Disinfection of Drinking Water by Helio-photocatalytic Process in Sahelian Zone: Chemical, Physical and Technological Aspects

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DEDICATION

A toi papa

Janvier 2007 est déjà loin mais tu me manques toujours.

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ABSTRACT

The field trials of the present study were carried out at the International Institute for Water and Environmental Engineering (2iE) at Ouagadougou – Burkina Faso, West Africa. It was financially supported by Swiss Agency for Development and Cooperation (SDC). The project aimed at contributing to the improvement of solar-based treatment processes of drinking water at underprivileged point-of-use level in developing countries. Its general objective was to evaluate the feasibility of solar-assisted photo-Fenton disinfection of wild enteric bacteria in several water sources characteristically different, under different reactors and seasons.

First of all, the evaluation of the possibility of using natural iron present in water as the catalyst of the photo-Fenton reaction was carried out. Afterward, the experimentation of the efficiency of the photo-Fenton process was made in treating small volume (Pet bottles, 1.5 L) and large volume (CPC, 25-50 L) of water as well as the evaluation of the post irradiation effect of the treatment (regrowth). The effect of nitrogenous compounds and the influence of the solar radiation parameters (irradiance vs. dose) on the efficiency of the processes were also evaluated. The photo-Fenton disinfection was evaluated in alkaline surface water and in well water sources during the raining season. Finally, the contribution of this study to the general comprehension of the mechanistic pathway of the photo-Fenton process was developed.

Methodologically, the inactivation of the wild enteric bacteria (total coliforms/*E. coli* and *Salmonella* spp.) in natural water containing dissolved ($Fe^{2+/3+}$) and solids irons forms (e.g. iron oxides) was conducted by solar disinfection (*hv*) and the contribution of the photo Fenton reactant ($Fe^{2+,\ 3+}/H_2O_2/hv$) after the addition of hydrogen peroxide (H_2O_2). The natural iron content of the well water (pH 5.4 or 6.3±0.1) was 0.07 mg/L, during the experiments the chemically added iron content was 0.6 mg/L. In the results, it was noticed that the photo-

Fenton systems carried out with added iron ($H_2O_2/Fe^{2+}/hv$) or natural iron content of the water (H_2O_2/hv) has led to total inactivation of both enteric bacteria. No enteric bacteria re-growth was observed in water treated by photo-Fenton one week after the treatment. The photo-treatment of well water (25 L) under direct solar radiation in the compound parabolic collector (CPC) has given the opportunity to evaluate the effects of solar radiation parameters on the efficiency of the processes. Significant influence of the solar irradiance ($W.m^{-2}$) and not of the dose ($Wh.m^{-2}$) was noticed during both photo-treatment methods

The photo-disinfection of natural alkaline surface water (pH 8.6±0.3) in the CPC was efficiently carried out under direct solar radiation. Despite the alkalinity of the water, the photo-Fenton disinfection was proven to take place. None of the enteric bacteria strains, totally inactivated by photo-Fenton, recovered their viability during the 24 hours of subsequent dark storage. The water was still free of pathogen one week after the photo-Fenton treatment. The oxido-reduction of nitrates and nitrites and the oxidation of ammonia were recorded during both photo-disinfection process (photo-Fenton and solar disinfection). The evaluation of the efficiency of the photo-disinfection during the raining season has brought out the negative impact of the weather variations of this season on the processes. The dilution of the water by rainwater highly affected their chemical composition. Very low iron contents comparatively to the ones recorded during the summer season were found in the water. In a CPC, 25 L of water were subjected during 4 h to both photo-disinfecting process and none of them have shown the total inactivation of both wild enteric bacteria (total coliforms/*E. coli* and *Salmonella* spp.) strains involved in the treatment.

Keywords: *Compound Parabolic Collector, Disinfection, Drinking Water, Enteric bacteria, H_2O_2, Inactivation, Photo-Fenton, Salmonella* spp.

RESUME

Les tests sur le terrain de la présente étude ont été réalisés à l'Institut International d'Ingénierie de l'Eau et de l'Environnement (2iE) à Ouagadougou - Burkina Faso, Afrique de l'Ouest. Ce projet de thèse été subventionné par la Direction du Développement et de la Coopération Suisse (DDC). Le projet avait pour but de contribuer à l'amélioration de la potabilisation de l'eau par des procédés de désinfection solaire au niveau communautaire dans les zones défavorisées des pays en développement. Et comme objectif principal l'évaluation de la faisabilité de la désinfection photo- Fenton sous irradiation solaire directe des bactéries entériques sauvages de plusieurs sources d'eau de caractéristiques différentes, sous différents réacteurs et à différentes saisons.

L'évaluation de la possibilité d'utiliser le fer naturel présent dans l'eau comme catalyseur de la réaction photo-Fenton a été effectuée en premier lieu. Suivie de l'évaluation de l'efficacité du processus de photo-Fenton dans le traitement de petit volume (bouteilles en PET, 1,5 L) et de grand volume (CPC, 25-50 L) d'eau, ainsi que de la détermination de l'efficacité du traitement par des tests post-irradiation (évaluation de la régénération des bactéries). L'effet des composés azotés et de l'influence des paramètres du rayonnement solaire (irradiance vs. dose) sur l'efficacité des processus de traitement a également été évalué. Par la suite, la désinfection photo- Fenton a été évaluée dans les eaux de surface alcaline et dans les sources d'eau de puits au cours de la saison des pluies. Enfin, la contribution de cette étude à la compréhension générale du mécanisme du processus de photo-Fenton a été développée.

Méthodologiquement, l'inactivation des bactéries entériques sauvages (coliformes totaux / *E. coli* et *Salmonella* spp) dans l'eau naturelle contenant du fer dissout ($Fe^{2+/3+}$) et les formes solides de fer (e.g. oxydes de fer) a été réalisée par désinfection solaire (*hv*) avec la contribution du réactif photo-Fenton ($Fe^{2+,3+}/H_2O_2/hv$) après ajout de peroxyde d'hydrogène (H_2O_2).

Au cours des expériences, la teneur en fer naturel de l'eau de puits (pH 5,4 ou 6,3 ± 0,1) était de 0,07 mg/L, tandis que la teneur en fer ajouté était de 0,6 mg/L. Des résultats, on note que les systèmes photo-Fenton réalisés avec du fer ajouté ($Fe^{2+}/H_2O_2/hv$) ou la teneur en fer naturel de l'eau (H_2O_2/hv) ont conduit à une inactivation totale des deux bactéries entériques. Aucune recroissance de bactéries entériques n'a été observée dans les eaux traitées par photo-Fenton une semaine après le traitement. Le photo- traitement de l'eau de puits (25 L) dans le collecteur parabolique compact (CPC), sous rayonnement solaire direct a permis d'évaluer les effets des paramètres du rayonnement solaire sur l'efficacité des processus. L'influence notable de l'irradiance solaire ($W.m^{-2}$) et non de la dose ($Wh.m^{-2}$) a été observée pendant les traitements avec les deux processus de photo-traitement (solaire et photo-Fenton).

La photo- désinfection d'eau de surface naturellement alcaline (pH 8,6 ± 0,3) dans le CPC a été efficacement réalisée sous rayonnement solaire direct. Le processus de désinfection par photo-Fenton a pu avoir lieu malgré l'alcalinité de l'eau. Aucune des souches de bactéries entériques, totalement inactivées par photo- Fenton, n'a pu retrouver ses capacités de viabilité au cours des 24 heures de stockage dans l'obscurité après le traitement. L'eau est resté saine, sans de pathogènes une semaine après le traitement photo- Fenton. L'oxydo- réduction des nitrates et des nitrites et l'oxydation de l'ammoniac ont été enregistrées durant les deux processus de photo-désinfection (solaire et photo-Fenton). L'évaluation de l'efficacité de la photo-désinfection pendant la saison des pluies a mis en évidence l'impact négatif des variations climatiques de cette saison sur les processus. La dilution de l'eau par l'eau de pluie a affecté significativement leur composition chimique. De très faibles teneurs en fer comparativement à celles enregistrées au cours de la saison estivale ont été trouvées dans l'eau. Aucune des souches de bactéries entériques sauvages (coliformes totaux/*E. coli* et *Salmonella* spp.) photo-traitées dans le CPC (25 L) pendant 4 h n'a été totalement inactivée.

Mots-clés: *Collecteur parabolique Compact, Désinfection de l'eau potable, Bactéries entériques, H_2O_2, Inactivation, Photo-Fenton, Salmonella spp.*

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AOP: Advanced Oxidation Processes

CPC: Compound Parabolic Collector/collecteur parabolique compact

L: liter

CFU/ml: Colony Forming Units per milliliter

NTU/FTU/FNU: Nephelometric turbidity units/Formazin Turbidity Units/Formazin Nephelometric Units

spp. Species

UV: Ultraviolet

Min: minutes

mg/L: milligrams per liter (ppm)

h: hours

EDT: Effective disinfection time

$W.m^{-2}$: Watt per square meter

$Wh.m^{-2}$: Watt hours per square meter

SODIS: Solar disinfection

NOM: Natural organic matter

JMP: Joint Monitoring Program for Water Supply and Sanitation

UNICEF: The United Nations Children's Fund

WHO: World Health Organization

MDG: Millennium Development Goal

1. INTRODUCTION AND OVERVIEW

1.1. DRINKING WATER CRISIS AND HOUSEHOLD WATER TREATMENT TECHNOLOGIES

Inadequate access to safe drinking water is a major cause of morbidity and mortality in developing countries (Hunter, 2009). The target date for the Millennium Development Goals (MDGs) is close to be delivered according to the Joint Monitoring Program (JMP) for Water Supply and Sanitation, but over 768 million people were still estimated living without access to safe source for drinking-water in 2011, including 185 million who relied on surface water to meet their daily drinking-water needs (UNICEF and WHO, 2013). If this situation remains unchanged, these numbers will remain unacceptably high in 2015. 605 million people will be without an safe drinking water source and 2.4 billion people will lack access to proper sanitation facilities (UNICEF and WHO, 2013). Sub-Saharan Africa and Oceania have the lowest drinking water

coverage (Fig. 1.1.). Efforts are needed to reduce urban-rural disparities and inequities associated with poverty, to dramatically increase coverage in countries in sub-Saharan Africa and Oceania. To promote global monitoring of drinking water quality, to bring sanitation 'on track' and to look beyond the MDG target towards universal coverage (UNICEF and WHO, 2012).

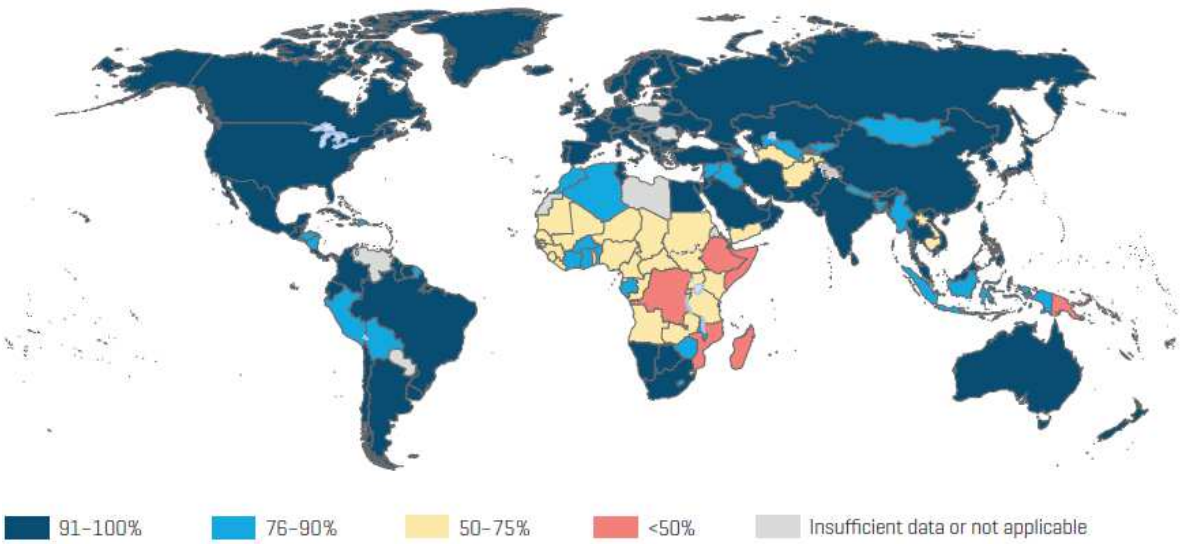

Figure 1.1: Proportion of the population using improved drinking-water sources in 2011: sub-Saharan Africa and Oceania has the lowest coverage. Source: (UNICEF and WHO, 2013).

Improving the sustainability of household water treatment (HWT) approximately 100 L or point-of-use water treatment (approximately 1000 L, community level), could significantly help solving the problems of poor quality drinking water in poor communities in developing countries. Sobsey (2002) described and detailed the functional and practical principle of several technologies for treatment of household water. The technologies with the aim to improve the microbial quality of household water and reduce waterborne disease include a number of physical and chemical treatment methods (Fig. 1.2.). The physical methods, include boiling, heating (fuel and solar), settling, filtering, exposing to the UV radiation in sunlight, and UV disinfection with lamps. The chemical methods include coagulation-flocculation and precipitation, adsorption, ion exchange and chemical disinfection with germicidal agents (primarily chlorine). Today, chlorine is the

most widely applied and most efficient method, but it has some disadvantages (e.g. availability, toxic byproducts formation).

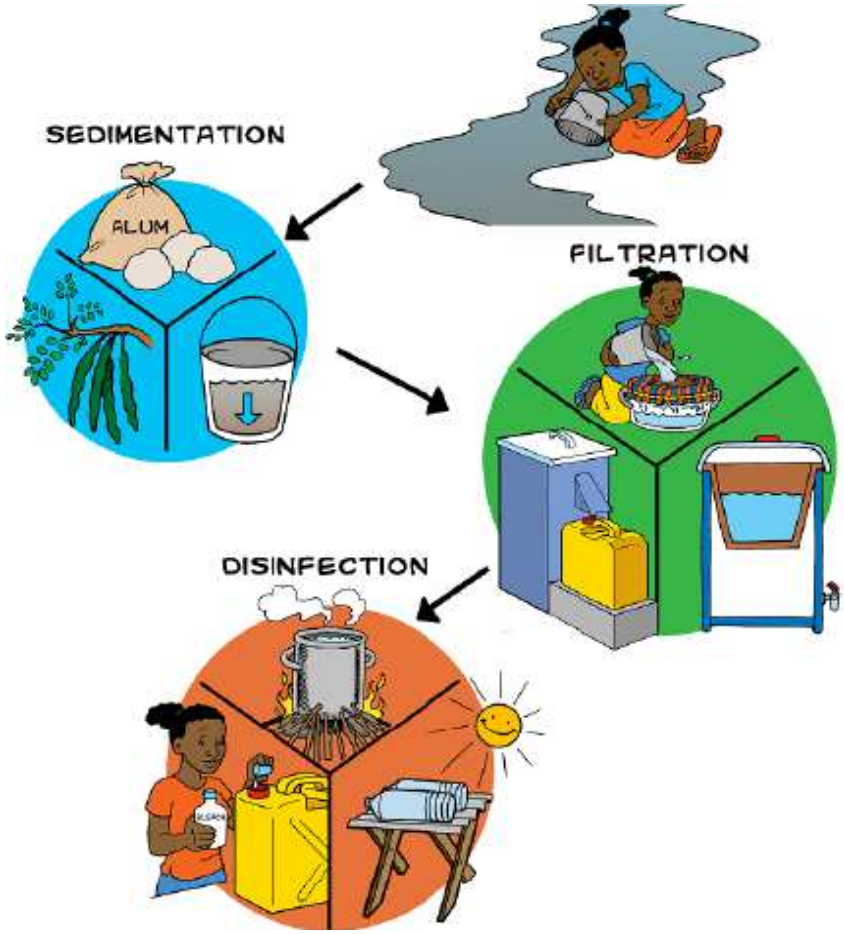

Figure 1.2: Household water treatment and storage. Source: (CAWST, 2011)

Some water treatment and storage systems use chemicals and other media and materials that cannot be easily obtained locally at reasonable cost (e.g. ozonation, ultrafiltration). They required relatively complex and expensive systems and procedures to treat the water. Such systems may be too inaccessible, complex and expensive to employ for treatment and storage of household or point-of-use water in some places and settings (Sobsey, 2002). The physical disinfection under solar radiation seems to be the more affordable, as it deals with a renewable energy source and

locally available materials (e.g. glass or transparent plastic bottles, CPC reactor). Nevertheless, this technique has some practical and technical disadvantages such as the availability in rural area of the proper reactor free of scratch or the availability of high and intense solar radiation to ensure an efficient disinfection. The regrowth of the inactivated pathogen during the storage after the solar disinfection is one of the major disadvantages of this process. The evaluation of the way to improve the yielding and sustainability of this process before its broad vulgarization could significantly help to face the problem of drinking water scarcity in some regions of the world.

1.2. LOCALIZATION OF THE STUDY AREA

It was recently reported that, approximately of 83% of the population without access to an improved drinking-water source were living in rural areas at the end of 2011 (UNICEF and WHO, 2013). In accordance, rural areas in Burkina Faso as in most of the Sub-Saharan African countries are subject to drastic shortage in drinking water coverage (Fig.1.3.). Most of the drinking water diseases outbreaks are recorded in rural and peri-urban area in developing countries (UNICEF and WHO, 2012). However, most of the developing countries are geographically located in such a way to receive high and intense solar radiation during the sunny day and have a long summer season. The advantageous solar radiation received by some developing countries during the summer season could be usefully exploited at household level for drinking water disinfection.

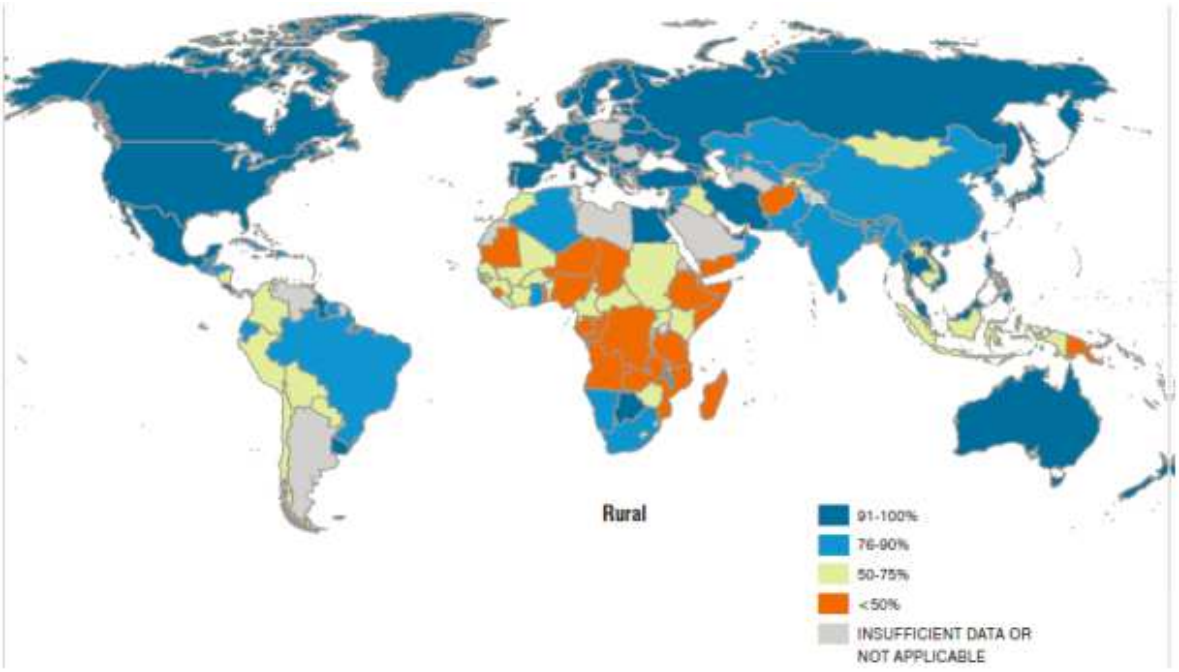

Figure 1.3: Drinking water coverage in rural areas, 2010. Source: (UNICEF AND WHO, 2012)

The practical works of this thesis has been carried out at Ouagadougou-Burkina Faso, West Africa. Ouagadougou is located at 12°21'26" of Latitude North and 1°32'7" of Longitude West. This location is subject to receive approximately 1900-2200 kWh/m^2 of annual average solar irradiance (Fig. 1.4.). Therefore, it could be considered as a good place for the experimentation of photo-disinfection of drinking water.

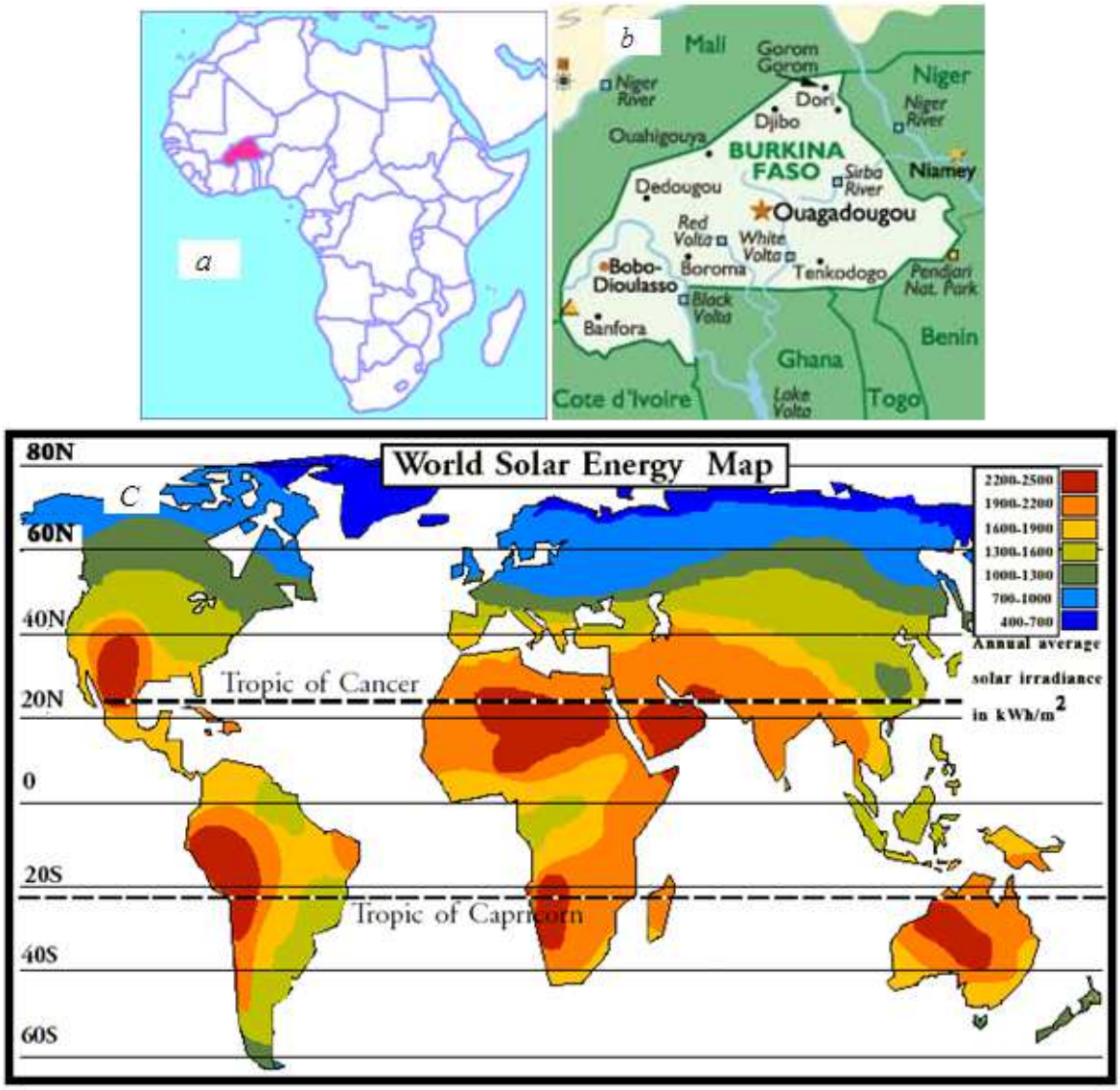

Figure 1.4.: Localization of Burkina Faso in Africa (a), Ouagadougou in Burkina Faso (b) and repartition of the world annual solar irradiance (c).

Sources:

a): http://www.niccolomaffeo.es/africa/mapas/paises/burkinafaso.htm, Accessed on: 12-07-2013

b):http://www.spirulinesolidaire.org/index.php?page=%2FBURKINA+FASO, Accessed on: 12-07-2013

c): http://www.skyscrapercity.com/showthread.php?p=19499307, Accessed on: 12-07-2013

1.3. SODIS: SOLAR DISINFECTION OF WATER

1.3.1. BACKGROUND AND PRINCIPLE OF SODIS

The bactericidal effect of solar radiation was scientifically reported for the first time by Downes and Blunt (1877). Almost a century later Acra *et al.* (1980) re-introduced this effect and proposed the practical application of sunlight for the disinfection of oral re-hydration solution. According to these results, they concluded that sunlight might be able to provide a low-cost, sustainable, and simple method for treating contaminated drinking water in developing countries with consistently sunny climates (Acra *et al.*, 1989). This process is broadly known today as SODIS (Solar Disinfection). It is one of the recommended methods by the WHO for household drinking water treatment or for the temporary treatment during an emergency situation, where people do not have access to an alternative method to obtain safe drinking water (WHO, 2007). Its principle is based on a synergistic effect of thermal and optical parameters of the solar radiation which lethally inactivates the water microorganisms. 1 to 2 L of water of low turbidity (< 30 NTU) are filled up in PET (polyethylene terephthalate) bottles or plastic bags and exposed during about 6 h of mid-latitude midday summer sunshine (Wegelin *et al.*, 1994; Byrne *et al.*, 2011; Spuhler *et al.*, 2013) or up to two days under cloudy weather (Fig. 1.5.) (Meierhofer and Wegelin, 2002).

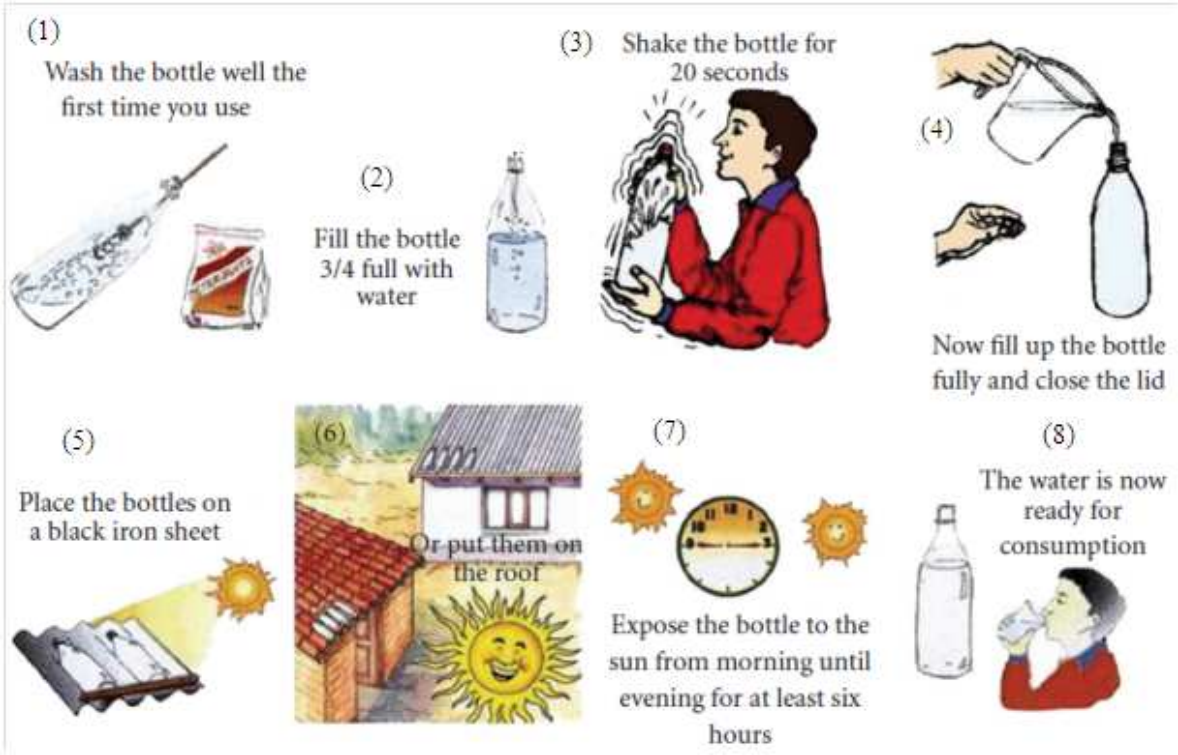

Figure 1.5: The SODIS process. Source: (MEIERHOFER AND WEGELIN, 2002)

1.3.2. LIMITATION OF SODIS AND PROSPECTS OF IMPROVING ITS PRODUCTION QUALITY

The synergetic (thermal and physical) inactivation of SODIS is strongly dependant on the local immediate climatic conditions and the volume and shape of the used recipient. This is due to the fact that the infra-red (IR, wavelenght from 780 nm to 500 µm) solar radiation is significantly attenuated by clouds, optical inactivation does limit the volumes that can be treated at a time because the penetration of UVA (wavelenght from 320 nm to 400 nm) radiation decreases exponentially with the water depth and can be scattered by turbidity. Moreover, the process does not remove natural or anthropogenic chemical pollutants contained in the water. In consequence,

the control of the required exposure time or turbidity for a given weather condition and water source can overburden some households, leading to under exposure, regrowth and limited acceptance among the population (Mäusezahl *et al.*, 2009; Spuhler *et al.*, 2013).

Several enhancement processes have been assessed during the past 20 years to overcome the climatic and volumetric dependency of SODIS. Tested physical improvement include: agitation (Reed *et al.*, 2000; Khaengraeng and Reed, 2005), aluminum foil, black painted back, (Kehoe *et al.*, 2001), the possibility of adding an artificial photosensitizers (PS), e.g. methylene blue (Wegelin *et al.*, 1994; Acra and Ayoub, 1997) or a photocatalyst e.g. TiO_2 (Rincon *et al.*, 2001; Marugan *et al.*, 2008; Malato *et al.*, 2009) or rose bengal (Jiménez-Hernández *et al.*, 2006). Most of these enhancement technologies were sophisticated, expensive and not adapted for a point of used water treatment. Moreover, most of the drawbacks of SODIS technology such as bacterial regrowth during the storage (24h) and volume limitation (1-2L) still merit the effort for further improvement.

1.3.3. THE MOST PROMISING ENHANCEMENT PROCESS FOR SODIS

To enhance SODIS for the application with less insecurity regarding changing weather conditions, it is strongly beneficial to improve the optical inactivation process, which means to maximize the generation of reactive oxygen species (ROS). In 2006, Rincon and Pulgarin (2006) tested the effect of Fe^{3+} and H_2O_2 on the photocatalytic disinfection of water compared to the one by TiO_2. During some control experiments, Rincon and Pulgarin (2006) observed that by an initial near neutral pH (6.5), Fe^{3+}/H_2O_2 (0.3 mg/L and 10 mg/L, respectively) systems had almost a strong bactericidal effect as TiO_2 system under irradiation and a stronger effect in the dark. Furthermore, they observed that only the addition of either Fe^{3+} or H_2O_2 alone to a basic SODIS

system does enhance the inactivation rates. Rincon and Pulgarin (2006) concluded that the presence of Fe^{3+} accelerates the simulated sunlight *E. coli* inactivation due to the photo-activation of iron aquo-complexes under UV and visible light. This SODIS enhancement by the simultaneous addition of iron salts ($Fe^{2+ \text{ or } 3+}$) and H_2O_2 (Moncayo-Lasso *et al.*, 2009) or by the addition of H_2O_2, leading to a derived process taking advantage of iron salts or other transition metals naturally present in the water source (Sciacca *et al.*, 2010; Bandala *et al.*, 2012; Soboleva *et al.*, 2012; Spuhler *et al.*, 2013) is known as the photo-Fenton process, deriving from the Fenton process.

1.4. THE FENTON AND PHOTO-FENTON PROCESSES

The history of Fenton chemistry dates back to 1894, when Fenton (1894) reported that Fe^{2+} could be activated by Fe^{2+} salts to oxidize tartaric acid. Afterwards, Fenton and related reactions have become of great interest for their relevance to biological chemistry, the chemistry of natural waters and the treatment of wastewater. Fenton and related systems encompass reactions of peroxides (usually H_2O_2) with metal ions leading to the fromation of reactive oxygen species (ROS) and reactive radical species (Eq. 1.1) (Spuhler *et al.*, 2013):

$$M^{n+} + H_2O_2 \rightarrow M^{(n+1)+} + OH^- + {}^{\bullet}OH \qquad (1.1)$$

1.4.1. THE FENTON REACTION

The Fenton reagent is a system containing a mixture of ferrous iron (Fe^{2+}) or ferric iron (Fe^{3+}) and H_2O_2. The Fenton reaction refers basically to the generation of $^{\bullet}OH$ aided by the activation of H_2O_2 by Fe^{2+} via the Haber-Weiss reaction (Eq. 1.2) (Haber and Weiss, 1934; Sychev and Isak, 1995; Malato *et al.*, 2009). It has been largely studied for the treatment of bio-recalcitrant

wastewater from industry or agriculture at low pH (2.5-3) (Pignatello *et al.*, 2006; Kenfack *et al.*, 2009).

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^{-} \qquad k = 53 - 76\ M^{-1}\ s^{-1} \qquad (1.2)$$

The Haber-Weiss reaction is a specific case of the Fenton reaction and is explained as the decomposition of H_2O_2 in the presence of Fe^{2+} in an aqueous solution, to water and oxygen, involving the formation of $^{\bullet}OH$ and other reactive species capable of oxidizing a wide variety of organic substrates (Eq. 1.2). In this system, the generated $^{\bullet}OH$ are reported to attack mainly organic pollutants via electrophilic addition, hydrogen abstraction or electron transfer. The oxidizing capacity of the generated radicals is nowadays getting popular as an advanced oxidation process (AOP) for the elimination of biorecalcitrant pollutants in industrial or agricultural wastewater (Kenfack *et al.*, 2009; Malato *et al.*, 2009). The rate constant *k* of this reaction is approximately 53 – 76 $M^{-1}\ s^{-1}$, depending on pH and other factors. To achieve a catalytic system, Fe^{2+} needs to be regenerated from Fe^{3+} and this can take place in presence of H_2O_2 leading to the generation of $HO_2^{\bullet}$ and H^+ (Eq. 1.3) (Sychev and Isak, 1995; Pignatello *et al.*, 2006; Malato *et al.*, 2009):

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+}\ HO_2^{\bullet} + H^{+} \qquad k = 1 - 2\ 10^{-2}\ M^{-1}\ s^{-1} \qquad (1.3)$$

But as the rate constant *k* for the reduction of Fe^{3+} by H_2O_2 to Fe^{2+} is of several magnitudes lower than it consumption in Eq. 1.2 (Haber and Weiss, 1934; Sychev and Isak, 1995; Gernjak *et al.*, 2004; Spuhler *et al.*, 2013), this reaction is the limiting step for the Fenton process.

1.4.1.1. INTERMEDIATE FENTON REACTIONS

In addition to equations 2 and 3 and in the absence of other interfering ions and organic substances, the following catalytic reactions (Eqs. 1.4-1.7) have to be regarded, *k* have been reported by (Sychev and Isak, 1995).

$$Fe^{2+} + HO_2^{\bullet} \rightarrow Fe^{3+} + HO_2^{-} \qquad k = 75 - 150\ 10^4\ M^{-1}\ s^{-1} \qquad (1.4)$$

$$Fe^{2+} + {}^{\bullet}OH \rightarrow Fe^{3+} + OH^{-} \qquad k = 25 - 58\ 10^7\ M^{-1}\ s^{-1} \qquad (1.5)$$

$$Fe^{3+} + HO_2^{\bullet} \rightarrow Fe^{2+} + O2 + H^{+} \qquad k = 33 - 210\ 10^4\ M^{-1}\ s^{-1} \qquad (1.6)$$

$$Fe^{3+} + O_2^{\bullet -} \rightarrow Fe^{2+} + O_2 \qquad k = 50 - 190\ 10^7\ M^{-1}\ s^{-1} \qquad (1.7)$$

For practical applications, Fenton and Fenton-like reactions are difficult to distinguish as in the presence of the above described catalytic cycle, ferrous Fe^{2+} and ferric Fe^{3+} iron species are always present simultaneously. Especially in a large molar excess of H_2O_2, when initial Fe^{2+} is transformed to Fe^{3+} quickly, it is meaningless, from a mechanistic point of view, to distinguish ferrous from ferric type Fenton reactions (Pignatello *et al.*, 2006). When dissolved Fe^{2+} is continuously regenerated from Fe^{3+} allowing a continuous generation of ${}^{\bullet}OH$, this can be referred to as a homogenous catalytic system. The regeneration of Fe^{2+} from Fe^{3+} can be accelerated irradiating the system (photo-Fenton process).

1.4.1.2. FENTON PROCESS AND DRINKING WATER DISINFECTION

The Fenton reagent is strongly enhanced by solar radiation, but was generally perceived to be limited to a very low operational pH, which seems not adapted for treating water for human consumption (Spuhler *et al.*, 2013). Murray and Parsons (2004) identified the pH and the Fe^{2+} dose as key parameters determining the removal efficiency of both, Fenton and photo-Fenton

processes. However, high removal efficiencies were achieved also within a pH range of 4 to 6, making the processes more attractive to drinking water treatment. Based on these results, they suggested that this process could be used to prevent the formation of disinfection by-products (DBPs) during water treatment by reducing the levels of precursor species prior to chlorination. The photo-Fenton reaction typically gives faster rates and a higher degree of mineralization than the thermal or dark reaction and can take advantage of light in the solar spectral region because of the photo-activity of some iron aquao-or organo- complexes (Malato *et al.*, 2009). Therefore, the photo-Fenton is of a general increasing interest of the scientific community for drinking water disinfection.

1.4.2. THE PHOTO-FENTON PROCESS

The enhancement of the Fenton kinetic under light illumination is known as photo-Fenton. The catalytic cycle of the system is maintained through the regeneration of Fe^{2+} from Fe^{3+}. This reaction is highly efficient at acidic pH (2.5-3). At this pH, Fe^{2+} exists predominantly as the hydrolysed hexaquo ion $Fe(H_2O)^{2+}$ (Eqs. 1.8-1.9) (Pignatello *et al.*, 2006; Malato *et al.*, 2009):

$$H_2O + Fe(H_2O)_6^{2+} \leftrightarrow Fe(H_2O)_5(OH)^+ + H_3O^+ \quad (1.8)$$

$$2H_2O + Fe(H_2O)_6^{2+} \leftrightarrow Fe(H_2O)_4(OH)_2 + 2H_3O^+ \quad (1.9)$$

Dropping water ligands from the formulas, $Fe(H_2O)_6^{2+}$, $Fe(H_2O)_5(OH)^+$ and $Fe(H_2O)_4(OH)_2$ can be written as Fe^{2+}, $Fe(OH)^+$ and $Fe(OH)_2$. Below pH 3, most of the present ferrous ion will be present as Fe^{2+}. At pH above 3, ferrous ions will tend to co-precipitate with Fe^{3+} oxyhydroxides, if the two ions are present together. However, the illumination of the system leading to the photo-Fenton system can accelerate the regeneration of Fe^{2+} from Fe^{3+} because some Fe^{3+}-hydroxy

complexes undergo photoreduction under UVA and visible radiation, producing $^{\bullet}OH$ and regenerating Fe^{2+}, via the ligand to metal charge transfer (LMCT) (Spuhler *et al.*, 2013):

$$[Fe^{3+}(L)_n] + h\nu \rightarrow [Fe^{3+}(L)_n]^* \rightarrow [Fe^{2+}(L)_{n-1}] + L^{\bullet}_{ox} \quad (1.10)$$

Fe^{3+} complexes have different light absorption properties for different ligands L affecting directly the quantum yields for the generation of Fe^{2+}, $^{\bullet}OH$ and other ROS.

1.4.2.1. PHOTO-FENTON PROCESS AT NEAR NEUTRAL PH FOR DRINKING WATER DISINFECTION

Solar water disinfection enhanced by a photo-Fenton process at near neutral pH and low temperatures is novelty from a scientific and technological point of view. This enhancement is achieved by increasing the rate of the $^{\bullet}OH$ radical and others ROS production. Fe^{3+} can form complexes with many substances and undergo photoreduction as described in Equation 1.10. Fe^{3+} complexes with organic ligands, which absorb light in the solar spectrum and are stable at environmental pH, allow to circumvent the pH dependency of photo-Fenton. Such ligand can be formed with some oxidation intermediates of degradation products of natural organic matter (NOM) and promote the conversion of Fe^{3+} to Fe^{2+}, in addition to the uni-molecular decomposition of the Fe^{3+}hydroxy complexes (Jiang *et al.*, 2010). The photolysis of these Fe^{3+}-organo complexes, which have generally a high molar absorption coefficient in UVA and visible light than the Fe^{3+} leads to the regeneration of Fe^{2+} and the formation of a ligand radical (Feng and Nansheng, 2000). Fe^{3+}-carboxylate complexes (Eq. 1.11) can have much higher quantum yields than Fe^{3+}-hydroxo complexes and undergo also LMCT.

$$[(RCOO^{-})Fe^{3+}] + h\nu \rightarrow Fe^{2+} + CO_2 + R^{\bullet} \quad (11)$$

During the photocatalytic cycle, both Fe^{2+} and organic radicals can than react with O_2 leading to the formation of ROS ($O_2^{\bullet-}$, $^{\bullet}OH$, H_2O_2), which may attack bacteria and other targets (Faust and Zepp, 1993; Feng and Nansheng, 2000).

1.4.2.2. IMPACT OF NOM ON PHOTO-FENTON DISINFECTION

Natural organic matter contains functional groups which can form complexes with Fe^{3+} or Fe^{2+}. These complexes not only increase the solubility of iron over the natural pH range, but they can also considerably contribute to the photo-Fenton reactions via a LMCT under solar radiation. The positive effect of NOM constituents (carboxylic acids) on photo-Fenton process, which allows to work at near neutral pH has been reported by (Georgi *et al.*, 2007; Lipczynska-Kochany and Kochany, 2008; Vermilyea and Voelker, 2009; Ruales-Lonfat *et al.*, 2013). Strong and photoactive complexes with Fe^{3+} are formed by carboxylate groups or polycarboxylates (e.g. oxalate, malonate and citrate), the most common functional groups of dissolved NOM (Zepp *et al.*, 1992; Pignatello *et al.*, 2006; Lipczynska-Kochany and Kochany, 2008). The Fe^{3+}-organo complex thereby undergoes LMCT (Eq. 1.11). Under photo-Fenton, the carboxylic acids groups can partially substitute the OH groups in Fe^{3+}-hydroxy complexes and stabilize these complexes at near neutral pH leading to the possible disinfection of drinking water. In the past few years, several authors have shown, that because of its chelating effect leading to the formation of photo-active Fe^{3+} or Fe^{2+}-organo-complex, NOM did not only accelerate the photo-Fenton system and allow to operate at near neutral pH but it can also auto-oxidized subsequently (Murray and Parsons, 2004; Georgi *et al.*, 2007; Vermilyea and Voelker, 2009; Spuhler *et al.*, 2013).

NOM can be a serious problem for drinking water treatments not only because they can be a substrate for bacterial regrowth after treatment, but also because under chlorination, this NOM is

often subject to the formation of carcinogenic halogenated disinfection by-products (DBP) such as trihalomethanes (Gallard and von Gunten, 2002; Dickenson *et al.*, 2008; Moncayo-Lasso *et al.*, 2008a; Guo and Chen, 2009). Photo-Fenton process at near neutral pH can considerably decrease the NOM content in drinking water sources (Murray and Parsons, 2004; Moncayo-Lasso *et al.*, 2008a; Vermilyea and Voelker, 2009). The degradation of NOM by the photo-Fenton reagent does not lead to the formation of DBP (Ribordy *et al.*, 1997). It seems, that is the chelating effect of some functional groups of the NOMs, which on one hand, contribute to the solubilization of Fe^{2+} and Fe^{3+} at near neutral pH and on the other hand are oxidized during this process either by ROS generated when solubilized Fe^{2+} reacts with H_2O_2 and on the other when undergoing a LMCT in the Fe^{3+}-organo complexes.

1.5. THE COMPOUND PARABOLIC COLLECTOR (CPC) SOLAR REACTOR

High-performance compound parabolic collector (CPC) in which large volumes of water can be treated at one time can be used for different solar water treatment technologies. The CPC is a batch solar reactor composed of borosilicate glass tubes, which are placed in the focus of the aluminum reflectors. The aluminum frame is mounted on tilted platforms orientated to the sun. The glass tubes are interconnected so that water flows directly from one tube to another and finally into a tank (recirculation). A centrifugal pump returns the water to the tubes exposed to the solar collector. Once the water has been completely treated, the total volume is redirected to this tank, which is closed to prevent recontamination. The advantage of recirculating CPC is definitively the large volumes, which can be treated at a time. Several authors have tested the efficiency of the CPC in enhancing the photo-disinfection of waste and drinking water (Rincon

and Pulgarin, 2007a; Kenfack *et al.*, 2009; Moncayo-Lasso *et al.*, 2009; Ubomba-Jaswa *et al.*, 2010; Byrne *et al.*, 2011; Sciacca *et al.*, 2011).

1.6. THE PHOTO-INACTIVATION MECHANISM

1.6.1. INACTIVATING REACTION UNDER DIRECT SOLAR RADIATION

Sunlight may cause direct damage to biomolecules when UVB radiation is absorbed by DNA (Smith *et al.*, 1987; Reed, 2004). It is more common for solar UVA and visible light to cause indirect damage. The central hypothesis of indirect optical inactivation is that photosensitizing compounds can absorb solar UVA and visible radiation and thereby get excited. The excited compounds can then either directly attack microorganism via the reaction with cellular biomolecules (type I) or produce ROS via energy transfer and redox reactions with surrounding oxygen in the water (type II) (Kohn and Nelson, 2006; Spuhler *et al.*, 2013).

The optical inactivation of microorganism in solar disinfection systems is mainly due to the generation of reactive oxygen species (ROS, type II pathway of photo-inactivation), even though the direct interaction of excited photosensitizers with biomolecules without the intermediate formation of ROS (type I) plays also a role depending on the water composition. The transition metal iron (in particular free or loosely bound iron salts Fe^{2+or3+}) as well as the long living ROS H_2O_2 and $O_2^{\bullet-}$, play an important role in the generation of short living and thus more reactive ROS (e.g. $^{\bullet}OH$ via the Fenton reaction) leading to the fatal damage during photo-oxidative stress (e.g. sub-lethal or lethal damage) in the process of solar disinfection of water (Eisenstark, 1998; Imlay, 2008).

1.6.1.1. MECHANISM OF TYPE I AND TYPE II PATHWAY OF PHOTOINACTIVATION

Type I reactions includes the attack of proteins and cell membrane components, especially membrane lipids (Gourmelon *et al.*, 1994), leading to lipid peroxidation chains (Cabiscol *et al.*, 2000) and the inactivation of the cells which could be due to increased permeability and/or the disruption of trans-membrane ion gradients, (Reed, 2004). ROS generated in the type II pathway can also monitor these attacks. The main ROS generated by photosensitization of organic dissolved matter are singlet oxygen ($^1O_2$), superoxide ($O_2^{\bullet-}$), hydroxyl radicals ($^{\bullet}OH$) or hydrogen peroxide (H_2O_2). When the concentration of active oxygen species increases to a level that exceeds the cell's defense capacity, this is called oxidative stress (Cabiscol *et al.*, 2000). The biological targets for these highly reactive oxygen species are DNA, RNA, proteins and lipids (Spuhler *et al.*, 2013).

1.6.1.2. THE PHOTOSENSITIZERS

Photosensitizers (PS) can be either endogenous (thus part of the cells themselves) such as porphyrins, cytochromes, cytochrome oxidase, aromatic amino acids, flavins, tryptophan, chlorophylls or chromophore groups of proteins or quinones (Hartman and Eisenstark, 1978; Curtis *et al.*, 1992; Rajendran *et al.*, 2004). Exogenous photosensitizers, which absorb light in the solar UV and visible range are often of organic nature, such as humic (Curtis *et al.*, 1992; Paul *et al.*, 2004) and fulvic acids (Cory *et al.*, 2008) or other dissolved natural organic material (Haag and Hoigné, 1985; Canonica *et al.*, 1995; Sandvik *et al.*, 2000; Zhan *et al.*, 2006; Zhan, 2009).

1.6.1.3. CHARACTERIZATION OF SOME ROS

Many studies have shown that $^1O_2$ can oxidize a variety of organic substances including biologically important compounds, such as amino acids (Zepp *et al.*, 1977) or other parts of

proteins (Davies, 2003; Rengifo-Herrera *et al.*, 2009). The bacterial membranes (lipids) are the most likely target for the often exogenously produced singlet oxygen and oxygen radicals (Curtis *et al.*, 1992; Spuhler *et al.*, 2013) leading to enhanced permeability and serious damage of the cells.

$O_2^{\bullet-}$ itself is relatively little reactive. When generated exogenously $O_2^{\bullet-}$ is sufficiently long living to diffuse into the cells but this process is slowed because $O_2^{\bullet-}$ is charged (Imlay, 2008). However, $O_2^{\bullet-}$ produced endogenously is responsible for the oxidation of [4Fe-4S] cluster present in cells, the subsequent release of Fe^{2+} and the simultaneous formation of H_2O_2 allowing the generation of toxic $^{\bullet}OH$ via intracellular Fenton reactions (Gutteridge, 1982; Imlay, 2003; Fridovich, 2011).

$^{\bullet}OH$ reacts at, or close to, a diffusion-controlled rate. Hence, any formed $^{\bullet}OH$ will react with whatever is present at its formation site and directly damage almost all biological molecules, including DNA (Halliwell and Gutteridge, 1992). If these radicals are produced sufficiently close to the bacterial membrane (because their high reactivity does not allow them to travel in the bulk water) they lead to the oxidation of lipids affecting the structural and functional integrity of the membranes (Kellogg and Fridovich, 1975). If they are produced inside the cells, they will immediately attack the biomolecules, especially DNA.

H_2O_2 on its own is also relatively little reactive, but has the ability to attack the cellular membrane, initiating lipid peroxidation chains that increase membrane permeability and affect the viability of the cells (Halliwell and Chirico, 1993). However, H_2O_2 is long-living and therefore penetrates membranes and diffuse into cells whenever it is present in the extracellular

habitat (Imlay, 2008). The presence of H_2O_2 in the cell can led to serious damage because of its ability to react with metal ions (e.g. Fe^{2+}) generate highly toxic $^{\bullet}OH$.

1.6.2. PATHWAY OF THE PHOTO-OXIDATIVE STRESS

The biological targets of ROS ($^1O_2$, H_2O_2, $O_2^{\bullet-}$ as well as $^{\bullet}OH$, type II photosensitization) and other radicals (type I) are DNA, RNA, proteins and lipids (Cabiscol *et al.*, 2000). Oxygen dependent type II photoreaction are probably the major component of optical bacterial damage because of the ROS induced membrane lipid peroxidation and DNA damage (Reed, 2004). Free radicals attack membrane lipids (Gourmelon *et al.*, 1994) such as the phospholipids chains (Gutteridge, 1982) or polyunsaturated fatty acids, initiating lipid peroxidation chains (Cabiscol *et al.*, 2000). Lipid peroxidation is a complex process characterized by three distinct phases: a slow induction period, a rapid autocatalytic phase and a slow termination phase (Gutteridge, 1982). Lipid peroxidation results in a decrease of the membrane fluidity altering the membrane properties and disrupting membrane-bound proteins (Cabiscol *et al.*, 2000). It action can also lead to increase membrane permeability, disruption of transmembrane ion gradients and finally inactivation (Reed, 2004). The attack of polyunsaturated fatty acids acts as an amplifier, more radicals are formed, and the polyunsaturated fatty acids are degraded to a variety of products. Some of them are aldehydes, which are on their turn very reactive and can damage molecules, such as proteins (Cabiscol *et al.*, 2000). When proteins are exposed to reactive oxygen species, modifications of amino acid side chains occur and, consequently, the protein structure is altered (Cabiscol *et al.*, 2000). The toxic effects of H_2O_2 and $O_2^{\bullet-}$ is due to their important role in the Fenton reaction and the generation of $^{\bullet}OH$ via this pathway (Gutteridge, 1982; Imlay, 2003;

2008). $^1O_2$, on its term is an effective initiator of lipid peroxidation chains and it has also been proposed, beside the generation of $^{\bullet}OH$ via the Fenton reaction (Kellogg and Fridovich, 1975).

1.6.3. OXIDATIVE STRESS THROUGH INTRACELLULAR FENTON

Under UVA and visible light, iron concentrations can be enhanced. Hoerter *et al.* (1996) and (2005) have shown that enterobactin is an endogenous chromophore for UVA and contributes to cell lethality under radiation in the solar spectrum via the destruction of its ligand, releasing Fe^{2+} into the cytoplasm of *E. coli* bacteria to catalyze the production of highly reactive hydroxyl radicals and other toxic oxygen species via the Haber-Weiss reaction. Pourzand *et al.* (1999) have shown that the UVA radiation also increases the level of intracellular reactive iron pools via the proteolysis of ferritin (Tyrrell *et al.*, 2000). UVA radiation also breaks down heme-containing proteins in the microsonial membrane to release free heme as an additional photosensitizing component (Tyrrell *et al.*, 2000). Also, ROS can directly interact with bound iron to make it available (Spuhler *et al.*, 2013).

Imlay (2008) described that during the oxidative-stress, $O_2^{\bullet-}$ could penetrate in the active sites of enzymes containing iron-sulfur clusters ($[4Fe\text{-}4S]^{2+}$). $O_2^{\bullet-}$ then binds the critical iron atom and oxidizes the cluster to a redox state that is unstable, the produced $[4Fe\text{-}4S]^{3+}$ then releases Fe^{2+} and is left in a inactive $[4Fe\text{-}4S]^{+}$ form. The $[4Fe\text{-}4S]^{2+}$ cluster can also be oxidized by H_2O_2 leading to the release of Fe^{3+}. And the oxidation of the cluster by $O_2^{\bullet-}$ leads simultaneously also to the generation of cellular H_2O_2 (Imlay, 2003; 2008):

$$[4Fe - 4S]^{2+} + O_2^{\bullet-} + 2H^{+} \rightarrow [4\,Fe - 4S]^{3+} + H_2O_2 \quad (1.12)$$

$$[4Fe - 4S]^{3+} \rightarrow [4Fe\text{-}_4S]^{+} + Fe^{2+} \quad (1.13)$$

Besides the Fe^{2+} release from iron-sulfur cluster, $O_2^{\bullet-}$ can also reduce and liberate Fe^{3+} from ferritin (Henle and Linn, 1997). The generated H_2O_2 and Fe^{2+} can through the Fenton reaction lead to the generation of highly reactive $^{\bullet}OH$ radical which will lethally oxidize the cell DNA (Gutteridge, 1982; Henle and Linn, 1997; Imlay, 2003).

1.7. SYSTEMATIC EVALUATION OF THE PHOTO-FENTON DISINFECTION

The first systematic study on photo-Fenton solar water disinfection allowing a mechanistic interpretation and which illustrates the possible pathways involved in photo-inactivation of *E. coli* in presence of Fe^{2+}, Fe^{3+}, H_2O_2 and the photo-Fenton reagent in milliQ water was carried out by (Spuhler *et al.*, 2010). According to the results, it was estimated that the differences in how Fe^{2+}, Fe^{3+}, H_2O_2 and the photo-Fenton reagent affect bacterial photo-inactivation involve not only classical photo-Fenton reaction and photo-oxidative cellular stress, but also excessive Fe^{2+} and H_2O_2 uptake by bacteria leading to intracellular dark Fenton reaction (photo-oxidative stress) and the aggregation of some iron species with bacteria leading to the formation of photo-active Fe-bacteria bound. Many gram-negative bacteria, such as *E. coli* possess highly specified binding proteins and siderophores to sequestrate Fe^{3+} for facultative anaerobic respiration (Braun, 2001). Besides, the membrane of gram-negative bacteria does also contain other proteins with many carboxylic end groups which are likely to show an affinity for Fe^{3+} and to support the formation of photoactive Fe^{3+}-bacteria bounds. Intracellular iron and H_2O_2 play both an important role in photo-oxidative stress (Imlay *et al.*, 1988; Imlay, 2003). The toxicity of Fe^{2+} for microorganisms due to its ability to generate radicals via intracellular Fenton reaction can also be observed in nature (Touati, 2000) and has been explored for the inactivation of waterborne viruses (Ryan *et*

al., 2002; You *et al.*, 2005) and bacteria (Auffan *et al.*, 2008; Lee *et al.*, 2008) via the corrosion and subsequent Fe^{2+} release from zerovalent iron (Spuhler *et al.*, 2013).

1.8. SOME APPLICATIONS OF THE PHOTO-FENTON AT NEAR NEUTRAL PH

The photo-Fenton process at near neutral pH and very low reagent concentration was tested for the elimination of NOM from river water (Murray and Parsons, 2004; Moncayo-Lasso *et al.*, 2008a; Ruales-Lonfat *et al.*, 2013; Spuhler *et al.*, 2013). Simultaneous to organic oxidation it was also observed that the process significantly enhanced the solar inactivation of some pathogenic bacteria (Moncayo-Lasso *et al.*, 2009). The enhancing effect of the photo-Fenton process for photo-inactivation of *E. coli* in drinking water at near neutral pH was for the first time reported by (Rincon and Pulgarin, 2006). Subsequently, the enhancement of bacterial photo-inactivation via photo-Fenton at near neutral pH was reported at lab scale, pilot scale and in natural waters at reagents' concentrations in the micro to millimolar range (Moncayo-Lasso *et al.*, 2009; Sciacca *et al.*, 2010; Spuhler *et al.*, 2010; Polo-Lopez *et al.*, 2012).

1.9. AIMS AND OUTLINE OF THE THESIS

Research conducted in the framework of this thesis aimed to determine the feasibility of the disinfection of large volumes of natural drinking water in Sub-Saharan region, via the photo-Fenton reagent and to test the technical implementation at field scale.

The following specific tasks were carried out:

- Evaluate the simple solar and enhanced photo-Fenton solar disinfection of natural waters with near to neutral pH (wells) and alkaline pH (dam) in PET bottles and CPC reactor.
- Characterize the physicochemical and wild enteric bacteria contents of the water sources and evaluate their influence on the sole solar (*hv*) and photo-Fenton ($Fe^{2+}/H_2O_2/hv$) disinfection.
- Evaluate the impact of the pH, temperature, irradiance and dose of the direct solar radiation on the inactivation rate of the wild enteric bacteria during the simple solar or enhanced photo-Fenton solar disinfection.
- Evaluate the optimal concentration of added hydrogen peroxide (H_2O_2) needed for an efficient photo-Fenton disinfection of natural drinking water.
- Evaluate the effect of the raining season on the efficiency of the photo-Fenton disinfection.
- Conduct post irradiation control of the water quality to evaluate the efficiency of the photo-disinfection.

To efficiently present this research work, this thesis is organized in five chapters. The introductive Chapter 1, contains the overview of the drinking water scarcity in the world and the potential of point-of-use and household level water treatment, followed by a broad presentation of the solar and photo-Fenton disinfection mechanisms.

In Chapter 2, the results of the research is presented for which natural wells water with near to neutral pH was inactivated in PET bottles considering several photocatalytic systems to evaluate the impact of the natural iron contents of the water on photo-Fenton disinfection before the experimentation of large volume of water (25L) in the CPC solar reactor.

Chapter 3, present the results from the evaluation of the impact of different irradiances and doses following several exposure sets of the water sample in a CPC solar reactor, during different day times. Considering the impact of the pH on the efficiency of the photo-Fenton and the proximity of the studied wells with agricultural areas, the monitoring of the variation of pH, NH_4^+, NO_3^- and NO_2^- during the photo-treatment was assessed to evaluate the impact of these parameters on the photo-Fenton treatment and vice-versa as well as the possible formation of the health related disinfecting byproducts.

Chapter 4, present the results on the first evaluation of the efficiency of photo-Fenton disinfection at alkaline pH for the disinfection of natural surface water (pH 8.6) collected from a dam in Ouagadougou, at field scale in a CPC. The evolution of pH during the photo-disinfection was monitored. The impact of some inorganic ions presents in the natural water sample on the efficiency of the photo-Fenton process was also evaluated.

Chapter 5, present the results from the experimentation during the raining season in order to present the impact of the high intermittence of the solar radiation on the efficiency of photo-disinfection (simple solar and enhanced photo Fenton solar disinfection) comparatively to the situation available during the summer time.

Each chapter contains its corresponding materials and methods section. At the end, a summarized conclusion on the progress on photo-disinfection is presented with a directions to future work, in order to improve the state-of-art knowledge of this promising field of water disinfection processes.

2. CHAPTER 2:

INACTIVATION BY SOLAR PHOTO-FENTON IN PET BOTTLES OF WILD ENTERIC BACTERIA OF NATURAL WELL WATER: ABSENCE OF RE-GROWTH AFTER ONE WEEK OF SUBSEQUENT STORAGE.

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Inactivation by solar photo-Fenton in pet bottles of wild enteric bacteria of natural well water: Absence of re-growth after one week of subsequent storage

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ABSTRACT

Iron photo-assisted inactivation of wild enteric bacteria (total coliforms/*E. coli* and *Salmonella* spp.) was carried out in water from the Sahelian wells having different pH (W1: 4.9 and W2: 6.3) and a natural iron content of 0.07 mg/L. We evaluate the efficiency of the disinfection on different systems containing both or only one Fenton reagent (H_2O_2/Fe^{2+}): (i) $H_2O_2/Fe^{2+}/h\nu$, (ii) $Fe^{2+}/h\nu$, (iii) $H_2O_2/h\nu$, and (iv) only light irradiation ($h\nu$) at lab and field scale. Generally, 0.6 mg/L of Fe^{2+} and/or 8.5 mg/L of H_2O_2 were used in the Fenton reagent. The systems $H_2O_2/Fe^{2+}/h\nu$ and $H_2O_2/h\nu$ led to total inactivation of *Salmonella* and *E. coli*. The natural iron content (0.07 mg/L) was enough to drive an efficient photo-Fenton process leading to total bacterial inactivation. Our results show that: (i) the iron salt present in Sahelian water is enough to perform a photo-Fenton disinfection of drinking water when adding H_2O_2, (ii) addition of external iron salts at near neutral pH has no additional effect on the bacterial photo-Fenton inactivation process. After one week of storage, no enteric bacteria re-growth was observed in treated waters. Mechanistic suggestions are presented to explain the observed results.

cf. Annex.

2.1. INTRODUCTION

The use of solar energy for water treatment is a new and promising alternative to the disinfection of drinking water. Solar disinfection (SODIS) was first used in 1980 to produce re-hydration solutions for children suffering from diarrhea in Beirut (Acra *et al.*, 1980). The SODIS treatment involves the use of transparent plastic bottles (Polyethylene Terephthalate (PET) of 1-1.5 L) filled with water and exposing them to sunlight for at least 6 hours depending on the meteorological conditions. The inactivation or death of pathogenic microorganisms is achieved by the synergistic effect of radiation and heat (Wegelin *et al.*, 1994; Sommer *et al.*, 1997; McGuigan *et al.*, 1999). Underexposure and bacterial re-growth result in incomplete bacterial inactivation (Reed, 2004; Mäusezahl *et al.*, 2009). Improvement of the SODIS treatment includes the use of black backs bag (Kehoe *et al.*, 2001) or the TiO_2 photocatalytic processes (Rincon and Pulgarin, 2003; Fernandez *et al.*, 2005; Marugan *et al.*, 2008). Recently, the photo-Fenton system ($Fe^{2+}/H_2O_2/h\nu$) has been shown to increase the solar photo-inactivation of *Escherichia coli* at near-neutral pH (Rincon and Pulgarin, 2007a; Moncayo-Lasso *et al.*, 2009; Spuhler *et al.*, 2010). Hydrogen peroxide (H_2O_2) significantly increased the photocatalytic inactivation process in natural water containing natural iron (Sciacca *et al.*, 2010). The Fenton reagent generates the highly reactive $^{\bullet}OH$ via the Haber-Weiss reaction (Sychev and Isak, 1995):

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^{-} \qquad k = 53 - 76\ M^{-1}s^{-1} \qquad (2.1)$$

During the Fenton process, Fe^{2+} can be regenerated from Fe^{3+} in the presence of H_2O_2:

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+}\ HO_2^{\bullet} + H^{+} \qquad k = 1 - 2 \times 10^{-2}\ M^{-1}s^{-1} \qquad (2.2)$$

But the Fenton process is limited by the Fe^{2+} regeneration of Fe^{3+} (Eq. 2.2). This drawback is partially countered by photo-Fenton reactions. In fact, under illumination, ferric-hydro-complexes or ferric-organo-complexes in solution can absorb photons and generate ligand-to-metal charge-transfer (LMCT) reaction in which Fe^{2+} generating $^{\bullet}OH$ (Eq. 2.3-2.5), (Pignatello *et al.*, 2006; Malato *et al.*, 2009).

$$Fe^{3+}(L_n) + h\nu \rightarrow Fe^{2+}(L_{n-1}) + L_{ox} + \quad (2.3)$$

$$[Fe(OH)]^{2+} + h\nu \rightarrow Fe^{2+} + {}^{\bullet}OH \quad (2.4)$$

$$[Fe(OOC - R)]^{2+} + h\nu \rightarrow Fe^{2+} + CO_2 + R^{\bullet} \quad (2.5)$$

The pH influences the efficiency of the photo-Fenton reagent, with an optimum level of pH 2.8-3 (Safarzadeh-Amiri *et al.*, 1996). Considering this acidity criteria, the photo-Fenton was in the past preferably used for the treatment of wastewaters, mainly for the degradation of bio-recalcitrant organic pollutants via the generated ROS (Kenfack *et al.*, 2009; Malato *et al.*, 2009). But the recent discovery of its efficiency at near-neutral pH in the presence of organic substances, (Spuhler *et al.*, 2010) gives the possibility of using it in the treatment of drinking water. The Sub-Sahelian African region receives about 2,500-3,000 hours of solar radiation annually with more than 2,300 $KWh.m^{-2}.an^{-1}$ irradiance in some parts (Kenfack *et al.*, 2009). This natural illumination with a UV-component represents a powerful intake to drive photo-Fenton reactions in the region.

Studies on the inactivation of model bacteria (*E. coli* K 12) by the photo-Fenton reagent in milliQ water (Rincon and Pulgarin, 2007a; Spuhler *et al.*, 2010) or milliQ water containing simulated NOM (Spuhler *et al.*, 2010) and some research on the disinfection of wild bacteria in natural water have been reported (Sciacca *et al.*, 2010; 2011). This study is related to the inactivation of

bacteria in natural waters. In this study, we address the intervention of the Fenton (Fe^{2+}/H_2O_2) reagent-driven bacterial inactivation and photo-Fenton ($Fe^{2+}/H_2O_2/hv$) processes on various wild bacteria in groundwater from wells in Burkina Faso, West Africa.

2.2. EXPERIMENTAL DETAILS

2.2.1. REAGENTS AND MATERIALS

Hydrogen peroxide, 30% (AnalaR Normapur, VWR) and Iron (II) Sulphate Heptahydrate ($FeSO_4.7H_2O$) were used to prepare the Fenton reagent. During the experiments, Catalase from bovine liver was used to inactivate the remaining H_2O_2, in the treated water before the bacterial culture. Hydrochloric acid fuming (HCl), 37% was used for glass-reactor cleaning. Catalase and HCl were from Fluka Analytical, SIGMA-ALDRICH ®.

2.2.2. WATER SAMPLING

Water samples were collected at two household wells from two sectors in Ouagadougou: well 1 (W1) at Tanghin, sector 30, and well 2 (W2) at Nonsing, sector 21. Water from these wells is used for cooking, laundry, bathing and occasionally for drinking purposes during the recurrent water shortage period. Samples for lab experiments were collected from the water sources with two PET bottles (1.5 L) one hour before the beginning of the process. One bottle was used to determine some physico-chemical parameters and the other for the disinfecting experiments. Water for field experiments was collected only from W2 with a 20-liter plastic jerrican. *In-situ* temperature was monitored.

2.2.3. BACTERIAL STRAIN AND GROWTH MEDIA

The wild bacterial strain monitored in this study was the fecal indicator bacteria coliforms/*E. coli*, and *Salmonella* spp. Microbiology Chromocult ® (Merck KGaA), was used for bacterial plating.

The Chromocult is a selective and differential growth media. It selectively inhibits growth of the non-enteric bacteria. As experiments were conducted with natural water, considering their initial enteric bacteria contents, no dilution was realized before the bacterial plating. 100 µl of sample water were inoculated in the growth medium. Considering the selectivity of Chromocult, the detection limit of enteric bacteria was 0 (zero) colony growths observed in the plate. The differential nature of the medium permits the distinction of *Salmonella* spp (colorless), *E.coli* (purple and pink) and the blue and salmon colored colonies of other coliforms bacteria. The Incubation period was 18-24 h at 37°C, allowing the growth of all previously mentioned enteric bacteria. However, in order to more strongly represent the decrease of the total coliforms, all the *E. coli* observed and others coliforms counted were presented together in this study.

2.2.4. ANALYTICAL METHODS FOR PHYSICAL PARAMETERS OF WATER

Temperature (T°C), pH and hydrogen peroxide evolution were monitored following Sciacca *et al.* (2010). Turbidity was measured with a PCcompact ® Turbidity/Trübung, (Aqualitic). The total iron content was measured with the spectrophotometer HACH 2000, by the FerroVer method 265. The detection limit of the spectrophotometer HACH 2000 was 0.02±0.01 mg/L.

2.2.5. HELIO-PHOTO-INACTIVATION EXPERIMENTS

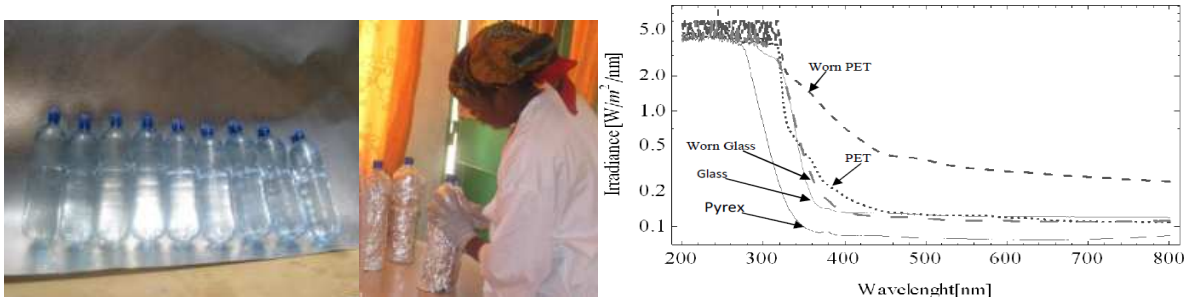
2.2.5.1. LABORATORY EXPERIMENTS USING A SOLAR SIMULATOR (SUNTEST)

The photo-inactivation experiments at laboratory scale were conducted following the process used by Spuhler *et al.* (2010) in a solar simulator (suntest). The disinfecting efficiency of four photo-assisted systems combined with both or only one Fenton reagent (H_2O_2/Fe^{2+}) in: (i) $H_2O_2/Fe^{2+}/hv$, (ii) Fe^{2+}/hv, (iii) H_2O_2/hv, and (iv) only light irradiation (*hv*) were evaluated in parallel with dark-control experiments: (5) $H_2O_2/Fe^{2+}/obs$, (6) Fe^{2+}/obs, (7) H_2O_2/obs, and (8) obscurity only (*obs*). In these systems, *hv:* refers to illumination and *obs:* to obscurity. Glassware

for analytical analysis and reactors were acid soaked after each experimental series to prevent iron cross-contamination (10% HCl, 3 days and nights). After preliminary experiments the H_2O_2 dose in this study was set at 8.5 mg/L. The concentration of the added iron was Fe^{2+} (0.6 mg/L) as evaluated by Spuhler *et al.* (2010). The initial pH of the water was 4.9 and 6.3 respectively for W1 and W2. Each experiment was repeated at least three times to ensure the reproducibility of the results.

2.2.5.2. FIELD EXPERIMENT USING PET BOTTLES

PET bottles were used for field experiments as they have a relative good absorbance (Fig.2.1-c). Only the water from W2 with a close-to-neutral pH (table 2.1) was used at this level. Following the results of the lab experiments, the system Fe^{2+}/*hv* was not evaluated at field scale and the other systems and their blank were evaluated in triplicate (Fig.2.1-a,b) over three successive days at the end of April 2010. Nine new PET bottles (1.5 L) representing three replications of the systems (H_2O_2/Fe^{2+}/*hv*, H_2O_2/*hv*, and (*hv*)) were filled with: Fe^{2+} (0.6 mg/L) and H_2O_2 (8.5 mg/L) added before their exposure to solar radiation. Blank control bottles were covered with Al-foil and kept in the dark. During the experiments, solar radiation was recorded following the process used by Sciacca *et al.* (2011). To evaluate the bacterial inactivation rate, samples of the treated water were collected at regular time intervals in a sterile 15 mL Eppendorf for plating. Some parameters of these water samples are presented in table 2.1. The PET bottles were used only once to ensure the same and relatively good light transmittance (Fig.2.1-c) in all the experiments.



(a) (b) (c)

Figure 2.1. (a) 9 Pet bottles for the triplicate simultaneous exposure of the systems $H_2O_2/Fe^{2+}/hv$, H_2O_2/hv, and hv (irradiation only). (b) Blank of each system, (c) Absorbance of different reactor materials (Pyrex, Glass, Pet).

2.2.6. POST-IRRADIATION EVENTS

At the end of the irradiation phase of the laboratory experiments, remaining samples from systems 1 and 2 were introduced into sterile 50 mL Eppendorf flasks and kept in the dark at room temperature varying from 25 – 30°C. For field experiments, each PET bottle from the exposed and blank tests was closed and kept in the dark. Re-growth experiments were realized on stored bottles after 24 h, 72 h and one week. Considering the real scale situation for treated water intended for populations, the samples water were kept in the dark without removing their remaining 2 to 3 mg/L of H_2O_2. This remaining amount of H_2O_2 ensures a residual effect on the treatment. However, it was no more detectable after 48 to 72 h.

2.2.7. DATA ANALYSIS

The three-way ANOVA Package of the Wolfram Mathematica 8.0 program was used to evaluate the influence of the acidity, bacteria types and irradiation used on the disinfection rate.

2.3. RESULTS

2.3.1. PHYSICO-CHEMICAL CHARACTERIZATION

Both water samples used in this study were collected at Ouagadougou during the months of March and April 2010, (dry season). They had an initial temperature of 29°C and low turbidity < 10 NTU. The maximum acceptable turbidity recommended for SODIS disinfection is 30 NTU, (McGuigan *et al.*, 1999; Reed, 2004). The W1 has an initial pH of 4.9 and a total iron content of 0.06 mg/L; while in W2 the initial pH value was 6.3 and the total iron content 0.07 mg/L. The concentration of both wild enteric bacteria (total coliforms/E. coli and Salmonella spp.) was approximately 10^5 CFU/ml in both water sources.

2.3.2. EXPERIMENT UNDER SIMULATED SOLAR RADIATION

As it has been reported before (McGuigan *et al.*, 1998; Spuhler *et al.*, 2010), the decrease in CFU/mL of the samples' enteric bacteria broadly follows the first order kinetics, based on log-linear plots (Fig.2.2). Inactivation rate constants observed during the inactivation process reported as k [min^{-1}] were calculated by linear regression (Table 2.2). Considering all the systems tested with W1 and W2, only the photo-Fenton ($Fe^{2+}/H_2O_2/hv$) and hydrogen peroxide systems (H_2O_2/hv) lead to a total inactivation of the fecal indicators bacteria. Irradiation only (*hv*) and Fe^{2+}/hv, as well as blank systems ($H_2O_2/Fe^{2+}/obs$, Fe^{2+}/obs, H_2O_2/obs, *obs*) show a lower decrease in their active enteric bacteria concentration within two hours' irradiation (Fig. 2.2)

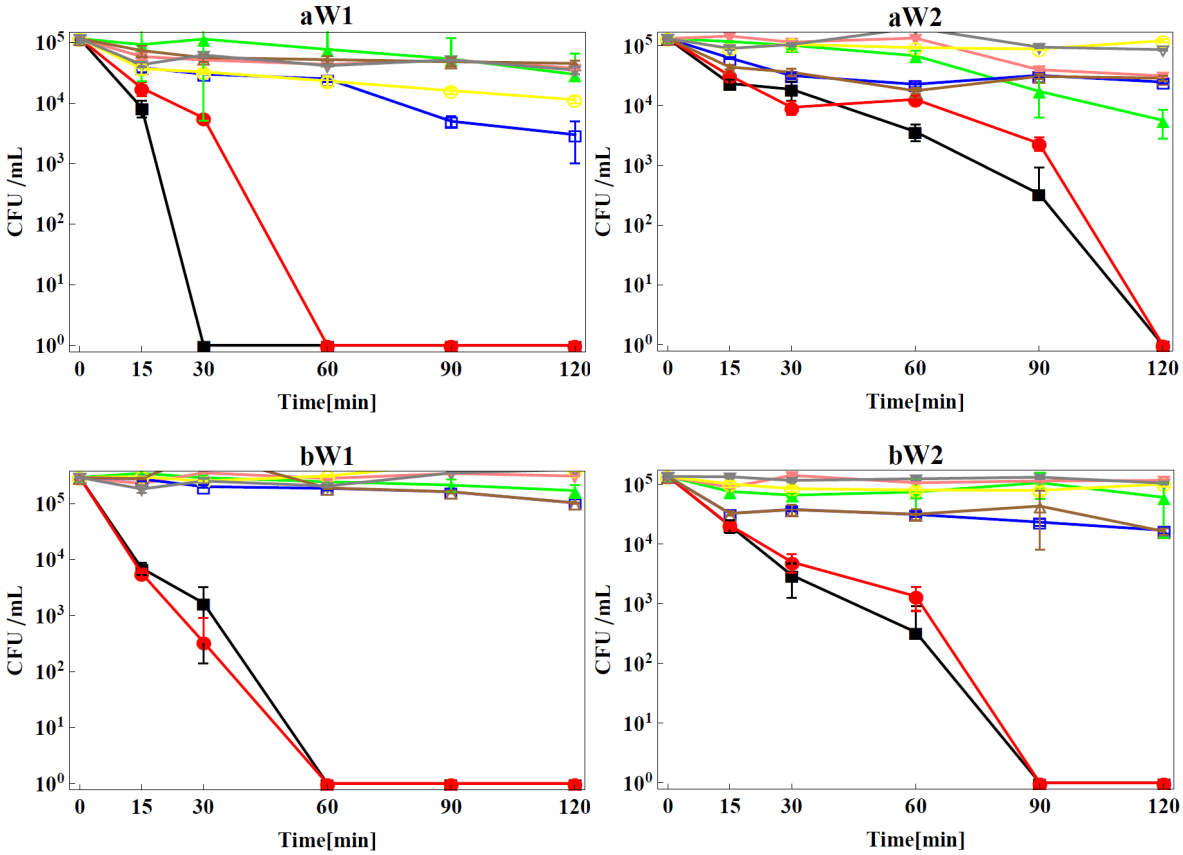


Figure 2.2.: Inactivation of the bacteria content in sample water from well 1(W1, pH: 4.9±0.05) and well 2 (W2, pH: 6.3±0.05) during photocatalytic treatment in the solar simulator Suntest. After the introduction of 90 mL of sample water to the 100 mL glass reactor, 8.5 mg/L of H_2O_2 and 0.6 mG/L of Fe^{2+} were added to the corresponding systems and their dark control. during the experiments, water temperature was< 45°C. (*a*) total coliforms/*E. coli*, (*b*) *Salmonella* spp. (■) $Fe^{2+}/H_2O_2/hv$, (●) H_2O_2/hv, (▲) Fe^{2+}/hv, (▼)*hv* only, (□)$Fe^{2+}/H_2O_2/obs$, (○)H_2O_2/obs, (△) $Fe^{2+}/$ *obs* and (▽) *obs* only. Graphics produced by the Listlogplot function of WOLFRAM MATHEMATICA software.

Table 2.1: Characteristics of some physico-chemical parameters of the water sample used in field experiments

Parameters	Experiment 1 (J1)	Experiment 2 (J2)	Experiment 3 (J3)
Turbidity (NTU)	8±0.2	8±0.2	9±0.1
pH	6.13±0.05	6.26±0.02	6.14±0.05
Temperature (°C)	28.9±0.2	31.1±0.2	29.3±0.2
Initial Iron content (mg/L)	0.07±0.02	0.07±0.02	0.07±0.02
Added Iron (mg/L)	0.6	0.6	0.6
Added H_2O_2 (mg/L)	8.5	8.5	8.5

2.3.2.1. INACTIVATION IN THE SYSTEMS $FE^{2+}/H_2O_2/HV$ AND H_2O_2/HV

Considering only the systems in which the total inactivation of the bacteria were achieved ($Fe^{2+}/H_2O_2/hv$ and H_2O_2/hv), it can be noticed that in W1 (pH=4.9) the photo-Fenton system ($Fe^{2+/}H_2O_2/hv$) induced a stronger inactivation of the enteric bacteria, particularly in the total coliforms/*E. coli* group. This group has shown an inactivation rate constant of (k = -0.3887±0.005 min^{-1}), (table 2.2) and their total inactivation was achieved after 30 min. In the H_2O_2/hv system, we have to take into account the natural presence of 0.07 mg/L of iron in water. Natural iron confers to this system the photocatalytic properties, leading to a high inactivation rate constant (k = -0.1953±0.003 min^{-1}). Total inactivation was achieved in about 60 min. In both systems, the *Salmonella spp.* concentration was totally inactivated after about 60 min.

The ranking of the two photocatalytic systems considering the inactivation rate constant (Table 2.2), gives the following for both wild enteric bacteria at pH 4.9 (W1):

$$k_c^{Fe^{2+}/H_2O_2/hv} > k_s^{H_2O_2/hv} > k_s^{Fe^{2+}/H_2O_2/hv} > k_c^{H_2O_2/hv}$$

At pH 6.3 (W2), the ranking was as follows:

$$k_s^{Fe^{2+}/H_2O_2/hv} > k_s^{H_2O_2/hv} > k_c^{Fe^{2+}/H_2O_2/hv} > k_c^{H_2O_2/hv}$$

C stands for total coliforms/*E. coli* and S for *Salmonella* spp.

The *Salmonella* spp. total inactivation was achieved before the total coliforms/*E. coli* group in both systems in approximately 90 and 120 min respectively.

2.3.2.2. INACTIVATION IN THE SYSTEMS *HV* AND IN ALL THE BLANK SYSTEMS ($H_2O_2/FE^{2+}/OBS$, FE^{2+}/OBS, H_2O_2/OBS, *OBS*)

For water from W1 (pH: 4.9), the irradiation (*hv*) alone and all the blank systems, gives just a slight inactivation of the total coliforms/*E. coli* after the 120 min of exposure. The *Salmonella* spp. content of the sample show an increase instead of a decrease at the end of the period of irradiation (120 min), with an increasing rate constant of (k =0.0013±0.005 min^{-1}). Its concentration increased also in some blank tests carried out in the dark (table 2.2). A slight decrease was observed both in the Fenton system $Fe^{2+}/H_2O_2/obs$ and the system Fe^{2+}/obs with the same inactivation rate constant (k = -0.0080±0.006 min^{-1}).

The inactivation of the total coliforms/*E. coli* under *hv* alone and in the W2 (pH: 6.3) blanks show a slight decrease as in W1. The *Salmonella* spp. content in the sample showed a slight increase in the system *hv* (k = 0.0001±0.005 min^{-1}). All the blank systems give rise to a slight decrease.

Table 2. 2: Inactivation rate constants k [min^{-1}] of each enteric bacteria group observed during the inactivation process, calculated by linear regression for the different photo-catalytic treatments and their corresponding blank conducted in the dark.

		Treatments / k [min^{-1}]							
Water Origin/ pH	Enteric Bacteria	$Fe^{2+}/H_2O_2/h\nu$	$H_2O_2/h\nu$	$Fe^{2+}/h\nu$	$h\nu$	$Fe^{2+}/H_2O_2/Obs$	H_2O_2/Obs	Fe^{2+}/Obs	Obs
Wells 1 pH: 4.9	Total coliforms/ *E. coli*	-0.3887±0.005	-0.1953±0.003	-0.0124±0.005	-0.0055±0.008	-0.0284±0.011	-0.0161±0.005	-0.0062±0.002	-0.0063±0.004
	Salmonella spp.	-0.2085±0.002	-0.2110±0.005	-0.0044±0.012	0.0013±0.005	-0.0080±0.006	0.0041±0.006	-0.0080±0.006	0.0045±0.003
Wells 2 pH: 6.3	Total coliforms/ *E. coli*	-0.1001±0.011	-0.0798±0.007	-0.0260±0.011	-0.0133±0.004	-0.0105±0.015	-0.0002±0.003	-0.0103±0.007	-0.0024±0.003
	Salmonella spp.	-0.1380±0.004	-0.1189±0.006	-0.0025±0.003	0.0001±0.005	-0.0125±0.002	-0.0016±0.002	-0.0125±0.002	-0.0013±0.005

2.3.2.3. INACTIVATION IN THE SYSTEMS Fe^{2+}/HV

The system Fe^{2+}/hv did not lead to a total inactivation of enteric bacteria in both waters (W1 and W2). But a slight inactivation was observed, as presented in fig. 2.2 and by the inactivation rate constants (Table 2.2).

The data shown in Fig. 2.2 and Table 2.2, for a single day experiment, present experiments carried out in triplicate. Experiments were repeated on three different days and a similar inactivation rate was observed.

2.3.2.4. INFLUENCE OF PH, BACTERIA SPECIES AND INACTIVATION SYSTEM ON THE DISINFECTION

A significant difference between the inactivation rate of the total enteric bacteria concentration of both wells (W1: pH 4.9 and W2: pH 6.3) was observed when applying $Fe^{2+}/H_2O_2/hv$ and H_2O_2/hv. The analysis of these processes by the three-way ANOVA program of Mathematica 8.0, allowed us to evaluate the influence of the acidity and other parameters, as presented in Table 2.3. With a Fisher ratio or influence factor (F) of about 48.66, it is possible to state that the pH has a strong impact on the photo-catalytic disinfection process (Malato *et al.*, 2009). The probability (P = 0.001%) gives rise to the assumption that the error may be due to noise. The impact of bacteria (total coliforms/*E. coli* or *Salmonella* spp.) or the treatment ($Fe^{2+}/H_2O_2/hv$ or H_2O_2/hv) on the disinfection process was less significant. The probability that the difference in the inactivation rate related to these two parameters could be due to experimental error was about 11% and 18% respectively for the bacteria species and treatment used. The cross-interactions between the three parameters did not significantly affect the data obtained.

Table 2.3: Three-way ANOVA analysis results

Parameters	DF	SS	MS	F	P
pH	1	21930.8	21930.8	48.66	0.001%
Bacteria species	1	1201.25	1201.25	2.65	11%
Treatments	1	826.875	826.88	1.83	18%
pH/Bacteria species	1	396.75	396.75	0.88	35%
pH/Treatments	1	6.13	6.13	0.01	91%
Bacteria species/Treatments	1	585.21	585.21	1.30	26%
Error	73	32901.8	450.71	-	-
Total	79	57848.8	-	-	-

DF: Degree of freedom, SS: Sum of square, MS: Means square, F: Fisher factor (influence factor) P: Probability.

2.3.2.5. POST-IRRADIATION EFFECT

To be sure that the inactivated bacteria was not just partly damaged, rather than killed, when using the photo-Fenton ($Fe^{2+}/H_2O_2/hv$) or hydrogen peroxide (H_2O_2/hv) systems under light irradiation, after completion of the runs the samples were transferred into sterile flasks and kept at 25-30°C in the dark (*obs*). Further spread plate counts were performed on the samples after 24 h, 72 h and up to one week. No re-growth of coliforms/*E. coli* or *Salmonella* spp. was observed, and this suggested irreversible inactivation.

2.3.3. FIELD EXPERIMENTS

Field experiments indicated that the inactivation rate of both bacteria types applying $Fe^{2+}/H_2O_2/hv$ and H_2O_2/hv was not significantly affected by the difference observed in the solar radiation and temperature each day. Variation in the inactivation rates were observed, however, when light alone was applied. As presented in all the graphs in Fig.2.3, any variation of the solar radiation influences the inactivation of the bacteria. Some of the coliforms/E. coli

inactivation curves have a shoulder. However, the decrease in CFU mL^{-1} of most of the enteric bacteria curves follows first-order kinetics, as in the lab experiments, based on log-linear plots.

2.3.3.1. INACTIVATION UNDER DIRECT SOLAR RADIATION ONLY (*HV*)

2.3.3.1.1. INACTIVATION OF TOTAL COLIFORMS/*E.COLI*

The temperature of 49°C and UV irradiation of more than 30 W/m^{-2} during the first exposure gave rise to the total inactivation of coliforms/*E.coli* within 90 min. On the second day, irradiation was reduced and decreased from 26 W/m^{-2} to 20 W/m^{-2} during the first exposure. Consequently, inactivation was achieved after 120 min only. No re-growth was observed after 24h of storage in the dark over these two days. On the third day, the reduction in temperature to less than 45°C and high fluctuation in the solar radiation (between 23 and 14 W/m^{-2}) did not permit a total inactivation of coliforms/*E.coli* in this system (*hv*). Inactivation of the blank (*obs*) kept in the dark with a water temperature of around 38°C was not significant during the time of experimentation (2 h). But after the 24 h dark-storage period, inactivation of about 97% was observed on the first and third day, and total inactivation on the second.

2.3.3.1.2. INACTIVATION OF *SALMONELLA* SPP.

The favorable conditions during the first day of experimentation enhanced considerably the inactivation of *Salmonella* spp.: about 96% of the population was inactivated during the 2 hours of exposure. On the second day, the relatively low irradiation gave rise to a total inactivation at the end of the illuminated process, but a recovery of the viability during the 24 h dark-storage period was observed (Fig.2.3-b2). The atmospheric conditions on the third day were not favorable and only 44 % was inactivated at the end of the exposure. Blank tests in the system *obs*, did not give a significant inactivation during the experiments and after the 24 h of dark storage, their concentration remained constant.

2.3.3.2. INACTIVATION UNDER ENHANCED SYSTEMS $Fe^{2+}/H_2O_2/HV$ AND H_2O_2/HV

$Fe^{2+}/H_2O_2/hv$ and H_2O_2/hv showed a significant increase in the inactivation rate of bacteria compared to the non-enhanced systems (*hv*) (Fig. 2.3). Moreover, the variation of the solar radiation over the three days did not significantly influence the inactivation kinetics. For both bacteria species, H_2O_2/hv showed a higher inactivation rate than that of the systems with added iron ($Fe^{2+}/H_2O_2/hv$). The added iron has precipitated in the system at pH > 6. An exception was observed in the case of total coliforms/*E. coli* on the third day (Fig 2.3-aJ3), because they were totally inactivated in both systems. This could be related to the variability of the daily solar radiation. Re-growth experiments for both systems during 24 h did not show any bacterial recovery.

For total coliforms/*E. coli*, the control systems ($Fe^{2+}/H_2O_2/obs$ and H_2O_2/obs) did not show significant inactivation during the experiments. However, the inactivation occurred after the 24 h of dark storage for the second and third day. It's occurred only in the system H_2O_2/obs the first day. As in the previous cases, the control systems did not lead to a significant inactivation of *Salmonella* spp. during the experiment. But after the dark storage period (24 h), total inactivation was observed in both systems on the second and third day. None of the systems led to total inactivation on the first day.

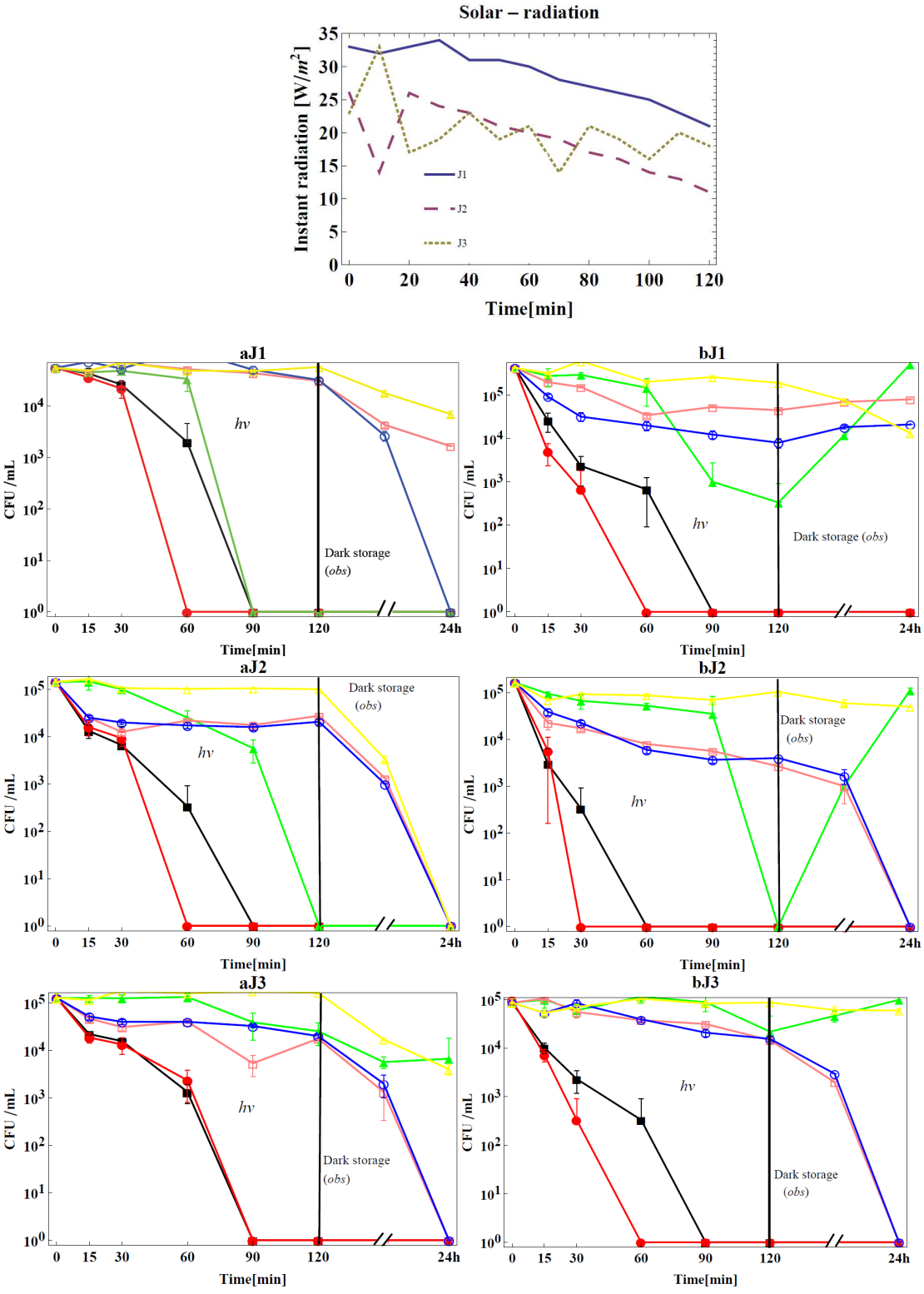


Figure 2.3: Inactivation of the bacteria contained in water sample from wells 2 (W2, pH: 6.3) during the field experiment under direct solar radiation. After the introduction of 1.5 L of water in to the 1.5 L pet reactor, 8.5 mG/L of H_2O_2 and 0.6 mG/L of Fe^{2+} were added to the corresponding systems and their dark control. (*a*) total coliforms/*E. coli*, (*b*) *Salmonella* spp., (J1) 28/04/2010, (J2) 29/04/2010, (J3) 30/04/2010, (■) $Fe^{2+}/H_2O_2/hv$, (●) H_2O_2/hv, (▲) *hv* only, (□) $Fe^{2+}/H_2O_2/obs$, (○) H_2O_2/obs, (△) *obs* only. Graphs produced by the Listlogplot function of WOLFRAM ATHEMATICA software.

2.4. DISCUSSIONS

2.4.1. IRRADIATION CHARACTERISTICS

The efficiency of the solar photocatalytic disinfection of water can be influenced by the sun's intensity, light absorption, initial bacterial concentration, water temperature and turbidity (Moncayo-Lasso *et al.*, 2009; Sciacca *et al.*, 2010; Spuhler *et al.*, 2010; Polo-Lopez *et al.*, 2011b). In the lab experiments, the photo inactivation was carried out under continuous irradiation at constant intensity, with a radiation intensity of 560 Wm^{-2}. This corresponds to 300-400 nm UVA or approximately 32 Wm^{-2} UVA, representing the average UVA radiation of Ouagadougou in summer (Kenfack *et al.*, 2009). During the lab experiments, the water temperature inside the batch reactor remained inferior to 45°C and thermal inactivation can be excluded (Wegelin *et al.*, 1994; Sommer *et al.*, 1997). Direct DNA damage by UVB can also be excluded as the used solar simulator emits negligible amounts of photons at wavelengths shorter than 300 nm (Rincon and Pulgarin, 2003) and the reactor material screened UVB (280-320 nm, fig: 2.1-c). It has to be noted that the water matrix used in this study is natural water, contrary to the laboratory milliQ-water which contains NOM and exogenous photosensitizers.

2.4.2. EXPERIMENTS UNDER SOLAR SIMULATOR

2.4.2.1. IRON SYSTEM (FE^{2+}/HV)

In solutions at low pH (2-3), the irradiation with UV of various hydroxylated Fe^{3+} species produces Fe^{2+} and the hydroxyl radical $OH^{\bullet}$ (Eq. 2.3-2.4) (Safarzadeh-Amiri *et al.*, 1996; Pignatello *et al.*, 2006). The generated $OH^{\bullet}$ radicals are highly oxidant, as explained above. But in natural water (pH: 4.9 and 6.3 respectively), the photoactive ferric hydrolyzed molecules [$Fe(OH)^{2+}$] are not soluble, as the predominant iron component of this pH is the iron-complex which under irradiation generates Fe^{2+}, with an organic radical instead of $OH^{\bullet}$ (Eq. 2.5) (Malato *et al.*, 2009). During the photocatalytic inactivation process in the Fe^{2+}/hv

system, the bactericidal effect of Fe^{2+} arises from its ability to diffuse into the cells, leading to the generation of $OH^{\bullet}$ via intracellular Fenton reactions when reacting with metabolic H_2O_2 (Imlay, 2008; Jang and Imlay, 2010). Spuhler *et al.* (2010), observed a lethal action of the system Fe^{2+}/*hv* during the inactivation of *E. coli* K12, in MilliQ Water (*hv* > 290 nm, pH: 5 to 5.5) resulting in a total inactivation after 120 min. However, in the present study neither the wild total coliforms/*E. coli* nor the *Salmonella* spp. were totally inactivated after the same exposure period in well water (W1, pH 4.9; W2: pH 6.3). The difference in this and Spuhler *et al.* (2010) results could be explained by the nature of the water and bacteria species and by the following pathway: (i) The inactivation process through the ROS generated after the excitation of the exogenous and endogenous photosensitizers was not sufficient to ensure the total inactivation of the wild enteric bacteria involved; (ii) after the active ROS production (20 – 30 min), injured bacteria have developed self- repair mechanisms (Rincon and Pulgarin, 2007a) and became more resistant to the light irradiation, and multiplied (iii) the wild bacteria are more resistant than the manufactured *E. coli* strain regularly used in lab experiments; (iv) the natural water matrix used here contains NOM and other minerals substances. These bacteria cannot create an osmotic stress as in MilliQ water which could weaken the bacteria and support the introduction of Fe^{2+} into the bacteria leading to intra-cellular Fenton ROS inactivation.

2.4.2.2. *SYSTEMS* $Fe^{2+}/H_2O_2/HV$ AND H_2O_2/HV

Natural water in the Sahelian region contains large quantities of iron as it flows on ferruginous substrates (Ben Yahmed, 2005; Sciacca *et al.*, 2010; 2011). It is introduced into the atmosphere by wind and is found in aerosols, fog, rain drops, ground water and lakes (Safarzadeh-Amiri *et al.*, 1996). A total initial iron concentration of about 0.06 mg/l and 0.07 mg/l was detected in W1 and W2 respectively. The high inactivation rate observed in the system H_2O_2/*hv* for both wells as in the systems Fe^{2+}/H_2O_2/*hv* in contrast to that of the

systems Fe^{2+}/*hv* or *hv* only can allow us to assume that the photo-Fenton also takes place in the system H_2O_2/*hv* by using the initial iron contained as the catalyst. From Fig. 2.2., and table 2.2, the difference in the iron content for the systems Fe^{2+}/ H_2O_2/*hv* (natural iron + added iron) and H_2O_2/*hv* (natural iron only) did not significantly influence the inactivation kinetic of both bacteria species in both wells. It can be underlined that it is only in the case of total coliforms/*E. coli* in water from W1 that the difference between the inactivation rates was significant for different photo-catalytic systems. In the remaining systems (*Salmonella* spp. of W1 and total coliforms/*E. coli* and *Salmonella* spp. of W2), the total inactivation was achieved approximately within similar times for both photo-catalytic systems and no significant differences were observed in their inactivation rate constants (k). Considering the initial iron content of water from the Sahelian region, it seems possible to achieve disinfection of water by photo-Fenton process without adding extra iron. This would be a great contribution, not only in reducing the chemical inputs required for the application of the photo-Fenton system for the treatment of drinking water, but also in reducing treatment costs.

2.4.2.3. ILLUMINATION ALONE (*HV*)

The total inactivation of total coliforms/*E. coli* or *Salmonella* spp. was not observed in the reactor under the effect of light irradiation alone. This could be related to the fact that the exposure time was just 2 hours, and not 5 or 6 hours as recommended for the SODIS process (Reed, 2004). As the present study was focused on the reduction of the solar disinfection exposure time by the photo-Fenton, the 2 hours exposure were sufficient to obtain a significant inactivation rate using Fe^{2+}/ H_2O_2/*hv* and H_2O_2/*hv*.

2.4.2.4. *PH*

Great differences and contradictions were observed in the W2 (pH 6.3) in both systems where total inactivation was achieved, as the *salmonella* spp. strain was the first to be totally inactivated in both systems in about 90 min, while that of total coliforms/*E. coli* took about

120 min (Fig. 2.2). This situation is in contrast with the assumption that *Salmonella* spp. is more resistant to photocatalytic inactivation than *E. coli* (Bosshard *et al.*, 2009; Sciacca *et al.*, 2010). Indeed, even in the W1 (pH: 4.9), in the system H_2O_2/*hv*, both enteric bacteria species were inactivated approximately at the same time and the inactivation rates constant of *Salmonella* spp. were slightly greater than those of total coliforms/*E. coli* (-0.2110±0.005 > -0.1953±0.003) (table 2.2). It is only in the system Fe^{2+}/ H_2O_2/*hv* that the *E. coli* was rapidly inactivated before the *Salmonella* spp.

2.4.2.5. CONTROL EXPERIMENTS: (H_2O_2/FE^{2+}/*OBS*, FE^{2+}/*OBS*, H_2O_2/*OBS*, *OBS*)

None of the control systems (blank) led to total inactivation of the wild enteric bacterial concentration, even though a slight inactivation was observed in some cases for both wells. These results correspond with previous studies (Rincon and Pulgarin, 2007a; Spuhler *et al.*, 2010). However, it was noticed that total coliforms/*E. coli* inactivation was more pronounced in W1 (pH: 4.9) than in W2 (pH: 6.3) (table 2.2), but that situation was the reverse for the *Salmonella* spp. inactivation, whose population even increased in some systems (H_2O_2/*obs* and *obs*).

2.4.3. EXPERIMENTS UNDER DIRECT SOLAR RADIATION

The decrease of the enteric bacterial amount and even its total inactivation in the control systems (Fe^{2+}/H_2O_2/*obs* and H_2O_2/*obs*), after the 24 hours of dark storage could be due to the scavenging action of H_2O_2 as it is also a powerful ROS (Spuhler *et al.*, 2010; Polo-Lopez *et al.*, 2011b). It should be noticed that at high pH (6.3) in the dark, the iron precipitation makes them unavailable to initiate the simple Fenton reaction (Eq: 2.1) (Malato *et al.*, 2009). The *hv* and *obs* systems are expected not to produce ROS during the 24 h dark storage, thus explaining why no more inactivation was observed in the remaining total coliforms/*E. coli* and for *Salmonella* spp. The increased concentration after the storage, could be due to the fact that during the dark storage without ROS production to injure them, the bacteria recover their

ability to grow and replicate (Rincon and Pulgarin, 2007a). For total coliforms/*E. coli*, no re-growth was observed after total inactivation. The re-growth of *Salmonella* spp. was due to resistance to solar disinfection (not enhanced), as recently reported (Bosshard *et al.*, 2009).

For the enhanced systems, H_2O_2/hv shows a better inactivation kinetic than that of the systems containing added iron ($Fe^{2+}/H_2O_2/hv$). It can be assumed that with the low Fe-concentration in natural water (0.07 mg/L), this amount was enough to start, in the presence of H_2O_2/hv, the photo-Fenton process and generate $OH^{\bullet}$ inactivating of the enteric bacteria. The higher inactivation kinetic of the systems H_2O_2/hv compared to $Fe^{2+}/H_2O_2/hv$ may suggest that a high iron concentration could have a negative effect on the photo-Fenton treatment of natural water close to neutral pH. This negative effect could be due to: (i) iron precipitation resulting in the lack of soluble iron in the medium to maintain the photocatalytic cycle, (ii) the reduction of light transmittance due the increased turbidity of water and its coloration due to high iron contents, (iii) excess iron concentration in the solution increasing the $OH^{\bullet}$ scavenging potential (Eq: 2.6) and concomitantly reduce the efficiency of the process (Pignatello *et al.*, 2006).

$$Fe^{2+} + OH^{\bullet} \rightarrow Fe^{3+} + OH^{-} \qquad (2.6)$$

On the third day, the *Salmonella* spp. showed approximately the same inactivation kinetic as that of the first day under the best atmospheric conditions. Inactivation on the second day, under the same temperature but lower irradiation (26-20 Wm^{-2}) was higher than on the first and third day. These observations are related to the results for total coliforms/*E. coli*, which show also approximately the same inactivation kinetic on the first and second day. It could be assumed that up to a certain level of irradiation and temperature, the influence of the two parameters (temperature and irradiation intensity) on the photo-Fenton inactivation process is no longer significant. The same observation was made previously by Ubomba-Jaswa *et al.* (Ubomba-Jaswa *et al.*) during the investigations into the effect of the UVA dose on the

inactivation of *E. coli* K12. However, a slight reduction of the inactivation kinetic of total coliforms/*E. coli* on the third day in the systems H_2O_2/*hv* (Rincon and Pulgarin, 2007a) is indicative that intermittent irradiation associated with low irradiation has negative influence on the bacterial inactivation rate.

2.4.4. INACTIVATION PATHWAYS

2.4.4.1. INACTIVATION IN THE ILLUMINATED SYSTEM

In all the illuminated systems, part of the observed photo-inactivation could be due to excitation of exogenous (ferric-hydro-complex or ferric-organo-complex) and endogenous (cytochrome, flavin, tryptophan) photosensitizers, (Pignatello *et al.*, 2006; Malato *et al.*, 2009) as well as the ROS-action ($^1O_2$, $O_2^{\bullet-}$, $OH^{\bullet}$ and H_2O_2) generated from the dissolved oxygen (O_2) contained naturally in the water via successive steps of one-electron reductions (Pignatello *et al.*, 2006; Imlay, 2008). The $O_2^{\bullet-}$ and/or the H_2O_2 have the ability to attack proteins and cell membrane components, especially membrane lipids, resulting in their peroxidation (Imlay, 2008). This peroxidation increases the cell membrane permeability and the disruption of the trans-membrane ion gradients (Reed, 2004), which can lead to the inactivation of the cells. H_2O_2 is a non-charged molecule and penetrates readily the cellular membranes (Spuhler *et al.*, 2010). The toxicity of H_2O_2 is due to the fact that they can induce the production of $OH^{\bullet}$ through the Fenton reaction (eq. 2.1) within the cell (Imlay, 2008). $OH^{\bullet}$ is a highly-reactive oxidant, which can degrade non-biodegradable chemical components (Kenfack *et al.*, 2009; Malato *et al.*, 2009), NOM (Moncayo-Lasso *et al.*, 2009; Spuhler *et al.*, 2010) and inactivate bacteria (Sciacca *et al.*, 2010; Spuhler *et al.*, 2010).

2.4.4.2. INACTIVATION OF DEFENSE MECHANISMS

The deficiency in the cellular defense against ROS, constituted by enzymes such as SOD/ SOR which control the $O_2^{\bullet-}$ or catalase which regulate the H_2O_2 concentration (Imlay, 2008), can result in an oxidative stress leading to the increase of the ROS content of the cells at the

level exceeding their defense capacity (Reed, 1997; Jang and Imlay, 2010). This deficiency in self defense mechanisms can arise from the exposure of these enzymes to thermal or optical inactivation (Wegelin *et al.*, 1994). Ghadermarzi and Moosavi-Movahedi (1996) suggested that inactivation arises when the temperature is around 45°C. Considering the temperature measured during the photo-inactivation process in this study, it can be assumed that the enzymes for self-defense mechanisms were not efficient, giving rise to the optical inactivation through UVA radiation to efficiently inactivate the bacterial contents of the water (Sommer *et al.*, 1997). The inactivation of the SOD/SOR and catalase leads to the increase of intracellular ROS with the direct attack of membrane and other proteins. Followed by the generation of the highly-reactive $OH^{\bullet}$ via intracellular Fenton reaction (Eq 2.1.). This reaction take place between the H_2O_2 and the iron liberated from the iron sulfur clusters ([4Fe-4S]) after the inactivation of clusters enzymes like dihydroxy-acid deshydratase, aconitase B and fumarases A and B (Jang and Imlay, 2010) by the $O_2^{\bullet-}$ (Imlay, 2006). The liberation of free iron in the cell comes from the specific oxidizing action of the superoxide on the centers [4Fe-4S] of these hydrolytic enzymes(Imlay, 2006; Jang and Imlay, 2010). The $OH^{\bullet}$ attacks lead to important cellular damage on DNA (Jang and Imlay, 2010). When the bacteria are not sufficiently exposed to illumination, they can recover viability by self-defense mechanisms in a short time (Rincon and Pulgarin, 2003). In this study, the systems Fe^{2+}/*hv* or *hv* have showed a slight initial inactivation in both wells in about 20 or 30 min depending on the enteric bacteria involved (Fig: 2.2.). After this time, their concentration in the water have increased and stabilized till the end of the two hours of irradiation. This situation could be explained by the recovery of the self-defense mechanisms suggested by Rincon and Pulgarin (2003). After such recovery, the actors of the defense constituted by SOD and catalase, which have been certainly weakened but not inactivated, have recovered their properties and eliminated the exceeding ROS ($O_2^{\bullet-}$, H_2O_2), giving rise to a recovery in the damaged bacteria, which

multiplied and stabilized in the medium, (see figure 2.2). In the systems, $Fe^{2+}/H_2O_2/hv$ and H_2O_2/hv, the photo-Fenton under light and high or low iron content increase the $OH^{\bullet}$ production. This increase led to an increased inactivation of wild enteric bacteria at high inactivation rates compared to those found for Fe^{2+}/hv or hv. The Fe^{3+}-bacteria interaction are enhanced in the presence of H_2O_2 with the increased production of $OH^{\bullet}$ and fast Fe^{2+}/Fe^{3+} interconversion under illumination (Spuhler *et al.*, 2010). $OH^{\bullet}$ is the most powerful oxidant generated inside the cells. It reacts instantly with no selectivity, at the diffusion limits, with sugars, amino acids, phospholipids, nucleotides and organic acids including DNA (Valentine *et al.*, 1998). Cellular defense mechanisms against a DNA attack by $OH^{\bullet}$ were not yet discovered.

2.5. CONCLUSIONS

This study showed that the H_2O_2/hv was as efficient as the photo-Fenton system ($Fe/H_2O_2/hv$) in significantly increasing the inactivation rate of the enteric bacteria contents of the water wells. The iron naturally present in the well water influences the reaction mechanism of H_2O_2/hv by favoring the photo-Fenton process. The efficiency of the system $H_2O_2/hv/$natural water in lab experiments under simulated solar radiation was confirmed in the field in PET bottles showing a better inactivation rate than ($Fe^{2+}/H_2O_2/hv/$natural water). Consequently, it can be assumed that iron as the catalyst of the photo-Fenton process is not necessary in high concentrations to inactivate enteric micro-organisms. The lower efficiency of the system $Fe^{2+}/H_2O_2/hv/$natural water may also indicate that an optimum concentration of iron for an efficient photo-Fenton process exists. Indeed, at near neutral pH (6.3), a part of added iron salts precipitate and negatively affects the color and turbidity and light transmittance and leads to a concomitant decrease in the disinfection efficiency. This result suggests the use of

Sahelian Fe-containing region to perform a photo-Fenton treatment of drinking water by adding H_2O_2 only.

The fastest inactivation kinetic of *Salmonella* spp. compared to that of the total coliforms/*E. coli* in the water with the near-to-neutral pH (W2, pH: 6.3) brings us to the assumption that the pH can significantly influence the resistance of these enteric bacteria to photo-catalytic inactivation. The results have to be confirmed by further research, however in order to establish the best disinfection process considering all the parameters affecting wild enteric bacteria inactivation.

3. CHAPTER 3:

RELEVANT IMPACT OF IRRADIANCE (VS. DOSE) AND EVOLUTION OF PH AND MINERAL NITROGEN COMPOUNDS DURING NATURAL WATER DISINFECTION BY PHOTO-FENTON IN A SOLAR CPC REACTOR.

3.1. INTRODUCTION

Microbial contamination of water sources by farming, breeding and/or domestic activities in developing countries, reduces the amount of available drinking water and increases waterborne diseases outbreak such as dysentery, typhoid and cholera, as recorded in these countries (UNICEF and WHO, 2012). Therefore, there is a need to develop low-cost water disinfection processes for rural and sub-urban areas. Disinfection is the process of removal of the pathogenic microorganisms present in water. Many developing countries are situated in the latitude lines of 30°N and 30°S and receive about 2000 to 3000 hours of solar illumination annually. Solar disinfection of water (SODIS) was first assessed in Beirut and afterwards in other tropical regions (Meierhofer and Wegelin, 2002; McGuigan *et al.*, 2011). SODIS principles imply the synergistic effect of sunlight and temperature on enteric bacteria inactivation. Inactivation is a process which consists to the inhibition of biological activity of an organism (e.g. bacteria) by the action of heat, chemical or other agent. As a result, SODIS treatment is used by more than 4.5 million people in more than 50 countries (Meierhofer and Wegelin, 2002).

Researches on SODIS enhancement were focused on (i) the use of black back bottles to speed up increase of the temperature (Kehoe *et al.*, 2001) either by using Pyrex bottles, instead of glass or polyethylene terephthalate (PET) bottles to increase UV-A radiation penetration, (ii) increase oxygenation of the system through the agitation of the bottles before exposition (Kehoe *et al.*, 2001), (iii) the use of azo dyes as dosimetric indicators to enhance the photocatalytic process during the disinfection (Bandala *et al.*, 2011), (iv) the enhancement of production of highly oxidant hydroxyl radicals ($OH^{\bullet}$), which increases the inactivation rate by

the addition of a photocatalyst i.e. TiO_2, (Rincon and Pulgarin, 2007a) or H_2O_2 and iron salts (Sciacca *et al.*, 2010; Spuhler *et al.*, 2010). In order to enhance the solar disinfection process, compound parabolic collector (CPC) solar reactor has been operated with the addition of catalyst (TiO_2 or iron salts) and/or oxidants (H_2O_2) (Rincon and Pulgarin, 2007a; Bichai *et al.*, 2012; Ndounla *et al.*, 2013). Also, previous photo-disinfection treatment carried out under solar exposure in a CPC suggested that the accumulated radiation dose had a great influence on bacterial inactivation rate (Ubomba-Jaswa *et al.*, 2009; Rodriguez-Chueca *et al.*, 2012). In the present work we assume that as the natural water under treatment contains dissolved and solid iron forms, the addition of H_2O_2 will generate under solar light a homogeneous and heterogeneous photo-Fenton system. Photo-Fenton, as well as dark Fenton reaction, was, in the past, considered to take place only within acidic pH values (Pulgarin and Kiwi, 1996; Cho *et al.*, 2004; Pignatello *et al.*, 2006). In these conditions the dark Fenton reaction (Eqs. 3.1-3.2) is limited by the low kinetic of Fe^{2+} production (Eq. 3.3) (Bandara *et al.*, 1997; Herrara *et al.*, 1998; Pignatello *et al.*, 2006). High production rates of additional Fe^{2+} and $OH^{\bullet}$ are generated during the photoreduction of Fe^{3+}-complexes in the solution (Eqs. 3.3-3.7). The primary step of the photoreduction of dissolved ferric iron is a ligand-to-metal charge-transfer (LMCT) reaction. Fe^{3+}-complexes undergo LMCT excitation to give Fe^{2+} and an oxidized ligand, L_{ox} (Eq. 3.3). At acidic pH, hydroxyl radicals ($OH^{\bullet}$) and Fe^{2+} are produced from Fe^{3+}-aquo-hydroxy-complexes ($Fe(OH)^{2+}$, $Fe(H_2O)^{3+}$), which absorb light in the UV/visible region (Eqs. 3.4–3.5) (Herrara *et al.*, 1998; Pignatello *et al.*, 2006; Malato *et al.*, 2009; Vedrenne *et al.*, 2012). Nevertheless, photo-Fenton treatment was successfully evaluated at near-to-neutral pH (Sciacca *et al.*, 2010; Carra *et al.*, 2012). Indeed a part of the natural organic matter (NOM) complexes Fe^{3+} and maintains it in solution. When a natural water sample containing Fe^{3+}-NOM complexes is photo-irradiated, the Fe^{3+} is reduced to Fe^{2+} and the NOM is oxidized (Eq. 3.6) . For example, the photolysis of Fe^{3+}-NOM carboxylate or Fe^{3+}-

polycarboxylate complexes through LMCT reaction, leads to the formation of Fe^{2+}, which is introduced in the photo-Fenton cycle and the concomitant oxidation of the organic ligand (Eq. 3.6-3.7), (Malato *et al.*, 2009; Vedrenne *et al.*, 2012; Rodríguez-Chueca *et al.*, 2013).

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\bullet} + OH^{-} \qquad k = 53 - 76\ M^{-1}s^{-1} \qquad (3.1)$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+}\ HO_2^{\bullet} + H^{+} \qquad k = 1 - 2 \times 10^{-2}\ M^{-1}s^{-1} \qquad (3.2)$$

$$Fe^{3+}(L)_n + h\nu \rightarrow Fe^{2+}(L)_{n-1} + L_{ox} \qquad (3.3)$$

$$Fe(OH)^{2+} + h\nu \rightarrow Fe^{2+} + OH^{\bullet} \qquad (3.4)$$

$$Fe(H_2O)^{3+} + h\nu \rightarrow Fe^{2+} + OH^{\bullet} + H^{+} \qquad (3.5)$$

$$Fe^{3+}\text{-}(NOM)_m + h\nu \rightarrow Fe^{2+}\text{-}(NOM)_{m-1} + NOM_{ox}^{+} \qquad (3.6)$$

$$R\text{-}OOC\text{-}Fe^{3+} + h\nu \rightarrow Fe^{2+} + R\text{-}COO^{\bullet} \rightarrow Fe^{2+} + CO_2 + R^{\bullet} \qquad (3.7)$$

In presence of irons oxides as is the case in the water under study in this work, the heterogeneous photo-Fenton system could also be considered. This heterogeneous action could take advantage of the indiscriminant siderophore transport system present in bacteria. The siderophore is a molecular receptor that binds and transports iron (Neilands, 1995; Stintzi *et al.*, 2000). Their impact on the heterogeneous photo-Fenton system could proceed from the fact that: (i) the siderophore and Fe^{3+} enter the bacterium together leading to internal Fenton reaction (Stintzi *et al.*, 2000; Spuhler *et al.*, 2010), (ii) a ligand exchange step occurs in the course of the transport and (iii) the implementation of the photo-Fenton reaction due to the semiconductor action of some forms of iron oxides naturally present in water. (Mazille *et al.*, 2010).

Several studies on solar and photocatalytic disinfection efficiency have been reported using *E. coli* K12 in lab (Spuhler *et al.*, 2010; Rodriguez-Chueca *et al.*, 2012) and at field scale with

natural water (Rincon and Pulgarin, 2007a; Ubomba-Jaswa *et al.*, 2009; Polo-Lopez *et al.*, 2011a). However, *E. coli* was determined to be not always an appropriate indicator for bacterial inactivation monitoring, due to its high sensitivity to photo-inactivation (Berney *et al.*, 2006; Sciacca *et al.*, 2010). Recently, some authors have evaluated the efficiency of photo-Fenton on the inactivation of several microorganisms (Sciacca *et al.*, 2011; Bandala *et al.*, 2012; Michael *et al.*, 2012; Polo-Lopez *et al.*, 2012; Rodríguez-Chueca *et al.*, 2013). Sciacca *et al.* (2011) have reported that photo-Fenton disinfection of wild total coliforms and *Salmonella* spp. in natural water containing NOM (turbidity: 800-1000 NTU (Nephelometric Turbidity Units)) was not efficient. This inefficiency was due to the consumption of the H_2O_2 by the NOM. It is a need before intending to address the photo-Fenton disinfection of drinking water to human consumption to evaluate it efficiency on disinfecting bigger volumes of natural clear water of turbidity less than 30 NTU, as recommended in SODIS references (Meierhofer and Wegelin, 2002).

Most wells intended to drinking water collection are situated in agricultural areas in the Sahelian region. Individual wells in agricultural areas throughout the world specifically contribute to nitrate-related toxicity problems and nitrate levels in the well water often exceed 50 mg/L (WHO, 2011b). The photochemical reduction of nitrite or nitrate and the chemical oxidation of ammonia are the most involved pathways during the interconversion of the nitrogen compounds in natural water. Some authors have reported that upon sunlight irradiation of natural waters in presence of humic substances, nitrate and nitrite salts are produced and reactive oxygen species (ROS) are generated (Kotzias *et al.*, 1987; Fanning, 2000). Nitrates in the soil are from various origins; it could be from humus degradation, fresh or composted natural organic matter that is used as fertilizer or from nitric nitrogen of chemical fertilizers. The infiltration of nitrates in wells' water can induce high concentrations, even greater than the restrictions of the World Health Organization (WHO) guidelines for

drinking water (50 mg/L). The toxicity of nitrate to humans is mainly attributable to its reduction to nitrite (WHO, 2011b). The health risk related to excess nitrate in drinking water is mostly related to its transformation (oxido-reduction), which can lead to more toxic compounds, such as nitrite and nitrosamines. Nitrosamines are formed by the combination of nitrite or nitrate with amines or amides. Most of them are classified as carcinogenic by the WHO. The WHO guideline for nitrite in drinking water is less than 3 mg/L (WHO, 2011a, b).

Moreover, the term ammonia includes the non-ionized (NH_3) and ionized (NH_4^+) species. Ammonia in the environment originates from metabolic, agricultural and industrial processes and from disinfection with chloramine. Natural levels in groundwater and surface water are usually below 0.2 mg/L, while anaerobic groundwaters may contain up to 3 mg/L. Farm animals excreta lead to higher levels in surface water. Ammonia in water is an indicator of possible bacterial, sewage and animal waste pollution. Taste and odor problems, as well as decreased disinfection efficiency, are to be expected if drinking-water containing more than 0.2 mg/L of ammonia. Toxicological effects are observed only at exposures above about 200 mg/kg body weight. Ammonia in drinking-water is not of immediate health relevance and therefore no health-based guideline value is proposed. (WHO, 2003; Weng *et al.*, 2011; WHO, 2011a).The current study aims mainly to evaluate the contrasting effect of solar radiation parameters (Irradiance vs. Dose) on the efficiency of photo-disinfection of wild total coliforms/*E. coli* and *Salmonella* spp. under the addition of H_2O_2 in a natural drinking water source containing dissolved a solid iron forms. The evaluation of the impact of different irradiances and doses was carried out following several exposure sets of the water sample in a CPC solar reactor, during different day times. This evaluation could suggest a schedule of the most favorable day periods which could be proposed to the users, if the vulgarization of the processes is considered.

Considering the impact of the pH on the efficiency of the photo-disinfection and the proximity of the studied wells with agricultural areas, the monitoring of the variation of pH, NH_4^+, NO_3^- and NO_2^- during the photo-treatment will be follow in this study in order to evaluate the impact of these parameters on the photo-disinfection treatment and vice-versa as well as the possible formation of the health related disinfecting byproducts.

3.2. MATERIALS AND METHODS

3.2.1. CHEMICAL REAGENTS

Hydrogen peroxide, 30% (AnalaR Normapur, VWR) was used to prepare the Fenton reagent. Hydrochloric acid fuming (HCl), 37% (Fluka Analytical, SIGMA-ALDRICH®) was used for glass-reactor cleaning. HACH specific reagents were used for total iron, nitrite, nitrate and ammonia detection. Microbiology Chromocult ® (Merck KGaA) was used for bacterial plating. Growth media was poured in pre-sterilized Petri Dish; 92x16mm (Sarstedt AG) for bacterial enumeration.

3.2.2. ANALYTICAL METHODS APPLIED FOR MEASURING THE PHYSICAL PARAMETERS OF WATER

A Universal meter WTW 340i equipped with a WTW SenTix 41-3 probe was used to measure the pH and temperature (T°C). The concentration of Hydrogen peroxide (H_2O_2) was monitored during the experiments via Merckoquant peroxide analytical test strips (Test Peroxides, Merck Merckoquant), while detection limit was 0.5 mg/L.

The HACH DR/2000 spectrophotometer methodologies used in this study to characterize some physico-chemical properties of the water sample follows the guidelines of the Standard Methods for Examination of Water (HACH, 2001). The water turbidity was evaluated with the program 750 (wavelength 450 nm) and the detection ranges were between 0 to 450 NTU/FTU (Nephelometric turbidity units/ Formazin Turbidity Units). The nitrate (NO_3^-)

contents was determined with the High Range (HR) program 355 or the Cadmium Reduction Method (NitraVer 5, Nitrate Reagent Powder Pillow, wavelength 500 nm) and the detection ranges were between 0 to 30.0 mg/L NO_3^--N. The program gives the results as the concentration of (X) NO_3^- in N (nitrogen) contents of the sample (NO_3^--N) and the exact concentration of NO_3^- in the water was calculated using the HACH Species Conversion Factors (SCF) specific for each component and program. The SCF of NO_3^--N is 4.427mg/L, then NO_3^- = X*4.427 mg/L. The nitrite (NO_2^-) contents was determine with the Low Range (LR) program 371 or Diazotization Method (NitriVer 3 Powder Pillows, detection ranges between: 0 to 0.300 mg/L NO_2^--N), for wavelength 507 nm. As for the calculation of nitrate, nitrite is subject to a SCF number (3.284), then its exact determination was through the calculation NO_2^- = X'*3.284 mg/L. The ammonia (NH_4^+) concentration was evaluated with the Nessler Method program 380 for wavelength 425 nm and detection ranges between (0 to 2.50 mg/L NH3-N). The result was of $NH4^+$ concentration in N contents in the water was obtained after the calculation with the ammonia SCF (1.288), $NH4^+$ = X"*1.288 mg/L. X, X' and X" was the number read on the spectrophotometer DR/2000 during each specific measure; the results presented in this paper are the average for each components recorded upon the experiments (HACH, 2001). On site at Ouagadougou (2iE), with the HACH process, the dissolved total iron content of the water sample was determined by the FerroVer Method (Powder Pillows), program 265, wavelength 510 nm and the detection ranges were between 0 to 3.00 mg/L. Further at Lausanne (EPFL) the solid total iron (iron oxides) was evaluated with the ICP-MS spectrometry, with sensitive detection limit ranges (0.1-0.9 µg/L).

3.2.3. WATER SAMPLE CHARACTERISTIC

The experiments presented in this study were carried out from February to March 2011 (dry/summer season) in Burkina Faso. The water samples were collected from a family well of Tanghin district of Ouagadougou. Ouagadougou is located at 12°21'26" of Latitude North

and 1°32'7" of Longitude West and the experiments were conducted at the site of 2iE Foundation. This location is subject to approximately 2500 hours of solar radiation per year (Kenfack *et al.*, 2009). Then it could be considered as a good place for the experimentation of solar and photo-Fenton disinfection of drinking water. Table 3.1 presents the initial concentration of some relevant physico-chemical parameters of the water and the microorganisms considered during the disinfection process. Sampling was performed one hour before the experiments. For the laboratory experiments, it was collected in 1.5 L PET bottles, while for field experiments; plastic jerricans of 20 liters were used.

Table 3.1: Some characteristics of the wells water sample used during the experiments

Parameters	**Contents**
Turbidity	5± 3 NTU
pH	5.4 ± 0.1
Temperature	29± 0.1°C
Disolve total iron	0.07± 0.02 mg/L
Solids total iron	0.23± 0.01 mg/L
Wild *E. coli*	10^4 CFU/mL
Wild *Salmonella* spp	10^5 CFU/mL

NTU=Nephelometric Turbidity Units, CFU/mL=Colony Forming Unit per milliliter, °C=Degre Celsus, mg/L=milligram per liter

3.2.4. LABORATORY EXPERIMENTS UNDER SIMULATED SOLAR RADIATION

To evaluate the effect of H_2O_2 concentration on the photo-Fenton inactivating rate, 90 mL of the sample were introduced in 8 glass reactors of 100 mL each containing different H_2O_2

concentrations (0, 2, 4, 5, 6, 7, 8 and 10 mg/L) and were irradiated in a solar simulator (Hanau Suntest). The radiation intensity applied for lab-scale experiments was 560 $W.m^{-2}$ (32 $W.m^{-2}$ in the UV-A) which is the average UV-A delivered by sun light in Ouagadougou during summer times (Kenfack *et al.*, 2009; Sciacca *et al.*, 2011).

To evaluate the bacterial inactivation rate, 1 mL of the sample was taken at time intervals (0, 10, 20, 30, 45, 60, 90, 120, 150, 180, 240, 300, 360 min) during the experiment (6 h) with a sterile 5 mL syringe connected to the reactor and placed in a sterile 1.5 mL Eppendorf microtube. From this 1 mL, 100 µL were sampled and poured in a Petri dish plate containing growth media (Chromocult agar). Plates were incubated for 18 -24 h at 37°C and bacteria were counted with a colony counter (Stuart SC6 Colony Counter). Specification and use as well as plating and counting with Chromocult agar have been described before (Ndounla *et al.*, 2013).

The final treated samples were kept in the dark for bacterial regrowth assessment after 24h, 72h and a week. To ensure the residual effect of the Fenton reaction in the treated water during the storage, the samples were kept in the dark without removing their remaining H_2O_2. This residual content was measured every day during the storage for the evaluation of its decay in the water before its possible consumption. The experiments were repeated five times to ensure reproducibility.

3.2.5. FIELD EXPERIMENTS UNDER DIRECT SOLAR RADIATION

Field experiments were conducted under solar radiation in a solar CPC with 25 L of well water at constant flow (2 L/min).

From the results of the determination of the optimized concentration of H_2O_2 to be used efficiently in drinking disinfection by photo-Fenton, evaluated at lab scale in the Suntest, the concentrations from 5 to 10 mg/L has shown approximately the same inactivation rate.

Preliminary experiments were conducted at field scale with 5 mg/L of H_2O_2 added on the water sample. Unfortunately the H_2O_2 concentration in this case, in contrast to the stability noticed at lab scale, was degraded certainly due to high fluctuation of water in the CPC, which increased the oxygenation. This fast degradation has lead to total consumption of H_2O_2 after only 3 to 4 hours of exposure. In order to ensure that there is H_2O_2 left after the disinfection process (6 h), to ensure the residual effect of the Fenton treatment, the experiments presented in this paper were carried out with 10 mg/L of H_2O_2. The remaining H_2O_2 in the treated water, when 10 mg/L was used, 2 to 3 mg/L; however the continuous evaluation of it persistence in the treated water has permitted to record that it was depleted totally after 48 hours.

Control experiments were carried out on samples without added H_2O_2. The efficiency of both photo-disinfection process (SODIS and photo-Fenton), was evaluated during three different time period of 6 hours: (i) 8 am to 2 pm (8-14h), (ii) 10 am to 4 pm (10-16h) and (iii) 12 pm to 6 pm (12-18h). The samples were subject to the variation of: (i) water temperature, (ii) instantaneous irradiation (irradiance) ($W.m^{-2}$) and (iii) cumulated global radiation (dose) ($Wh.m^{-2}$). Blank tests took place in the dark, a 100 mL samples also containing H_2O_2. Experiments were repeated three times to ensure reproducibility.

The batch photoreactor (Fig.3.1) used in this study was a Compound Parabolic Collector (CPC): SOLARDETOX ACADUS-2003 device model delivered by Ecosystem SA, (Barcelona, Spain). It has a useful exposition surface of 2.12 m^2, out of a total surface of 2.54 m^2. The photoreactor active volume was 15.1 L within a total volume of 16.07 L and a working volume between 18 L and 50 L. It is made of 16 borosilicate cylindrical glass tubes of 32 mm diameter, 1.5 m length and 1.4 mm of width, through which water is circulating and exposed to solar irradiation. Tubes are disposed on aluminum cylindro-parabolic mirrors in such a way that the distribution of the UV irradiation by the mirrors is equal around the tube

circumference. This configuration implies no light concentration, but allows working with diffuse light. A polypropylene stirring tank of 50 L is connected in series with the CPC module and constitutes a re-circulating tank. Hence, the pilot plant behaves as a plug-flow reactor in which water is circulating using a centrifugal pump with a flow of 24.2 $L.min^{-1}$.The reactor is mounted on a two-position fixed platform inclinable at 10° and 35° allowing operating at the approximate local latitude of Ouagadougou-Burkina Faso (12.2° N), at 10° angle position for the most of the time. The solar UV radiation was reported during the experiments by a UV-A radiometer ACADUS 85 UV fixed on the CPC photoreactor at the same inclination of 10°. Solar irradiance intensity per square meter ($W.m^{-2}$) was then monitored between 300 and 400 nm.

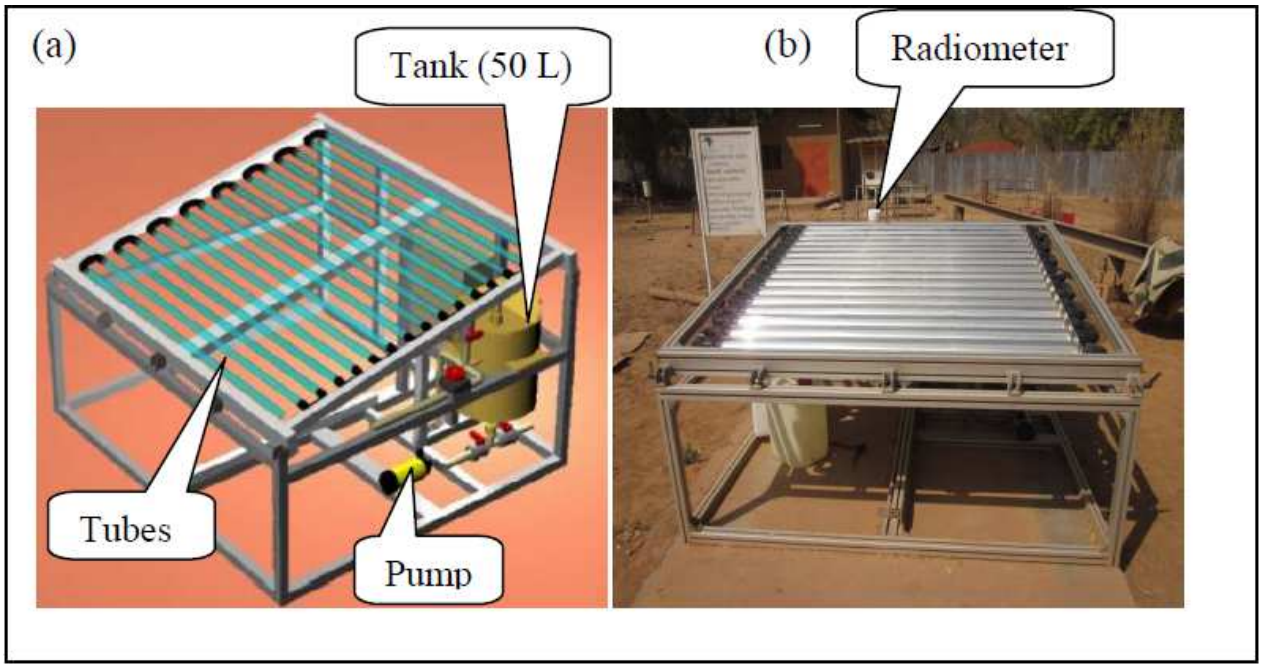


Figure 3.1. The schematic (a) and physical (b) representation of the compound parabolic collector (CPC) solar reactor.

3.2.6. DATA ANALYSIS

The Effective Disinfection Time (EDT), which is the time (h) required to get the total inactivation in water of a targeted bacteria in defined conditions (Rincon and Pulgarin, 2004b)

will be used for the comparative study on the influence of the irradiance on the bacteria inactivation rate.

AI: Average irradiance during the EDT ($W.m^{-2}$). (8)

Dose for total inactivation = AI*EDT ($Wh.m^{-2}$). (9)

The evaluation of the impact of the H_2O_2 concentration and the bacteria species on the efficiency of the enhanced-photo-disinfection was conducted by an analysis of variance (ANOVA). This analysis was carried out with the two-way ANOVA Package of the Wolfram Mathematica 8.0 program. The determination of the discriminating power (Fisher ratio (F)) of the H_2O_2 or the bacteria species on the inactivation rate will permit to point out the significance of each of these parameters on the photo-disinfection process. The accuracy of the results will be evaluated by the noise level (Probability (P)).

3.3. RESULTS AND DISCUSSION

3.3.1. LAB EXPERIMENTS IN A SOLAR SIMULATOR: INFLUENCE OF H_2O_2 CONCENTRATION ON BACTERIAL INACTIVATION

The temperature of the water in the Suntest increased from 28°C to 45°C during the experiments. It is well know that temperature above 45°C has a bactericidal effect (Meierhofer and Wegelin, 2002). According to this, it can be assumed that the bacterial inactivation obtained in the Suntest was not due to the thermal effect of IR irradiation. The studied well water already contained dissolved iron: 0.07± 0.02 mg/L of $Fe^{2+, 3+}$ and solid iron oxides: 0.23± 0.01 mg/L, hence the additional reagent required to induce the photo-Fenton reaction is only H_2O_2. Adsorption of bacteria to iron oxides was recorded in MilliQ water (Spuhler *et al.*, 2010). This adsorption favors the siderophore iron transport action (Stintzi *et al.*, 2000). In such conditions, heterogeneous photo-Fenton reaction can take place leading to bacterial inactivation (Moncayo-Lasso *et al.*, 2008b; Mazille *et al.*, 2010). Spuhler *et al.*

(2010) have conducted experiments of photo-disinfection of E. coli suspended in MiliQ water, with 0.6 mg/L of iron $Fe^{2+ \text{ or } 3+}$ and 10 mg/L of H_2O_2 added in corresponding systems ($Fe/H_2O_2/hv$ or H_2O_2/hv) before the exposure to irradiation (*hv*). For this systematic disinfection in MilliQ water, the inactivation rate of the E. coli in the photo-Fenton system ($Fe/H_2O_2/hv$) recorded, was significantly higher than the one of the system enhanced with sole H_2O_2 (H_2O_2/hv). Afterwards, Ndounla *et al.* (2013) carried out the photo-disinfection of natural well water with an initial dissolved total iron content of 0.07 ±0.02 mg/L by adding (0.6 mg/L of Fe^{2+} and 8.5 mg/L of H_2O_2) on similar system ($Fe/H_2O_2/hv$ or H_2O_2/hv). For this natural water, similar inactivation rate was recorded for both systems for the disinfection of wild E.coli and Salmonella spp. leading to the assumption that even in presence of low dissolved iron contents, the Fenton (Eq.3.1-3.2) and photo-Fenton (Eq.3.3-3.7) reactions, could take place with the contribution of solid iron oxides present in the water. They are efficient for bacterial inactivation via a heterogeneous photo-Fenton process (Moncayo-Lasso *et al.*, 2008b; Mazille *et al.*, 2010).

To ensure the quality of treated water, H_2O_2 concentrations in the photo-Fenton process should be optimized for natural water source (Kenfack *et al.*, 2009; Malato *et al.*, 2009; Sciacca *et al.*, 2011). For this reason this part of the study aims to evaluate the minimal H_2O_2 concentration which could be used for significant drinking water disinfection by photo Fenton.

The influence of H_2O_2 concentration on the photo-Fenton inactivation rate is presented on Fig.3.2 Trace (■), representing the disinfection conducted without H_2O_2, showed the lower inactivation rate for both enteric bacterial species (*Salmonella* spp., coliforms/*E. coli*). The inactivation rate constant k, presented in Table 3.2, confirmed the effect observed on the curves, with k=-0.008±0.002 and -0.005±0.001 min^{-1} for coliforms/*E. coli* and *Salmonella* spp., respectively. These inactivation rates have drastically increased in both cases in presence

of H_2O_2. With only 2 mg/L of H_2O_2 (Trace (●)), more than 50% increase of the inactivation rate was noticed in both cases with respectively k=-0.016±0.003 and k=-0.083±0.002 min^{-1} for coliforms/*E. coli* and *Salmonella* spp. Beyond 4 mg/L of H_2O_2, the *Salmonella* spp. content of all the systems was totally inactivated in approximately 90 min. The inactivation rate of coliforms/*E. coli* and *Salmonella* spp., as presented in Table 3.2, underline that they were greater than the correspondent ones, under simulated solar light alone. The high sensitivity of *Salmonella* spp. here is in contrast with the report of several authors on treatment under direct solar radiation. Berney *et al.* (2006) and Sciacca *et al.* (2010) have noticed that *Salmonella* spp. was more resistant to photo-inactivation than *E. coli* and other enteric bacteria. The inactivation rate constant of coliforms/*E. coli* observed for 5 and 10 mg/L of H_2O_2, were approximately the same, being k=-0.032±0.001 min^{-1} and -0.034±0.001 min^{-1}. The first order kinetics decrease in CFU/mL was observed in the curves of both enteric bacteria treated by photo-Fenton with up to 4 mg/L of H_2O_2, then k was calculated by linear regression (Spuhler *et al.*, 2010). Fig. 3.2 shows that control experiments (Trace (▪)) that took place with 10 mg/L of H_2O_2 in dark did not lead to enteric bacteria inactivation. Post-irradiation evaluation after 24 hours of dark storage has revealed the regrowth of both enteric bacteria in the water, after their exposure to solar simulator, in absence of H_2O_2. Only *Salmonella* spp. regrowth was observed in the water illuminated in presence of 2 mg/L of H_2O_2. Beyond 4 mg/L of H_2O_2, none of the enteric bacteria has shown regrowth after photo-treatment. It could be assumed that treated water under a solar simulator, with concentration of 4 mg/L of H_2O_2, ensures not only efficient inactivation of the enteric bacteria, but also prevents the subsequent regrowth.

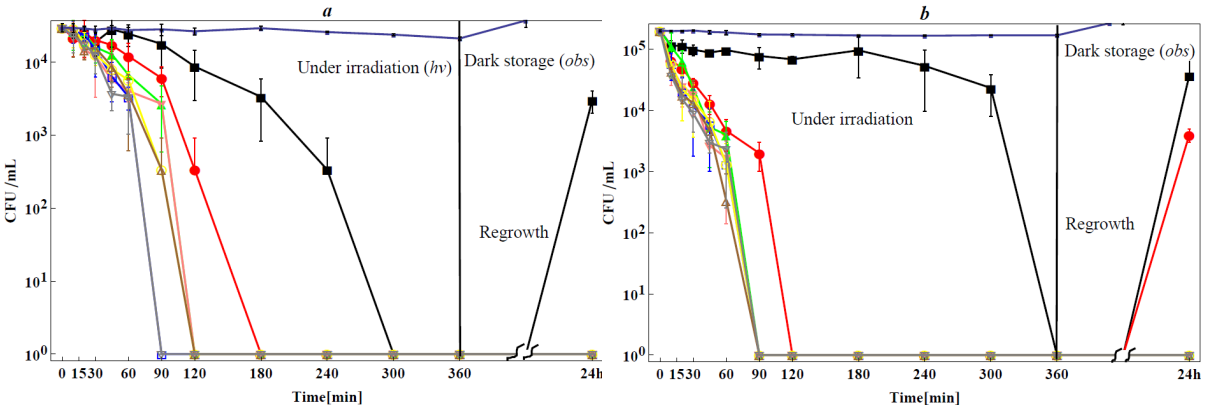


Figure 3.2: Inactivation kinetics of the wild enteric bacteria of natural well water containing natural iron (dissolved: 0.07± 0.02 mg/L OF $Fe^{2+,3+}$ and solid iron oxides (0.23± 0.01 mg/L) treated with different concentrations of H_2O_2. (*a*) total coliforms/*E. coli*, (*b*) *Salmonella* spp. (■) 0 mg/L *hv*, (●) 2 mg/L *hv*, (▲) 4 mg/L *hv*, (▼) 5 mg/L *hv*, (□) 6 mg/L *hv*, (○) 7 mg/L *hv*, (△) 8 mg/L *hv*, (▽) 10 mg/L *hv* and (▪) 10 mg/L *dark*.

Table 3.2: inactivation rate constant k [m^{-1}] of the enteric bacteria present in water treated by photo-Fenton with simulated solar radiation at different H_2O_2 (mg/l) concentrations.

Microorganisms	H_2O_2 [mg/L] & k [min^{-1}]							
	0	2	4	5	6	7	8	10
E. coli and Coliforms	-0.008±0.002	-0.016±0.003	-0.022±0.003	-0.032±0.001	-0.028±0.002	-0.029±0.001	-0.029±0.001	-0.034±0.001
Salmonella spp.	-0,005±0.001	-0.083±0.002	-0.063±0.002	-0.120±0.002	-0.119±0.001	-0.117±0.003	-0.142±0.003	-0.160±0.003

The discrimination power (Fisher ratio (F)) obtained from ANOVA analysis for the evaluation of the impact of H_2O_2 concentration on the photo-Fenton inactivation rate is up to 51.5, leading to the evidence that this concentration has a strong impact on the process with a very small probability ($P < 0.001\%$) that the effect may be due to noise (Table 3.3). The bacteria species, either wild *E. coli* or *Salmonella* spp., did not significantly influence the inactivation rate under the photo-Fenton disinfection as shown by the low Fisher ratio ($F = 0.11$). The probability that this effect is due to noise is very large ($P=74\%$). With an influence factor of 6.1, the interaction between the treatment and bacteria type seems to have just a slight impact on the photo-Fenton disinfection rate.

Table 3.3: results of the two way analysis of variance between treatment/bacteria species

Parameters	DF	SS	MS	F	P
Treatment	7	399154	57022	51.51	0.001%
Bacteria species	1	119.83	119.83	0.11	74%
Treatment /Bacteria	7	47703.9	6814.84	6.1	0.01%
Error	76	84127.5	1106.94	-	-
Total	91	531105	-	-	-

DF: Degree of Freedom, SS: Sum of Square, MS: Mean Square, F: Fisher factor, P: probability.

3.3.2. FIELD SCALE EXPERIMENTS IN A CPC SOLAR REACTOR

3.3.2.1. TREATMENT UNDER SOLAR IRRADIATION ALONE

The photo-disinfection of both enteric bacteria under uniquely solar radiation from 8 to 14h is presented on Fig.3.3-a. The total coliforms/*E. coli* strains (Fig.3.3-a., trace (○)) were totally inactivated after the first three hours of exposure when the average irradiance was 20 W.m^{-2} (Fig.3.3-a' trace (−)) for a accumulated dose of 120 Wh.m^{-2} (Fig.3.3-a' trace (+)). The *Salmonella* spp. strains (Fig.3.3-a., trace (●)) resisted till the fifth hour of exposure (dose of 250 Wh.m^{-2}) with an average irradiance (AI) of 20 W.m^{-2} and then were totally inactivated.

For the experiments conducted from 10 to 16h, the exposure began when the sun irradiance (Fig.3.3-b' traces (−)) was three times higher than the one measured when started at 08:00. For *Salmonella* spp. (Fig.3.3-b., trace (●)) the fast temperature increase from 29°C to 45°C in less than one hour and half (Fig.3.3-b', trace (X)) coupled to average irradiance of 32 W.m^{-2} (Fig.3.3-b' traces (−)) during the 3h, required for total inactivation (EDT), led to a cumulated energy of 200 Wh.m^{-2}. Compared to the previous period (5h with a dose of 250 Wh.m^{-2}), the

Salmonella spp. were totally inactivated here in 3h for a dose of 200 $Wh.m^{-2}$, but at higher AI exposure. The total coliforms/*E. coli* (Fig.3.3-b., trace (○)) were completely inactivated in 30 min before the *Salmonella* spp. Regrowth of *Salmonella* spp. were also observed in this case as in the previous one during the post-irradiation tests (Table 3.5). Considering that the temperature level remained also below 50°C here, the impact of temperature on the inactivation process was not significant.

In experiments carried out from 12 to 18h, the average irradiance (37 $W.m^{-2}$) available at noon (Fig.3.3-c' trace (−)) together with a fast temperature increase from 29 to 44°C in one hour (Fig.3.3-c' trace (X)) drastically affect the total coliforms/*E. coli* concentration, leading to their total inactivation in 90 min (Fig.3.3-c., trace (○)). However, the sudden decrease of irradiance to 18 $W.m^{-2}$ after the first hour of exposure negatively affects the *Salmonella* spp. inactivation (Fig.3.3-c., trace (●)) even though the cumulative dose was still increasing (Fig.3.3-c' Traces (+)). Indeed, after a slight decrease noticed at the beginning of the process, *Salmonella* spp. has remained stable at approximately 10^5 CFU/mL till the end of the experiment (6h). The resistance of *Salmonella* spp. to sole solar photo-disinfection, when the irradiance is low is significantly noticed here (Table 3.5) and confirmed the observation reported by Berney *et al.* (2006) and Sciacca *et al.* (2010). These results point out that irradiance and temperature are more crucial for *Salmonella* spp. than for total coliforms/*E. coli* during solar treatment in a CPC.

The synergy between the irradiance (related to UV and visible part of sunlight) and thermal action (T°C) (related to infrared rays) has lead to both enteric bacteria inactivation as already noticed by several authors (Meierhofer and Wegelin, 2002). However, *Salmonella* spp. regrowth occurred after 24 hours of dark storage for all the illumination periods and for the samples taken at 90, 120, 150, 180, 240, 300 and 360 min. Therefore, it can be assumed that to ensure a lethal impact in solar disinfection of such resistant strains in a CPC reactor,

temperature up to 50°C is required, as recommended by SODIS reference for 1-2 liters. However, the weather variation can't always ensure such conditions, even in the Sahelian region, as it can be observed with the irradiance fluctuations presented by the traces (−) of Fig.3.3-(a', b', c'). The crucial impact of the available irradiance on the photo-Fenton disinfection of natural water will be addressed in the next part of this paper.

3.3.2.2. ENHANCED PHOTO-DISINFECTION BY ADDITION OF H_2O_2 IN WATER CONTAINING NATURALLY DISSOLVED AND SOLID IRON FORMS

For this part of the study, the disinfection of natural well water in a CPC solar reactor was preliminary tested at field scale with 5 mg/L of H_2O_2 as explained in the methodology section 2.5. However, it was noticed that during these preliminary runs that H_2O_2 was totally consumed after 3 - 4 hours of irradiation. This consumption was probably due to intensive O_2 supply by water recirculation in the CPC into the solar simulator. Indeed, H_2O_2 degradation is favored by O_2 concentration. Oxygen associated to photosensitizers (i.e. NOM) present in water led to reactive oxygen species (ROS) generation (Reed, 1997; Kehoe *et al.*, 2001), increasing H_2O_2 consumption, following the reaction presented in Eqs. 3.10 -3.12 and the Haber-Weiss reaction (Eqs. 3.13-3.14) (Pignatello *et al.*, 2006; Malato *et al.*, 2009). In order to ensure that there is H_2O_2 left after the disinfection process, additional experiments were carried out with 10 mg/L of H_2O_2 initial concentration. The remaining H_2O_2 after the treatment was enough to ensure a residual effect during the dark storage (24 hours). Experiments performed with 10 mg/L of H_2O_2 lead to a remaining H_2O_2 concentration of 3 - 4 mg/L after 6 hours of irradiation. This residual H_2O_2 could possibly ensure the Fenton activity in presence of the dissolved and solid iron of the water in the dark. Fig. 3.3 (a, b, c), shows the inactivation of wild enteric bacteria (open symbols for total coliforms/*E. coli* and full symbols for *Salmonella* spp.) contents of the natural wells water in the CPC during different periods of the day and different disinfection experimental trials. Direct solar radiation (*hv*) (○, ●), H_2O_2

enhanced photo-disinfection ($Fe^{2+}/H_2O_2/hv$) (□, ■) and the dark Fenton ($Fe^{2+}/H_2O_2/obs$) (△, ▲). The initial concentration of the H_2O_2 added was 10 mg/L.

$$NOM + hv \rightarrow NOM^{*} + e^{-} \quad (3.10)$$

$$O_2 + e^{-} \rightarrow O_2^{\bullet -} \quad (3.11)$$

$$O_2^{\bullet -} + H^{+} \rightarrow HO_2^{\bullet} \quad (3.12)$$

$$HO_2^{\bullet} + H_2O_2 \rightarrow O_2 + H_2O + OH^{\bullet} \quad (3.13)$$

$$O_2^{\bullet -} + H_2O_2 \rightarrow O_2 + OH^{\bullet} + OH^{-} \quad (3.14)$$

The decrease of both enteric bacteria under H_2O_2 enhanced photo-disinfection follows the first order kinetic while under the uniquely solar illumination the curves presented a shoulder leading to a concave shape (Meierhofer and Wegelin, 2002; Berney *et al.*, 2006). Fig. 3.3 (a', b', c'), presents the evolution of water temperature (X) [T°C], solar irradiance (−) [$W.m^{-2}$] and cumulated total dose (+) [$Wh.m^{-2}$].

The *Salmonella* spp. Effective Disinfection Time (EDT), or in another words, the required time to achieve the total inactivation of *Salmonella* spp. in the water sample, was annotated on the graphs of (Fig.3.3-a', b', c'). 10 mg/L of H_2O_2 was added to the water sample to initiate the photo-Fenton process, in presence of its natural iron contains of (dissolved: 0.07± 0.02 mg/L of $Fe^{2+,\ 3+}$ and solid iron oxides (0.23± 0.01 mg/L). We confirmed the meaningful contribution of the photo-Fenton process with the fact that when natural water is diluted, 2-3 folds of the bacteria inactivation rate significantly diminished even if the amount of added H_2O_2 is kept at 10 mg/L (results not shown). Despite the low average irradiance (AI) available, the photo-Fenton disinfection treatment conducted from 8h to 14h led to an enhancement of the simple solar disinfection of 33% for total coliforms/*E. coli* strain (Fig.3.3-a., trace (□)) and of 40% for the *Salmonella* spp. (trace (■)). The EDT of 180 min (3h) was

noticed for the photo-Fenton inactivation of *Salmonella* spp., for an AI of 20 $W.m^{-2}$ and a cumulated dose of 60 $Wh.m^{-2}$ (Table 3.4 and Fig.3.4). Temperature increased from 29 to 45°C during the EDT (Fig.3.3-a' Trace (X)). The post-irradiation evaluation revealed that none of both enteric bacteria regrew during the subsequent 24 hours of dark storage (Table 3.5). From these results it can be assumed that neutral photo-Fenton does not need temperatures higher than 50°C to disinfect efficiently as it is required under sole solar illumination, in classical SODIS applications (Meierhofer and Wegelin, 2002; Bichai *et al.*, 2012).

An important enhancement on the enteric bacteria inactivation rate of uniquely solar treatment (10-16h) was observed, when the photo-Fenton treatment was applied. An increase of 50% and 80% was respectively noticed for *Salmonella* spp. (Fig.3.3-b., Trace (■)) and total coliforms/*E. coli* (Fig.3.3-b., trace (□)). This increase in disinfection rates, compared to the one recorded in the experiments that took place from 8 to 14h, is probably due to the higher AI available at the beginning of the process (28 $W.m^{-2}$, Table 3.4). This leads us to suggest that AI and temperature increase have a greater impact on photo-Fenton than on solar irradiation only for enteric bacteria inactivation.

The high AI (35 $W.m^{-2}$) recorded during the first 45 min (0.75 h) (EDT) in the experiments conducted from 12 to 18h, has led to total inactivation of *Salmonella* spp. A dose of 26 $Wh.m^{-2}$ was accumulated during this EDT (Table 3.4 and Fig.3.4). From all the AI and doses recorded in Table 3.4 for *Salmonella* spp., it can be noticed that high irradiances, but not necessarily high doses, are needed to get an efficient achievement of inactivation (Fig.3.3-c. traces (□) and (■)) and durable lethal impact on enteric bacteria (Table 3.5) during the neutral photo-Fenton treatment. The enhancement of total coliforms/*E. coli* inactivation rate by neutral photo-Fenton compared to the one of the simple photo-disinfection by solar light, during the same illumination interval starting at 12h, was of 67%. Rincon and Pulgarin (2004b) have reported a similar effect of the significant impact of the irradiance during the TiO_2

photocatalytic disinfection of *E. coli*. In contrast, Ubomba-Jaswa *et al.* (2009) have reported the significant impact of the dose during the solar disinfection in the CPC reactor for effective *E. coli* K12 disinfection achievement. Nevertheless, solar disinfection of the wild enteric bacteria carried out in a similar reactor during this study reveals that the irradiance significantly influence the inactivation rate of the process (Fig.3.3. traces (○), (●) and (−)). The lethal oxidative action of the neutral photo-Fenton on these enteric bacteria strains as reported previously by several authors (Bandala *et al.*, 2009; Sciacca *et al.*, 2010; Spuhler *et al.*, 2010; Ndounla *et al.*, 2013) leads to the assumption that it could efficiently disinfect the *Salmonella* spp. and other resistant strains to ensure the sustainability of a CPC solar drinking water treatment of higher volumes of water.

The membrane peroxidation by external ROS attacks produced by photo-Fenton, due to natural dissolved and solid iron forms present in water and added H_2O_2, leads to an increased permeability and the disruption of the trans-membrane ion gradients (Spuhler *et al.*, 2010). The microorganism (*E. coli* or *Salmonella* spp.) death is related to the damages of their nuclear constituents (DNA) by intracellular highly ROS (H_2O_2, $O_2^{\bullet-}$, $OH^{\bullet}$) generated after the inactivation of their antioxidant enzymes (catalases, superoxide dismutases, alkylhydroperoxidase and thiol peroxidase) by the thermal (temperature increase) and optical (UV A and B) effects (Arenas *et al.*, 2011; Byrne *et al.*, 2011; Polo-Lopez *et al.*, 2012). The iron liberated from the iron sulfur clusters ([4Fe-4S]) after the inactivation by UV (A and B) of some clusters' enzymes induced the intracellular Fenton (Eq.1) in presence of H_2O_2 leading to increase generation of highly reactive $OH^{\bullet}$. $OH^{\bullet}$ attacks on cellular DNA lead to irreversible damages and consequently, to cell inactivation (Jang and Imlay, 2010; Spuhler *et al.*, 2010; Sobota and Imlay, 2011). Additionally, the photo-degradation of humic substances and nitrogen compounds in both photo-disinfection treatments leads to the additional

generation of $OH^{\bullet}$ (E.q, 3.13-3.15) (Kotzias *et al.*, 1987; Fanning, 2000; Brito *et al.*, 2010) which can contribute to the bacterial inactivation.

3.3.2.3. FENTON DISINFECTION (DARK EXPERIMENTS)

Enteric bacterial inactivation carried out in the dark (*obs*), shows a limited decrease of their contents during the whole experiment, as it could be observed in all graphs of Fig.3.3., (traces (△) and (▲)), for total coliforms/E. coli and *Salmonella* spp. respectively. The Fenton ($Fe/H_2O_2/obs$) process was conducted in the dark simultaneously with the photo-Fenton ($Fe/H_2O_2/hv$) ones. The significant effect of the illumination (*hv*) during the neutral photo-Fenton disinfection observed in this study is highlighted from these results.

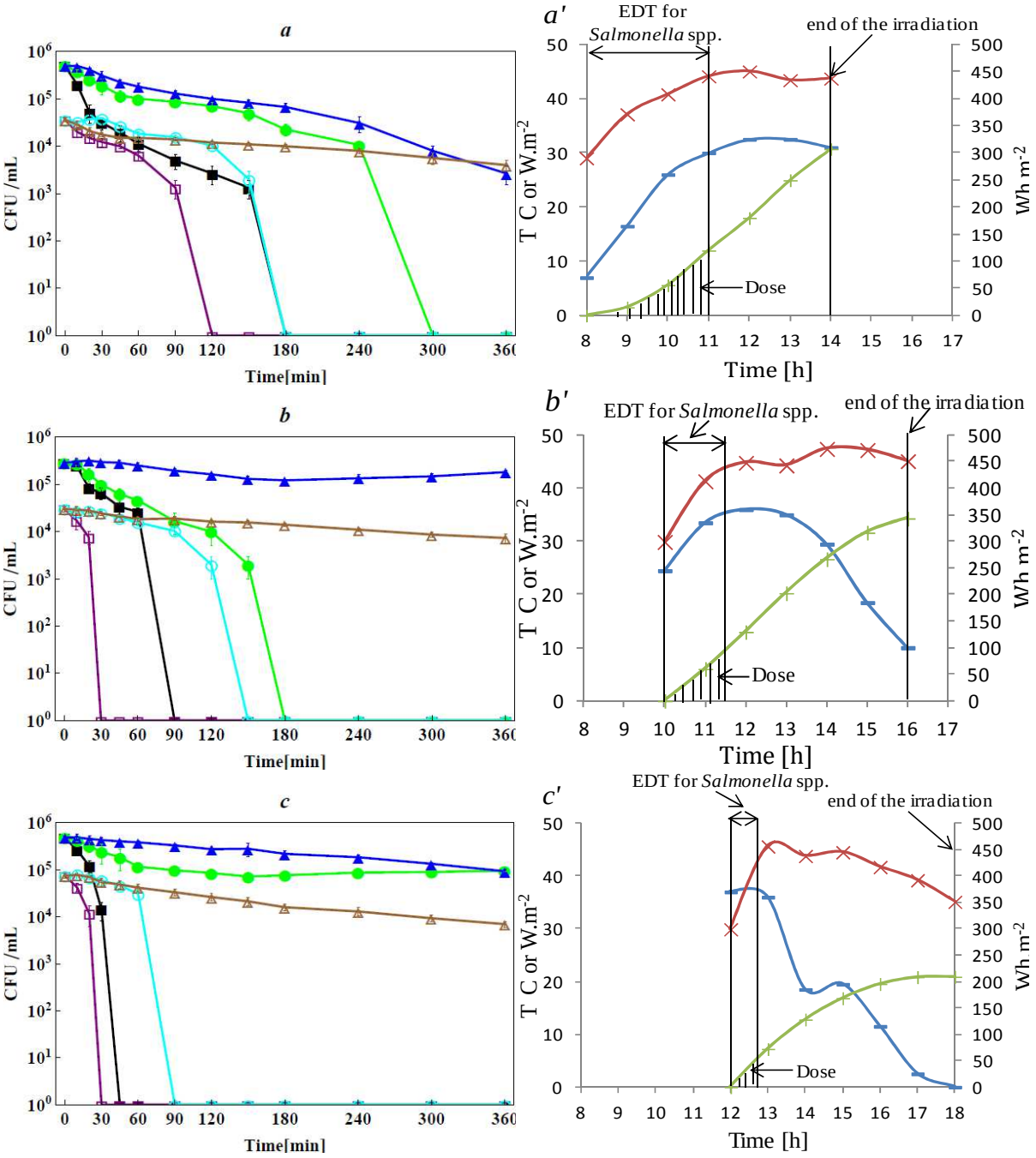


Figure 3.3. Inactivation under different day periods (*a*, *b*, *c*) of the wild enteric bacteria of well water containing natural iron (dissolved: 0.07± 0.02 mg/L of $Fe^{2+,3+}$ and solid iron oxides (0.23± 0.01 mg/L) and addition of 10 mg/L of H_2O_2). (*a'*, *b'*, *c'*) evolution of water temperature ([T°C], (X)), irradiance ([W.m^{-2}], (−)) and cumulated total dose ([Wh.m^{-2}], (+)) during the treatments. EDT (photo-Fenton): Effective Disinfection Time for *Salmonella* spp. (time required for the total inactivation of *Salmonella* spp. under photo-Fenton treatment). day time periods (*aa'*: 8-14h), (*bb'*: 10-16h), (*cc'*: 12-18h) total coliforms/*E. coli* (□) and *Salmonella* spp. (■) under photo-Fenton (natural $Fe^{2+,3+}/H_2O_2/hv$), total coliforms/*E. coli* (○) and *Salmonella* spp. (●) under direct solar radiation (natural $Fe^{2+,3+}/hv$), total coliforms/*E. coli* (△) and *Salmonella* spp. (▲) IN the dark Fenton (natural $Fe^{2+,3+}/H_2O_2/obs$).

Table 3. 4: Influence of irradiance on the efficient disinfection time and dose for the photo-Fenton treatment of *Salmonella* spp.

	[b]AI [W.m^{-2}]	[a]EDT [h]	[c]Dose [Wh.m^{-2}]
8h-14h	20	3	60
10h-16h	28	1.5	42
12h-18h	35	0.75	26

[a]Efficient disinfection Time (time required to achieve the total inactivation in water of a target bacteria (e.g *Salmonella* spp. in this case)), [b]Average irradiance during the EDT, [c]Solar cumulated energy during the EDT.

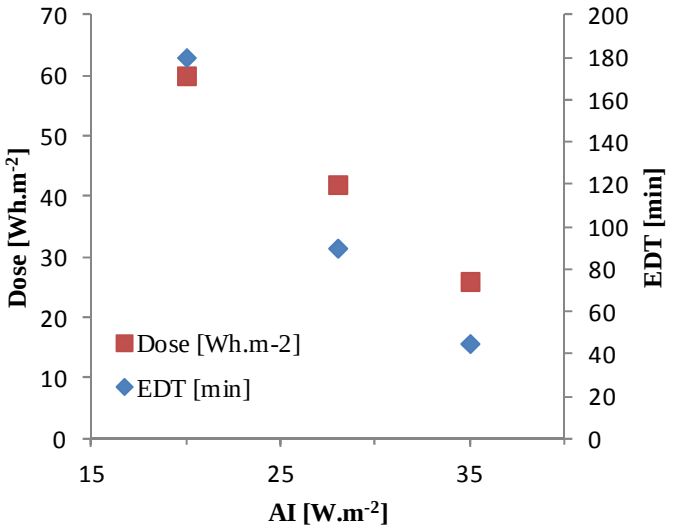


Figure 3. 4. Efficient disinfection time (EDT) [min] and dose required for *Salmonella* spp. Total inactivation as a function of average irradiance (AI) [W.m^{-2}] available, (■) cumulated total dose [Wh.m^{-2}], (♦) EDT (photo-Fenton) [min]: time required for the total inactivation of *salmonella* spp. under photo-Fenton treatment.

3.3.2.4. DURABILITY OF THE DISINFECTION PROCESS: POST-IRRADIATION EVENTS

The evaluation of the sustainability of the photo-disinfection in absence of H_2O_2 was carried out by the monitoring of post-irradiation events. The results presented in Table 3.5 pointed out that all the strains of total coliforms/*E. coli* have remained in their lethal state after 24 hours of dark storage while that of *Salmonella* spp. have recovered their culturability as presented by their positive status. This *Salmonella* spp. regrowth could be due to the fact that

the temperature did not rise up to 50°C, as recommended for SODIS applications in bottles (Meierhofer and Wegelin, 2002). The results of the post-irradiation regrowth observed (+) or not (-) after the neutral photo-Fenton treatment are also shown in Table 3.5. No enteric bacterial regrowth was observed after 24 h of dark storage. This sustainability of the disinfection is highly significant, as some of the samples stocked in the dark for testing were collected before the total inactivation of their enteric bacterial content, (cf. sampling periods in Table 3.5 and graphics of Fig.3.3). Notice that the absence of bacterial regrowth observed during the 24h of dark storage, was maintained during the subsequent 72 and 168 h (one week). For water treated by simple direct solar radiation, only the total coliforms bacteria/*E. coli* did not show regrowth during the storage. Regrowth of *Salmonella* spp. was observed in all the samples (Table 3.5), after 24 h of dark storage. These results have confirmed the resistance of the *Salmonella* spp. strain to simple solar disinfection treatment as previously reported by some authors (Berney *et al.*, 2006; Sciacca *et al.*, 2010).

Table 3.5. Enteric bacterial regrowth evaluation (total coliforms bacteria/*E. coli* and *Salmonella* spp.), after the photo-disinfection and neutral photo-Fenton treatments of natural well water with or without H_2O_2 (10 mg/L) under direct solar light. The results were similar for all the illumination intervals (8 -14h; 10 - 16h; 12 - 18h).

Sampling periods (min)	Without H_2O_2 (only direct solar radiation)		With H_2O_2 (Photo-Fenton)	
	Coliforms bacteria/*E. coli*	*Salmonella* spp.	Coliforms bacteria/*E. coli*	*Salmonella* spp.
90	−	+	−	−
120	−	+	−	−
150	−	+	−	−
180	−	+	−	−
240	−	+	−	−
300	−	+	−	−
360	−	+	−	−

− no re-growth of bacteria even after one week + re-growth of bacteria after 24h.

3.3.3. PH EVOLUTION DURING THE IRRADIATION PROCESS

The evaluation of the pH variation during the photo disinfection conducted in this study, showed an increase of 1.5 and 2.5 pH units range, respectively, for solar treatment (without H_2O_2) and neutral photo-Fenton as presented in Fig. 3.5, this pH increase follows the same tendency in all the times interval (8-14h, 10-16h, 12-18h). The rising phase was recorded during the first two hours with an increase from 5.5 to 7.9 in almost all the photo-Fenton treatments and 5.5 to 6.8 or 7.2 for the solar treatment. This rising phase was followed by a stable phase in the new high pH values. This pH increase could be linked to several chemical pathways in action in the treated water during the photo-disinfection. Some of these pathways are (i) the degradation of bacterial nitrogen compounds, such as amino-acids and proteins, producing alkaline by-products, (ii) the shifting of the CO_2-carbonate equilibrium by water

heating and CO_2 degassing, (iii) the photo-reduction of NO_2^- and NO_3^- in solution leading OH^- production (Eqs: 3.15-3.16) (Kotzias *et al.*, 1987; Fanning, 2000) and (iv) the consumption of H^+ or the generation of OH^- in the solution during, the Fenton and photo-Fenton process as described in the equations 3.1, 3.12, and 3.14, (Bandara *et al.*, 1997; Pignatello *et al.*, 2006; Malato *et al.*, 2009). This last mechanism could be the one impacting the higher pH increase noticed in the neutral photo-Fenton process, with regards to the one observed in the uniquely solar treatment. The pH change to alkaline during the photo-disinfection could be useful to upgrade the one originally acidic groundwater in Sahelian region to the level 6.5 – 8 recommended for human consumption by the WHO (WHO, 2011a).

$$NO_3^- + H_2O + h\nu \rightarrow NO_2^- + OH^- + OH^{\bullet} \quad (3.15$$

$$NO_2^- + H_2O + h\nu \rightarrow NO + OH^- + OH^{\bullet} \quad (3.16)$$

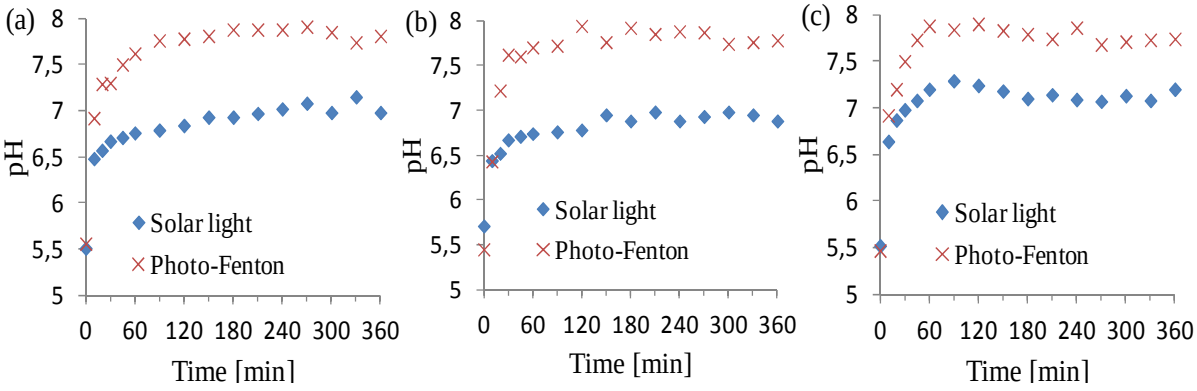


Figure 3.5. Variations of pH during the disinfection by solar light exposure and neutral photo-Fenton of total coliforms bacteria/*E. coli* and *Salmonella* spp. Recorded at different day time's intervals: (a) 8-14h, (b) 10-16h and (c) 12-18h.

3.3.4. NITRITE, NITRATE AND AMMONIA VARIATION DURING THE TREATMENT

The concentration of nitrogen components was determined during both photo-disinfection treatment trials. As presented in Figure 3.6, in absence (solar treatment) or presence of H_2O_2

(photo-Fenton), the rise in NO_2^- and NO_3^- concentration was recorded and concurrently the decrease in NH_4^+ concentration was also noticed. The photochemical reduction of NO_3^- in natural waters leads to NO_2^-, OH^-, and $OH^\bullet$, while that of NO_2^- leads to NO, OH^- and $OH^\bullet$ (Eq 3.15-3.16) (Kotzias *et al.*, 1987; Fanning, 2000). However, the WHO report revealed that the presence of the ammonium cation in raw water may result in drinking-water containing nitrite as the result of catalytic action (WHO, 2003). Brito *et al.* (2010) have proposed a pathway of ammonia photo-oxidation by $OH^\bullet$ leading to NO_2^- and NO_3^- generation (Eq. 3.17). Hence, the variation recorded during the experiments could be attributed to these oxido-reduction interactions. The generation of the highly oxidant $OH^\bullet$ will not certainly react only towards ammonia oxidation but could also intervene on bacteria inactivation.

$$NH_4^+ \leftrightarrow NH_3 + OH^\bullet \rightarrow NH_2OH \rightarrow NOH \rightarrow NO \rightarrow NO_2^- \leftrightarrow NO_3^- \qquad (3.17)$$

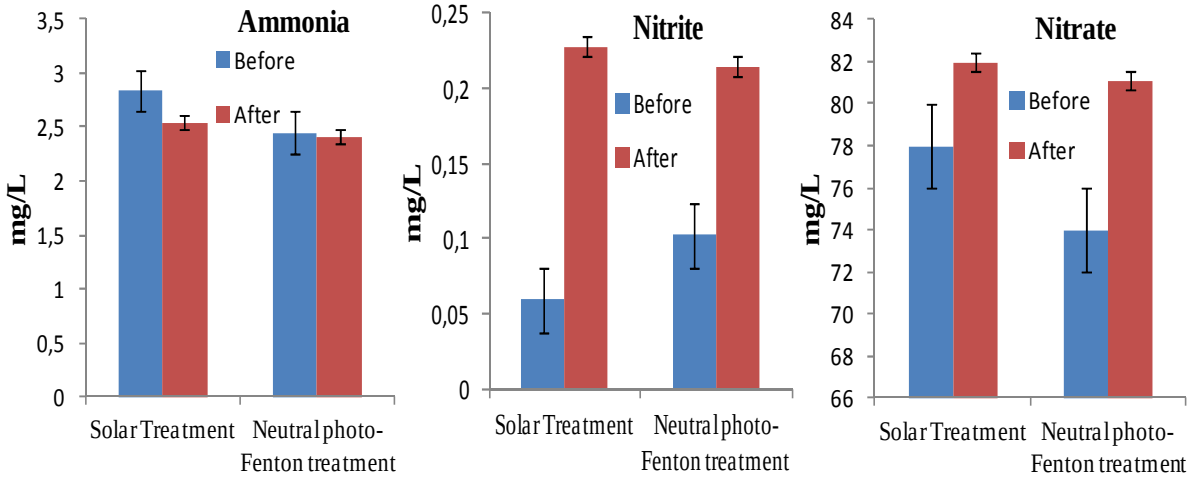


Figure 3. 6. Evolution of Ammonia, Nitrite and Nitrate concentration during the neutral photo-Fenton and sole solar treatment of natural well water. (notice that the difference in the initial concentration of each chemical component in the results presented here are related to the fact that new water sample was collected and used for each new experiment).

The World Health Organization (WHO) classifies ammonia as an esthetic quality component without a direct importance for health in the concentrations regularly recorded in natural drinking-water. Therefore, no health-based guideline has been prescribed for it. The initial concentration of the nitrates followed in this study, was higher than that of the health-based

guideline for drinking water recommended by the WHO (50 mg/L). During the applied photo-Fenton treatment in this study, increased nitrite concentrations remain far below the health-based ones recommended by the WHO norms for drinking water, of maximum 3 mg/L (WHO, 2003, 2011b). When nitrate levels in drinking-water exceed 50 mg/l, drinking-water will be the major source of total nitrate intake, especially for bottle-fed infants. The major biological effect of nitrite in humans is its involvement in the oxidation of normal Hemoglobin (Hb) to Methemoglobin (metHb), which is then unable to transport oxygen to the tissues. The reduced oxygen transport becomes clinically manifested when metHb concentrations reach 10% of normal Hb concentrations and above; the condition, called methaemoglobinaemia, causes cyanosis and, at higher concentrations, asphyxia. The normal metHb level in humans is less than 2%; in infants under 3 months of age, it is less than 3%. However, high nitrate concentration, above 100 mg/l, is an important cause of metHb formation (WHO, 2011b). Considering health risk relate to high nitrogen components presence in drinking water (methaemoglobinaemia or cancer) (Weng *et al.*, 2011), further research should be conducted to intensively monitor and study the mechanism of its formation and persistence in the photo-treated water.

3.4. CONCLUSIONS

The photo-disinfection of natural well water was successfully carried out at real scale in a solar CPC reactor under several time intervals of 6 hours (8-14h, 10-16h, 12-18h). 25L of water were treated with H_2O_2 which generate in-situ the photo-Fenton system (H_2O_2/natural $Fe^{2+,\ 3+}$/*hν*) or (uniquely solar radiation). All the samples treated without H_2O_2 addition showed *Salmonella* spp. regrowth after 24h of dark storage. The resistance of the *Salmonella* spp. strain to uniquely solar disinfection treatment was recorded. The H_2O_2 addition has significantly enhanced the inactivation rate of the disinfection in all cases, without the need to reach 50°C as required for classical SODIS bottles process. No enteric bacteria regrowth was

noticed one week after the in-situ generated photo-Fenton treatment. Significant influence of the solar irradiance but not the dose was noticed during the process. The experiments revealed that higher irradiance level leads to lower EDT and dose to achieve bacterial disinfection. High average irradiance (AI) of 35 $W.m^{-2}$ led to the total inactivation of *Salmonella* spp. with a dose of 26 $Wh.m^{-2}$. In contrast, low irradiance of 20 $W.m^{-2}$ required a dose of 60 $Wh.m^{-2}$.

The pH becomes more alkaline during both neutral photo-Fenton and solar treatment. A rise of 1.5 and 2.5 in pH range was recorded respectively in solar treatment and neutral photo-Fenton. This pH increase was not detrimental to the photo-Fenton and bare solar disinfection, but could be beneficial for the Sahelian ground-waters which are originally acidic and could then simultaneously be upgraded through photo-Fenton disinfection.

This study has revealed significant variation of the nitrogen compounds state during both photo-disinfection processes (Solar, Photo-Fenton). The recorded oxido-reduction of nitrates and nitrites and the oxidation of ammonia following the variation of their concentration during the treatments (increase of nitrates and nitrite and decrease of ammonia), has pointed out the importance of evaluating the sustainability of these disinfection processes before recommending them for human consumption. The WHO report on nitrate (NO_3^-) in drinking water reveal that several wells water in the world naturally contain more than 50 mg/L of NO_3^-, as the one found in this study. Considering this fact, it's a need to efficiently determine in further research project on photo-disinfection the generation rate of the nitrite (NO_2^-) produced through the reduction of nitrate or the oxidation of ammonia and evaluate if it eventually remains below 3 mg/L. The characterization of this nitrite and other nitrogen byproducts formation (such as nitrosamine), could help evaluate the health impact of the photo-disinfection of the drinking water before thinking the vulgarization of the photo-Fenton disinfection.

4. CHAPTER 4:

ENHANCEMENT OF THE SOLAR PHOTO-DISINFECTION OF NATURAL ALKALINE WATER (PH 8.6) WITH H_2O_2 IN THE COMPOUND PARABOLIC COLLECTOR (CPC) AT DIFFERENT DAY PERIODS IN SAHELIAN REGION.

4.1. INTRODUCTION

Pathogenic enteric bacteria are related to the outbreak of several water-borne diseases (e.g. diarrhea, cholera, dysenteries and typhoid) in developing countries. The use of solar radiation to disinfect drinking water has been successfully evaluated by several authors under the Solar Disinfection (SODIS) process (Sommer *et al.*, 1997; Sobsey, 2002; Boyle *et al.*, 2008). Burkina Faso, like many other developing countries, is situated in the latitude lines of 30°N and 30°S and receives about 2,000 to 3,000 hours of solar illumination annually. This energy could be productively used to improve solar drinking water disinfection. SODIS implies the synergistic effect of sunlight and temperature (Wegelin *et al.*, 1994). Clinical field trials on the evaluation of the efficiency of SODIS towards the reduction of occurrences of diarrhea have been conducted in Kenya (Conroy *et al.*, 2001; Du Preez *et al.*, 2011), India (Rose *et al.*, 2006), Iran (Mahvi, 2007) and Cambodia (McGuigan *et al.*, 2011). The development of an enhanced solar disinfection process in the Sahelian region could be useful to efficiently solve the problem of potable drinking water scarcity.

The enhancement of the SODIS efficiency has been reported by several authors, with the aim of developing a low-cost process capable of producing a higher volume in less time than the 1-2 liters per bottle in 6 hours proposed by SODIS references (Reed, 2004; Rincon and Pulgarin, 2006; Ubomba-Jaswa *et al.*, 2010). SODIS enhancement by the photo-Fenton process has been considered by some authors to be an affordable and efficient process which could be used to speed up the SODIS process and to increase the volume of water produced (Sciacca *et al.*, 2010; Spuhler *et al.*, 2010). Fenton and related systems encompass reactions of peroxides (usually H_2O_2) with metal ions leading to the formation of reactive oxygen species (ROS) and reactive radical species (Eq. 4.1). These metal ions are mostly transition metals which could be found naturally or due to industrial activities in natural waters such as

manganese, zinc, chromium, copper, iron, etc. In most of the researches carried recently on Fenton and photo-Fenton, the metal ion involved in the reaction is iron (Cho *et al.*, 2004; Bandala *et al.*, 2012; Rodríguez-Chueca *et al.*, 2013). Therefore, the catalytic Fenton reaction in the dark, generally active at acidic pH, is known to favor the generation of the hydroxyl radicals ($^{\bullet}OH$) and Fe^{2+} ions (Eq.4.2). However the kinetics of the reaction is limited by the slow regeneration of Fe^{2+} from Fe^{3+} (Eq.4.3). Under illumination, the Fenton reagent (Fe^{2+}/H_2O_2) leads to the photo-Fenton reagent ($Fe^{2+}/H_2O_2/h\nu$) with an increased production of $^{\bullet}OH$ in the treated water during the process, and the regeneration of Fe^{2+} from Fe^{3+} is reached via a ligand-to-metal charge-transfer (LMCT) reaction (Eq.4.4). At acidic pH, the most abundant iron photo-active complexes are the Fe^{3+}-hydroxy-complexes ($Fe(OH)^{2+}$ and $Fe(H_2O)^{3+}$), which can lead to the production of $^{\bullet}OH$ and Fe^{2+}. In the presence of a Fe^{3+}-organo-complex, the reaction leads to Fe^{2+} and an oxidized organo-complex (Eq.4.5). Both reactions (Eq.4.4 and Eq.4.5) take place when the complex absorbs light in the UV and the visible region (Pignatello *et al.*, 2006; Malato *et al.*, 2009). Surface water contains a large amount of natural organic matter (NOM). This NOM can form photo-active Fe^{3+}-complexes even at neutral and basic pH. A part of NOM is also able to interact as a photosensitizer, leading to the production of reactive oxygen species (ROS) ($^{\bullet}OH$, $HO_2^{\bullet}$, $O_2^{\bullet-}$) (Canonica *et al.*, 1995). Domestic, agricultural and industrial activities favor the introduction in the water cycle of some inorganic ions such as HCO_3^-, CO_3^{2-} SO_4^{2-}, S^{2-}, F^-, HPO_4^{2-}, NO_2^-, NO_3^- and NH_4^+. These ions can either lead to the precipitation of iron, scavenging of $^{\bullet}OH$ or coordinate to dissolve Fe^{3+} and Fe^{2+} in more or less un-reactive complexes (Pignatello *et al.*, 2006). These reactions, when they occur, can affect the photo-inactivation process.

$$M^{n+} + H_2O_2 \rightarrow M^{(n+1)+} + OH^- + {}^{\bullet}OH \qquad (4.1)$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^- \qquad k = 53 - 76\ M^{-1}s^{-1} \qquad (4.2)$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} HO_2^{\bullet} + H^+ \qquad k = 1 - 2 \times 10^{-2}\ M^{-1}s^{-1} \qquad (4.3)$$

$$Fe(OH)^{2+} + h\nu \rightarrow Fe^{2+} + {}^{\bullet}OH \qquad (4.4)$$

$$R\text{-}OOC\text{-}Fe^{3+} + h\nu \rightarrow R\text{-}COO^{\bullet} + Fe^{2+} \rightarrow Fe^{2+} + CO_2 + R^{\bullet} \qquad (4.5)$$

As mentioned above, the photo-Fenton process was firstly more efficiently used at acidic pH (2.5 – 3) for biorecalcitrant chemical compound degradation (Herrera *et al.*, 1998; Kenfack *et al.*, 2009; Malato *et al.*, 2009). Recently, it has been successfully tested at near-neutral pH for drinking water disinfection (Rincon and Pulgarin, 2006; Ndounla *et al.*, 2013). Moncayo-Lasso *et al.* (2009) have simultaneously inactivated *E. coli* and degraded NOM in river water with a compound parabolic solar reactor (CPC). The current study is the first conducted on alkaline surface water with natural iron content at field scale in CPC with the aim of evaluating the efficiency of the photo-Fenton treatment in theoretically unfavorable alkaline conditions. The pH evolution during photo-disinfection and the effect of the solar radiation parameters (day period of illumination, irradiance and dose) on the efficiency of the photo-disinfection is assessed. The impact of some inorganic ions present in the natural water sample on the efficiency of the photo-Fenton process is also evaluated.

4.2. MATERIALS AND METHODS

4.2.1. PHYSICO-CHEMICAL MEASUREMENTS AND CHEMICAL REAGENTS

The HACH DR/2000 spectrophotometer methodologies used in this study to characterize some physico-chemical components of the water sample (turbidity, iron, nitrite, nitrate, phosphate, sulphate, fluoride, sulfide and ammoniac) follows the guidelines of the Standard Methods for Examination of Water (HACH, 2001). The HACH methods used for the determination of each component and its detection limit are presented in table 4.1. However, the bicarbonate and carbonate ions concentration were determined by titration. A universal

meter WTW 340i equipped with a WTW SenTix 41-3 probe was used to measure the pH and temperature. The Hydrogen peroxide (H_2O_2) concentration was followed during the experiments by a Peroxide Merckoquant (Merk) test with a detection limit of around 0.5 mg/L. Microbiology Chromocult ® (Merck KGaA), was used for bacterial plating. Growth media were poured into a Pre-sterilized Petri Dish, 92x16mm (Sarstedt AG). Hydrogen peroxide, 30% (AnalaR Normapur, VWR) was used to prepare the Fenton reagent. And Hydrochloric acid fuming (HCl), 37% (Fluka Analytical, SIGMA-ALDRICH®) was used for glass-reactor cleaning.

Table 4.1: summary of the HACH analytical methods used to characterize some components of the water sample (HACH, 2001).

Components	**HACH DR/2000 Methods/Programs**	**Detection limits**
Turbidity	Program 750 (wavelength 450 nm)	0 - 450 NTU (Nephelometric turbidity units)
Total Iron	FerroVer Method, program 265 (Powder Pillows, wavelength 510 nm)	0 - 3.00 mg/L
Nitrate	High Range (HR), program 355 or the Cadmium Reduction Method (wavelength 500 nm)	0 - 30.0 mg/L NO_3^--N
Nitrite	Low Range (LR), program 371 or Diazotization Method (wavelength 507 nm)	0 - 0.300 mg/L NO_2^--N
Ammonia	Nessler Method, program 380 (wavelength 425 nm)	0 - 2.50 mg/L NH_3-N
Phosphate	PhosVer 3 (Ascorbic Acid) Method, program 490 (Powder Pillows, wavelength 890 nm)	0 - 2.50 mg/L PO_4^{3-}
Sulfate	SulfaVer 4 Method, program 690 (Powder Pillows, wavelength 450 nm)	0 - 70 mg/L SO_4^{2-}
Fluoride	SPADNS Method, program 190 (wavelength 580 nm)	0 - 2.00 mg/L F^-
Sulfide	Methylene Blue Method, program 690 (wavelength 665 nm)	0 - 0.600 mg/L S_2^-

4.2.2. CHARACTERISTICS OF THE WATER SAMPLE

The water used during the experiments was collected from April to May 2011(summer season) at dam 3 in Ouagadougou, Burkina Faso. Ouagadougou is located at 12°21'26" Latitude North and 1°32'7" Longitude West and receives approximately 2,500 hours of solar radiation per year. The experiments were conducted at the site of 2iE Foundation. The water sample is used by part of the local population for household purposes (cooking, drinking and washing) and has a pH 8.6±0.3. Its physico-chemical parameter concentrations are presented in Table 4.2. The enteric bacteria contents of this water were approximately 10^4 CFU/mL for each entity involved in this study (total coliforms/*E. coli*, and *Salmonella* spp). The sampling collection was realized one hour before the experimentation in a 20-liter plastic jerrican.

4.2.3. BACTERIAL STRAIN AND GROWTH MEDIA

The wild bacterial strain monitored in this study was the fecal indicator bacteria coliforms/*E. coli*, and *Salmonella* spp. Microbiology Chromocult ® (Merck KGaA), was used for bacterial plating. Chromocult is a selective and differential growth media. It selectively inhibits growth of the non-enteric bacteria. As experiments were conducted with natural water, considering their initial enteric bacteria contents, no dilution was realized before the bacterial plating. 100 µl of sample water were inoculated into the growth medium. Considering the selectivity of Chromocult, the detection limit of enteric bacteria was 0 (zero) colony growths observed in the plate. The differential nature of the medium permits the distinction of *Salmonella* spp (colorless), *E.coli* (purple and pink) and the blue- and salmon-colored colonies of other coliforms from bacteria. However, in order to emphasize the decrease of the total coliforms, all the *E. coli* observed and others coliforms counted are presented together in this study as total coliforms/*E. coli*.

4.2.4. THE EXPERIMENTS

All the experiments (solar radiation and photo-Fenton), were conducted under direct sunlight in a Compound Parabolic Collector (CPC). The CPC is a SOLARDETOX ACADUS-2003 batch photoreactor device model delivered by Ecosystem SA (Barcelona, Spain). 25 L of surface water was disinfected during each treatment at constant flow (2 L/min). Preliminary experiments were realized to evaluate the efficiency exposure time (4 and 2 hours respectively) which could be proposed to the population concerned if the vulgarization of the photo-Fenton disinfection is taken into account. Afterwards, the photo-disinfection was carried out in the CPC during 6 different time intervals: (i) 8 am to 12 pm (8-12h), (ii) 10 am to 12 pm (10-12h), (iii) 12 pm to 2 pm (12-14h) (iv) 1 pm to 3 pm (13-15h), (v) 2 pm to 4 pm (14-16h) and (vi) 3 pm to 5 pm (15-17h) for both processes (uniquely solar radiation and photo – Fenton). The evaluation of the influence of direct solar radiation parameters (irradiance and cumulated dose) on the efficiency of both photo-disinfection processes is reported. During the exposure, pre-sterilized glass flasks of 100 mL were used at regular time intervals (0, 10, 20, 30, 45, 60, 90, 120, 150, 180 and 240 min) to collect the treated water samples to be analyzed. 100 µL were taken with a micropipette from the flask and poured into a Petri dish plate containing growth media (Chromocult agar). Plates were incubated for 18-24 h at 37°C and the colonies counted with a colony counter (Stuart SC6 Colony Counter). To check the durability of photodisinfection after exposure, all the flasks were further kept in the dark for post-irradiation controls after 24 hours. Fenton tests were conducted simultaneously in the dark on 100 mL of samples containing 10 mg/L of H_2O_2. The concentration of some physico-chemical parameters of the water (HCO_3^-, CO_3^{2-}, SO_4^{2-}, S^{2-}, F^-, HPO_4^{2-}, NO_2^-, NO_3^-, NH_4^+, turbidity and the total iron content) were evaluated before and after the treatment. The pH and temperature evolution during the treatments were successively

recorded. The experiments were repeated three times to ensure reproducibility. The Wolfram Mathematica 8.0 and MS-Excel programs were used for data analysis and graph fitting.

4.3. RESULTS AND DISCUSSION

4.3.1. EVOLUTION OF SOME PHYSICO-CHEMICAL CHARACTERISTICS OF THE WATER SAMPLE

The surface water used in this study was collected from Ouagadougou's dam 3 during the dry season. Table 4.2 presents its main physico-chemical characteristics before and after photo-Fenton treatment. Water composition has a great influence on the photo-disinfection treatment and light penetration is minimal in highly turbid water (Joyce *et al.*, 1996; Kehoe *et al.*, 2001). To carry out an useful photo-disinfection, it is recommended to conduct it in water with less than 30 NTU turbidity (Byrne *et al.*, 2011). The water treated in this study was clear with only 8±3 NTU. The initial water temperature was ranged between 28-29.5±0.5°C; and it pH was 8.6±0.3. After the first records of the efficiency of photo-Fenton disinfection at near-neutral pH (Rincon and Pulgarin, 2006), several authors have conducted investigations into the efficiency of the process in natural water, some with neutral pH (Moncayo-Lasso *et al.*, 2009; Sciacca *et al.*, 2010; Ndounla *et al.*, 2013). The alkalinity of the natural water treated in this study will enable us to produce the first record of the efficiency of the photo-Fenton at alkaline pH. The Sahelian African soils are ferruginous, which leads permanently to natural iron contents in the water. Taking this into account, only 10 mg/L of H_2O_2 was added in the water sample before the photo-Fenton process.

The concentration of mineral nitrogen compounds (nitrate, nitrite, ammonia) in the surface water used in this study was far below the World Health Organization (WHO) norms for drinking water: 3 mg/L for nitrite, 50 mg/L for nitrate and undetermined for ammonia (WHO,

2011a). Their variations during the photo-treatment were not relevant. The photochemical reduction of nitrite or nitrate and the chemical oxidation of ammonia could lead to $OH^{\bullet}$ radical generation (Eq 4.6-4.8) (Kotzias *et al.*, 1987; Fanning, 2000; Brito *et al.*, 2010). $OH^{\bullet}$ radical is highly oxidant and has a lethal action on the enteric bacteria. Therefore, the redox activities of the nitrogen compounds in the water during photo-disinfection could significantly affect the rate of the photo-inactivation.

$$NO_3^- + H_2O + h\nu \rightarrow NO_2^- + OH^- + OH^{\bullet} \quad (4.6)$$

$$NO_2^- + H_2O + h\nu \rightarrow NO + OH^- + OH^{\bullet} \quad (4.7)$$

$$NH_4^+ \leftrightarrow NH_3 + OH^{\bullet} \rightarrow NH_2OH \rightarrow NOH \rightarrow NO \rightarrow NO_2^- \leftrightarrow NO_3^- \quad (4.8)$$

Inorganic ions can have an interfering effect on the Fenton reagent. Depending on their concentrations, Fenton and photo-Fenton oxidations of organic compounds are inhibited in varying degrees by inorganic ions (e.g. phosphate, sulphate, chloride) (Pignatello *et al.*, 2006). However, the concentration of sulphate, sulfide, fluoride and phosphate were under the restriction of the WHO guidelines for drinking water (WHO, 2011a). Photo-disinfection did not significantly affect their variation during the treatments (Table 4.2). Rincon and Pulgarin (2007b) have reported that the mixture of these components at high concentrations can positively influence the kinetic of the photo-disinfection, while when individually taken into account, they have a negative impact on the photocatalytic process (Pignatello *et al.*, 2006; Rincon and Pulgarin, 2007b).

Phosphate has a doubly detrimental effect by precipitating iron and by scavenging hydroxyl radicals (Malato *et al.*, 2009). The bicarbonate (HCO_3^-) concentration was extremely high in the surface water treated in this study and the presence of carbonate (CO_3^{2-}) ions was also recorded (Table 4.2). It should be taken in account that the carbonates are generated from bicarbonates in water, when its pH is greater or equal to 8.3. As a buffer complex HCO_3^-

/CO_3^{2-}, the bicarbonate and carbonate ions could greatly influence the photocatalytic process (Pignatello *et al.*, 2006; Rincon and Pulgarin, 2007b) by their quenching effect on the hydroxyl radicals in a H_2O_2/light System (Kochany and Lipczynskakochany, 1992). Due to their buffering effect, they also have a great impact on the pH variation during photo-disinfection. Relevant differences were not noticed in their concentration before and after photo-disinfection.

Table 4.2: Physico-chemical characteristics of the water sample measured before and after the photo-Fenton disinfection treatment.

Parameters	Before the treatment	After the treatment
Temperature (°C)	28-29.5±0.5	-
pH	8.6±0.3	-
Turbidity (NTU	8±3	8±3
Total Iron (mg/L)	0.10±0.05	0.11±0.06
Nitrite (NO_2^-) (mg/L)	0.011±0.003	0.012±0.002
Nitrate (NO_3^-) (mg/L)	3.26±0.2	4.02±0.3
Ammonia (NH_4^+) (mg/L)	0.11±0.05	0.17±0.04
Sulphate (SO_4^{2-}) (mg/L)	12±1	12±1
Sulfide (S^{2-}) (mg/L)	0.007±0.002	0.008±0.001
Fluoride (F^-) (mg/L)	0.50±0.02	0.50±0.03
Phosphate (PO_4^{2-}) (mg/L)	0.07±0.01	0.15±0.02
Bicarbonate (HCO_3^-) (mg/L)	148.10±0.05	137.86±0.04
Carbonate (CO_3^{2-}) (mg/L)	3.8±0.1	3.7±0.2

4.3.2. ENTERIC BACTERIA INACTIVATION IN ALKALINE WATER BY FENTON, SOLAR LIGHT AND PHOTO-FENTON

The inactivation of wild enteric bacteria contents of the natural surface water in the CPC during the photo-disinfection treatment by solar radiation (*hv*), photo-Fenton ($Fe^{2+}/H_2O_2/hv$) and dark Fenton ($Fe^{2+}/H_2O_2/obs$) are presented in this paper. The initial natural iron content of the sample water was 0.10±0.05 mg/L and the H_2O_2 added was 10 mg/L. The open symbols represent the total coliforms/*E. coli* and the full symbols are for *Salmonella* spp. The traces (△) and (▲) represent Fenton evaluation conducted in 100 ml glass flasks kept in the dark. The traces (○) and (●) illustrate the enteric bacteria decreased under solar radiation, and (□) and (■) presents the inactivation under photo-Fenton treatment. The decrease of both enteric bacteria under both photo-disinfection methods follows the first order kinetic (McGuigan *et al.*, 1998). The water temperature evolution during the treatment and the available irradiance and dose recorded during the exposure are also presented.

4.3.2.1. FENTON SYSTEM

As can be observed in all the traces (△) and (▲)) in Figs.4.1, 4.2 and 4.3, neither the total coliform/*E. coli* strain nor that of *Salmonella* spp. decreased of one magnitude order were noticed during the exposure to the Fenton system. However, it is surprising to notice that the Fenton process (Eqs. 4.1-4.2) led to the total inactivation of the weakened total coliform/E. coli strain during the subsequent 24-hour dark storage. In contrast, the more resistant strain of *Salmonella* spp. (Berney *et al.*, 2006) attained a higher active bacteria population than that present at the beginning of the treatment during the same dark storage time.

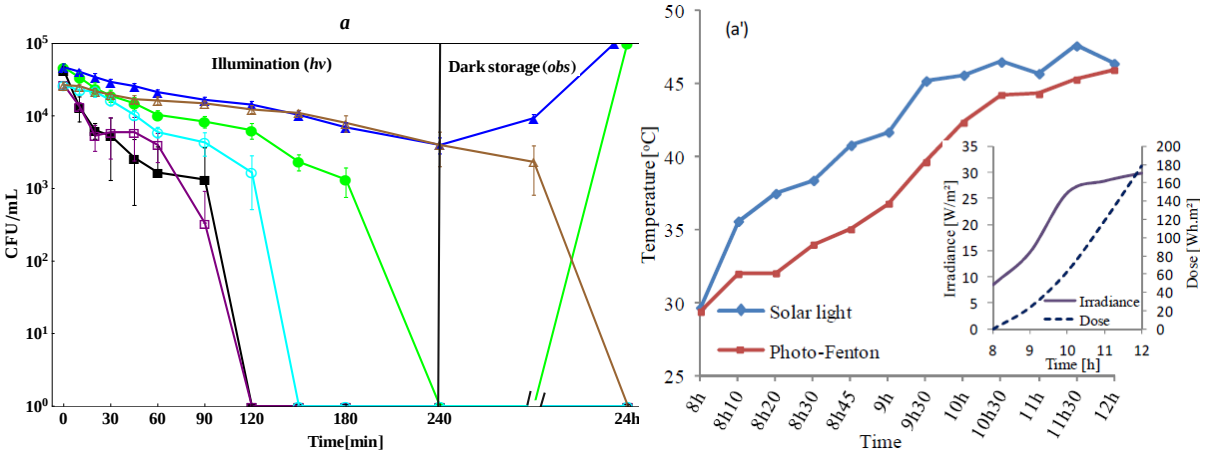


Figure 4.1: (*a*) Inactivation of wild enteric bacteria in natural surface water carried out from 8-12h under direct solar illumination (*hv*). Post irradiation events (24-hour dark storage). pH 8.6±0.3, natural iron content (Fe): 0.10±0.05 mg/L, addition of 10 mg/L of H_2O_2 in the water for photo-Fenton process. Total coliforms/*E. coli* (□) and *Salmonella* spp. (■) under photo-Fenton (natural $Fe^{2+/3+}/H_2O_2/hv$), total coliforms/*E. coli* (○) and *Salmonella* spp. (●) under direct solar radiation (natural $Fe^{2+/3+}/hv$), total coliforms/*E. coli* (△) and *Salmonella* spp. (▲) in the dark (Fenton process) (natural $Fe^{2+/3+}/ H_2O_2/obs$). (*a'*) evolution of the water temperature [T°C] during both treatments (solar and photo-Fenton); insert: solar irradiance [$W.m^{-2}$, (▬)] and cumulated total dose [$Wh.m^{-2}$, (╌╌)] available during the experiment.

4.3.2.2. SOLAR RADIATION SYSTEM

The generation of the reactive oxygen species (ROS) from NOM, which intervene together with the direct action of photons in the lethal attack of the bacteria, is highly influenced by the light intensity or irradiance ($W.m^{-2}$). However, the solar inactivation of wild enteric bacteria of natural surface water at different daytime intervals is not only influenced by average irradiance (AVI) but also by the temperature increase recorded during each exposure period in Figs.4.1, 4.2, 4.3, trace (○) and (●). It is noticeable that the synergy between the temperature rise and the average irradiance (AVI), and not the cumulated dose, significantly affected the photo-disinfection process. Indeed, during the exposure period 15-17h, in the presence of very low AVI of 8 $W.m^{-2}$ for a cumulated dose of 21 $Wh.m^{-2}$ and a temperature of less than 40°C (Fig.4.3-f'), none of the enteric bacteria in the water were inactivated under uniquely solar radiation (Fig.4.3-f).

During the periods 8-12h (Fig.4.1-a) and 14-16h (Fig.4.3-e), the temperature increased to approximately 45°C and the AVI were 17 and 20 $W.m^{-2}$ respectively. This led to the total

inactivation of both enteric bacteria. 150 min (period 8-12h) and 90 min (period 14-16h) were required for total coliform/*E. coli* strain inactivation. 240 min (period 8-12h) and 120 min (period 14-16) were recorded for *Salmonella* spp. strain inactivation. The high dose required during the period 8-12h for *Salmonella* spp. total inactivation (180 $Wh.m^{-2}$) proved that the availability of high doses does not lead to high inactivation kinetics. Only 80 $Wh.m^{-2}$ was required during the exposure period 14-16h for both enteric bacteria's total inactivation. The dose recorded for the successive exposure periods 10-12h (Fig.4.2-b), 12-14h (Fig.4.2-c) and 13-15h (Fig.4.2-d) was respectively 90, 50 and 50 $Wh.m^{-2}$ for both enteric bacteria's inactivation in approximately 90 min in all the cases. This result again confirms the previous observation about the irrelevant impact of the dose in the photo-disinfection of drinking water sources.

The AVI recorded during the successive daytime periods 10-12h, 12-14h and 13-15h was respectively 29, 27 and 28 $W.m^{-2}$. These high AVI have approximately the same order of magnitude as the ones recorded in the Sahelian region during the dry season (summer season) (Kenfack *et al.*, 2009). Associated to the temperature rise of more than 40°C during the first hour of exposure, in almost all the cases they have led to the total inactivation of both enteric bacteria strains in approximately 90 min. Therefore, high AVI induced a similar required time (and dose) for total inactivation. The total coliform/*E. coli* strain was the only one to be inactivated in 60 min during the exposure period 13-15h. This fast inactivation kinetic was certainly due to the fact that the temperature increase was very fast at the beginning of its exposure.

It is generally accepted that, the photonic flux greatly affect the ROS production and the concomitant oxidative stress in the bacteria, leading to inactivation or death while the increased temperature inactivates the enzymes which were supposed to protect them from this stress (Cabiscol *et al.*, 2000). As neither the temperature increase nor the ROS generation

through the photonic action of irradiance were sufficiently available during the exposure period 15-17h, the steady state of the active enteric bacteria recorded in the water at the end of the exposure could be attributed to the absence of lethal oxidative stress. These results highlight the fact that it is the synergetic effect of the photonic and the thermal parameters of the solar radiation (intense solar radiation coupled to increased temperature to approximately 45°C), which lead to the enteric bacteria inactivation during the solar disinfection (Wegelin *et al.*, 1994; Sommer *et al.*, 1997; McGuigan *et al.*, 1998). Unfortunately, during the post-irradiation storage in the dark, the *Salmonella* spp. strains recovered their viability and grew to more than their initial level in all the treated water. This regrowth of *Salmonella* spp. is the negative side of the bare solar disinfection. It revealed that their inactivation was not irreversible. It will be necessary to evaluate whether the photo-Fenton could ensure the irreversibility of all the enteric bacteria inactivation.

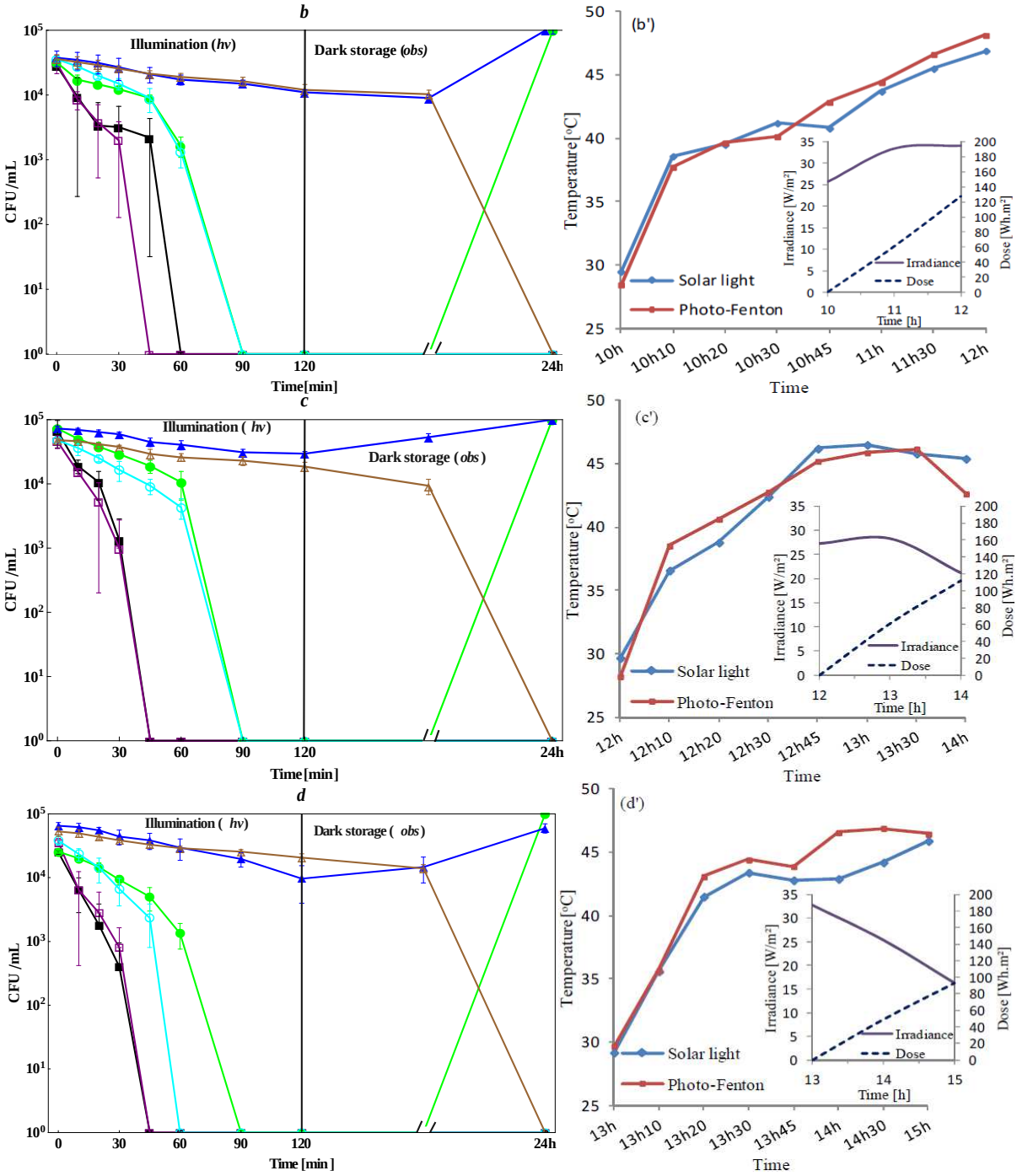


Figure 4.2: (*b*, *c*, *d*) Inactivation of wild enteric bacteria in natural surface water carried out from 10-12h, 12-14h and 13-15h respectively under direct solar illumination (*hv*). Post irradiation events (dark storage during 24h). pH 8.6±0.3, natural iron (Fe) content: 0.10±0.05 mg/L, addition of 10 mg/L of H_2O_2 in the water for photo-Fenton process. Total coliforms/*E. coli* (□) and *Salmonella* spp. (■) under photo-Fenton (natural $Fe^{2+/3+}/H_2O_2/hv$), total coliforms/*E. coli* (○) and *Salmonella* spp. (●) under direct solar radiation (natural $Fe^{2+/3+}/hv$), total coliforms/*E. coli* (△) and *Salmonella* spp. (▲) in the dark (Fenton process) (natural $Fe^{2+/3+}/ H_2O_2/obs$). (*b'*, *c'*, *d'*) evolution of the water temperature [T°C] during both treatments (solar and photo-Fenton); insert: solar irradiance [$W.m^{-2}$, (—)] and cumulated total dose [$Wh.m^{-2}$, (---)] available during the experiment.

4.3.2.3. PHOTO-FENTON SYSTEM

The photo-Fenton process in alkaline water is normally subject to the formation of iron hydroxides and iron oxides which precipitate. However drastic inactivation kinetics were recorded in all the enhanced by H_2O_2 addition photo-disinfection treatment (Figs.4.1, 4.2 and 4.3; trace (□) and (■)). Comparatively to the situation observed under uniquely solar radiation in the previous section, it could be assumed that photo-Fenton reaction could significantly take place at alkaline pH. The ROS ($^{\bullet}OH$, $HO_2^{\bullet}$, $O_2^{\bullet-}$) production from photosensitized NOM (Canonica *et al.*, 1995), and Fe^{2+} regeneration from photo-active Fe^{3+}-organo-complex (Pignatello *et al.*, 2006) has certainly contributed to these observed results.

The high photon-flux generated by the AVI available during the exposure periods 10-12h, 12-14h and 13-15h, which was respectively 29, 27 and 28 $W.m^{-2}$, associated to the temperature rise of more than 40°C during the first hours of exposure led to a drastic inactivation of both enteric bacteria in approximately 45 min in all cases (Fig. 4.2, trace (□) and (■)). However, during the exposure period 10-12h, the *Salmonella* spp. total inactivation took a little more time. The effect of the low solar radiation available in the morning and afternoon was highlighted by the recorded irradiance: 17 and 20 $W.m^{-2}$ respectively for the exposure periods 8-12h and 14-16h (Fig. 4.1-4.3). A relevant inactivation kinetics was however, recorded in both cases. The total inactivation was achieved for both enteric bacteria after 120 min during the exposure period 8-12h. Drastic inactivation in 20 min of total coliforms/*E. coli* strain was recorded for the exposure period 14-16h, while that of *Salmonella* spp. was achieved after 60 min.

The inactivation of the weakened strains of total coliforms/*E. coli* was noticed at the end of the photo-Fenton process, during the exposure period 15-17h, in contrast to the negative results recorded for the same period under bare solar radiation (Fig. 4.3, trace (□) and (■)). Even though the AVI was very low during this period (8 $W.m^{-2}$), no regrowth was recorded

after the dark post-irradiation storage (24h). As noticed in the previous section, the cumulated dose of the solar radiation did not significantly influence the inactivation process. The dose recorded for the total inactivation of both enteric bacteria during the exposures was approximately 50 $Wh.m^{-2}$ for the periods 12-14h, 13-15h and 14-16h. It was 120 and 60 $Wh.m^{-2}$ respectively for the exposure periods 8-12h and 10-12h. It is important to notice the residual effect of the H_2O_2 by Fenton reaction during the storage. However, the remaining H_2O_2 amount (3-4 mg/L) in the treated water disappears completely from the water after two or three days of storage. This H_2O_2 depletion was also reported in our previous paper (Ndounla *et al.*, 2013).

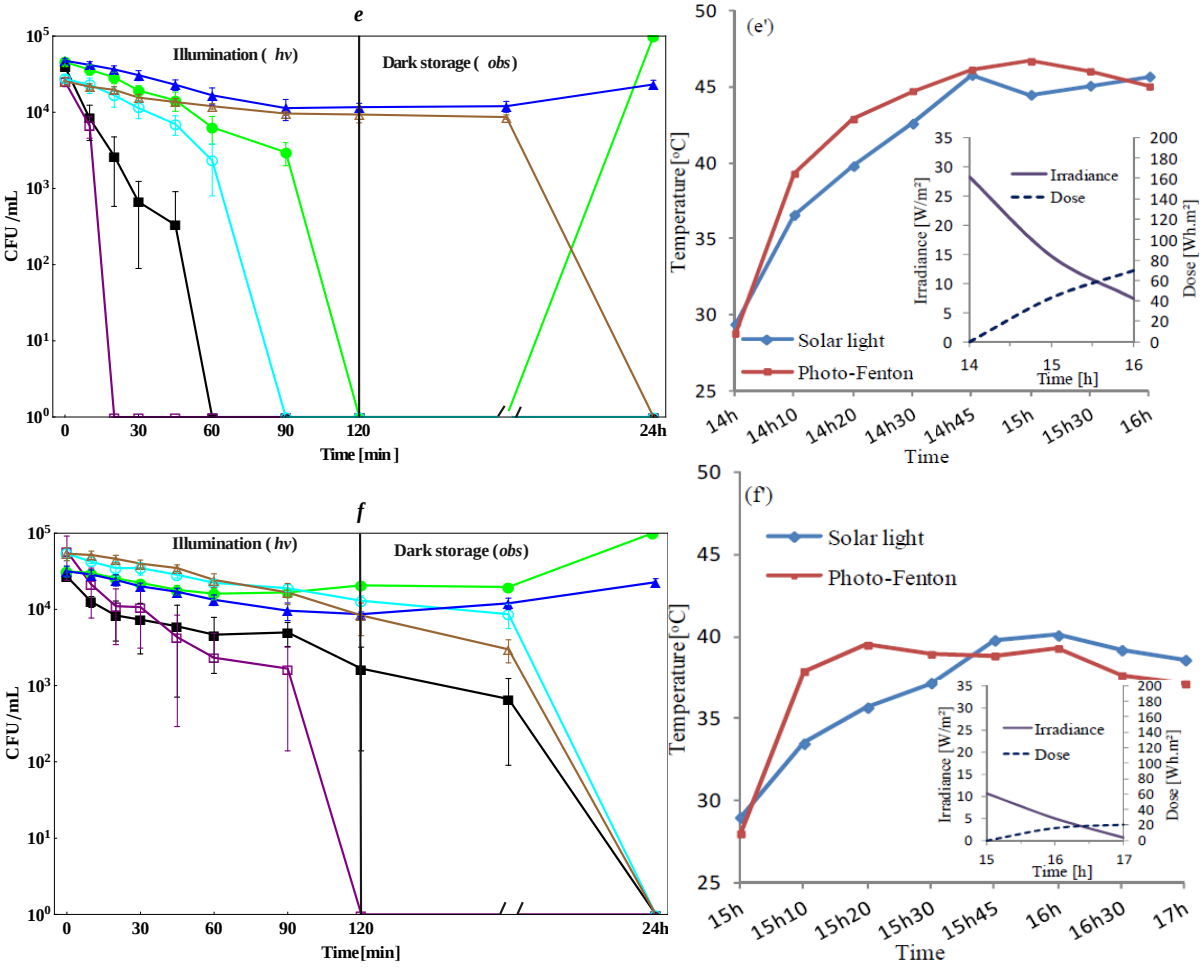


Figure 4.3: (*e*, *f*) Inactivation of wild enteric bacteria in natural surface water carried out from 14-16h and 15-17h respectively under direct solar illumination (*hv*). Post irradiation events (dark storage during 24h). pH 8.6±0.3, natural iron (Fe) content: 0.10±0.05 mg/L, addition of 10 mg/L of H_2O_2 in the water for photo-Fenton process. Total coliforms/*E. coli* (□) and *Salmonella* spp. (■) under photo-Fenton (natural $Fe^{2+/3+}/H_2O_2/hv$), total coliforms/*E. coli* (○) and *Salmonella* spp. (●) under direct solar radiation (natural $Fe^{2+/3+}/hv$), total coliforms/*E. coli* (△) and *Salmonella* spp. (▲) in the dark (Fenton process) (natural $Fe^{2+/3+}/ H_2O_2/obs$). (*e'*, *f'*) evolution of the water temperature [T°C] during both treatments (solar and photo-Fenton); insert: solar irradiance [$W.m^{-2}$, (—)] and cumulated total dose [$Wh.m^{-2}$, (---)] available during the experiment.

4.3.3. POST IRRADIATION EVENTS

4.3.3.1. POST IRRADIATION EVENTS AFTER SOLAR RADIATION DISINFECTION

The *Salmonella* spp. conversion from nonculturable to culturable strains was noticed in all the waters which were disinfected for 2 hours by uniquely solar radiation. After the recovery of

their culturability during the dark storage, they increased to more than their initial contents. These *Salmonella* spp. strains' recovery during favorable conditions lead to the assumption that the effect of solar radiation was bacteriostatic and not bactericidal (Rincon and Pulgarin, 2007b). A longer exposure time is therefore needed to ensure the bactericidal effect of the uniquely solar radiation on *Salmonella* spp. strains to ensure its irreversible inactivation. The total coliform/*E. coli* strain was not inactivated during the exposure period 15-17h. However, after being weakened by illumination, it was totally inactivated during the subsequent 24 h of dark storage. In contrast, the remaining *Salmonella* spp. strains took advantage of the favorable conditions of the dark storage to recover their capacity for growth. Such capacity is part of the resistance of *Salmonella* spp. to photo-inactivation (Berney *et al.*, 2006).

4.3.3.2. POST IRRADIATION EVENTS AFTER PHOTO-FENTON DISINFECTION

None of the total coliform/*E. coli* and *Salmonella* spp. strains inactivated under the photo-Fenton treatment in this study recovered viability during storage (Figs.4.1, 4.2 and 4.3, trace (□) and (■)). No regrowth of any of the strains was observed after the 24 hours of dark storage. Considering this irreversible inactivation, it can be assumed that the photo-Fenton disinfection of drinking water could be efficiently used to produce higher volumes of water in shorter time than requiered by SODIS bottles system.

4.3.4. PH EVOLUTION DURING THE EXPERIMENTS

The pH increase recorded in all the photo-disinfection (solar and photo-Fenton) processes as presented in Fig. 4.4, are in contrast with the decrease report by several authors during the photocatalytic treatment with TiO_2 (Rincon and Pulgarin, 2007b; Malato *et al.*, 2009). This increase is probably due to (i) the buffer action of the complex HCO_3^-/CO_3^{2-} which maintains the solution in the alkaline region and especially (ii) the degradation of the nitrogen component of the water during their photo-degradation (Eq.4.6-4.7), leading to the generation

of the OH^- and consequently increased alkalinity (Kotzias *et al.*, 1987; Fanning, 2000). However, this pH increase has not negatively affected the inactivation kinetic of the photo-Fenton process.

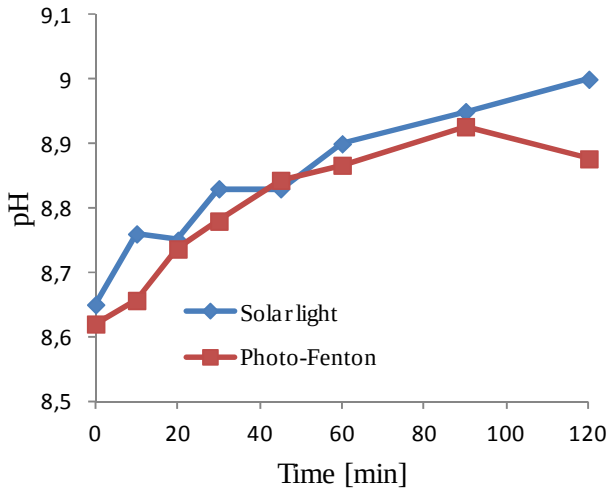


Figure 4.4: Evolution of pH during the photo-disinfection treatments: (♦) under solar radiation treatment without h_2o_2 addition, (■) under photo-Fenton treatment with 10 mg/L of H_2O_2.

4.3.5. COMPARATIVE EVALUATION OF THE ENTERIC BACTERIA INACTIVATION BY PHOTO-FENTON AT ALKALINE OR NEAR-NEUTRAL PH IN NATURAL WATERS

In this study, the presence of several iron species in natural water: soluble ($Fe^{2+ \text{ or } 3+}$) and solids (e.g. iron oxides) has lead to heterogeneous conditions for photo-Fenton process. This heterogeneous conditions could take advantage of several pathways: (i) the high adsorption of iron oxides by bacteria (Spuhler *et al.*, 2010), (ii) the effect of the bacteria siderophores which increases iron dissolution (Stintzi *et al.*, 2000) and leads to high photo-Fenton activity, (iii) the enhancing semi-conductor effect of some types of solid iron oxides on photo-disinfection (Moncayo-Lasso *et al.*, 2008b; Mazille *et al.*, 2010) or (iv) the natural organic matter (NOM) or humic substances complexation with iron to maintain their solubility in solution (Pignatello *et al.*, 2006; Lipczynska-Kochany and Kochany, 2008). The heterogeneous photo-Fenton degradation of phenolic compounds was recently carried out at alkaline pH. Under this

condition, 0.001 mol of the phenolic compound was degraded in 1 hour (Martínez *et al.*, 2005; Ayodele *et al.*, 2012). Avogadro's number (N_A = 6.022 10^{23} mol^{-1}) is the number of constituent species in one mole of a given substance, (usually atoms or molecules). If we consider the degradation of 0.001 $mol.h^{-1}$ as that of 6.022 10^{20} molecules, the target in natural waters disinfected in this study is far below this level with a maximum record at 10^6 CFU/ml. It could be assumed that the $^{\bullet}OH$ radical generated by the homogenous (Fe-org) and heterogeneous (Fe oxides) photo-Fenton in alkaline or near-neutral water sources was high enough to ensure the drastic inactivation of both enteric bacteria strains as recorded. Figure 4.5 shows the inactivation curves of the total coliforms/ *E. coli* and *Salmonella* spp. by photo-Fenton in well-water (W, pH 5.4 ± 0.1) or in surface-water (S, pH 8.6 ± 0.3). The solar radiation parameters (irradiance/dose) and temperature rise during the experiments were similar to those presented in the previous section at the same time intervals for both water sources. In contrast to the fact regularly observed at acidic or near-neutral pH, *Salmonella* spp. strains seem to be less resistant to photo-Fenton inactivation at alkaline pH. Their total inactivation occurred under several exposure periods in this study at the same time as that of total coliforms/ *E. coli* (Fig 4.5.a-c). The slight delay (15 min) noticed between its inactivation kinetics and that of total coliforms/ *E. coli* during the exposure period 10-12h is not worth recording as a relevant fact. It can also be noticed that the global effective inactivation time of *Salmonella* spp. in alkaline water is lower than the one recorded when it was inactivated under acidic or near-neutral conditions. It could therefore be assumed that the pH of the water could significantly affect the sensitivity of the bacteria to photo-inactivation, as is also the case for chlorination (WHO, 2011a).

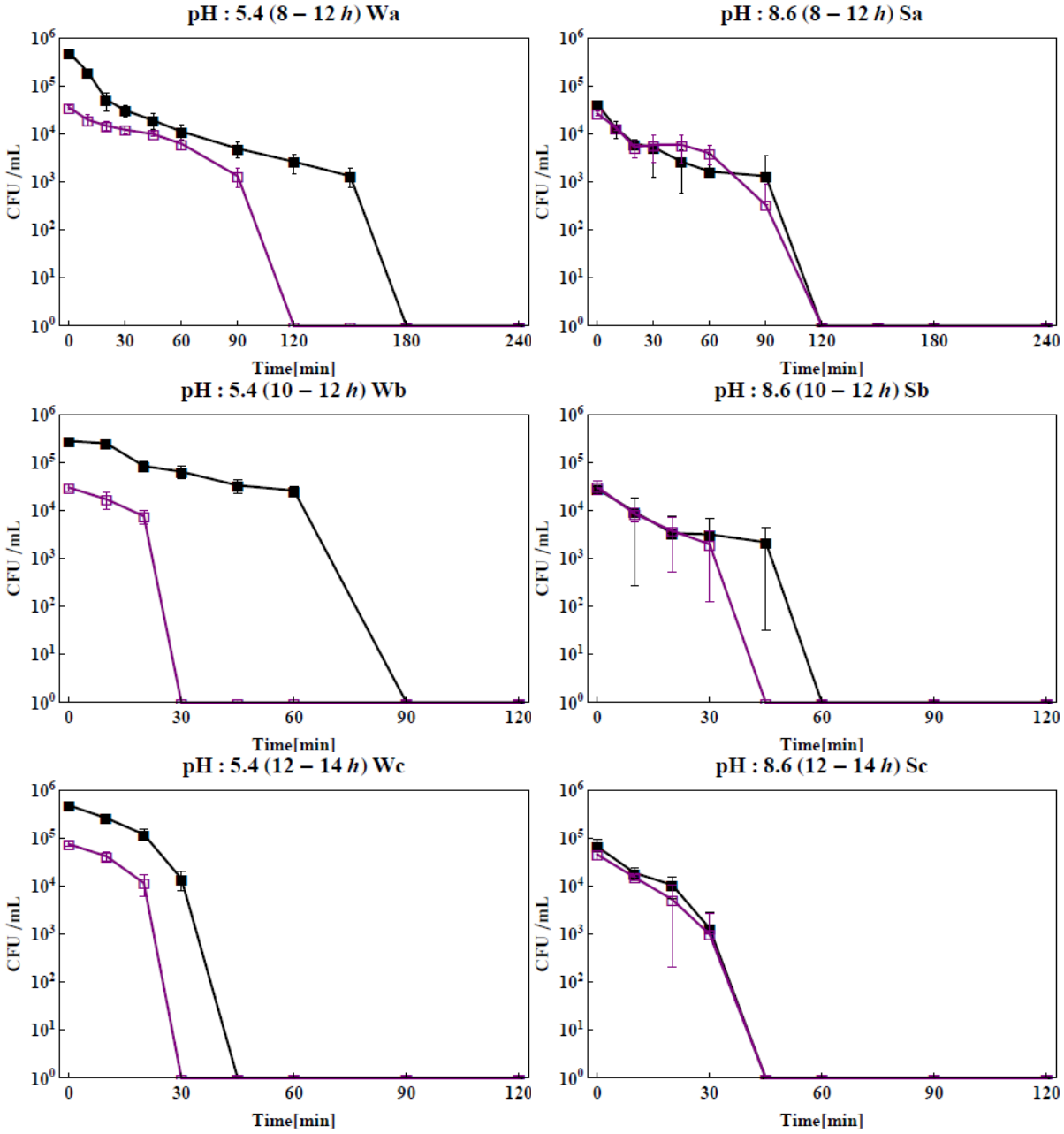


Figure 4.5: Wild enteric bacteria inactivation by photo-Fenton (natural $Fe^{2+/3+}/H_2O_2/hv$) in natural wells (W) and surface (S) water under direct solar radiation at different time intervals of the day (8-12h, 10-12h and 12-14h). (□) total coliforms/*E. coli*, (■) *Salmonella* spp. Some parameter of the sample water presented here: the surface water contains 0.10±0.05 mg/L natural iron (Fe) and pH 8.6±0.3, while the wells water contains natural iron (dissolved: 0.07± 0.02 mg/L of $Fe^{2+,3+}$ and solid iron oxides (0.23± 0.01 mg/L), pH 5.4±0.01. 10 mg/L of H_2O_2 was added in each sample to initiate the photo-Fenton process

4.3.6. INACTIVATION MECHANISMS

4.3.6.1. INACTIVATION MECHANISMS UNDER SOLAR RADIATION TREATMENTS

Dissolved oxygen in the water seems to have a direct impact on the bactericidal action of solar disinfection (Reed, 1997). The water recirculation in the CPC during the photo-disinfection process is subjected to high fluctuation following the oxygen availability at different point of the reactor. The NOM present in natural water act as photosensitizers. Under irradiation, the photosensitizers become electronically excited and react with O_2, leading to reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), superoxide ($HO_2^{\bullet}/^{\bullet}O_2^-$), H_2O_2 and $OH^{\bullet}$ radicals (Canonica *et al.*, 1995; Reed, 1997). The catalases and other enzymes which should protect the cells from oxidative stress are photo and thermo-sensitive. The temperature increase of up to 45°C during photo-disinfection inactivates these enzymes, leaving the cells susceptible to internal ROS attack and subsequent inactivation. $OH^{\bullet}$ radicals is the more bactericidal ROS (Jang and Imlay, 2010; Spuhler *et al.*, 2010). A delay in the photo-disinfection kinetic is observed when several environmentally unfavorable factors are present (Rincon and Pulgarin, 2007b), such as the low temperature and solar radiation available during the exposure period 15-17h. The *Salmonella* spp. reactivation after the photo-treatment is probably due to the fact that they were not sufficiently exposed to the lethal action of the ROS during the 2 hours of exposure.

4.3.6.2. INACTIVATION MECHANISMS UNDER PHOTO-FENTON TREATMENTS

Natural organic matter contains functional groups which can form complexes with Fe^{3+} or Fe^{2+}. These complexes not only increase the solubility of iron over the natural pH range, but can also considerably contribute to the photo-Fenton reactions via a LMCT under solar radiation. The positive effect of NOM constituents (e.g. carboxilic acids) on photo-Fenton process, which allow us to work at near-neutral pH and, as noticed here, at alkaline pH (pH 8.6±0.3) too, has recently been reported by several authors (Georgi *et al.*, 2007; Lipczynska-

Kochany and Kochany, 2008; Vermilyea and Voelker, 2009). The Fe^{2+} generation from the Fe^{3+}-organo-complex in the photo-Fenton experiments carried out in this study react with the H_2O_2 added to the water and lead to the generation of lethal $^{\bullet}OH$ radical (Eq.4.3-4.5) (Pulgarin *et al.*, 1995; Pignatello *et al.*, 2006; Malato *et al.*, 2009). The association of the ROS production with the reaction between the photosensitized NOM and dissolved O_2 supplied by the water recirculation in the CPC, and the highly generation of •OH by photo-Fenton in the water causes high oxidative stress in the enteric bacteria. In a normal situation, the cells can escape oxidative stress by producing catalases enzymes to inactivate them (Cabiscol *et al.*, 2000). It is known that the enzymes are photo and thermosensitive and are inactivated with increased temperatures (Ghadermarzi and Moosavi-Movahedi, 1996; McGuigan *et al.*, 1998). The simultaneous temperature increase and photonic action during the ROS production has inactivate the enzyme production. In the absence of the protective enzymes, the self-defense mechanisms of the cells are inhibited, thus encouraging the production of the OH• and other ROS ($^1O_2$, $HO_2^{\bullet}$, $^{\bullet}O_2^-$, H_2O_2) into the cell and lead to their irreversible inactivation. The post-irradiation events show no enteric bacteria recovery, thus confirming their irreversible inactivation.

4.4. CONCLUSIONS

The photo-disinfection treatment was efficiently enhanced by photo-Fenton system at alkaline pH. Solar irradiation is the key factor for both photo-disinfection processes (solar and photo-Fenton). The photo-Fenton disinfection at alkaline pH is not efficient if conducted under a low solar irradiance range of less than $12W.m^{-2}$ and a temperature of less than 40°C. None of the enteric bacteria strains (total coliform/*E. coli* and *Salmonella* spp.), totally inactivated by the photo-Fenton treatment have succeeded in recovering the culturability during the subsequent 24 hours of dark storage. The resistivity of the Salmonella spp. strains noticed at near-neutral pH in our previous study was not confirmed at alkaline pH in this one. These

strains were highly sensitive at alkaline pH and their total inactivation was recorded at the same time as those of the total coliforms/*E. coli*. The photo-Fenton ensures the irreversible inactivation of the enteric bacteria, while under a uniquely solar radiation exposure of 4 or 2 hours the inactivated *Salmonella* spp. strains have recovered their viability during the 24 hours of subsequent dark storage. To efficiently disinfect the water under uniquely solar radiation, it is important to expose it for more than 4 hours under high solar irradiance and impose a temperature increase of up to 45°C to ensure the simultaneous irreversible inactivation of total coliform/*E. coli* and *Salmonella* spp. Considering the enhancement ability of the photo-Fenton treatment and its irreversible lethal action on enteric bacteria, before vulgarization of the process we recommend additional research into improvements to ensure the safety of the treated water.

5. CHAPTER 5:

EVALUATING THE EFFICIENCY OF THE PHOTO FENTON DISINFECTION OF NATURAL DRINKING WATER SOURCE DURING THE RAINING SEASON IN THE SAHELIAN REGION

5.1. INTRODUCTION

In order to develop a useful household water treatment process for the populations, not connected to safe drinking water supply in developing countries, several researches have been conducted on the photo-disinfection of natural water sources in most of those countries (Mäusezahl *et al.*, 2009; Du Preez *et al.*, 2011; McGuigan *et al.*, 2011; Marques *et al.*, 2013; Ndounla *et al.*, 2013; Ruales-Lonfat *et al.*, 2013). As solar disinfection (SODIS) and photo-Fenton treatment take both advantage of the free energy of sunlight, most of those researches were conducted during the summer season. This season has the most favorable optical conditions for solar radiation (McGuigan *et al.*, 1999; Navntoft *et al.*, 2008; Sciacca *et al.*, 2010; Marques *et al.*, 2013; Ndounla *et al.*, 2013). The evaluation of the efficiency of the processes during the raining season taking in account the effect of the intermittence of a cloudy and/or sunny sky on it could be useful to improve the database of this point-of-use treatment method.

During the raining season, wells and surface water are highly diluted. This dilution of the chemical component of the water could significantly influence both photo-disinfection processes. In a previous study (Ndounla *et al.*, 2013), we have evaluated the enhancement of the SODIS process by the photo-Fenton reagent ($Fe^{2+}/H_2O_2/h\nu$) on natural well water. The inactivation rate of the wild enteric bacteria (total coliforms/*E. coli* and *Salmonella* spp.) naturally present in the water was followed to evaluate the efficiency of the processes. In the previous work, the iron considered for the Fenton reagent was either the added (0.6 mg/L) or the initial natural iron contents of the sample water (0.07 mg/L) (Table 5.1). The experiment was carried out on PET (polyethylene terephthalate) bottles and the results have shown that

the processes was highly efficient and the one conducted with the low natural iron of the water was the more efficient comparatively to the one with chemically added iron.

It is a fact that iron is the fourth most abundant element in the Earth's crust and the most abundant transition metal in soil. The ability of iron to undergo cyclic oxidation and reduction is an important aspect for photo-Fenton process. The photo-reactivity of iron as transition metal especially in natural water in presence of organic ligands, is of high environmental importance (Cieśla *et al.*, 2004). In general, the reduced forms of iron ions (Fe^{2+}) produce $^{\bullet}OH$ at a far faster rate upon reaction with H_2O_2 than the oxidized forms (Fe^{3+}) during the Fenton and Fenton-like reaction, which ideally operate at acidic pH (2.5-3), (Eqs. 5.1-5.2). Therefore, reducing agents catalyze the regeneration of Fe^{2+} from Fe^{3+} and accelerate the generation of $^{\bullet}OH$ for photo-Fenton process (Eq. 5.3) (Sychev and Isak, 1995; Kruszewski, 2003; Pignatello *et al.*, 2006):

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^{-} \qquad k = 53 - 76\ M^{-1}\ s^{-1} \qquad (5.1)$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+}\,HO_2^{\bullet} + H^{+} \qquad k = 1 - 2\ 10^{-2}\ M^{-1}\ s^{-1} \qquad (5.2)$$

$$Fe^{3+} + Donor^{red} \rightarrow Fe^{2+} + Donor^{ox} \qquad (5.3)$$

The Fenton reaction in Equation 5.1 is the primary source for $^{\bullet}OH$ and produces Fe^{3+}. For the process to become catalytic, Fe^{2+} needs to be regenerated from Fe^{3+}, which precipitates at neutral pH in absence of organic complexing matter. The regeneration of Fe^{2+} from Fe^{3+} can be accelerated by irradiating the system (photo-Fenton process) because some Fe^{3+}-complexes undergo photoreduction under UVA and visible radiation, producing $^{\bullet}OH$ and regenerating Fe^{2+} via ligand to metal charge transfer (LMCT) (Eq. 5.4):

$$[Fe^{3+}(L)_n] + h\nu \rightarrow [Fe^{3+}(L)_n]^* \rightarrow [Fe^{2+}(L)_{n-1}] + L^{\bullet}_{ox} \qquad (5.4)$$

The redox cycling of Fe^{2+} and Fe^{3+} depends strongly on the presence and nature of the natural organic matter it influence the geochemical cycles and strongly affects the chemical and biological processes, which are sensitive to the iron speciation (Cieśla *et al.*, 2004). The organic matter which form strong complexes with Fe^{3+} at near neutral pH and which undergo rapid photochemical reactions in sunlight are poly-carboxylates (Zuo and Hoigne, 1992; Faust and Zepp, 1993; Ruales-Lonfat *et al.*, 2013). Under illumination, the photochemical activities of these complexes favor the solubility of iron at near neutral pH leading the optimal condition for photo-Fenton reactions. The carboxylate group [R-COO^{-}] is one of the most common functional groups of the dissolved organic compounds present in natural waters (Feng and Nansheng, 2000), 2000). The Fe^{3+} -carboxyl or -polycarboxyl complexes ([Fe^{3+}-($^{-}$OOC)-R]$^{2+}$) undergo a photo-dissolution via a LMCT as described in equation 4-5 (Faust and Zepp, 1993; Pignatello *et al.*, 2006; Georgi *et al.*, 2007).

$$[(RCOO^{-})Fe^{3+}] + h\nu \rightarrow Fe^{2+} + CO_2 + R^{\bullet} \qquad (5.5)$$

As the presence of natural organic matter, the turbidity of the water is one of the most important parameter which can influence the efficiency of the photo-disinfection process. It can protect microorganism from UVA radiation and thus significantly decrease the efficiency of photo-disinfection. Water with turbidity higher than 30 NTU should be pre-filtered for conventional SODIS. Highly turbid or unfiltered water may not withstand any significant optical inactivation, because the sunlight is completely absorbed within the first few millimeters of water (McGuigan *et al.*, 1999). However, turbidity usually increases the maximum water temperature achieved within the bottle, resulting from the absorption of visible and IR radiation by the suspended particles (Sciacca *et al.*, 2011). Water temperature is raised until it could be accounted for the complete disinfection (Joyce *et al.*, 1996; Sciacca *et al.*, 2011). However, for high turbidity, the beneficial effect of increased temperature is likely to be far less important than the negative effects resulting from a decrease in penetration of

the inactivating radiation (Reed, 2004). Another component which could significantly influence the rate of a photo-disinfecting process is the bicarbonate ions concentration (Rincon and Pulgarin, 2007b). In fact, carbon dioxide that is stored in water will be present as either carbonate or bicarbonate ions. These ions are an important part of natural buffers that prevent the water from becoming too acidic or too basic. This parameter is highly important when process the photo-disinfection at near neutral pH.

For a sustainable application of photo-disinfection in drinking water treatment it is crucial to know whether the reciprocity of irradiance and dose, applies for bacterial inactivation by sunlight, because solar irradiation can vary considerably during a day due to clouds or other factors. This study will focus on the evaluation of the efficiency of the solar (natural $Fe^{2+}/h\nu$) and photo-Fenton (natural $Fe^{2+}/H_2O_2/h\nu$) disinfection of natural wells waters during the raining season in Sahelian region (Ouagadougou-Burkina Faso, West Africa). Comparative evaluation of the observed results with the one previously conducted during the summer season will be addressed.

5.2. MATERIALS AND METHODS

5.2.1. REAGENTS AND PHYSICO-CHEMICAL ANALYSIS

Universal meter WTW 340i equipped with a WTW SenTix 41-3 probe was used to measure the pH and temperature. Microbiology Chromocult ® (Merck KGaA), was used for bacterial plating (Ndounla *et al.*, 2013). Growth media was poured in pre-sterilized Petri Dish, 92x16mm (Sarstedt AG). Hydrogen peroxide, 30% (AnalaR Normapur, VWR) was used to prepare the Fenton reagent. Hydrogen peroxide (H_2O_2) concentration was followed during the experiments by Peroxide Merckoquant (Merk) test with a detection limit around 0.5 mg/L. Hydrochloric acid fuming (HCl), 37% (Fluka Analytical, SIGMA-ALDRICH®) was used for glass-reactor cleaning. The bicarbonate and carbonate ions concentration were determined by

titration. The HACH DR/2000 spectrophotometer methodologies were used to characterize some physico-chemical components of the water sample (Turbidity, iron, nitrite, nitrate, and ammoniac) following the guidelines of the Standard Methods for Examination of Water (HACH, 2001). The water turbidity was evaluated with the program 750 (wavelength 450 nm) and the detection limits were 0 to 450 NTU (Nephelometric turbidity units). The total Iron content was determined by the FerroVer Method (Powder Pillows), program 265 and detection limits 0 to 3.00 mg/L. The nitrate (NO_3^-) contents was determined with the High Range (HR) program 355 (NitraVer Method 5, Nitrate Reagent Powder Pillow, wavelength 500 nm) and the detection limits were 0 to 30.0 mg/L NO_3^--N. The HACH Species Conversion Factors (SCF) specific for the calculation of the nitrate in nitrogen (NO_3^- -N) is 4.427. The nitrite (NO_2^-) contents was determined with the Low Range (LR) program 371 (NitriVer 3 Powder Pillows Method, limit of detection: 0 to 0.300 mg/L NO_2^--N), the SCF of NO_2^--N is 3.284. The ammonia (NH_4^+) concentration was evaluated with the Nessler Method program 380. Its detection limit was from 0 to 2.50 mg/L NH_3-N with an SCF of 1.288. The results presented in this paper are the average for each components recorded upon the experiments

5.2.2. LOCALIZATION OF THE STUDY SITE

The experiments presented in this study were carried out from July to October 2011 (raining season) in Burkina Faso. The water samples were collected at two household wells from two district of Ouagadougou: well 1 (W1) at Tanghin, and well 2 (W2) at Nonsin. Ouagadougou is located at 12°21'26" of Latitude North and 1°32'7" of Longitude West. The experiments were conducted at the site of 2iE Foundation. This location is subject to receive approximately 1900-2200 kWh/m^2 of annual average solar irradiance. For the processes, water was collected from wells with a 20-liter plastic jerrican.

5.2.3. THE BACTERIAL STRAINS

As in our previous research carried out during the summer season (Ndounla *et al.*, 2013), the bacterial strains followed in this study are the wild fecal indicator enteric bacteria coliforms/*E. coli*, and *Salmonella* spp. naturally present in the sample water. Considering the selectivity of growth media Chromocult, the detection limit of enteric bacteria was 0 (zero) colony growths observed in the plate after the exposure. The incubation period was 18-24 h at 37°C.

5.2.4. THE EXPERIMENTS

The experiments were carried out at field scale under direct sunlight in a Compound Parabolic Collector (CPC). The CPC is a SOLARDETOX ACADUS-2003 batch photo-reactor device model delivered by Ecosystem SA, (Barcelona, Spain). The irradiance and cumulated total dose of the solar radiation was monitored during the experiments with an ACADUS 85 UV radiometer connected to the CPC. 25 L of surface water was disinfected during each treatment at constant flow (2 L/min). Considering the weather disturbance due to rain fall, the photo-disinfection was operated following the availability of the sunshine during 2 different time intervals of the day: (i) 8 am to 12 pm (8-12h), (ii) 12 pm to 4 pm (12-16h) for both processes (sole solar radiation and photo – Fenton). Experiments were repeated four times to ensure reproducibility. During the exposure, pre-sterilized glass flask of 100 mL, were used at regular time intervals (0, 10, 20, 30, 45, 60, 90, 120, 150, 180 and 240 min) to collect the treated water sample to be analyzed. 100 µL were taken with micropipette from the flask and poured in a Petri dish plate containing growth media (Chromocult agar). Plates were incubated for 18 -24 h at 37°C and the colonies counted with a colony counter (Stuart SC6 Colony Counter). To check the durability of photo-disinfection after the exposure, the entire flasks were further kept in the dark for post-irradiation controls. Regrowth experiments were realized on stored bottles after 24 h. Considering the real scale situation for treated water

intended for populations, the samples water were kept in the dark without removing their remaining 2 to 3 mg/L of H_2O_2. This remaining amount of H_2O_2 ensures a residual effect on the photo-Fenton treatment. The monitoring of this residual content has shown that it was no more detectable after 48 to 72 h. The concentration of some physico-chemical parameters of the water (HCO_3^-, CO_3^{2-}, NO_2^-, NO_3^-, NH_4^+, turbidity and total iron were evaluated before and after the treatment. The pH and temperature evolution during the treatments were successively recorded. The Wolfram Mathematica 8.0 and MS-Excel programs were used for data analysis and graphs fitting.

5.3. RESULTS

5.3.1. PHYSICO-CHEMICAL CHARACTERISTIC OF WATER

The experiments were conducted on two natural wells water of Ouagadougou (Burkina-Faso, West Africa) from July to October 2011 (raining season). The photo disinfection of water sample from both wells were previously conducted during the summer season and their results were published (Ndounla *et al.,* 2013). They had an initial temperature range of 30°C. Rain water infiltration significantly diluted the chemical composition of the wells water used in this study. In fact, in contrast to the ones obtained during the summer season, lower concentration were recorded in almost all the cases as presented in table 5.1. Both water sample were clear with a turbidity range between 1-4 NTU far below the critical limit for solar disinfection (30 NTU) (Meierhofer and Wegelin, 2002). The dilution of the water highly affects the total iron ($Fe^{2+,\ 3+}$), concentration and only 0.03-0.05±0.01 mg/L and 0.01-0.04±0.02 mg/L were recorded respectively for W1 and W2. The evaluation of the impact of this low iron concentration on the efficiency of the photo-Fenton disinfection will be useful on establishing the vulgarization perspective of this treatment for Sahelian region. Acidic pH ranges (4.6-5.7) were recorded in both wells water as it was the case during the summer season (dry season).

The difference noticed between bicarbonate concentrations (1.22-4.88±1 mg/L for W1 and 34.16-42.2±1 mg/L for W2), have significantly influenced the pH variation during the photo-disinfection. The pH increase during the first 30min in all the cases was followed by a decrease for both solar and photo-Fenton disinfection of waters from W1 in contrast to the plateau recorded after the increase in waters from W2 (Fig. 5.1). CO_2 degassing process has a significant impact on the alkalinity of the water recorded at the end of the photo-disinfection. The evolution in W2, put in evidence the impact of the buffering effect of HCO_3^- in the pH regulation during the photo-disinfection. In contrast, in W1 the pH decrease recorded after the initial increasing state is certainly due to the fact that the low HCO_3^- content of the water was not enough to ensure sufficient buffering effect and maintain it at high level as it is the case in W2. As suggested by Rincon and Pulgarin (2007b), the effect of the HCO_3^- concentration in chemical variation of the solution will certainly influence the inactivation rate of both photo-disinfection process.

Table 5.1: Physico-chemical parameters of the samples water

	Tanghin (W1)		Nonsin (W2)	
	Raining season	Summer season[1]	Raining season	Summer season[1]
pH	4.6-5.1±0.02	4.9-5.5	5.6-5.7±0.02	6.1-6.3
Initial temperature (°C)	30±1	29-30±1	30±1	29-30±1
Temperature (°C) after 2 h of treatment	40-44±1	> 45	40-44±1	> 45
Total Iron (mg/L)	0.03-0.05±0.02	0.05-0.09	0.01-0.04±0.01	0.07
Turbidity (NTU)	1-4±2	<10	1-4±1	<10
Bicarbonate (HCO_3^-) (mg/L)	1.22-4.88±1	-	34.16-42.7±1	-
Carbonate (CO_3^-) (mg/L)	0[2]	-	0[2]	-
Nitrite (NO_2^-) (mg/L)	0.02-0.06±0.01	-	0.02±0.01	-
Nitrate (NO_3^-) (mg/L)	82.1-95.5±0.2	-	89.7-96.1±0.2	-
Ammonia (NH_4^+) (mg/L)	0.9-1.1±0.1	-	0.13-0.15±0.01	-

[1](Ndounla *et al.*, 2013), [2]CO_3^- are generated from HCO_3^- in water, when its pH is greater or equal to 8.3. The water samples used in this study have an acidic pH, leading to no carbonates ions.

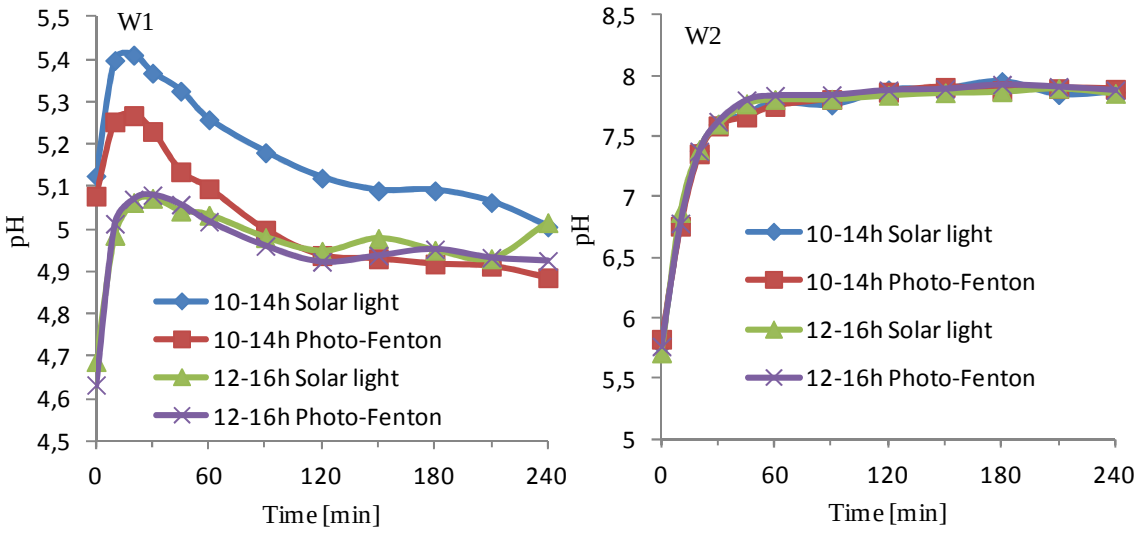


Figure 5.1: pH evolution during the photo-disinfection during raining season of wells 1 (Tanghin) and 2 (Nonsin) under solar light exposure and neutral photo-Fenton recorded at different day time's intervals. Bicarbonate (HCO_3^-) concentration: in W1 (1.22-4.88±1 mg/L); in W2 (34.16-42.7±1 mg/L).

5.3.2. DISINFECTION EXPERIMENTS IN THE CPC (FIELD SCALE)

At field scale, 25L of water were disinfected in the CPC solar reactor by solar (*hv*) or photo-Fenton ($H_2O_2/Fe^{2+}/hv$) disinfection. Considering the natural iron contents of the water (Table 5.1), the photo-Fenton disinfection was induced by adding 10 mg/L of H_2O_2 in the water sample prior to the exposure. The curves in Fig.5.2-W1-W2 and Fig.5.3-W1-W2, presents the inactivation kinetic of both enteric bacteria involved in this study under solar and photo-Fenton disinfection. On each figure, the empty symbols are for total coliforms/*E. coli* strains while the full symbols are for *Salmonella* spp. strains. The solar disinfection process is illustrated by the traces (○/●) while those of the photo-Fenton disinfection are traces (□/■). The intermittence between high solar illumination and cloudy sky has affected considerably the irradiance recorded during the treatments (Fig.5.2-W1'-W2' and Fig.5.3-W1'-W2'). The impact of irradiance ($W.m^{-2}$) on the cumulated dose, temperature rise and consequently the disinfecting processes are presented in the following sections.

5.3.2.1. EXPERIMENTS CONDUCTED FROM 10-14H

The irradiance recorded for sole solar radiation disinfection experiments carried out in sample water from W1 during the time interval's 10-14h were fluctuating between 25 and 30 $W.m^{-2}$. The temperature rise remains below 40°C during the major part of the exposure period. This situation was certainly due to the regular intermittence between a cloudy and sunny sky, which has reduced the IR ability limiting the thermal effect of the radiation. The cumulated total dose recorded at the end of this exposure period was 250 $Wh.m^{-2}$ (Fig.5.2-W1'). However, these conditions were not sufficient to ensure the total inactivation of the enteric bacteria contents of the treated water. Only the total coliforms/*E. coli* strains were totally disinfected at the end of the 4 hours exposure (Fig.5.2-W1, traces (○)). The *Salmonella* spp. strains concentration is remained up to 10^4 CFU/mL in the treated water (Fig.5.2-W1, traces (●)). The irradiance available for sole solar radiation disinfection of sample water from W2 during this time's interval was higher than the one available for W1. However, it fluctuated between 34 and 42 $W.m^{-2}$ during the first 2 hours of exposure and between 34 and 30 $W.m^{-2}$ during the last 2 hours. This higher irradiance associated to the increase of temperature to 45°C, which can enhance the thermal effect, has significantly affected the inactivation kinetic of the total coliforms/*E. coli* strain. Comparatively to the previous situation observed with W1, their total inactivation was achieved in approximately 120 min (Fig.5.2-W2, traces (○)). Nevertheless, as in the previous case, the fluctuation of the solar irradiance has negatively affected the *Salmonella* spp. inactivation as more than 10^4 CFU/mL of this strain is remained alive in the treated water at the end of the exposure (Fig.5.2-W2, traces (●)). The cumulated total dose of solar radiation recorded at the end of the exposure for both sole solar and photo-Fenton disinfection was approximately 270 $Wh.m^{-2}$ (Fig.5.2-W2').

As recorded during the sole solar disinfection, the intermittence of cloudy and sunny sky during the exposure period 10-14h has negatively affected the photo Fenton disinfection

process too. The high fluctuation of irradiance between 13 and 45 $W.m^{-2}$ has led to approximately 250 $Wh.m^{-2}$ of total cumulated dose at the end of the exposure. Similarly, temperature rises up to approximately 40°C as the one recorded under sole solar disinfection was noticed (Fig.5.2-W1'). The negative effects of these weather conditions on the photo-Fenton process are pointed out by the sole inactivation of the total coliforms/*E. coli* strain at the end of the 4 hours exposure (Fig.5.2-W1, traces (□)). The *Salmonella* spp. strain remained as in the previous cases more than 10^3 CFU/mL in the treated water at the end of the exposure (Fig.5.2-W2, traces (■)).

During the photo-Fenton disinfection of sample water from W2 at the same time period, the general situation was approximately the same as the previous one with W1. The cumulated total dose at the end of the experiments was 270 $Wh.m^{-2}$ (Fig.5.2-W2'). The temperature increase to approximately 43°C during the exposure associated to high fluctuation of the irradiance from 32 to 18 $W.m^{-2}$. In these conditions, total coliforms/*E. coli* strain sustained their total inactivation in approximately 60 min. The slight enhancement of the photo-Fenton process in W2 comparatively to the situation in W1 is difficult to explain as W2 has higher contents in HCO_3^- (Table 5.1) which in principle quenches the oxidative species (especially $^{\bullet}OH$) generated during the photo-Fenton process (Rincon and Pulgarin, 2007b). The overall inefficiency of the photo-Fenton process in both wells water could also be related to the diluted state of the sample water during this raining season (very low iron contents) as presented in table 5.1 comparatively to its composition during the summer season (Ndounla *et al.*, 2013).

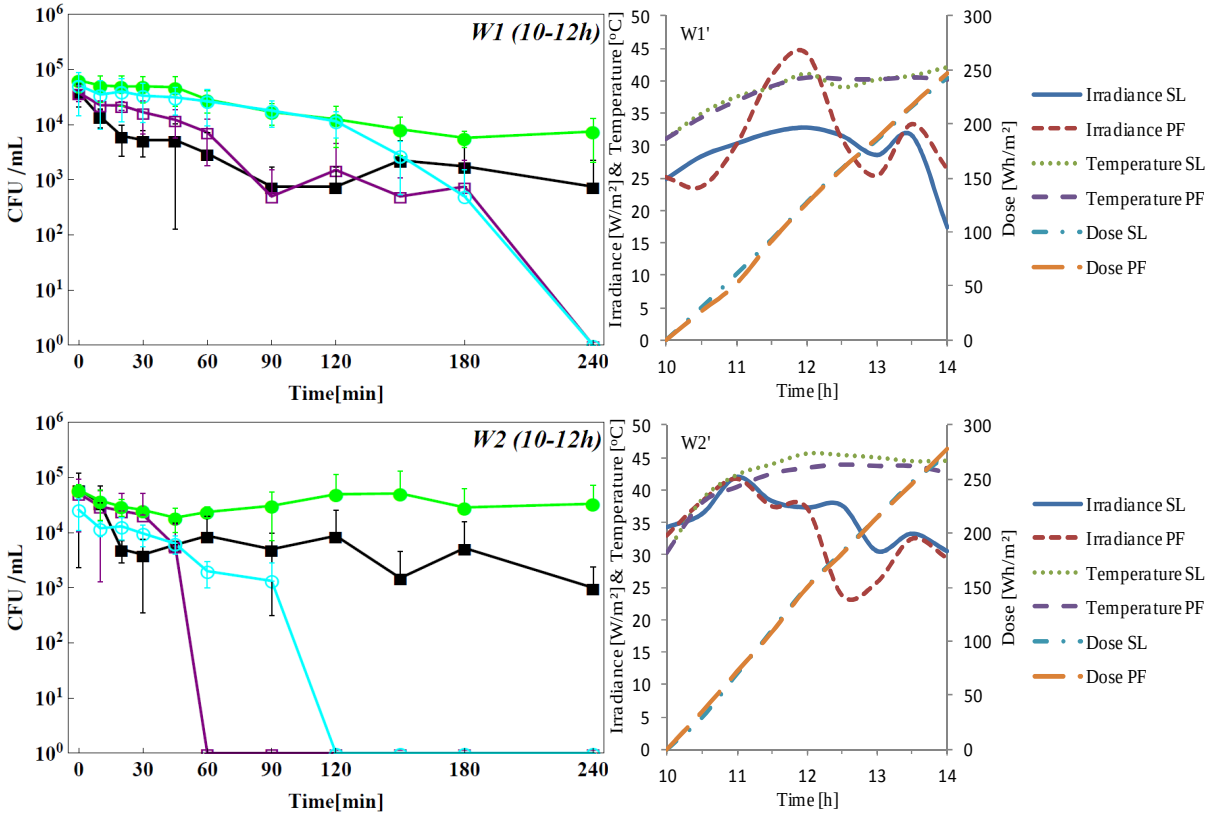


Figure 5.2: Inactivation of the wild enteric bacteria of wells 1 and 2, from 10-14h under direct solar radiation during the raining season. Addition of 10 mg/L of H_2O_2 to the water before the photo-Fenton experiments considering the natural iron (Fe) contents of wells both waters 0.03-0.05±0.02 mg/L for W1 and 0.01-0.04±0.01 mg/L for W2. Total coliforms/*E. coli* (□) and *Salmonella* spp. (■) under photo-Fenton (natural $Fe^{2+,3+}/H_2O_2/hv$), total coliforms/*E. coli* (○) and *Salmonella* spp. (●) under direct solar radiation (natural $Fe^{2+,3+}/hv$). (') evolution of water temperature [T°C], irradiance [$W.m^{-2}$] and cumulated total dose [$Wh.m^{-2}$] during the treatments; SL: direct solar radiation, PF: photo Fenton.

5.3.2.2. EXPERIMENTS CONDUCTED FROM 12-16H

During the uniquely solar and photo-Fenton disinfection of water from W1 conducted from 12-16h, the weather conditions were approximately the same for both cases. Temperatures were still below 40°C during the experiments (Fig.5.3-W1'). The weather conditions were particularly unstable during this time period, associated to the diluted composition of the water, they have negatively influenced both photo-disinfection processes. None of the enteric bacteria strains were totally inactivated at the end of the both processes (Fig.5.3-W1). The stable irradiance at approximately 40 $W.m^{-2}$ during the first hours of the solar disinfection carried out with W2 water from 12-16h, following by its decay to approximately 25 $W.m^{-2}$

after 2h30 has induced the total coliforms/*E. coli* strain inactivation (Fig.5.3-W2, traces (○)). After a slight increase in both photo-disinfection systems, the temperature of the water remained below 45°C. The cumulated total dose recorded at the end of the exposure was 220 and 270 $Wh.m^{-2}$, for sole solar disinfection and photo-Fenton treatment respectively. The bad weather conditions recorded here, did not favor the synergetic effect between the irradiance (related to UV and visible part of sunlight) and thermal action (T°C) in both photo-disinfection processes. The *Salmonella* spp. strains concentration remained up to 10^4 CFU/mL in the sample at the end of the exposure (Fig.5.3-W2, traces (●)).

At the beginning of the photo-Fenton disinfection during this time interval, the irradiance available was more than 45 $W.m^{-2}$, leading to the total inactivation of the total coliforms/*E. coli* strains in 60 min. The slight fluctuation of the irradiance between 37 and 30 $W.m^{-2}$ during the second hour of exposure followed by its drastic decrease to approximately 12 $W.m^{-2}$ at the end of the experiment did not favor the inactivation of the resistant strains of *Salmonella* spp. and approximately 10^4 CFU/mL remained in the water at the end of the exposure. The inefficiency of the photo-Fenton process in the treatment of wells water during the raining season, as presented on these results, is not certainly related only to the optical parameter of the solar radiation alone, but also to the dilution of photo-actives chemical component of the water. Iron and others metallic ions were highly diluted by the rain water during this season (Table 5.1), it can be suggested that the iron contents of the water was not enough to ensure the fast turnover of the Fenton reagent in the illuminated system.

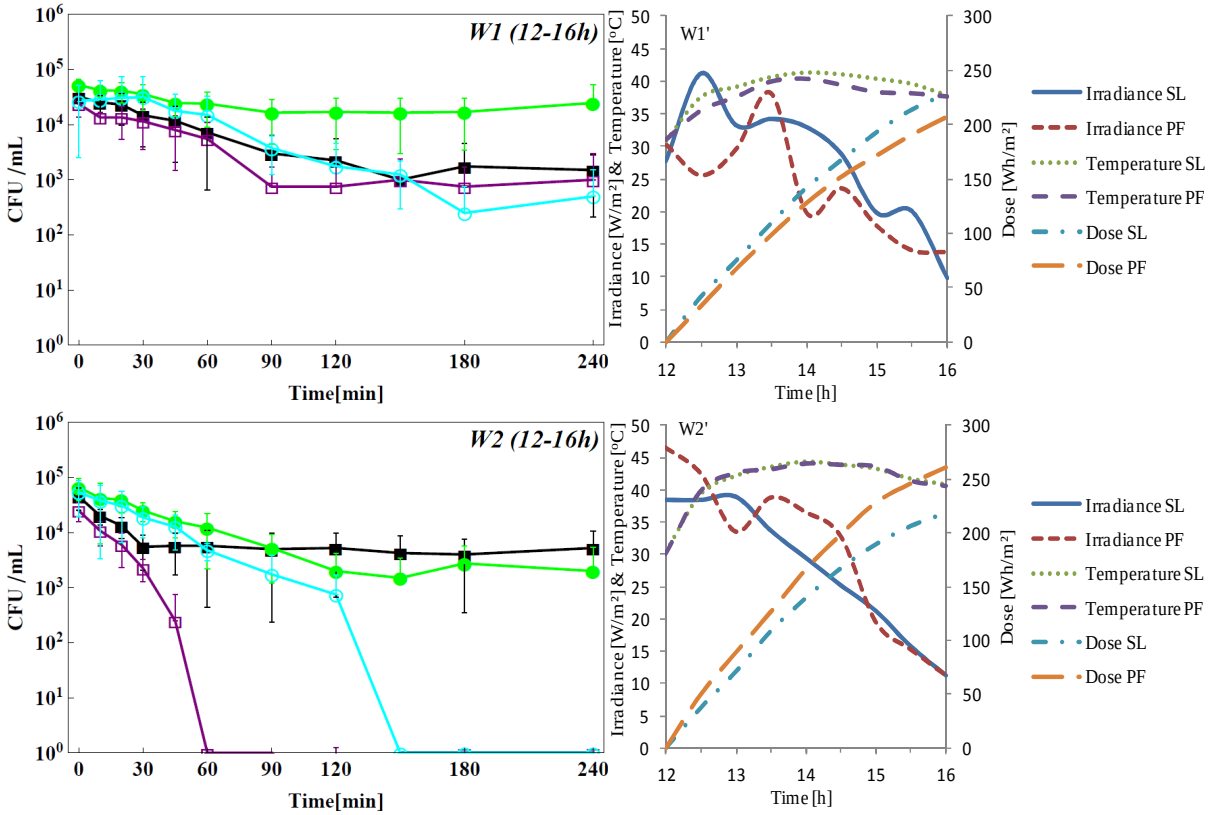


Figure 5.3: Inactivation of the wild enteric bacteria of wells 1 and 2, from 12-16h under direct solar radiation during the raining season. Addition of 10 mg/L of H_2O_2 to the water before the photo-Fenton experiments considering the natural iron (Fe) contents of both wells waters 0.03-0.05±0.02 mg/L for W1 and 0.01-0.04±0.01 mg/L for W2. Total coliforms/*E. coli* (□) and *Salmonella* spp. (■) under photo-Fenton (natural $Fe^{2+,3+}/H_2O_2/h\nu$), total coliforms/*E. coli* (○) and *Salmonella* spp. (●) under direct solar radiation (natural $Fe^{2+,3+}/h\nu$). (') evolution of water temperature [T°C], irradiance [$W.m^{-2}$] and cumulated total dose [$Wh.m^{-2}$] during the treatments; SL: direct solar radiation, PF: photo fenton.

5.3.3. POST-DISINFECTION EVENTS

The bacteriological analysis of the sample water from the flask kept in the dark for 24 hours after specific exposure time (table 5.2) brings out the positive effect of the continuous Fenton reaction in the dark after the photo-Fenton disinfection. Considering the duration of the water exposure to solar irradiation before their storage in the dark, the total inactivation of the entire enteric bacteria contents of the sample were recorded at different times following the photo-disinfection process involved. After 10 min of exposure only, none of the enteric bacteria strains were recorded totally inactivated during the storage for all the water samples in both

photo-disinfection processes. Reported by Berney *et al.* (2006) as resistant to photo-disinfection, the *Salmonella* spp. strains have confirmed this state after the exposure to sole solar radiation by recovering their viability capacity during the storage in all the cases. In contrast the total coliforms bacteria/*E. coli* strains after being shocked by the ROS under exposure to sole solar radiation for maximum 90 min in water from W2 and 150 min in W1 were lethally affected and loss their viability during the dark storage. However, considering the fact that the *Salmonella* spp. strains exposed to sole solar radiation did not show continuous and total inactivation during the storage, the water treated by this process during the raining season could not be considered as totally disinfected and cannot be recommended for consumption.

It is however, interesting to notice that the continuous Fenton process during the dark storage led to complete the photo-Fenton inactivation initiated during the exposure to intermittent solar radiation in almost all the stored sample. The total inactivation of both enteric bacteria during the storage was recorded after 20 min of exposure in most of the cases. However, more exposure time was required in water from W2 for the resistant strain of *Salmonella* spp. during the exposure period 10-14h, as a minimum of 45 min exposure was needed to get their total inactivation during the subsequent 24h of dark storage. The complete inactivation of both enteric bacteria in all the samples subjected to photo-Fenton disinfection during the 24 hours of dark storage bring to the assumption that the continuous Fenton reaction in the dark could efficiently help to fulfill the photo-Fenton disinfection of drinking water source during the raining season without the need of extra chemical addition.

Table 5.2: Enteric bacterial regrowth evaluation (total coliforms bacteria/*E. coli* and *Salmonella* spp.), after the photo-disinfection and neutral photo-Fenton treatments of natural well water with or without H_2O_2 (10 mg/l) under direct solar light.

Exposure time (min)	Tanghin (W1)								Nonsin (W2)							
	10-14h				12-16h				10-14h				12-16h			
	Solar Disinfection		Photo Fenton		Solar Disinfection		Photo Fenton		Solar Disinfection		Photo Fenton		Solar Disinfection		Photo Fenton	
	TC/*E.c.*	S.spp.	TC/*E.c.*	S.spp.	TC/*E.c.*	S.spp.	TC/*E.c.*	S.spp.	TC/*E.c.*	S.spp.	TC/*E.c.*	S.spp.	TC/*E.c.*	S.spp.	TC/*E.c.*	S.spp.
0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
20	+	+	-	-	+	+	-	-	-	+	-	+	+	+	-	-
30	+	+	-	-	+	+	-	-	-	+	-	+	+	+	-	-
45	+	+	-	-	+	+	-	-	-	+	-	-	+	+	-	-
60	+	+	-	-	+	+	-	-	-	+	-	-	+	+	-	-
90	+	+	-	-	+	+	-	-	-	+	-	-	-	+	-	-
120	+	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-
150	-	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-
180	-	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-
240	-	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-

−: no regrowth of bacteria after 24h, +: regrowth of bacteria after 24h, TC/*E.c.*: total coliforms/*E. coli*, *S.*spp.: *Salmonella* spp.

5.3.4. OXIDO -REDUCTION OF NITROGEN COMPOUNDS IN THE WATERS DURING THE TREATMENTS

The health risk related to high nitrite or nitrate in drinking water is mostly methaemoglobinaemia or in critical cases cancer (Weng *et al.*, 2011). The evaluation of the impact of photo-disinfection treatment on the nitrogen component of water destined to human consumption is then highly important before considering the vulgarization of the process to the users. The catalytic transformation of ammonium cation in raw water may lead to increased nitrite concentration in drinking-water (WHO, 2003). This could be linked to the ammonia photo-oxidation by $OH^{\bullet}$ which generates NO_2^- and NO_3^- (Eq. 5.6) (Brito *et al.*, 2010). However, it is also reported that, the photochemical reduction of NO_3^- in natural

waters leads to NO_2^-, OH^-, and $OH^\bullet$, while that of NO_2^- leads to NO, OH^- and $OH^\bullet$ (Eq 5.7-5.8) (Kotzias *et al.*, 1987; Fanning, 2000). The impact of this redox activities is illustrated here by the results of the comparative evaluation of the initial (before the photo-disinfection) and final (after the photo-disinfection) concentration of this nitrogen component in the sample water during the treatment of both wells water. It can be noticed as presented on Figure 5.4 that the redox activities interacting between them have led to several variations of their final concentration. The increase of nitrite concentrations in all the cases after both photo-disinfection processes is however remarkable. Fortunately, this increase remains far below the health-based guidelines recommended by the WHO for drinking water (3 mg/L) (WHO, 2011b). The major biological effect of nitrite in humans is its involvement in the oxidation of normal Hemoglobin (Hb) to Methemoglobin (metHb), which is then unable to transport oxygen to the tissues. High nitrate concentration, above 100 mg/l, is an important cause of metHb formation (WHO, 2011b). Nitrates in the soil are from various origins; it could be from humus degradation, fresh or composted natural organic matter. Their infiltration in wells' water can induce high concentrations (WHO, 2011b), as the one recorded in both wells' water used in this study which is greater than the restrictions of the World Health Organization (WHO) guidelines for drinking water (50 mg/l).

$$NH_4^+ \leftrightarrow NH_3 + OH^\bullet \rightarrow NH_2OH \rightarrow NOH \rightarrow NO \rightarrow NO_2^- \leftrightarrow NO_3^- \quad (5.6)$$

$$NO_3^- + H_2O + h\nu \rightarrow NO_2^- + OH^- + OH^\bullet \quad (5.7)$$

$$NO_2^- + H_2O + h\nu \rightarrow NO + OH^- + OH^\bullet \quad (5.8)$$

Very low amount of ammonia has been detected in both wells' waters used in this study. In accordance, no health-based guideline has been prescribed for ammonia in drinking water by the World Health Organization (WHO) because it classifies it as an esthetic quality

component without a direct importance for health in the concentrations regularly recorded in natural drinking-water (WHO, 2003).

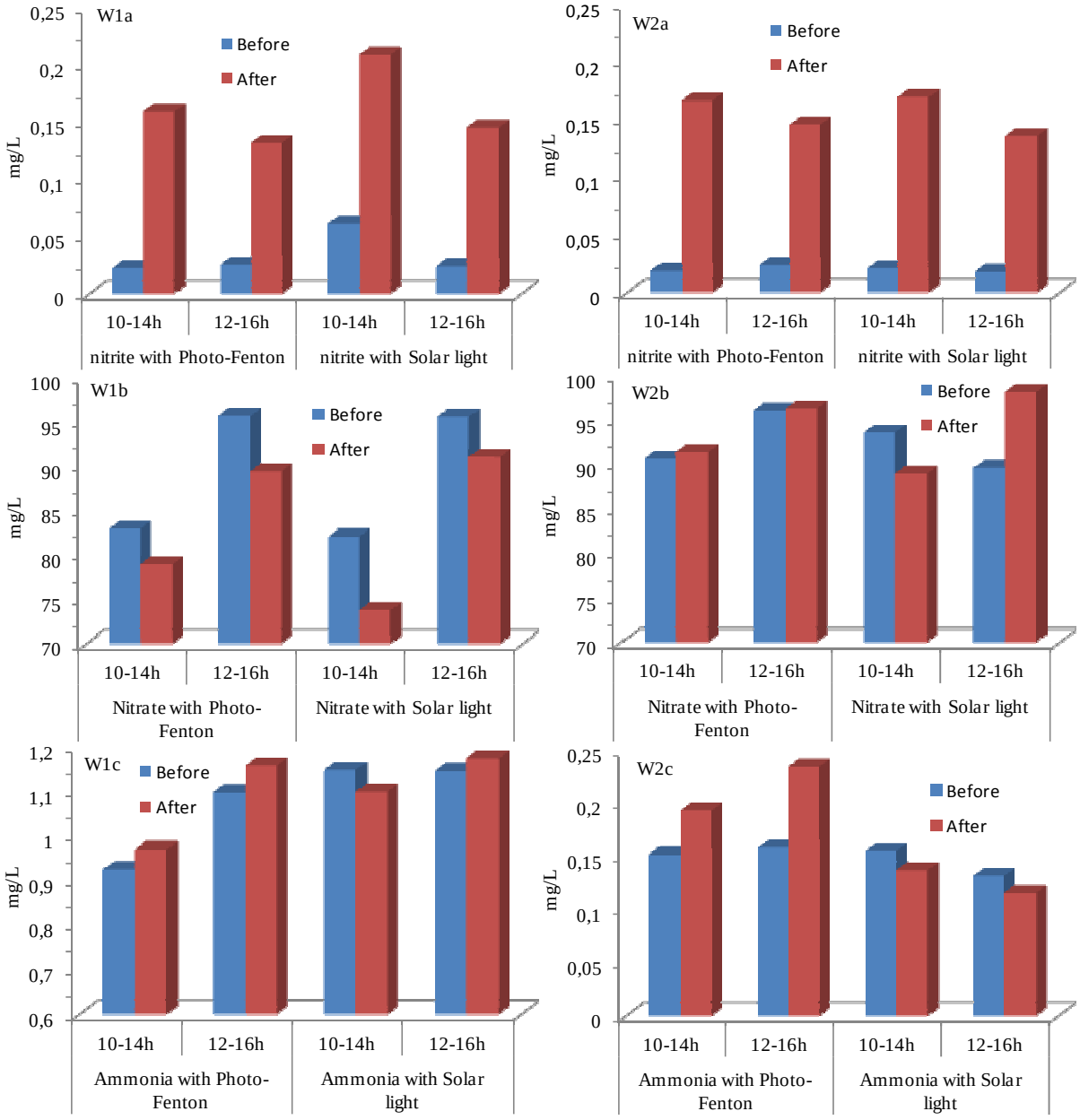


Figure 5.4: Evolution of nitrite (a), nitrate (b) and ammonia (c) concentration during the sole solar and photo-Fenton disinfection of two natural wells' water from Tanghin (W1) and Nonsin (W2) district of Ouagadougou. (Notice that the difference in the initial concentration of each chemical component in the results presented here are related to the fact that new water sample was collected and used for each new experiment).

5.3.5. PHOTO-DISINFECTION OF ENTERIC BACTERIA DURING THE SUMMER SEASON COMPARATIVELY TO RAINING SEASON

During summer season 2011, significant inactivation rate of both wild enteric bacteria were obtained during the photo-Fenton disinfection of water from W1 in the CPC. Total inactivation of both enteric bacteria (Salmonella spp. included) was also obtained during the solar disinfection in CPC of water from W1 at the same period (c.f. Chapter 3, Fig. 3.3). The deficiency noted in both photo-disinfection processes during the raining season 2011 presented in this study in comparison to the previous successful ones conducted during the summer season, highlights the parameters which influence in first order, the efficiency of these processes at field scale. During the summer season 2011, the total inactivation of the entire enteric bacterial content of the water sample (Salmonella spp. included) were achieved in 120 min under photo-Fenton disinfection initiated at 8 o'clock in the morning for 6 hours exposure. The high, intensive and stable irradiance available in this season, was leading to fast temperature rise and consequently a synergistic action of optical and thermal inactivation were noticed. This effect was enhanced when the photo-disinfection experiments were initiated at 10 or 12 o'clock. The total inactivation of the enteric bacteria contents of the water samples was achieved after approximately 90 and 45 min respectively for the experiments which were began at 10 and 12 o'clock (c.f. Chapter 3, Fig. 3.3). From the observed results and considering the impact of the worst weather conditions on efficiency of the photo-Fenton in this study, the availability of high, intense and stable irradiance acting as optical effect could be consider as the principal factors of influence of the process. Noticed that, this irradiance has significant impact on the temperature increase and consequently on the thermal effect. In last, the cumulated total dose of the solar radiation recorded during the effective disinfection time (EDT) of both enteric bacteria during the summer season was very low (c.f. Chapter 3, Fig. 3.3) comparatively to the one recorded for the total coliforms/E. coli only during the raining season in this study. These observations confirm that the dose did not have

a particular influence on the photo-disinfection as previously noticed (c.f. Chapter 3, Fig. 3.3). Therefore, this parameter cannot be considered in first order, for monitoring of the photo-Fenton disinfection.

5.3.6. EFFECT OF INTERMITTENCE ON INACTIVATION MECHANISMS

As optical and thermal inactivation mechanisms are involved simultaneously to induce lethal damage on bacteria under sole solar disinfection, the bacterial inactivation cannot be linearly proportional to the light intensity only. Considering the situation where several intermittence of cloudy and sunny time's are involved in the photo-disinfecting system as the one observed in this study, the capacity of the bacteria or other microorganism to escape the ROS attack can significantly affect the kinetic of the process. In presence of resistant strains such as *Salmonella* spp. the probability to have a negative result as the one obtained her is really high. The inactivation of the total coliforms/*E. coli* strains were recorded in some cases certainly due to the fact that it's the most weakened strain of the enteric bacteria involved in the solution and were then more sensible to the ROS available. Rincon and Pulgarin (2004a) have made the same assertion on the negative effect of the intermittence of several short exposure times on the efficiency of photo-disinfection. They have noticed that this situation favor the bacteria ability to repair light-induced oxidative stress.

5.3.7. PATHWAY OF THE ROS ATTACKS

When concentration of ROS increases to a level that exceeds the cell's defense capacity, this is called oxidative stress (Cabiscol *et al.*, 2000). When this increase is caused by the generation of ROS due to solar radiation and the simultaneous photo-inactivation of catalase and SOD as well as the internal photo-release of Fe^{2+}, this can be referred to photo-oxidative stress Reed *et al.* (2000). Both, a transient iron ($Fe^{2+\ or\ 3+}$) overload as well as increased internal H_2O_2 concentrations can be induced by solar radiation and produces strongly

favorable conditions for the production into the cell of highly reactive $^{\bullet}OH$ and other toxic oxygen species (Eqs. 5.1-5.4). Even though the internal generation of the $Fe^{2+ \text{ or } 3+}$ or H_2O_2 depends on irradiation, the resulting reaction of Fe^{2+} with H_2O_2 can also take place in the dark, once the reactive are available unless the photo-induced cellular dysfunctions are repaired. However, the synergistic effect of the intracellular presence of H_2O_2 and UVA and visible light which arises from the photo-inactivation of catalase and SOD (enzymes) and the toxic effect of the intracellular presence of H_2O_2 and iron has an highly lethal effect on cellular DNA (Eisenstark, 1998; Imlay, 2008). The potential toxicity of the reactive oxygen species (ROS) such as H_2O_2, to cells is directly linked to the intracellular concentration of iron, as a partner of the internal Fenton reaction.

The rate of the generation of $^{\bullet}OH$ and the cellular damage which is consequently caused in presence of ROS, such as H_2O_2 and $O_2^{\bullet-}$, depends on the availability of free or loosely bound iron available to undergo the Fenton reaction with H_2O_2, as well as the site of formation of the resulting radical. In effect, under solar radiation Fe^{2+} can be directly liberated when UVA light damages iron containing proteins such as enterobactin or ferritin (Hoerter *et al.*, 1996; Tyrrell *et al.*, 2000). Further, similarly to $O_2^{\bullet-}$, H_2O_2 can cause the oxidation of iron sulfur clusters ([4Fe-4S]) and the release of Fe^{3+}, which if reduced to Fe^{2+}, contributes to intracellular Fenton reactions (Imlay, 2003). A major portion of the toxicity of H_2O_2 in *E. coli* is attributed to DNA damage mediated by a Fenton reaction that generates $^{\bullet}OH$ and a whole series of other radical reactions, when H_2O_2 reacts with Fe^{2+} via the intracellular Fenton reaction Haber and Weiss; (Imlay and Linn, 1988). While H_2O_2 is a relatively stable oxidant, $^{\bullet}OH$ is an extremely powerful oxidant that reacts at nearly diffusion-limited rates near the site of its formation and is very effective for initiating lipid peroxidation chains. Therefore, iron as a partner for the Fenton reaction plays also an important role in the photo-oxidative stress induced cellular damage by catalyzing the propagation reaction (Gutteridge,

1982). The presence of an excessive intracellular Fe^{2+} concentrations can lead to the generation of $^{\bullet}OH$ via intracellular Fenton reactions when reacting with metabolic H_2O_2 (Imlay and Linn, 1988; Imlay, 2003; Imlay, 2008). Therefore, Fe^{2+} has highly bactericidal properties in some circumstances as it can be observed in well waters which contain little oxygen and therefore stable Fe^{2+} (Touati, 2000).

5.4. CONCLUSION

The weather variation, especially during the raining season, did not lead to sufficient conditions to ensure the normal solar disinfection. The efficiency of the photo-Fenton was also significantly reduced under these bad weather conditions. However, the opportunity to achieve the full disinfection of the enteric bacteria with the continuous Fenton process during the dark storage, gives the presumption to consider the possibility of exploiting the complementarities of the photo-Fenton and Fenton processes to ensure an efficient disinfection of the drinking water source in Sahelian region during the raining season.

The *Salmonella* spp. strains exposed to sole solar radiation did not show continuous and total inactivation during the storage as it was the case for total coliforms/*E. coli.* Therefore, water treated by this process during the raining season could not be considered as totally disinfected and cannot be recommended for consumption. The impact of the available irradiance on the efficiency of the photo-Fenton disinfection of natural water was highlighted during the exposure under high intermittent solar radiation. Considering it high impact on the photo-disinfecting process conducted during the summer season, it should be consider as the principal parameter of the process.

The negative effect of the very low iron contents of the entire sample water treated in this study; it is assumed that the Fenton reagent was not enough to ensure significantly the photo-Fenton reaction during the exposure. From this observation, we suggest for further evaluation

of the photo-Fenton disinfection of water during the raining season, to update the iron contents of the sample before the treatment at the optimum level recorded in such water during the summer season to ensure the availability of the Fenton reagent in the water. However, considering the fact that the continuous Fenton, reaction during the dark storage ensure with the residual H_2O_2 contents of the water the total inactivation of it entire enteric bacteria contents, the photo-Fenton treated water with very low natural iron content carried out during the raining season could be kept for complete disinfection during the storage (24h).

The possible impact of the HCO_3^- concentration of both wells' water on the evolution of the pH during the photo-disinfection was recorded, with a drastic decrease noticed after the initial fast increase in presence of low HCO_3^- concentration while a plateau was observed in presence of higher concentration in all the cases. However, further research on the different parameters of the photo-disinfection which could influence the pH evolution during the process should be conducted to confirm this assertion. The redox activities of the nitrogen components of the water during both photo-disinfection processes has led to increase concentration of nitrite in all the cases and variations were noticed in that of nitrate and ammonia. Fortunately, the nitrite increase remains far below the restriction of the WHO. However, it should be useful to investigate on the impact of this redox activities on the byproducts of the photo-disinfected water.

6. GENERAL CONCLUSIONS AND PERSPECTIVES

6.1. GENERAL CONCLUSIONS

Throughout this study, several trial experiments of the solar photo-disinfection were carried out. After the systematic assessment of the processes at laboratory scale on a solar simulator, specific experiments were conducted at field scale in a compound parabolic collector (CPC) solar reactor. It has been noticed at laboratory scale in the solar simulator, that the efficiency of the photo-Fenton was more significant in natural waters with low natural iron concentration compared to the system containing simulated water with added iron. It was also noticed that the enhancement of the photo-disinfection by photo-Fenton under solar simulator was efficient with very low H_2O_2 concentration (4 mg/L). However, at field scale in the CPC, the high oxygenation of the water due to its recirculation has lead to a fast degradation of H_2O_2. It was no more detectable after 2 to 3 hours of irradiation, when it was performed with an initial

concentration of 5 mg/L. To overcome this lack of H_2O_2 in the system, an optimal concentration of 10 mg / L was efficiently used in all the remaining experiments. Significant influence of the solar irradiance but not the dose was noticed during the process. Higher irradiance level leads to less effective disinfection time and dose required in order to achieve bacterial disinfection. The photo-disinfection treatment was efficiently enhanced by photo-Fenton at alkaline pH. Solar irradiance was recorded as the key factor for both photo-disinfection processes (bare solar and solar enhanced with photo-Fenton). However, the effectiveness of the processes decreased when carried out under a low solar irradiance (of less than $12W.m^{-2}$) and a temperature of less than 40°C.

Moreover, it was observed that the photo-Fenton process ensures the irreversible inactivation of the enteric bacteria. The resistance of the *Salmonella* spp. strain under uniquely solar disinfection treatment was also recorded. All the sample treated without addition of H_2O_2 (photo-Fenton) showed *Salmonella* spp. regrowth after 24h of dark storage. The neutral photo-Fenton treatment has significantly enhanced the inactivation rate of the disinfection in all cases, without the need to reach 50°C, as required for classical SODIS bottles process. None of the enteric bacteria strains (total coliform/*E. coli* and *Salmonella* spp.), showed regrowth one week after the photo-Fenton treatment. Therefore, to efficiently disinfect the water under uniquely solar radiation, it is important to expose it for more than 4 hours under high solar irradiance and impose a temperature increase of up to 45°C, to ensure the simultaneous irreversible inactivation of total coliform/*E. coli* and *Salmonella* spp.

The pH becomes more alkaline during both neutral photo-Fenton and solar treatment. This pH increase was not detrimental to the photo-Fenton and bare solar disinfection, and could be beneficial for the Sahelian groundwaters which are originally acidic. The water quality could simultaneously be improved through photo-Fenton disinfection. Moreover, an effect of the pH on the sensitivity of the enteric bacteria *Salmonella* spp. to photo-disinfection was observed

during this study. A better inactivation rate than for coliforms/*E. coli* at near-to-neutral pH (W2, pH: 6.3) (cf. Chapter 2) and at alkaline pH (8.6) (cf. Chapter 4) was shown. This side effect could be useful for the application of the process. The regrowth of *Salmonella* spp. strains during the storage after bare solar disinfection negatively affected it efficiency. With the record of their sensitivity at near-to neutral or alkaline pH, the possibility of improving the quality of the water during the photo-disinfection, by enhancing the pH, should be considered.

Significant variations of the nitrogen compounds state during both photo-disinfection processes (uniquely solar, photo-Fenton) were recorded. The oxido-reduction of nitrates and nitrites and the oxidation of ammonia during the treatment (increase of nitrates and nitrite and decrease of ammonia), has highlighted the possibility of photo-disinfection by products generation during the treatment.

6.2. PERSPECTIVES

Before the tested system with photo-Fenton can be applied for the treatment of drinking water at large scale, following researches should be conducted.

- The cost estimation of the photo-Fenton treatment at point-of use level with the optimal volume of water which could be treated, the cost effectiveness (price/liter), the optimal set-up (e.g. semi-enhanced and household-level, associated costs/liter) and operation and maintenance requirements.
- The evaluation of the optimal and/or minimum iron concentration to ensure an effective photo-Fenton disinfection during both seasons (summer/dry and raining)

- The evaluation of the water quality after the photo-Fenton treatment to ensure its acceptance by the users followed by the evaluation of the sustainability of the process in a target population.
- The characterization of nitrite and other nitrogen byproducts formation during the photo-disinfection and the fate of heavy metals (e.g. arsenic) during the process with the evaluation of their possible health impact.
- The effect of the NOM and the chemical composition of natural water especially HCO_3^- contents, on the photo-Fenton process, followed by the evaluation of their impact on the pH evolution during the photo-disinfecting treatment.
- Evaluate of the possible sources of H_2O_2 production at point-of-use level as well as the conception and production of hydrogen peroxide pellets in easily usable dose for photo-Fenton treatment which could help to facilitate its vulgarization.

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Submitted Articles

2013 :	**Ndounla J.,** Kenfack S., Wéthé J., and Pulgarin C. Relevant impact of irradiance (vs. dose) and evolution of pH and mineral nitrogen compounds during natural water disinfection by photo-Fenton in a

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ANNEXE

Article from Chapter 2.

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Inactivation by solar photo-Fenton in pet bottles of wild enteric bacteria of natural well water: Absence of re-growth after one week of subsequent storage

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ABSTRACT

Iron photo-assisted inactivation of wild enteric bacteria (total coliforms/*E. coli* and *Salmonella* spp.) was carried out in water from the Sahelian wells having different pH (W1: 4.9 and W2: 6.3) and a natural iron content of 0.07 mg/L. We evaluate the efficiency of the disinfection on different systems containing both or only one Fenton reagent (H_2O_2/Fe^{2+}): (i) $H_2O_2/Fe^{2+}/h\nu$, (ii) $Fe^{2+}/h\nu$, (iii) $H_2O_2/h\nu$, and (iv) only light irradiation ($h\nu$) at lab and field scale. Generally, 0.6 mg/L of Fe^{2+} and/or 8.5 mg/L of H_2O_2 were used in the Fenton reagent. The systems $H_2O_2/Fe^{2+}/h\nu$ and $H_2O_2/h\nu$ led to total inactivation of *Salmonella* and *E. coli*. The natural iron content (0.07 mg/L) was enough to drive an efficient photo-Fenton process leading to total bacterial inactivation. Our results show that: (i) the iron salt present in Sahelian water is enough to perform a photo-Fenton disinfection of drinking water when adding H_2O_2, (ii) addition of external iron salts at near neutral pH has no additional effect on the bacterial photo-Fenton inactivation process. After one week of storage, no enteric bacteria re-growth was observed in treated waters. Mechanistic suggestions are presented to explain the observed results.

1. Introduction

The use of solar energy for water treatment is a new and promising alternative to the disinfection of drinking water. Solar disinfection (SODIS) was first used in 1980 to produce re-hydration solutions for children suffering from diarrhea in Beirut [1]. The SODIS treatment involves the use of transparent plastic bottles (Polyethylene Terephthalate (PET) of 1–1.5 L) filled with water and exposing them to sunlight for at least 6 h depending on the meteorological conditions. The inactivation or death of pathogenic microorganisms is achieved by the synergistic effect of radiation and heat [2–4]. Underexposure and bacterial re-growth result in incomplete bacterial inactivation [5,6]. Improvement of the SODIS treatment includes the use of black backs bag [7] or the TiO_2 photocatalytic processes [8–10]. Recently, the photo-Fenton system ($Fe^{2+}/H_2O_2/h\nu$) has been shown to increase the solar photo-inactivation of *Escherichia coli* at near-neutral pH [11–13]. Hydrogen peroxide (H_2O_2) significantly increased the photocatalytic inactivation process in natural water containing natural iron [14]. The Fenton reagent generates the highly reactive •OH via the Haber–Weiss reaction [15]:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^- \qquad k = 53\text{–}76\,M^{-1}\,s^{-1} \quad (1)$$

During the Fenton process, Fe^{2+} can be regenerated from Fe^{3+} in the presence of H_2O_2:

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+}HO_2{}^{\bullet} + H^+ \qquad k = 1\text{–}2 \times 10^{-2}\,M^{-1}\,s^{-1} \quad (2)$$

But the Fenton process is limited by the Fe^{2+} regeneration of Fe^{3+} (Eq. (2)). This drawback is partially countered by photo-Fenton reactions. In fact, under illumination, ferric-hydro-complexes or ferric-organo-complexes in solution can absorb photons and generate ligand-to-metal charge-transfer (LMCT) reaction in which Fe^{2+} generating •OH (Eqs. (3)–(5)), [16,17].

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

$$[Fe(OH)]^{2+} + h\nu \rightarrow Fe^{2+} + {}^{\bullet}OH \quad (4)$$

$$[Fe(OOC\text{–}R)]^{2+} + h\nu \rightarrow Fe^{2+} + CO_2 + R^{\bullet} \quad (5)$$

The pH influences the efficiency of the photo-Fenton reagent, with an optimum level of pH 2.8–3 [18]. Considering this acidity criteria, the photo-Fenton was in the past preferably used for

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the treatment of wastewaters, mainly for the degradation of bio-recalcitrant organic pollutants via the generated ROS [16,19]. But the recent discovery of its efficiency at near-neutral pH in the presence of organic substances [13] gives the possibility of using it in the treatment of drinking water. The Sub-Sahelian African region receives about 2500–3000 h of solar radiation annually with more than 2300 K Wh m^{-2} an^{-1} irradiance in some parts [19]. This natural illumination with a UV-component represents a powerful intake to drive photo-Fenton reactions in the region.

Studies on the inactivation of model bacteria (*E. coli* K 12) by the photo-Fenton reagent in milliQ water [11,13] or milliQ water containing simulated NOM [13] and some research on the disinfection of wild bacteria in natural water have been reported [14,20]. This study is related to the inactivation of bacteria in natural waters. In this study, we address the intervention of the Fenton (Fe^{2+}/H_2O_2) reagent-driven bacterial inactivation and photo-Fenton ($Fe^{2+}/H_2O_2/hv$) processes on various wild bacteria in groundwater from wells in Burkina Faso, West Africa.

2. Experimental details

2.1. Reagents and materials

Hydrogen peroxide, 30% (AnalaR Normapur, VWR) and Iron (II) Sulphate Heptahydrate ($FeSO_4{\cdot}7H_2O$) were used to prepare the Fenton reagent. During the experiments, Catalase from bovine liver was used to inactivate the remaining H_2O_2, in the treated water before the bacterial culture. Hydrochloric acid fuming (HCl), 37% was used for glass-reactor cleaning. Catalase and HCl were from Fluka Analytical, SIGMA-ALDRICH®.

2.2. Water sampling

Water samples were collected at two household wells from two sectors in Ouagadougou: well 1 (W1) at Tanghin, sector 30, and well 2 (W2) at Nonsing, sector 21. Water from these wells is used for cooking, laundry, bathing and occasionally for drinking purposes during the recurrent water shortage period. Samples for lab experiments were collected from the water sources with two PET bottles (1.5 L) 1 h before the beginning of the process. One bottle was used to determine some physico-chemical parameters and the other for the disinfecting experiments. Water for field experiments was collected only from W2 with a 20-L plastic jerrican. In situ temperature was monitored.

2.3. Bacterial strain and growth media

The wild bacterial strain monitored in this study was the fecal indicator bacteria coliforms/*E. coli*, and *Salmonella* spp. Microbiology Chromocult® (Merck KGaA), was used for bacterial plating. The Chromocult is a selective and differential growth media. It selectively inhibits growth of the non-enteric bacteria. As experiments were conducted with natural water, considering their initial enteric bacteria contents, no dilution was realized before the bacterial plating. 100 μL of sample water were inoculated in the growth medium. Considering the selectivity of Chromocult, the detection limit of enteric bacteria was 0 (zero) colony growths observed in the plate. The differential nature of the medium permits the distinction of *Salmonella* spp (colorless), *E. coli* (purple and pink) and the blue and salmon colored colonies of other coliform bacteria. The Incubation period was 18–24 h at 37 °C, allowing the growth of all previously mentioned enteric bacteria. However, in order to more strongly represent the decrease of the total coliforms, all the *E. coli* observed and others coliforms counted were presented together in this study.

Table 1
Characteristics of some physico-chemical parameters of the water sample used in field experiments.

Parameters	Experiment 1 (J1)	Experiment 2 (J2)	Experiment 3 (J3)
Turbidity (NTU)	8 ± 0.2	8 ± 0.2	9 ± 0.1
pH	6.13 ± 0.05	6.26 ± 0.02	6.14 ± 0.05
Temperature (°C)	28.9 ± 0.2	31.1 ± 0.2	29.3 ± 0.2
Initial iron content (mg/L)	0.07 ± 0.02	0.07 ± 0.02	0.07 ± 0.02
Added iron (mg/L)	0.6	0.6	0.6
Added H_2O_2 (mg/L)	8.5	8.5	8.5

2.4. Analytical methods for physical parameters of water

Temperature (T °C), pH and hydrogen peroxide evolution were monitored following Sciacca et al. [14]. Turbidity was measured with a PCcompact® Turbidity/Trübung, (Aqualitic). The total iron content was measured with the spectrophotometer HACH 2000, by the FerroVer method 265. The detection limit of the spectrophotometer HACH 2000 was 0.02 ± 0.01 mg/L.

2.5. Helio-photo-inactivation experiments

2.5.1. Laboratory experiments using a solar simulator (suntest)

The photo-inactivation experiments at laboratory scale were conducted following the process used by Spuhler et al. [13] in a solar simulator (suntest). The disinfecting efficiency of four photo-assisted systems combined with both or only one Fenton reagent (H_2O_2/Fe^{2+}) in: (i) $H_2O_2/Fe^{2+}/hv$, (ii) Fe^{2+}/hv, (iii) H_2O_2/hv, and (iv) only light irradiation (*hv*) were evaluated in parallel with dark-control experiments: (5) $H_2O_2/Fe^{2+}/obs$, (6) Fe^{2+}/obs, (7) H_2O_2/obs, and (8) obscurity only (*obs*). In these systems, *hv:* refers to illumination and *obs:* to obscurity. Glassware for analytical analysis and reactors were acid soaked after each experimental series to prevent iron cross-contamination (10% HCl, 3 days and nights). After preliminary experiments the H_2O_2 dose in this study was set at 8.5 mg/L. The concentration of the added iron was Fe^{2+} (0.6 mg/L) as evaluated by Spuhler et al. [13]. The initial pH of the water was 4.9 and 6.3 respectively for W1 and W2. Each experiment was repeated at least three times to ensure the reproducibility of the results.

2.5.2. Field experiment using PET bottles

PET bottles were used for field experiments as they have a relative good absorbance (Fig. 1c). Only the water from W2 with a close-to-neutral pH (Table 1) was used at this level. Following the results of the lab experiments, the system Fe^{2+}/hv was not evaluated at field scale and the other systems and their blank were evaluated in triplicate (Fig. 1a and b) over three successive days at the end of April 2010. Nine new PET bottles (1.5 L) representing three replications of the systems ($H_2O_2/Fe^{2+}/hv$, H_2O_2/hv, and (*hv*)) were filled with: Fe^{2+} (0.6 mg/L) and H_2O_2 (8.5 mg/L) added before their exposure to solar radiation. Blank control bottles were covered with Al-foil and kept in the dark. During the experiments, solar radiation was recorded following the process used by Sciacca et al. [20]. To evaluate the bacterial inactivation rate, samples of the treated water were collected at regular time intervals in a sterile 15 mL Eppendorf for plating. Some parameters of these water samples are presented in Table 1. The PET bottles were used only once to ensure the same and relatively good light transmittance (Fig. 1c) in all the experiments.

2.6. Post-irradiation events

At the end of the irradiation phase of the laboratory experiments, remaining samples from systems 1 and 2 were introduced into sterile 50 mL Eppendorf flasks and kept in the dark at room

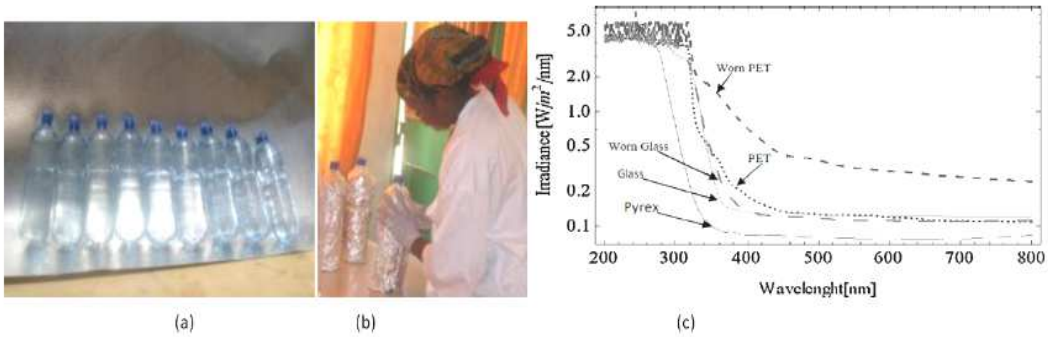

Fig. 1. (a) 9 PET bottles for the triplicate simultaneous exposure of the systems $H_2O_2/Fe^{2+}/hv$, H_2O_2/hv, and hv (irradiation only). (b) Blank of each system, (c) Absorbance of different reactor materials (Pyrex, Glass, PET).

temperature varying from 25 to 30 °C. For field experiments, each PET bottle from the exposed and blank tests was closed and kept in the dark. Re-growth experiments were realized on stored bottles after 24 h, 72 h and one week. Considering the real scale situation for treated water intended for populations, the water samples were kept in the dark without removing their remaining 2–3 mg/L of H_2O_2. This remaining amount of H_2O_2 ensures a residual effect on the treatment. However, it was no more detectable after 48–72 h.

2.7. Data analysis

The three-way ANOVA Package of the Wolfram Mathematica 8.0 program was used to evaluate the influence of the acidity, bacteria types and irradiation used on the disinfection rate.

3. Results

3.1. Physico-chemical characterization

Both water samples used in this study were collected at Ouagadougou during the months of March and April 2010 (dry season). They had an initial temperature of 29 °C and low turbidity < 10 NTU. The maximum acceptable turbidity recommended for SODIS disinfection is 30 NTU, [4,6]. The W1 has an initial pH of 4.9 and a total iron content of 0.06 mg/L; while in W2 the initial pH value was 6.3 and the total iron content 0.07 mg/L. The concentration of both wild enteric bacteria (total coliforms/*E. coli* and *Salmonella* spp.) was approximately 10^5 CFU/mL in both water sources.

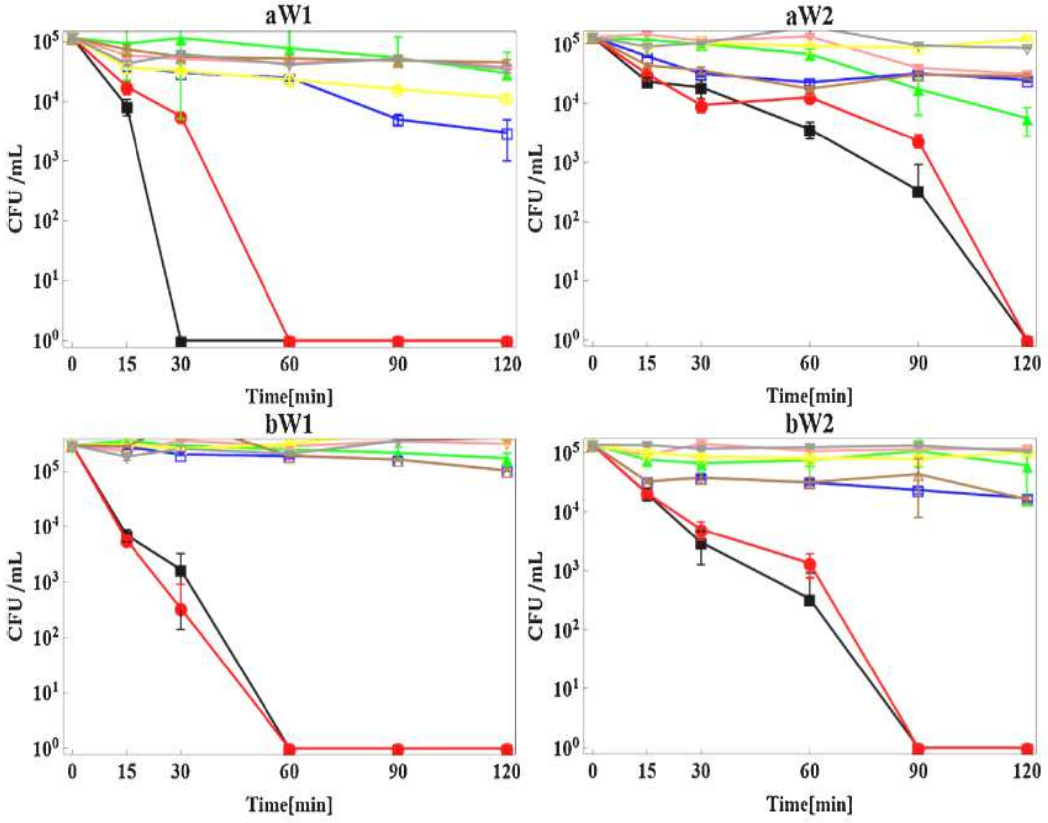

Fig. 2. Inactivation of the bacteria content in sample water from well 1(W1, pH: 4.9 ± 0.05) and well 2 (W2, pH: 6.3 ± 0.05) during photocatalytic treatment in the solar simulator Suntest. After the introduction of 90 ml of sample water to the 100 ml glass reactor, 8.5 mg/L of H_2O_2 and 0.6 mg/L of Fe^{2+} were added to the corresponding systems and their dark control. During the experiments, water temperature was <45 °C. (*a*) Total coliforms/*E. coli*, (*b*) *Salmonella* spp. (■) $Fe^{2+}/H_2O_2/hv$, (●) H_2O_2/hv, (▲) Fe^{2+}/hv, (▼)hv only, (□)$Fe^{2+}/H_2O_2/Obs$, (○)H_2O_2/Obs, (△) Fe^{2+}/Obs and (▽) *Obs* only. Graphics produced by the ListLogPlot function of Wolfram Mathematica software.

3.2. Experiment under simulated solar radiation

As it has been reported before [13,21], the decrease in CFU/mL of the samples' enteric bacteria broadly follows the first order kinetics, based on log-linear plots (Fig. 2). Inactivation rate constants observed during the inactivation process reported as k [min^{-1}] were calculated by linear regression (Table 2). Considering all the systems tested with W1 and W2, only the photo-Fenton ($Fe^{2+}/H_2O_2/hv$) and hydrogen peroxide systems (H_2O_2/hv) lead to a total inactivation of the fecal indicators bacteria. Irradiation only (hv) and Fe^{2+}/hv, as well as blank systems ($H_2O_2/Fe^{2+}/obs$, Fe^{2+}/obs, H_2O_2/obs, obs) show a lower decrease in their active enteric bacteria concentration within 2 h' irradiation (Fig. 2).

3.2.1. Inactivation in the systems $Fe^{2+}/H_2O_2/hv$ and H_2O_2/hv

Considering only the systems in which the total inactivation of the bacteria were achieved ($Fe^{2+}/H_2O_2/hv$ and H_2O_2/hv), it can be noticed that in W1 (pH = 4.9) the photo-Fenton system ($Fe^{2+}/H_2O_2/hv$) induced a stronger inactivation of the enteric bacteria, particularly in the total coliforms/*E. coli* group. This group has shown an inactivation rate constant of ($k = -0.3887 \pm 0.005\ min^{-1}$), (Table 2) and their total inactivation was achieved after 30 min. In the H_2O_2/hv system, we have to take into account the natural presence of 0.07 mg/L of iron in water. Natural iron confers to this system the photocatalytic properties, leading to a high inactivation rate constant ($k = -0.1953 \pm 0.003\ min^{-1}$). Total inactivation was achieved in about 60 min. In both systems, the *Salmonella* spp. concentration was totally inactivated after about 60 min.

The ranking of the two photocatalytic systems considering the inactivation rate constant (Table 2), gives the following for both wild enteric bacteria at pH 4.9 (W1):

$$k_c^{Fe^{2+}/H_2O_2/hv} > k_s^{H_2O_2/hv} > k_s^{Fe^{2+}/H_2O_2/hv} > k_c^{H_2O_2/hv}$$

At pH 6.3 (W2), the ranking was as follows:

$$k_s^{Fe^{2+}/H_2O_2/hv} > k_s^{H_2O_2/hv} > k_c^{Fe^{2+}/H_2O_2/hv} > k_c^{H_2O_2/hv}$$

C stands for total coliforms/*E. coli* and S for *Salmonella* spp.

The *Salmonella* spp. total inactivation was achieved before the total coliforms/*E. coli* group in both systems in approximately 90 and 120 min respectively.

3.2.2. Inactivation in the systems hv and in all the blank systems ($H_2O_2/Fe^{2+}/obs$, Fe^{2+}/obs, H_2O_2/obs, obs)

For water from W1 (pH: 4.9), the irradiation (hv) alone and all the blank systems, gives just a slight inactivation of the total coliforms/*E. coli* after the 120 min of exposure. The *Salmonella* spp. content of the sample show an increase instead of a decrease at the end of the period of irradiation (120 min), with an increasing rate constant of ($k = 0.0013 \pm 0.005\ min^{-1}$). Its concentration increased also in some blank tests carried out in the dark (Table 2). A slight decrease was observed both in the Fenton system $Fe^{2+}/H_2O_2/obs$ and the system Fe^{2+}/obs with the same inactivation rate constant ($k = -0.0080 \pm 0.006\ min^{-1}$).

The inactivation of the total coliforms/*E. coli* under hv alone and in the W2 (pH: 6.3) blanks show a slight decrease as in W1. The *Salmonella* spp. content in the sample showed a slight increase in the system hv ($k = 0.0001 \pm 0.005\ min^{-1}$). All the blank systems give rise to a slight decrease.

3.2.3. Inactivation in the systems Fe^{2+}/hv

The system Fe^{2+}/hv did not lead to a total inactivation of enteric bacteria in both waters (W1 and W2). But a slight inactivation was observed, as presented in Fig. 2 and by the inactivation rate constants (Table 2).

Table 2
Inactivation rate constants k [min^{-1}] of each enteric bacteria group observed during the inactivation process, calculated by linear regression for the different photo-catalytic treatments and their corresponding blank conducted in the dark.

Water origin/ pH	Enteric bacteria	Treatments/ k [min^{-1}]							
		$Fe^{2+}/H_2O_2/hv$	H_2O_2/hv	Fe^{2+}/hv	hv	$Fe^{2+}/H_2O_2/Obs$	H_2O_2/Obs	Fe^{2+}/Obs	Obs
Wells 1 pH: 4.9	Total coliforms/*E. coli*	−0.3887 ± 0.005	−0.1953 ± 0.003	−0.0124 ± 0.005	−0.0055 ± 0.008	−0.0284 ± 0.011	−0.0161 ± 0.005	−0.0062 ± 0.002	−0.0063 ± 0.004
	Salmonella spp.	−0.2085 ± 0.002	−0.2110 ± 0.005	−0.0044 ± 0.012	0.0013 ± 0.005	−0.0080 ± 0.006	0.0041 ± 0.006	−0.0080 ± 0.006	0.0045 ± 0.003
Wells 2 pH: 6.3	Total coliforms/*E. coli*	−0.1001 ± 0.011	−0.0798 ± 0.007	−0.0260 ± 0.011	−0.0133 ± 0.004	−0.0105 ± 0.015	−0.0002 ± 0.003	−0.0103 ± 0.007	−0.0024 ± 0.003
	Salmonella spp.	−0.1380 ± 0.004	−0.1189 ± 0.006	−0.0025 ± 0.003	0.0001 ± 0.005	−0.0125 ± 0.002	−0.0016 ± 0.002	−0.0125 ± 0.002	−0.0013 ± 0.005

Table 3
Three-way ANOVA analysis results.

Parameters	DF	SS	MS	F	P
pH	1	21,930.8	21,930.8	48.66	0.001%
Bacteria species	1	1201.25	1201.25	2.65	11%
Treatments	1	826.875	826.88	1.83	18%
pH/bacteria species	1	396.75	396.75	0.88	35%
pH/treatments	1	6.13	6.13	0.01	91%
Bacteria species/treatments	1	585.21	585.21	1.30	26%
Error	73	32,901.8	450.71	–	–
Total	79	57,848.8	–	–	–

DF: degree of freedom; SS: sum of square; MS: means square; F: Fisher factor (influence factor); P: probability.

The data shown in Fig. 2 and Table 2, for a single day experiment, present experiments carried out in triplicate. Experiments were repeated on three different days and a similar inactivation rate was observed.

3.2.4. Influence of pH, bacteria species and inactivation system on the disinfection

A significant difference between the inactivation rate of the total enteric bacteria concentration of both wells (W1: pH 4.9 and W2: pH 6.3) was observed when applying $Fe^{2+}/H_2O_2/hv$ and H_2O_2/hv. The analysis of these processes by the three-way ANOVA program of Mathematica 8.0, allowed us to evaluate the influence of the acidity and other parameters, as presented in Table 3. With a Fisher ratio or influence factor (F) of about 48.66, it is possible to state that the pH has a strong impact on the photo-catalytic disinfection process [16]. The probability (P = 0.001%) gives rise to the assumption that the error may be due to noise. The impact of bacteria (total coliforms/*E. coli* or *Salmonella* spp.) or the treatment ($Fe^{2+}/H_2O_2/hv$ or H_2O_2/hv) on the disinfection process was less significant. The probability that the difference in the inactivation rate related to these two parameters could be due to experimental error was about 11% and 18% respectively for the bacteria species and treatment used. The cross-interactions between the three parameters did not significantly affect the data obtained.

3.2.5. Post-irradiation effect

To be sure that the inactivated bacteria was not just partly damaged, rather than killed, when using the photo-Fenton ($Fe^{2+}/H_2O_2/hv$) or hydrogen peroxide (H_2O_2/hv) systems under light irradiation, after completion of the runs the samples were transferred into sterile flasks and kept at 25–30 °C in the dark (*obs*). Further spread plate counts were performed on the samples after 24 h, 72 h and up to one week. No re-growth of coliforms/*E. coli* or *Salmonella* spp. was observed, and this suggested irreversible inactivation.

3.3. Field experiments

Field experiments indicated that the inactivation rate of both bacteria types applying $Fe^{2+}/H_2O_2/hv$ and H_2O_2/hv was not significantly affected by the difference observed in the solar radiation and temperature each day. Variation in the inactivation rates were observed, however, when light alone was applied. As presented in all the graphs in Fig. 3, any variation of the solar radiation influences the inactivation of the bacteria. Some of the coliforms/*E. coli* inactivation curves have a shoulder. However, the decrease in CFU mL^{-1} of most of the enteric bacteria curves follows first-order kinetics, as in the lab experiments, based on log-linear plots.

3.3.1. Inactivation under direct solar radiation only (hv)

3.3.1.1. Inactivation total coliforms/E. coli. The temperature of 49 °C and UV irradiation of more than 30 W/m^{-2} during the first exposure gave rise to the total inactivation of coliforms/*E.coli* within 90 min. On the second day, irradiation was reduced and decreased from 26 W/m^{-2} to 20 W/m^{-2} during the first exposure. Consequently, inactivation was achieved after 120 min only. No re-growth was observed after 24 h of storage in the dark over these two days. On the third day, the reduction in temperature to less than 45 °C and high fluctuation in the solar radiation (between 23 and 14 W/m^{-2}) did not permit a total inactivation of coliforms/*E.coli* in this system (*hv*). Inactivation of the blank (*obs*) kept in the dark with a water temperature of around 38 °C was not significant during the time of experimentation (2 h). But after the 24 h dark-storage period, inactivation of about 97% was observed on the first and third day, and total inactivation on the second.

3.3.1.2. Inactivation of Salmonella spp.. The favorable conditions during the first day of experimentation enhanced considerably the inactivation of *Salmonella* spp.: about 96% of the population was inactivated during the 2 h of exposure. On the second day, the relatively low irradiation gave rise to a total inactivation at the end of the illuminated process, but a recovery of the viability during the 24 h dark-storage period was observed (Fig. 3b2). The atmospheric conditions on the third day were not favorable and only 44% was inactivated at the end of the exposure. Blank tests in the system *obs*, did not give a significant inactivation during the experiments and after the 24 h of dark storage, their concentration remained constant.

3.3.2. Inactivation under enhanced systems $Fe^{2+}/H_2O_2/hv$ and H_2O_2/hv

$Fe^{2+}/H_2O_2/hv$ and H_2O_2/hv showed a significant increase in the inactivation rate of bacteria compared to the non-enhanced systems (*hv*) (Fig. 3). Moreover, the variation of the solar radiation over the three days did not significantly influence the inactivation kinetics. For both bacteria species, H_2O_2/hv showed a higher inactivation rate than that of the systems with added iron ($Fe^{2+}/H_2O_2/hv$). The added iron has precipitated in the system at pH > 6. An exception was observed in the case of total coliforms/*E. coli* on the third day (Fig. 3aJ3), because they were totally inactivated in both systems. This could be related to the variability of the daily solar radiation. Re-growth experiments for both systems during 24 h did not show any bacterial recovery.

For total coliforms/*E. coli*, the control systems ($Fe^{2+}/H_2O_2/obs$ and H_2O_2/obs) did not show significant inactivation during the experiments. However, the inactivation occurred after the 24 h of dark storage for the second and third day. It's occurred only in the system H_2O_2/obs the first day. As in the previous cases, the control systems did not lead to a significant inactivation of *Salmonella* spp. during the experiment. But after the dark storage period (24 h), total inactivation was observed in both systems on the second and third day. None of the systems led to total inactivation on the first day.

4. Discussions

4.1. Irradiation characteristics

The efficiency of the solar photocatalytic disinfection of water can be influenced by the sun's intensity, light absorption, initial bacterial concentration, water temperature and turbidity [12–14,22]. In the lab experiments, the photo inactivation was carried out under continuous irradiation at constant intensity, with a radiation intensity of 560 W m^{-2}. This corresponds to 300–400 nm UVA or approximately 32 W m^{-2} UVA, representing the average UVA radiation of Ouagadougou in summer [19]. During the lab experiments,

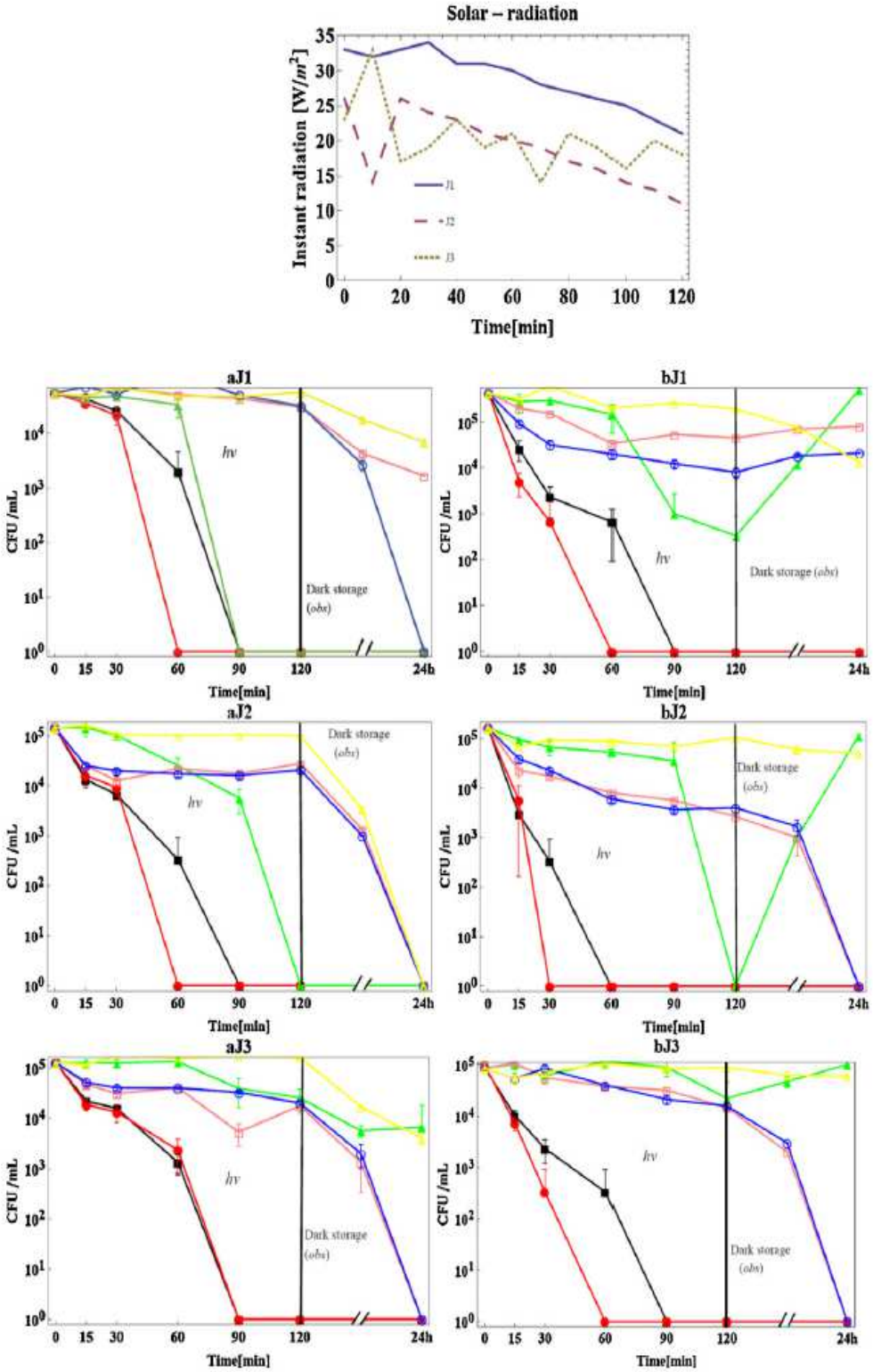

Fig. 3. Inactivation of the bacteria contained in water sample from wells 2 (W2, pH: 6.3) during the field experiment under direct solar radiation. After the introduction of 1.5 L of water in to the 1.5 L PET reactor, 8.5 mg/L of H_2O_2 and 0.6 mg/L of Fe^{2+} were added to the corresponding systems and their dark control. (*a*) Total coliforms/*E. coli*, (*b*) *Salmonella* spp., (J1) 28/04/2010, (J2) 29/04/2010, (J3) 30/04/2010, (■) $Fe^{2+}/H_2O_2/hv$, (●) H_2O_2/hv, (▲) *hv* only, (□) $Fe^{2+}/H_2O_2/Obs$, (○) H_2O_2/Obs, (△) *Obs* only. Graphs produced by the ListLogPlot function of Wolfram Mathematica software.

the water temperature inside the batch reactor remained inferior to 45 °C and thermal inactivation can be excluded [2,3]. Direct DNA damage by UVB can also be excluded as the used solar simulator emits negligible amounts of photons at wavelengths shorter than 300 nm [8] and the reactor material screened UVB (280–320 nm, Fig. 1c). It has to be noted that the water matrix used in this study is natural water, contrary to the laboratory milliQ-water which contains NOM and exogenous photosensitizers.

4.2. Experiments under solar simulator

4.2.1. Iron system (Fe^{2+}/hv)

In solutions at low pH (2–3), the irradiation with UV of various hydroxylated Fe^{3+} species produces Fe^{2+} and the hydroxyl radical OH• (Eqs. (3) and (4) [17,18]. The generated OH• radicals are highly oxidant, as explained above. But in natural water (pH: 4.9 and 6.3 respectively), the photoactive ferric hydrolyzed molecules [$Fe(OH)^{2+}$] are not soluble, as the predominant iron component of this pH is the iron-complex which under irradiation generates Fe^{2+}, with an organic radical instead of OH• (Eq. (5)) [16]. During the photocatalytic inactivation process in the Fe^{2+}/*hv* system, the bactericidal effect of Fe^{2+} arises from its ability to diffuse into the cells, leading to the generation of OH• via intracellular Fenton reactions when reacting with metabolic H_2O_2 [23,24]. Spuhler et al. [13], observed a lethal action of the system Fe^{2+}/*hv* during the inactivation of *E. coli* K12, in MilliQ Water (*hv* > 290 nm, pH: 5–5.5) resulting in a total inactivation after 120 min. However, in the present study neither the wild total coliforms/*E. coli* nor the *Salmonella* spp. were totally inactivated after the same exposure period in well water (W1, pH 4.9; W2: pH 6.3). The difference in this and Spuhler et al. [13] results could be explained by the nature of the water and bacteria species and by the following pathway: (i) The inactivation process through the ROS generated after the excitation of the exogenous and endogenous photosensitizers was not sufficient to ensure the total inactivation of the wild enteric bacteria involved; (ii) after the active ROS production (20–30 min), injured bacteria have developed self-repair mechanisms [11] and became more resistant to the light irradiation, and multiplied (iii) the wild bacteria are more resistant than the manufactured *E. coli* strain regularly used in lab experiments; (iv) the natural water matrix used here contains NOM and other minerals substances. These bacteria cannot create an osmotic stress as in MilliQ water which could weaken the bacteria and support the introduction of Fe^{2+} into the bacteria leading to intra-cellular Fenton ROS inactivation.

4.2.2. Systems Fe^{2+}/H_2O_2/hv and H_2O_2/hv

Natural water in the Sahelian region contains large quantities of iron as it flows on ferruginous substrates [14,20,25]. It is introduced into the atmosphere by wind and is found in aerosols, fog, rain drops, ground water and lakes [18]. A total initial iron concentration of about 0.06 mg/L and 0.07 mg/L was detected in W1 and W2 respectively. The high inactivation rate observed in the system H_2O_2/*hv* for both wells as in the systems Fe^{2+}/H_2O_2/*hv* in contrast to that of the systems Fe^{2+}/*hv* or *hv* only can allow us to assume that the photo-Fenton also takes place in the system H_2O_2/*hv* by using the initial iron contained as the catalyst. From Fig. 2 and Table 2, the difference in the iron content for the systems Fe^{2+}/H_2O_2/*hv* (natural iron + added iron) and H_2O_2/*hv* (natural iron only) did not significantly influence the inactivation kinetic of both bacteria species in both wells. It can be underlined that it is only in the case of total coliforms/*E. coli* in water from W1 that the difference between the inactivation rates was significant for different photo-catalytic systems. In the remaining systems (*Salmonella* spp. of W1 and total coliforms/*E. coli* and *Salmonella* spp. of W2), the total inactivation was achieved approximately within similar times for both photocatalytic systems and no significant differences were observed in their inactivation rate constants (*k*). Considering the initial iron content of water from the Sahelian region, it seems possible to achieve disinfection of water by photo-Fenton process without adding extra iron. This would be a great contribution, not only in reducing the chemical inputs required for the application of the photo-Fenton system for the treatment of drinking water, but also in reducing treatment costs.

4.2.3. Illumination alone (hv)

The total inactivation of total coliforms/*E. coli* or *Salmonella* spp. was not observed in the reactor under the effect of light irradiation alone. This could be related to the fact that the exposure time was just 2 h, and not 5 or 6 h as recommended for the SODIS process [6]. As the present study was focused on the reduction of the solar disinfection exposure time by the photo-Fenton, the 2 h exposure were sufficient to obtain a significant inactivation rate using Fe^{2+}/H_2O_2/*hv* and H_2O_2/*hv*.

4.2.4. pH

Great differences and contradictions were observed in the W2 (pH 6.3) in both systems where total inactivation was achieved, as the *salmonella* spp. strain was the first to be totally inactivated in both systems in about 90 min, while that of total coliforms/*E. coli* took about 120 min (Fig. 2). This situation is in contrast with the assumption that *Salmonella* spp. is more resistant to photocatalytic inactivation than *E. coli* [14,26]. Indeed, even in the W1 (pH: 4.9), in the system H_2O_2/*hv*, both enteric bacteria species were inactivated approximately at the same time and the inactivation rates constant of *Salmonella* spp. were slightly greater than those of total coliforms/*E. coli* ($-0.2110 \pm 0.005 > -0.1953 \pm 0.003$) (Table 2). It is only in the system Fe^{2+}/H_2O_2/*hv* that the *E. coli* was rapidly inactivated before the *Salmonella* spp.

4.2.5. Control experiments: (H_2O_2/Fe^{2+}/obs, Fe^{2+}/obs, H_2O_2/obs, obs)

None of the control systems (blank) led to total inactivation of the wild enteric bacterial concentration, even though a slight inactivation was observed in some cases for both wells. These results correspond with previous studies [11,13]. However, it was noticed that total coliforms/*E. coli* inactivation was more pronounced in W1 (pH: 4.9) than in W2 (pH: 6.3) (Table 2), but that situation was the reverse for the *Salmonella* spp. inactivation, whose population even increased in some systems (H_2O_2/*obs* and *obs*).

4.3. Experiments under direct solar radiation

The decrease of the enteric bacterial amount and even its total inactivation in the control systems (Fe^{2+}/H_2O_2/*obs* and H_2O_2/*obs*), after the 24 h of dark storage could be due to the scavenging action of H_2O_2 as it is also a powerful ROS [13,22]. It should be noticed that at high pH (6.3) in the dark, the iron precipitation makes them unavailable to initiate the simple Fenton reaction (Eq. (1)) [16]. The *hv* and *obs* systems are expected not to produce ROS during the 24 h dark storage, thus explaining why no more inactivation was observed in the remaining total coliforms/*E. coli* and for *Salmonella* spp. The increased concentration after the storage, could be due to the fact that during the dark storage without ROS production to injure them, the bacteria recover their ability to grow and replicate [11]. For total coliforms/*E. coli*, no re-growth was observed after total inactivation. The re-growth of *Salmonella* spp. was due to resistance to solar disinfection (not enhanced), as recently reported [26].

For the enhanced systems, H_2O_2/*hv* shows a better inactivation kinetic than that of the systems containing added iron (Fe^{2+}/H_2O_2/*hv*). It can be assumed that with the low Fe-concentration in natural water (0.07 mg/L), this amount was enough to start, in the presence of H_2O_2/*hv*, the photo-Fenton process and generate OH• inactivating of the enteric bacteria. The higher inactivation kinetic of the systems H_2O_2/*hv* compared to Fe^{2+}/H_2O_2/*hv* may suggest that a high iron concentration could have a negative effect on the photo-Fenton treatment of natural water close to neutral pH. This negative effect could be due to: (i) iron precipitation resulting in the lack of soluble iron in the medium to maintain the photocatalytic cycle, (ii) the reduction of

light transmittance due the increased turbidity of water and its coloration due to high iron contents, (iii) excess iron concentration in the solution increasing the OH• scavenging potential (Eq. (6)) and concomitantly reduce the efficiency of the process [17].

$$Fe^{2+} + OH^{\bullet} \rightarrow Fe^{3+} + OH^{-} \quad (6)$$

On the third day, the *Salmonella* spp. showed approximately the same inactivation kinetic as that of the first day under the best atmospheric conditions. Inactivation on the second day, under the same temperature but lower irradiation (26–20 W m^{-2}) was higher than on the first and third day. These observations are related to the results for total coliforms/*E. coli*, which show also approximately the same inactivation kinetic on the first and second day. It could be assumed that up to a certain level of irradiation and temperature, the influence of the two parameters (temperature and irradiation intensity) on the photo-Fenton inactivation process is no longer significant. The same observation was made previously by Ubomba-Jaswa et al. [27] during the investigations into the effect of the UVA dose on the inactivation of *E. coli* K12. However, a slight reduction of the inactivation kinetic of total coliforms/*E. coli* on the third day in the systems $H_2O_2/h\nu$ [11] is indicative that intermittent irradiation associated with low irradiation has negative influence on the bacterial inactivation rate.

4.4. Inactivation pathways

4.4.1. Inactivation in the illuminated system

In all the illuminated systems, part of the observed photo-inactivation could be due to excitation of exogenous (ferric-hydro-complex or ferric-organo-complex) and endogenous (cytochrome, flavin, tryptophan) photosensitizers, [16,17] as well as the ROS-action ($^1O_2$, $O_2^{\bullet-}$, OH• and H_2O_2) generated from the dissolved oxygen (O_2) contained naturally in the water via successive steps of one-electron reductions [17,23]. The $O_2^{\bullet-}$ and/or the H_2O_2 have the ability to attack proteins and cell membrane components, especially membrane lipids, resulting in their peroxidation [23]. This peroxidation increases the cell membrane permeability and the disruption of the trans-membrane ion gradients [6], which can lead to the inactivation of the cells. H_2O_2 is a non-charged molecule and penetrates readily the cellular membranes [13]. The toxicity of H_2O_2 is due to the fact that they can induce the production of OH• through the Fenton reaction (Eq. (1)) within the cell [23]. OH• is a highly reactive oxidant, which can degrade non-biodegradable chemical components [16,19], NOM [12,13] and inactivate bacteria [13,14].

4.4.2. Inactivation of defense mechanisms

The deficiency in the cellular defense against ROS, constituted by enzymes such as SOD/SOR which control the $O_2^{\bullet-}$ or catalase which regulate the H_2O_2 concentration [23], can result in an oxidative stress leading to the increase of the ROS content of the cells at the level exceeding their defense capacity [24,28]. This deficiency in self defense mechanisms can arise from the exposure of these enzymes to thermal or optical inactivation [2]. Ghadermarzi and Moosavi-Movahedi [29] suggested that inactivation arises when the temperature is around 45 °C. Considering the temperature measured during the photo-inactivation process in this study, it can be assumed that the enzymes for self-defense mechanisms were not efficient, giving rise to the optical inactivation through UVA radiation to efficiently inactivate the bacterial contents of the water [3]. The inactivation of the SOD/SOR and catalase leads to the increase of intracellular ROS with the direct attack of membrane and other proteins. Followed by the generation of the highly reactive OH• via intracellular Fenton reaction (Eq. (1)). This reaction take place between the H_2O_2 and the iron liberated from the iron sulfur clusters ([4Fe–4S]) after the inactivation of clusters enzymes like dihydroxy-acid deshydratase, aconitase B and fumarases A and B [24] by the $O_2^{\bullet-}$ [30]. The liberation of free iron in the cell comes from the specific oxidizing action of the superoxide on the centers [4Fe–4S] of these hydrolytic enzymes[24,30]. The OH• attacks lead to important cellular damage on DNA [24]. When the bacteria are not sufficiently exposed to illumination, they can recover viability by self-defense mechanisms in a short time [8]. In this study, the systems $Fe^{2+}/h\nu$ or $h\nu$ have showed a slight initial inactivation in both wells in about 20 or 30 min depending on the enteric bacteria involved (Fig. 2). After this time, their concentration in the water have increased and stabilized till the end of the 2 h of irradiation. This situation could be explained by the recovery of the self-defense mechanisms suggested by Rincon and Pulgarin [8]. After such recovery, the actors of the defense constituted by SOD and catalase, which have been certainly weakened but not inactivated, have recovered their properties and eliminated the exceeding ROS ($O_2^{\bullet-}$, H_2O_2), giving rise to a recovery in the damaged bacteria, which multiplied and stabilized in the medium, (see Fig. 2). In the systems, $Fe^{2+}/H_2O_2/h\nu$ and $H_2O_2/h\nu$, the photo-Fenton under light and high or low iron content increase the OH• production. This increase led to an increased inactivation of wild enteric bacteria at high inactivation rates compared to those found for $Fe^{2+}/h\nu$ or $h\nu$. The Fe^{3+}-bacteria interaction are enhanced in the presence of H_2O_2 with the increased production of OH• and fast Fe^{2+}/Fe^{3+} interconversion under illumination [13]. OH• is the most powerful oxidant generated inside the cells. It reacts instantly with no selectivity, at the diffusion limits, with sugars, amino acids, phospholipids, nucleotides and organic acids including DNA [31]. Cellular defense mechanisms against a DNA attack by OH• do not to exist.

5. Conclusions

This study showed that the $H_2O_2/h\nu$ was as efficient as the photo-Fenton system ($Fe/H_2O_2/h\nu$) in significantly increasing the inactivation rate of the enteric bacteria contents of the water wells. The iron naturally present in the well water influences the reaction mechanism of $H_2O_2/h\nu$ by favoring the photo-Fenton process. The efficiency of the system $H_2O_2/h\nu$/natural water in lab experiments under simulated solar radiation was confirmed in the field in PET bottles showing a better inactivation rate than ($Fe^{2+}/H_2O_2/h\nu$/natural water). Consequently, it can be assumed that iron as the catalyst of the photo-Fenton process is not necessary in high concentrations to inactivate enteric micro-organisms. The lower efficiency of the system $Fe^{2+}/H_2O_2/h\nu$/natural water may also indicate that an optimum concentration of iron for an efficient photo-Fenton process exists. Indeed, at near neutral pH (6.3), a part of added iron salts precipitate and negatively affects the color and turbidity and light transmittance and leads to a concomitant decrease in the disinfection efficiency. This result suggests the use of Sahelian Fe-containing region to perform a photo-Fenton treatment of drinking water by adding H_2O_2 only.

The fastest inactivation kinetic of *Salmonella* spp. compared to that of the total coliforms/*E. coli* in the water with the near-to-neutral pH (W2, pH: 6.3) brings us to the assumption that the pH can significantly influence the resistance of these enteric bacteria to photo-catalytic inactivation. The results have to be confirmed by further research, however in order to establish the best disinfection process considering all the parameters affecting wild enteric bacteria inactivation.

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