-) at ambient temperature. (b) *Salmonella* sp.; (··Δ··) without H_2O_2 under aluminium sheet and (··□··) at ambient temperature, (-Δ-) with H_2O_2 under aluminium sheet and (-□-) at ambient temperature. Final temperature monitoring reached in bottles under aluminium sheet (–▲–) and in dark bottles (–■–). [H_2O_2] was below 3 mg L^{-1} after 6 h and below 1 mg L^{-1} after 24 h of sterile storage. pH = 7.5. Minimum and maximum values of bacterial measurements are reported on each curves and the trend is the mean value.

5 and 6, which were exposed to the sun but protected with aluminium foil sheets). However, when the maximum temperature reached only 37.8 °C (bottle No. 12), re-growth of *Salmonella* sp. was observed (Fig. 2b). H_2O_2 was mainly consumed after 6 h, since its concentration was approximately below 2 mg L^{-1} and it was near 0.5 mg L^{-1} after 24 h.

Furthermore, total coliforms (*E. coli* included) seem to be the weakest microorganisms since even without H_2O_2 addition and ambient temperature (max temperature: 37.8 °C), they were not able to survive for 72 h of storage. The monitoring of total coliforms in reference solutions confirms the difficulty of *E. coli* to survive in non-host environments such as surface water (Winfield and Groisman, 2003).

Fig. 2a and b shows that the addition of H_2O_2 in the dark leads to a noticeable bacteria inactivation after 2 and 4 h for total coliforms and *Salmonella* sp. respectively. Several studies asserted that a significant toxic effect of hydrogen peroxide on bacteria is not observable at concentration lower than 15 mg L^{-1} in the dark (Rincòn and Pulgarin, 2004, 2006; Moncayo-Lasso et al., 2009). Nevertheless, extracellular hydrogen peroxide is known to weaken cellular walls, making bacteria more sensitive to oxidative stress and could explain the inactivation observed at lower concentrations (Imlay and Stuart, 1986; Hyslop et al., 1995; Rincòn and Pulgarin, 2006). Then, H_2O_2 is not directly considered as toxic for bacteria, but its dissociation products like ·OH radicals and superoxide ($HO_2^{\cdot}/\cdot O_2^-$) are active oxygen species, which are toxic for the cells (Halliwell and Gutteridge, 1984; Labas et al., 2009). They can induce reactions on lipids, proteins and DNA producing a detrimental effect on the microorganisms (Rincòn and Pulgarin, 2006; Moncayo-Lasso et al., 2009). Moreover, the simultaneous presence of dissolved iron complexes at neutral pH and H_2O_2 could induce Fenton reactions leading to an additional formation of ·OH radicals. Actually, iron organo-complexes formed with natural organic matter (NOM) are potentially able to act as catalyst because of the homogeneous Fenton-like system at neutral pH already carried out in previous studies (Arslan et al., 2000).

3.2. Effect of H_2O_2 addition under solar light exposition on total coliforms and Salmonella sp. (Fig. 1, PET bottle Nos. 1, 2, 3 and 4)

Fig. 3a and b shows SODIS experiments under UV-A average intensities around 28.6 W m^{-2}. Both total coliforms and *Salmonella* sp. were inactivated after 3 and 4 h respectively, under solar exposure, which raised temperatures within the bottles up to 45–48.2 °C. In spite of the fact that *Salmonella* sp. reached 0 cultivable cell for 4 h of SODIS treatment and that the solar exposition was

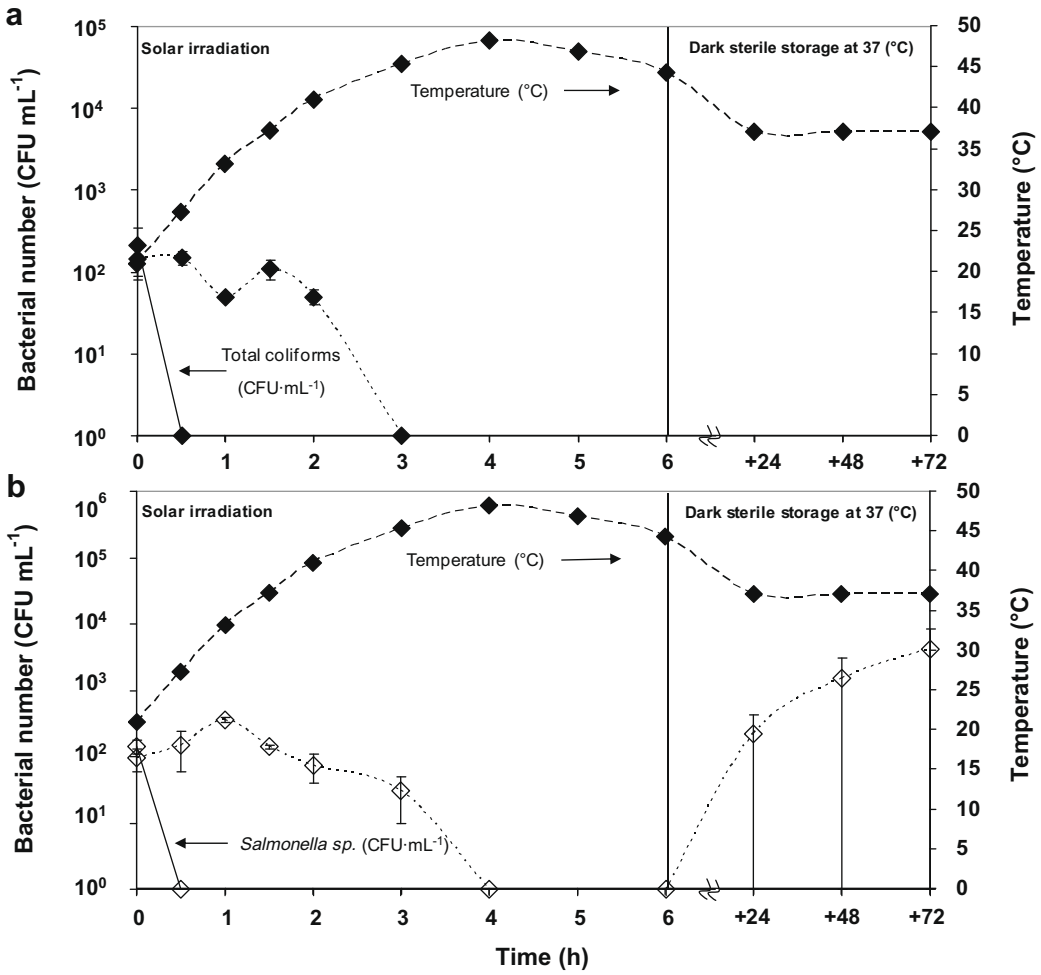

Fig. 3. Bacterial inactivation under solar light in presence and absence of H_2O_2. (a) Total coliforms. (··♦··) SODIS and (-♦-) SODIS plus 10 mg L^{-1} of H_2O_2. (b) *Salmonella* sp. (··◇··) SODIS and (-◇-) SODIS plus 10 mg L^{-1} of H_2O_2. PET bottles were exposed from 10:00 to 16:00 h under an average irradiance of 28.6 W m^{-2} (maximum of 36 W m^{-2}). Temperature monitoring reached in SODIS bottles (–♦–). [H_2O_2] was below 2 mg L^{-1} after 6 h and below 0.5 mg L^{-1} after 24 h of sterile storage. pH = 7.5.

prolonged during an additional 2 h (Fig. 3, trace –◇–), a sharp re-growth was observed as soon as the bottles were stored in the dark. In contrast, total coliforms (and *E. coli*) did not regrow.

When 10 mg L^{-1} of H_2O_2 were added into PET bottles exposed to the solar light, a dramatic enhancement of bacterial inactivation rate was observed leading to total inactivation in less than 30 min without further re-growth after 3 days of dark storage for both total coliforms and *Salmonella* sp. As in the dark experiments, H_2O_2 concentration was decreased to 2 mg L^{-1} after 6 h of solar irradiation. This concentration was below 0.5 mg L^{-1} after 24 h of storage.

It is known that bacterial inactivation in PET bottles is induced by the synergetic effect of solar irradiation and temperature above 45 °C (Wegelin et al., 1994). Indeed, bacteria are very sensitive to UV-B wavelengths (280–315 nm) because their DNA can absorb this radiation, which produces photochemical changes such as thymine bases dimerization, affecting negatively the normal replication of DNA. But UV-B is mainly absorbed through PET bottles (until ~310 nm) (McGuigan et al., 1998). Furthermore, the UV-A irradiation (315–400 nm) and visible light, mainly blue light (400–500 nm), produce also a detrimental effect on the bacteria, chemically altering some proteins (Harris et al., 1987). Solar disinfection of natural waters can also be positively affected by its NOM content.

Indeed, humic substances and organic chromophores which are part of NOM, act as photo-sensitizers. Under UV–Vis irradiation, these photo-sensitizers can generate photochemical processes, leading the production of ROS such as singlet oxygen ($^1O_2$), superoxide ($HO_2^{\cdot}/O_2^{\cdot-}$), and ·OH radicals, which are toxic to the cells (Paul et al., 2004; Canonica, 2007; Moncayo-Lasso et al., 2008) (Fig. 4). However, it has been previously described that subsequent bacterial reactivation can occur when the microorganisms are exposed to more favourable environmental conditions after illumination, via dark repairing processes (Leyer and Johnson, 1993; Rincòn and Pulgarin, 2007a). Photoreactivation of bacteria exposed to solar light has also been suggested to explain the recovering viability after a *viable but non-cultivable* (VBNC) state induced by solar illumination (Liltved and Landfald, 1996).

This is why SODIS process needs the synergic action of both temperature and light to inactivate bacteria. However, in our conditions of study, 4 h of SODIS treatment did not permit to avoid the subsequent re-growth in the dark of *Salmonella* sp. (Fig. 3b) that exhibits resistance to simultaneous solar light irradiation and temperatures rising up to 48.2 °C. In this case, SODIS might also induce a VBNC state, which corresponds to a damaged state recoverable under more favourable conditions (Rincòn and Pulgarin, 2006, 2007a).

3.3. Possible reaction pathways with H_2O_2 addition under solar exposition

Solar irradiation and hydrogen peroxide could lead to photocatalytic reactions depending of the presence of transient metals

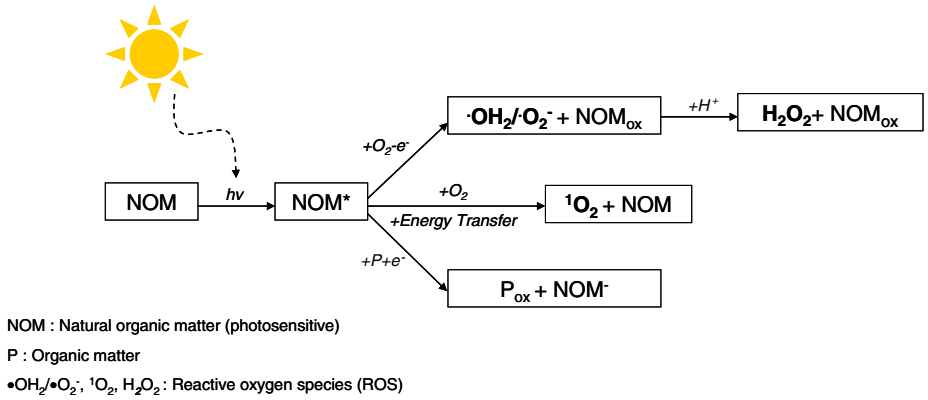

Fig. 4. Potential reactions of photosensitive NOM promoting an oxidation process through ROS production (Moncayo-Lasso et al., 2008, 2009; Canonica, 2007; Paul et al., 2004).

such as iron (0.3 mg L^{-1} in our case) and copper able to act as photo-catalysts in so-called solar photo-Fenton reactions which generate ·OH (Eqs. (1)–(3)).

At our natural pH of 7.5, iron species in an aqueous solution are principally present as dissolved organic- and aqua-complexes of ferric ions (Pignatello et al., 2006; Moncayo-Lasso et al., 2008). Under visible light, the photo-reduction of central ferric ions via a ligand-to metal-transfer reaction also leads to the production of ·OH and organic (L·) radicals as follows (Lee and Yoon, 2004; Malato et al., 2009):

$$[Fe^{3+}L] + h\nu \rightarrow [Fe^{3+}L]^* + Fe^{2+} + \cdot L \quad (4)$$

$$[Fe(H_2O)^{3+}] + h\nu \rightarrow Fe^{2+} + \cdot OH + H^+ \quad (5)$$

$$[Fe(OH)]^{3+} + h\nu \rightarrow Fe^{2+} + \cdot OH \quad (6)$$

After photo-dissociation, generated ferrous ions could initiate a new Fenton reaction producing additional quantities of highly reactive ·OH radicals in presence of H_2O_2 (Eqs. (1)–(3)) at natural neutral pH as observed by Vermilyea and Voelker (2009). In Fig. 3a and b, total coliforms and *Salmonella* sp. exposed to solar light and in presence of 10 mg L^{-1} of H_2O_2 are inactivated in a significantly shorter period than under the simple solar exposition (SODIS). Certainly, in the natural presence of iron ions, H_2O_2 leads to an extra production of toxic ROS and, consecutively, to a complete inactivation without further dark re-growth, even for a more resistant *Salmonella* sp. This observation is fundamental for a practical application of this technique. In this case, the natural presence of 0.3 mg L^{-1} of iron could be considered as sufficient to generate photo-Fenton reaction, a potential way of bacterial inactivation. Similar experiments have been conducted in a laboratory batch photoreactor using deionized water and at pilot scale in a CPC using natural surface water of Leman Lake by Rincòn and Pulgarin (2006, 2007a,b). In both cases, 0.3 mg L^{-1} of iron and 10 mg L^{-1} of H_2O_2 were enough to inactivate total *E. coli* charge and maintain bacterial water quality for 24 h in the dark (also called as efficient disinfection time after 24 h or EDT_{24}). In our case, EDT of 72 h was reached in a SODIS system treating water naturally containing 0.3 mg L^{-1} of dissolved iron with the addition of only 10 mg L^{-1} of H_2O_2.

4. Conclusions

SODIS process was strongly enhanced by the addition of hydrogen peroxide in natural waters containing dissolved iron. Re-growth of bacteria and especially *Salmonella* sp. was inhibited under these conditions. This could be attributed to the presence of iron ions in solution, giving rise to a homogeneous photo-catalytic reaction, the photo-Fenton reaction. The larger production of ROS could then increase the oxidative stress and subsequent death of bacteria.

It is important to notice that H_2O_2 persists on concentrations below 2 mg L^{-1} after the solar treatment. Moreover, previous researches have demonstrated that helio-photo-Fenton process also reduces the organic matter present in water without formation of halomethanes DBPs (Ribordy et al., 1997). However, further experiments must be carried out in order to clarify the role of naturally present iron and NOM in this process. The obtained results also suggest that total coliforms (including *E. coli*) is not a suitable indicator for monitoring and testing the efficiency of a solar disinfection process such as SODIS since this microorganism seems to be more sensitive than other human pathogens. Therefore, *Salmonella* sp. could be a better option because of its higher resistance to solar treatment.

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