

Bacteria and fungi inactivation using Fe³⁺/sunlight, H₂O₂/sunlight and near neutral photo-Fenton: a comparative study.

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ABSTRACT

Wastewater reuse is becoming increasingly important for water sustainability, and is essential for the enhancement of access to safe water for human needs like drinking water and crop irrigation. The adequate treatment of contaminated wastewater is needed so that it may be used to recharge water resources. Therefore, reduction and control of waterborne pathogens are required for appropriate water reuse. Advanced Oxidation Processes, which generate hydroxyl radicals, are promising treatments for water disinfection. The purpose of the current study was to evaluate and compare the effectiveness of three solar treatments; Fe³⁺/sunlight, H₂O₂/sunlight, and solar photo-Fenton at near-neutral pH, for the inactivation of *F. solani* and *E. coli* in water. Different concentrations of Fe³⁺ (0 to 50 mg/L), H₂O₂ (0 to 10 mg/L) and Fe³⁺/H₂O₂ (1/2.5, 5/10, 10/10, 50/10 mg/L) were evaluated in bottle reactors (200 mL) for 5 h under natural solar light in the Southeast of Spain. The order of efficacy for disinfection of both kinds of microorganisms was: photo-Fenton > H₂O₂/sunlight > Fe³⁺/sunlight.

The results for bacteria inactivation show that the highest rate was observed using photo-Fenton system with 5 mg/L of Fe³⁺ and 10 mg/L of H₂O₂, which gave a 5-log inactivation of *E. coli* in 10 min (0.96 kJ/L). The best results for *Fusarium* inactivation were found using 2.5 mg/L of Fe³⁺ and 5mg/l of H₂O₂, which gave a 3.4-log decrease in 3 h of solar exposure (14.47 kJ/L). Moreover, sunlight with H₂O₂ alone showed good potential for water disinfection with only low doses of H₂O₂ (10 mg/L) required for 6-log inactivation of *E. coli* and a 3-log inactivation of *F. solani*.

In all cases studied, the inactivation pattern and rate is observed to be highly dependent on the type of microorganism. The spores of *F. solani* were more resistant than the vegetative cells of *E. coli* to the solar treatments.

Keywords: *Fusarium solani*, *Escherichia coli*, solar photo-Fenton, solar disinfection, hydrogen peroxide.

INTRODUCTION

According to United Nations, the world human population is expected to reach over 10 billion in the next few decades. One of the most serious global problems will be water scarcity and lack of access to safe water. Although, access to clean, safe water for human consumption was declared a human right by the United Nations in July 2010, the perspective to 2025 is that 1.8 billion people will be living in countries or regions with absolute water scarcity, and two thirds of the world's population could be living under water stressed-conditions [1]. The most important issue is the disinfection of drinking water. According to the WHO and UNICEF, polluted drinking water and lack of sanitation is responsible for the death of approximately 4500 - 5000 children every day, and 884 million people still lack access to improved sources of drinking water [2].

The second most critical issue is the disinfection of water for agriculture. According to the Food and Agriculture Organization of the United Nations (FAO), agriculture is the largest global consumer of water. The 80% of land cultivated is today still exclusively rain fed, and supplies over 60% of the world's food. However, this activity could triple or quadruple in the coming decades to provide food for the growing human population [3].

The effective and sustainable treatment of polluted water is one of the most attractive strategies to combat water quality problems. Wastewater must be treated before discharge because it contains domestic, industrial and agriculture chemical pollutants and also is loaded with a wide range of pathogenic microorganisms. These pathogens may belong to completely different kingdoms like the human bacterial pathogen *Escherichia coli*, which is an indicator of fecal contamination and fungal phytopathogens like *Fusarium solani*, which causes a significant crop loss. The guidelines of safety standards for wastewater reuse are different depending on the intended final use for the treated water. Standards for water reuse for agriculture irrigation are more tolerant than for drinking water. Taking into account that agriculture is the largest global consumer of fresh water, wastewater reuse for agriculture can reduce the pressure exerted by human activities on existing fresh water resources and augment water supply in water-scarce and semi-arid zones.

Irrigation water and wastewater effluents accumulate pathogens like bacteria and fungi almost everywhere. The water is a vehicle for these pathogens generating plant and human diseases.

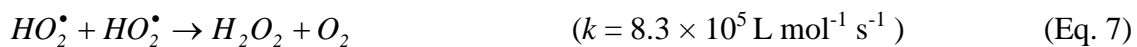
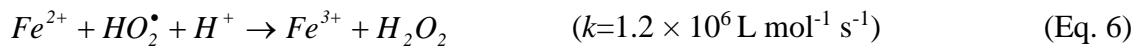
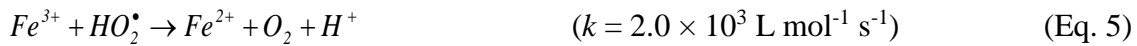
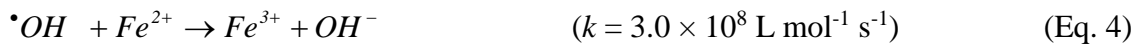
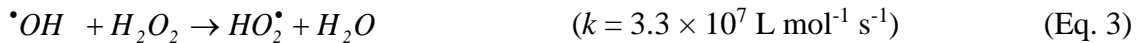
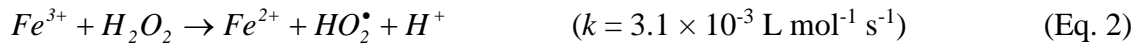
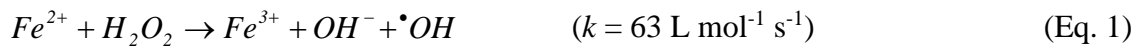
Agriculture is probably the most affected field by fungal pathogens like *Fusarium* spp [4], which is especially harmful in intensive greenhouse agriculture due to the optimal conditions of both humidity and temperature found [5]. *Fusarium* is a ubiquitous soil borne filamentous fungi which can be transmitted via water, and is known to be plant, animal, and human pathogen [6]. *Fusarium* genus produces three forms of spore: macroconidia, microconidia and chlamydospores. This genus has been reported to be highly resistant to chemical and photocatalytic treatments due to the formation of a resistant spore [8]. Furthermore, *Fusarium* spp also produces human diseases e.g., skin diseases, especially in immunodeficient, or eye infections due to fungal contamination of contact lenses [9]. Some *Fusarium* species can increase their virulence producing fumonisins and trichothecenes toxins in water. These micotoxins are associated with a variety of respiratory neurological and other systemic symptoms, causing several human fungal infections [10].

E. coli is a faecal indicator organism and its presence in water indicates possible contamination with other enteric pathogens like *Salmonella* spp, *Yersinia* spp, *Shigella* sp, etc. which are found in the gastrointestinal tract of infected mammals. These pathogens can produce diseases when contaminate fruit or other agricultural products are consumed. Diarrhea is the main symptom of enteric bacteria, however depending on the strain virulence or the person immunologic state of the person, severe illness or death can occur. Therefore it is important to develop efficient methods to inactivate *Fusarium* spores and enteric bacteria prior to water reuse.

Traditional methods for water disinfection like boiling, filtering and chlorine tablets, have been shown to be inefficient against some resistant pathogens and can't guarantee complete disinfection. Some disinfection treatments can produce disinfection by-products (chlorination [11] and ozonation [12]), which are phytotoxic to plants and hazardous for human. Solar disinfection has been used in developing countries to disinfect drinking water and is effective against low resistance microorganisms like bacteria. This method, so called SODIS (solar disinfection), consists of exposing the water to solar radiation in transparent containers (1-2 L) and the combination of UVA and heat leads to inactivation of pathogenic microorganisms in the water [16] However, resistant microorganisms like sporulated fungi and bacteria, or protozoa are more resistant to solar disinfection [13,14, 15]. The biocidal effect of sunlight is due to optical and thermal processes, and a strong synergistic effect occurs for water

temperatures exceeding 45°C and UV radiation. The ROS generated in water by UV light can cause oxidative damage in proteins, lipids and nucleic acids [17].

The inactivation of microorganisms using UV light can be enhanced using Advanced Oxidation Processes (AOPs). These processes involve the generation of hydroxyl radicals (OH•). AOPs have been reported as promising techniques to remove hazardous organic compounds and microorganisms from contaminated water. Solar driven AOPs should be lower cost and may be applied for the sustainable treatment of drinking water and irrigation water [18]. With solar driven AOPs, the inactivation of microorganisms by UVA light is accelerated by the formation of reactive oxygen species, such as OH• radicals. Photo-induced AOPs can be divided into heterogeneous and homogeneous processes. Titanium dioxide photocatalysis is an example of a heterogeneous process and has been the most studied for the inactivation of microorganisms inactivation [18]. However, photo-Fenton has attracted great interest due to its high efficiency for OH• generation. The Fenton process is described by the following equations [19]:



where k is the second order rate constant.

The production of OH• is greatly increased by UV-Vis radiation up to a wavelength of 600 nm. This reaction (Eq. 8) closes the catalytic cycle and is called photo-Fenton [20]:



Under these conditions, the photolysis of Fe^{3+} complexes promotes Fe^{2+} regeneration and iron may be considered a true catalyst [19].

Another photo-induced process which has more recently generated interest for the inactivation of pathogens in water is the synergistic effect of H_2O_2 and solar radiation. It is well known that the photolysis of H_2O_2 occurs when it is irradiated by photons of wavelengths lower than 300 nm yielding OH^\bullet as shown in equation 9 [21,22]:



However, solar radiation at Earth's surface does not contain photons with wavelengths below 280 nm, so solar energy is inefficient for OH^\bullet generation by this pathway. The combined effect of H_2O_2 with solar radiation was reported for first time for phage T7 inactivation in 1977 [23]. Up to now, very few contributions have reported the damaging effects of H_2O_2 /solar light on microorganisms in water. There is experimental evidence of disinfection capacity of near UV or visible light and hydrogen peroxide using different targets such as *Escherichia coli*, and *Streptococcus mutans* [24,25]. Nevertheless, recent studies have shown very good disinfection efficiencies in resistant fungal spores (*Fusarium equiseti* and *Fusarium solani*) using low concentrations of hydrogen peroxide (≤ 10 mg/L) in the presence of natural solar radiation in solar reactors with compound parabolic collectors (CPCs) [4,26].

The synergistic effect between hydrogen peroxide and solar photons is attributed to the generation of OH^\bullet through Fenton reactions inside microbial cells due to the natural iron content and diffusion of H_2O_2 across the cell membranes [26]. Increased ferrous ion concentrations may occur in cells irradiated with near UV photons due to increased membrane permeability to Fe^{2+} [27]. The critical factor seems to be the availability of the cellular labile iron pool (LIP), which also may be favored by cells under UV light irradiation [28]. The trace concentrations of “free” iron catalyse the production of hydroxyl radicals via Fenton/Haber-Weiss reaction cycle.

The purpose of the current study was to evaluate and compare the effectiveness of three solar treatments; Fe^{3+} /sunlight, H_2O_2 /sunlight and solar photo-Fenton at near-neutral pH for the

inactivation of *F. solani* and *E. coli* in distilled water. For this, different concentrations of Fe^{3+} (0 to 50 mg/L), H_2O_2 (0 to 10 mg/L), and $\text{Fe}^{3+}/\text{H}_2\text{O}_2$ (1/2.5, 5/10, 10/10, 50/10 mg/L) were evaluated in bottle reactors (200 mL) for 5 h under natural solar light in the Southeast of Spain. In the literature, it has been shown that the use of photo-Fenton system is efficient for inactivation of bacteria like *E. coli* [27], and the denaturation of prion protein [29] using UV-lamps. To our knowledge the solar photo-Fenton system has not been applied to fungi spores inactivation. Thus, the main novelty of this study is to demonstrate the disinfection capacity of solar photo-Fenton at near neutral pH treatment and to compare with H_2O_2 /sunlight for the inactivation of *E. coli* and *F. solani* spores in water.

MATERIALS AND METHODS

Fungal strain enumeration and quantification

A wild strain of *Fusarium solani* belonging to fungal library of the University of Almería, isolated from the Andarax River in Almeria, Spain, was used like fungal spore model to conduct experiments. This fungus was chosen because it is a common phytopathogen in soil and the water distribution system affecting crops and it has been demonstrated to be highly resistant to photocatalytic treatment. The same strain and enumeration-quantification methods have been described elsewhere [4,5,8]. Fungal colonies were transferred to sporulation agar (Cultimed, Spain) containing potassium chloride (Panreac, Spain) in Petri dishes, and kept at 25 °C for 15-30 days exposed to UV-C radiation from a mercury lamp (40 W). Under these conditions, the sporulation process is induced as in response to stress conditions while the mycelium generation is reduced. Microconidia were recovered by washing the plates with distilled water, and afterwards filtered through rock wool to separate spores from the mycelium fragment detached during the process. Spore concentration was determined by direct counting with a Neubauer plate (Brand, Germany) using a phase contrast microscope (Nikon, Japan) and diluted in the solar bottle reactor to the desired spore concentration. The initial concentration used for these experiments was $\sim 10^3$ CFU/mL. This concentration was selected because it is the one normally found in the environment.

The concentration of fungal spores in water was measured using the plate counting technique. 50-250-500 μL of samples were plated out on acidified malt agar (Sigma Aldrich, USA) to

reach the detection limit (DL), 2 CFU/mL. Fungal colonies were counted after 48 hours of incubation at 28 °C in dark. Samples and analyses were replicated three times.

Bacterial strain enumeration and quantification

Escherichia coli K-12 ATCC 23631 was inoculated from a stocks in Luria broth nutrient medium (Miller's LB Broth, Sigma–Aldrich, USA) and incubated at 37 °C by constant agitation in a rotator shaker under aerobic conditions. Bacteria were collected after 20 h of incubation which corresponds to the initial bacterial stationary phase, yielding a concentration of 10⁹ CFU/mL. *E. coli* suspensions were harvested by centrifugation at 3000 rpm for 10 min. Finally, the bacterial pellet was re-suspended in Phosphate Buffer Saline (PBS) and diluted in the reactor to the required 10⁶ CFU/mL cell density initial concentration. The samples taken during the experiment were enumerated using the standard plated counting method through a serial 10 fold dilutions in PBS and volumes of 20 µL were plated in triplicate on Luria agar Petri dishes (Sigma–Aldrich, USA). Colonies were counted after incubation of 24 h at 37 °C. The detection limit of this experimental method was found to be 4 CFU/mL.

Reagents

Different concentrations of hydrogen peroxide (H₂O₂, Riedel-de Häen, Germany at 35 wt %) were used; 2.5, 5 and 10 mg/L. Ferric nitrate (Fe(NO₃)₃·9H₂O Merck, Germany) was used as iron source. Several concentrations were studied; 1, 2.5, 5, 10 and 50 mg/L. pH was adjusted using NaOH (J.T.Baker, Holland) at near neutral pH 5.5-6 before *E. coli* inoculation due to their low resistance to acid solutions. Titanium (IV) oxysulphate solution (Riedel-de Häen, Germany) was used to measure H₂O₂ concentration by a spectrophotometric method. Luria broth nutrient medium (Miller's LB Broth, Sigma–Aldrich, USA) was used to grow the initial inoculums of *E. coli*, and Luria Agar for plated different dilutions of bacteria. Fungal spores inoculums were obtained into sporulation agar (Cultimed, Spain) containing potassium chloride (Panreac, Spain), and fungal colonies during experiment were counted on acidified Malt agar (Sigma Aldrich, USA).

Solar experiments

All assays were carried out in 250-mL DURAN-glass (Schott) batch bottle reactors in triplicate under natural solar radiation at Plataforma Solar de Almería. Bottle reactors were

magnetically stirred at 100 rpm, and exposed to sunlight for 5 hours in sunny days. Control bottles were stored in the dark during the treatment. Glass covers were used instead of plastic lids, to allow the solar radiation entering in the bottle reactor from all directions. UV-A transmission in the reactor (borosilicate glass) is 90% (cut-off at 280 nm). Total irradiate volume was 0.2 L and the illuminated surface, calculated as the cross section area of the bottle reactor facing the sun, was 0.0095 m².

The distilled water used had a conductivity < 10 µS/cm, Cl⁻ = 0.7 – 0.8 mg/L, NO₃⁻ = 0.5 mg/L and dissolved organic carbon < 0.5 mg/L. Reagents and microbial suspension were added to solar bottle reactor and diluted to achieve the desired initial concentrations. In *E. coli* assays with iron (Fe³⁺ and photo-Fenton assays) pH was adjusted using NaOH (J.T. Baker, Holland) at 5.5-6 before *E. coli* inoculation due to their low resistance to acid solutions. This was not necessary in solar disinfection and H₂O₂ assays with *E. coli*. In *Fusarium solani* experiments with Fe³⁺ and photo-Fenton, the pH ranged from 6.4 to 3.0 (depending on the iron salt concentration used); this pH was not adjusted because of the resistance of *Fusarium* to that pH. Temperature, pH and dissolved oxygen (DO) were measured during the experiment with WTW probes (Germany, series multi 720).

After agitation for 15 min in dark, the first sample (0-min) was taken and the reactors were exposure to sunlight. Samples were taken at regular intervals depending on each pathogen. The same first sample was kept in the dark at room temperature and plated again at the end of the experiment as a ‘‘control sample’’ to ensure strain good quality. Results were analyzed through a one-way ANOVA (P<0.05, confidence >95%, Origin v7.03, OriginLab Corp., 30 Northampton, USA), reporting a 95% confidence level for the average colony concentration and error.

Re-growth counts of pathogens were determined for all experiments by leaving the last two samples at room temperature for 24 h and 48 h. Plate counting method was used for each pathogen to determine bacterial and fungi colonies forming units.

Several experimental conditions were evaluated: (i) Only solar disinfection under natural sunlight without reagent addition; (ii) Photo-assisted inactivation with only H₂O₂ at several

concentrations: 2.5 - 5 - 10 mg/L; (iii) Photo-assisted inactivation with only Fe³⁺ at concentrations of 1 - 2.5 - 5 - 10 - 50 mg/L; (iv) and photo-Fenton process at different reagent ratio concentrations: 1/2.5 - 2.5/5 - 5/10 - 10/10 - 50/10 of Fe³⁺/H₂O₂ mg/L.

H₂O₂ measurement

H₂O₂ concentration was measured by a colorimetric method based in the absorbance of the yellow complex formed between titanium (IV) oxysulfate and H₂O₂ by using a spectrophotometer (PG Instruments Ltd T-60-U) at 410 nm. Titanium (IV) oxysulphate solution (Riedel-de Hæn, Germany) was used as received. The absorbance vs. concentration was linear in the range 0.1–100 mg/L [4]. The concentrations chosen (2.5, 5 and 10 mg/L) were previously evaluated in the dark observing that the pathogens viability was unaffected. A strong spore decrease was observed only concentrations equal to or higher than 500 mg/L of H₂O₂ [4]. Catalase was added to water samples to eliminate residual hydrogen peroxide, for which 1 ml samples were mixed with 100 mL of 2300 U/mg bovine liver catalase at 0.1 g/L (Sigma Aldrich, USA). Control tests in the dark with *E. coli*, H₂O₂ and catalase at the same solar experiments conditions showed no effect (positive or negative) of catalase on bacterial count results (data not shown).

Iron measurement

Ferric nitrate (Fe(NO₃)₃·9H₂O Merck, Germany) was used as iron source. The iron concentrations were measured according to ISO 6332. All samples were filtered with 0.20 µm CHROMAFIL[®] Xtra PET-20/25 (PANREAC, Spain) and measured with a spectrophotometer (PG Instruments Ltd T-60-U) at 510 nm.

Solar radiation

UV radiation was measured with a global UV-A radiometer (295-385 nm, Model CUV3, Kipp & Zonen, Netherlands) on a horizontal platform, with a typical sensibility of 264 µV W⁻¹m⁻². The radiometer provides data in terms of incident W/m², which is defined as the solar radiant energy rate incident on a surface per unit area. UV dose (W/m²) is dependent on UV intensity and time, and it is given by Equation 10.

$$Dose = I \times \Delta t \quad (\text{Eq. 10})$$

Where I is the average irradiation intensity, W/m^2 , and Δt is the experimental time in hours. Moreover, the inactivation kinetic can be plotted as function of cumulative energy per unit of volume (Q_{UV} , kJ/L) received in the photo-reactor, and calculated by Equation 11.

$$Q_{UV} = \sum_n \overline{UV}_{n-1} \frac{A_r}{V_t} (t_n - t_{n-1}) \quad (\text{Eq. 11})$$

Where t_n is the experimental time for n sample, \overline{UV}_{n-1} is the average solar ultraviolet radiation measured during the period $(t_n - t_{n-1})$, A_r is the illuminated surface, and V_t the total water volume. Q_{UV} is commonly used to compare results under different conditions [26].

RESULTS

Effect of Fe^{3+} /solar irradiation on pathogen inactivation

E. coli

Figure 1 shows inactivation of *E. coli* in water with different added concentrations of Fe^{3+} (0, 1, 5, 10, 50 mg/L) during 5 hours of exposure to natural solar irradiation. Viable bacteria cells in distilled water with 50 mg/L of Fe^{3+} remained constant in the dark for 5 h (data not shown). Consequently, the mere presence of iron did not affect *E. coli* cells cultivability under the experimental conditions of this work. Therefore, the bacterial inactivation observed in fig. 3 was due to the joint effect of solar light and Fe^{3+} .

The experiments were carried out at pH 5.5-6. It was fixed to near-neutral pH to maintain *E. coli* viability, since at pH below 4.5 *E. coli* cells are not viable [30]. The *E. coli* viability was monitored for pH < 4 in the dark for 5 h, and we observed a complete loss of bacterial concentration (data not shown). In all experiments (Fig. 3), pH ranged from 5 to 6.74. Temperatures never exceeded 33.1 °C, and DO ranged from 6.98 to 8.39 mg/L , conditions which do not affect bacterial viability.

Figure 1 & Table 1

Results shown in Fig. 3 present a decrease from initial *E. coli* concentration (10^5 - 10^6 CFU/mL) to detection limit (DL = 4 CFU/mL) in all cases except for 50 mg/L of Fe^{3+} , which did not enhance the results found for solar disinfection. The dissolved iron measured at the beginning of each experiment is shown in Table 1. The experiments with higher amounts of dissolved iron present the best disinfection performances, regardless the initial quantity of added iron salt. In the presence of 1 mg/L of Fe^{3+} (0.94 mg/L dissolved iron) bacterial inactivation till DL (5-log decrease) was obtained for $Q_{UV} = 2.1$ kJ/L. In the case of 5 mg/L of Fe^{3+} (0.36 mg/L dissolved iron), 4.3-log abatement of bacteria was observed for 4.3 kJ/L of Q_{UV} . Best disinfection results were observed when 10 mg/L of Fe^{3+} was added (1.42 mg/L dissolved iron), where a 4.7-log *E. coli* reduction was reached with only 1.2 kJ/L of Q_{UV} . Solar disinfection required 4.9 kJ/L of Q_{UV} to reach DL, while 50 mg/L of Fe^{3+} (0.24 mg/L dissolved iron) lead only to 2-log *E. coli* reduction.

E. coli photo-induced inactivation is attributed to the presence of light-absorbing species of iron added in the medium. It is known that Fe^{3+} aquacomplexes are able to play an important role for the photogeneration of hydroxyl radicals (Eq. 8). The attack of hydroxyl radicals over external cell walls gives account for loses of viability during solar experiments. The interesting point in such system, compared to the photo-Fenton process, is that no addition of hydrogen peroxide is needed.

It is well known that the nature of the iron species dissolved in water is highly dependent on pH. In strongly acid solution, iron exists as the hexa-aquo ion ($\text{Fe}(\text{H}_2\text{O})^{3+}_6$); when pH raises this ion precipitates as amorphous ferric oxyhydroxides, which have a reduced reactivity. The presence of hydrolyzed species was revealed in our experiments by turbidity and slight yellow-orange color of samples especially for higher amounts of Fe^{3+} added (50 mg/L).. This colored and turbid sample screens sunlight, and the very low amount of dissolved iron (0.24 mg/L) leads to the worse inactivation efficacy found at 50 mg- Fe^{3+} /L.

***F. solani* spores**

Figure 2 shows the *F. solani* microconidia inactivation in distilled water with several Fe^{3+} concentrations (1, 2.5, 5, 10 and 50 mg/L) under natural sunlight. In this case, pH was not adjusted to 5.5-6 because *F. solani* tolerates well pH 3 and 2. This resistance to acidic

conditions was evaluated in distilled water in the dark (data not shown). pH of experiments shown in fig. 2 ranged from 2.56 to 5.5, which depends on the iron concentration used as $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ was added as the iron source. Temperatures were measured through the experiments and did not exceed 43 °C. The thermal inactivation in *F. solani* spores occurs at temperature higher than 45 °C (data not shown), thus thermal killing was discarded in this experimental work. DO ranged from 6.8 to 8.78 mg/L.

Figure 2 & Table 2

Complete spore inactivation (from several thousands of CFU per mL to detection limit) was achieved with the low iron concentrations, 1, 2.5 and 5 mg/L of Fe^{3+} , while the higher concentrations (10 and 50 mg/L of Fe^{3+}) and solar radiation alone lead to worse disinfection results (Fig. 2). At 50 mg/L of Fe^{3+} , *F. solani* concentration remained constant for 5 h of solar exposure, although the water had the most acid pH and the highest dissolved iron measured of all experiments done. Solar radiation alone and with 10 mg/L of Fe^{3+} achieved only 1-log reduction in spore concentration. Complete inactivation (3-log decrease) in the presence of 5 mg/L of Fe^{3+} required a cumulative solar UVA energy of 26.7 kJ/L. A 3.5-log killing was observed for 2.5 mg/L of Fe^{3+} when the system received 20.8 kJ/L. With 1 mg/L, total spore depletion (to the DL) was achieved with 28.2 kJ/L (3-log decrease).

Total iron dissolved in water for this case (Table 2) was higher than for *E. coli* tests due to the acid pH used for *F. solani*. Lower disinfection efficiencies were found for higher iron concentrations (50 mg/L). Results show that fungicidal action of sunlight with Fe^{3+} has similar effects than the solar radiation alone. Better disinfection efficacies were found with lower Fe^{3+} concentrations; the best inactivation result was found using 2.5 mg/L, which needed 20.8 kJ/L for complete spore inactivation.

Water disinfection with H_2O_2 /solar irradiation

E. coli

Figure 3 shows the effect of adding H_2O_2 to distilled water under natural solar irradiation over *E. coli* using 2.5, 5 and 10 mg/L of H_2O_2 as compared with solar radiation alone. Table 3 shows the data measured during these experiments. pH was not adjusted, it ranged from 5.9 to 6.2. Temperatures were lower than 37 °C in all cases, and DO varied from 7.12 to 8.49 mg/L.

All these conditions do not have detrimental effects on bacterial viability. H₂O₂ concentration decreased with experimental time during the 5 hours of each experiment (Table 3). The viable bacteria count with 10 mg/L H₂O₂ in the dark remained constant for 5 h (data not shown) demonstrating that this concentration had no effect on the viability of the *E. coli* cells.

Figure 3 & Table 3

In all cases studied we observed a disinfection enhancement when H₂O₂ was added as compared with solar disinfection alone. The inactivation efficiency increased with increasing H₂O₂ concentration, although there was no direct correlation between H₂O₂ demand and inactivation achieved. 10 mg/L of H₂O₂ yielded the best results, with a complete inactivation (4.9-log bacterial decrease), which required 0.97 kJ/L of solar UVA and consumed 2.54 mg/L of H₂O₂. A 5.4-log decrease was attained with 5 mg/L of H₂O₂ after receiving 3.15 kJ/L and consuming 0.26 mg/L of H₂O₂. With 2.5 mg/L of H₂O₂, a 5.3-log decrease (down to the DL) was observed after 5 h of solar exposure (Q_{UV} = 5.5 kJ/L) with total depletion of H₂O₂ during the treatment. On the other hand, solar disinfection required 7.92 kJ/L to reach complete bacterial inactivation (5.7-log reduction).

F. solani

Figure 4 shows *F. solani* spore inactivation with 2.5, 5 and 10 mg/L of H₂O₂. Complete spore inactivation was achieved following the same kinetics, and same final results for all H₂O₂ concentrations evaluated; complete inactivation was not observed with solar disinfection only. These spores are not affected by these low amounts of H₂O₂ (2.5, 5 and 10 mg/L) in the dark [4]. Table 4 shows the H₂O₂ concentration measured during the experiment. Temperatures never exceeded 37 °C. pH ranged 6.2 to 6.4, and DO was ranged from 6.4 to 8.1 mg/L. In all cases 15.4 kJ/L were needed to observe a 3-log reduction of microconidia of *F. solani*, which occurred within 3 h of solar exposure. However, the H₂O₂ consumption at the end of each experiment varied: when 10 mg/L of H₂O₂ was added, it was consumed 2.69 mg/L, while in the case of 5 and 2.5 mg/L of H₂O₂ added, it was consumed 0.83 and 0.62 mg/L, respectively. Only 1-log reduction in spore concentration was observed for solar disinfection tests, according to previous findings [4].

Figure 4 & Table 4

Although the efficiency order found in this case is $2.5 > 5 > 10$ mg/L of H_2O_2 , to our knowledge, the best option would be 5 mg/L because using a very low H_2O_2 concentration like 2.5 mg/L would end with complete hydrogen peroxide decomposition according to the own H_2O_2 chemistry, which is also favoured by a thermal increase [21]. With 5 mg/L of H_2O_2 , the inactivation of *F. solani* microconidia will be guaranteed for the same treatment time and also H_2O_2 will not be totally consumed before achieving the complete spores' inactivation as shown in Table 4.

The H_2O_2 demand depends on the quality and temperature of the water and not just on the microorganism concentration. Therefore there is not direct correlation between H_2O_2 consumption and inactivation rate. The optimal concentration of H_2O_2 will have to be determined (experimentally) as a function of chemical composition of the water, microbiological loading and temperature.

Disinfection with near neutral pH photo-Fenton

E. coli

Figure 5 shows the inactivation of *E. coli* with photo-Fenton treatment. Several concentration ratios between Fe^{3+}/H_2O_2 were evaluated 1/2.5, 5/10, 10/10, 50/10 mg/L. All experiments showed complete inactivation from 10^6 CFU/mL to the detection limit. The highest bacterial inactivation efficiency was achieved with 5 mg/L of Fe^{3+} -10 mg/L of H_2O_2 , obtaining 4.5-log reduction with 0.96 kJ/L of cumulated UV-A radiation, followed by 10 mg/L of Fe^{3+} -10 mg/L of H_2O_2 which reached total bacterial inactivation (4.9-log decrease) with 2.31 kJ/L of Q_{UV} . Solar disinfection also obtained the detection limit achieving 5.2-log inactivation bacteria with 4.83 kJ/L. 1 mg/L of Fe^{3+} -2.5 mg/L of H_2O_2 required 5.47 kJ/L of Q_{UV} to reach complete bacterial inactivation (4.8-log bacterial decrease), and with the reagent ratio of 50 mg/L of Fe^{3+} and 10 mg/L of H_2O_2 , 18.58 kJ/L was required to reach the detection limit (5.2-log reduction).

Figure 5 & Table 5

The experiments in this section were done at pH 5.5-6, i.e. near-neutral pH, due to the the loss of *E.coli* viability under acid conditions (below pH 4.5) [30]. At near neutral pH the initial

dissolved iron concentration was observed to be very low (Table 5). For 1 mg/L of Fe^{3+} , the total iron dissolved in the system was 0, which could explain the low inactivation efficiency, observing also a complete disappearance of H_2O_2 from the beginning of the experiment. However, for 5 and 10 mg/L of Fe^{3+} , the low iron dissolved in the water (0.51 and 0.41 mg/L of Fe^{3+} , respectively) reacted with H_2O_2 , which remained in the system during the experiment being sufficient to enhance the inactivation of bacterial cells. Finally, in the case of 50 mg/L of Fe^{3+} , the dissolved iron concentration was only 0.31 mg/L, and the rest of iron added to the reactor was precipitated, reducing the inactivation of *E. coli* by the scattering light effect as it was explained for Fe^{3+} /solar irradiation tests. All experiments' pH ranged from 5.06 to 6.63 (Table 5), temperatures never exceeded 42 °C, and DO ranged from 7.01 to 8.43 mg/L; under these conditions *E. coli* cells are viable.

Between all the reagents concentrations evaluated, only 5 and 10 mg/L of Fe^{3+} with 10 mg/L of H_2O_2 improved the inactivation kinetics of *E. coli* compared to solar disinfection results. Higher and lower concentrations ratio (50 mg/L Fe^{3+} -10 mg/L H_2O_2 , and 1 mg/L of Fe^{3+} -2.5 mg/L of H_2O_2) lead to complete inactivation although they required solar UVA energy doses higher than those observed for solar disinfection only.

F. solani

F. solani microconidia inactivation was also evaluated using photo-Fenton treatment at several iron and hydrogen peroxide concentrations ratio (Figure 6). Although the disinfecting effect of solar light is enhanced by the system $\text{Fe}^{3+}/\text{H}_2\text{O}_2$, not all the tested concentration ratios had good disinfection results as they did not reach the detection limit. It was observed that total inactivation was reached using the following concentration, 1 mg/L of Fe^{3+} -2.5 mg/L of H_2O_2 ; 2.5 mg/L of Fe^{3+} -5 mg/L of H_2O_2 ; and 5 mg/L of Fe^{3+} -10 mg/L of H_2O_2 . The best results were obtained with a concentration ratio of 2.5 mg/L of Fe^{3+} -5 mg/L of H_2O_2 , which reached the complete disinfection of microconidia (3.4-log decrease) with 14.47 kJ/L. With 1 mg/L Fe^{3+} -2.5 mg/L of H_2O_2 , DL was achieved only when the system had accumulated 20.79 kJ/L (3.3-log decrease). A 3.2-log inactivation was observed with 5 mg/L of Fe^{3+} -10 mg/L of H_2O_2 under 26.67 kJ/L of solar UVA energy. Nevertheless, microconidia were not inactivated with higher concentration ratios (10, 35 and 50 mg/L) of Fe^{3+} and H_2O_2 (10 mg/L) probably due to the scattering light effect mentioned in previous section, nor with solar irradiation alone.

Figure 6 & Table 6

Temperatures of all experiments were below 43 °C. pH ranged from 2.6 to 4.1, and DO varied from 6.0 to 8.8 mg/L. The *F. solani* strain is viable under these conditions. The initial dissolved iron in all cases were higher than those found for *E. coli* experiments because the pH ranged from 4 to 3, and therefore, the iron added was mostly dissolved during the 5 hours of solar exposure (Table 6).

Comparison of the different solar treatments

E. coli

The best inactivation results obtained with each treatment evaluated before are compared in Figure 8. Solar photo-Fenton with 5 mg/L of Fe³⁺ and 10 mg/L of H₂O₂; H₂O₂/solar light with 10 mg/L of H₂O₂; and Fe/solar light with 10 mg/L of Fe³⁺ were compared with solar disinfection alone. The highest *E. coli* inactivation rate was obtained with photo-Fenton using 5 mg/L of Fe³⁺ and 10 mg/L of H₂O₂ attaining the detection limit with only 0.96 kJ/L of Q_{UV} . This cumulative energy is obtained in a normal sunny day in only 5 min of solar exposure. These results are quite similar to those obtained with sunlight and 10 mg/L of H₂O₂. The inactivation results obtained with 10 mg/L of Fe³⁺/solar light system showed a higher efficiency reaching the detection limit with 1.2 kJ/L. Solar disinfection required 4.8 kJ/L to reach complete abatement of *E. coli*. Therefore, it can be concluded that no significant differences has been observed between all processes evaluated except for solar disinfection.

Figure 7

Fusarium solani

Figure 8 shows the comparison of the best results obtained with each inactivation process tested on *F. solani*. The best results were found using solar photo-Fenton, with 2.5 mg/L of Fe³⁺ and 5 mg/L of H₂O₂. In this case, the detection limit was reached with a cumulative UVA energy of 14.5 kJ/L. H₂O₂/solar light required slightly higher energy of 15.4 kJ/L to achieve the same inactivation result. Although both processes have different kinetics, both solar photo-Fenton and H₂O₂/solar treatment reach the DL close to $Q_{UV} = 15$ kJ/L. The reasons for this will be discussed in next section. Meanwhile, the photo-induced process Fe³⁺/solar light had a lower efficacy (it required 20.79 kJ/L to achieve the detection limit) followed by the

solar disinfection, which did not reach the detection limit after 5 h of solar exposure. Similarly to the results observed with *E. coli*, the highest efficiency was obtained with photo-Fenton process followed by H₂O₂/solar light and the less efficient was Fe³⁺/solar light after solar disinfection.

Figure 8

DISCUSSION

Exposure to oxidative stress can induce a wide series of responses in microorganisms ranging from increase mitosis to apoptosis, and finally, necrosis [31]. This stress is induced by reagent oxidative species (ROS) such as O₂, H₂O₂ or derived oxygen species generated during photolysis [32]. Different inactivation mechanisms are involved in the cell death during photolysis [27]. Furthermore, when an advanced oxidation treatment like solar photo-Fenton is used for inactivation of microorganisms in water, hydroxyl radicals (OH[•]) are generated and the disinfection process is accelerated.

ROS cause damage to cells by direct attack to the external cell membrane by initiating lipid peroxidation chain reactions. This increases membrane permeability, subsequently alters normal function of the cells and affects their viability [33]. Depending on the source and location of the ROS generated, they have different adverse effects. Exogenous short-living ROS formed outside of the cell act on the external membranes of cells. Internal or endogenous ROS may be generated by three ways; (i) by direct action of UV-Vis solar radiation, which generate superoxide and H₂O₂ [16,17], (ii) via internal Haber-Weiss/Fenton reactions which may occur with internal iron [34], and (iii) exogenous long-living radicals which can diffuse into the cells generating injure inside the cells. An example of this case is H₂O₂; which can cross membranes freely and may react with free iron present in the cells, generating OH[•] by Fenton reactions [32]. For this reason, H₂O₂ may be very toxic to cellular aerobic metabolism, cleaving DNA molecule in amine bases [21]. Fe²⁺ is another example of exogenous species which induce internal damage. The presence of Fe²⁺ can induce damaging effects in the cells because it can cross membranes easily and injure cells internally via reactions with metabolic H₂O₂ [4,27,32].

In this experimental work, we observed the detrimental effect produced by the solar radiation over *E. coli* and *Fusarium* (Fig. 3-8). Microorganisms may present defence mechanisms

against the ROS generated under solar exposure, like catalase and superoxide dismutase (SOD), which are the most common enzymes scavenging intracellular H₂O₂. *E. coli* contains two catalases, one is expressed under the stationary phase and the other acts when there is an over exposure to extracellular H₂O₂ [35]. They dissociate H₂O₂ into H₂O and O₂ (Eq. 12) [21]:



When the enzymatic defence system of microorganisms is damaged, the cells die eventually due to the accumulated damage on different components. During solar exposure, cell death is partially due to the inactivation of catalase by UV-A radiation [27, 32].

The detrimental effects produced by solar radiation are superimposed on the damaging effects of solar photo-Fenton, solar/H₂O₂ and solar/Fe³⁺. In these cases, there are additional ways of generation of oxidative radicals under each process which will be discussed below.

The treatment Fe³⁺/solar light enhanced solar disinfection for *E. coli*. Fe²⁺ and OH• are formed according to Eq. 8. This reaction is produced for wavelengths higher than 313 nm, and the aquacomplex of Fe³⁺ (mainly Fe(OH)²⁺) generates hydroxyl radicals and reduce the iron from Fe³⁺ to Fe²⁺ [36]. Moreover, some authors [37] report that the iron can easily diffuse into the bacterial cells, especially Fe²⁺, generating injury by the Haber-Weiss reaction thanks to the presence of hydrogen peroxide from the cellular aerobic metabolism.

The efficiency of the Fe³⁺/light for bacterial inactivation has been described in the literature using UV-lamps as opposed to natural sunlight. This previous work demonstrated that irradiated suspensions of *E. coli* in the presence of 0.6 mg/L of Fe³⁺ enhanced the bactericidal action over UV light alone. They observed a bacterial inactivation from 10⁷ CFU/mL to detection limit in 180 minutes [27]. Our results, under natural sunlight with *E. coli* (Fig. 3), show complete inactivation for all conditions where dissolved iron was equal or above 0.4 mg/L. For 1.42 mg/L of dissolved iron we observed complete *E. coli* inactivation after 15 min (1.2 kJ/L) of solar exposure.

The same mechanisms explain the inactivation of *Fusarium* spores with Fe³⁺/solar light (Fig. 2). The concentrations of dissolved iron that yielded to best inactivation results were 0.6 and 2.5 mg/L, which required more than 20 kJ/L of Q_{UV} and around 4 hours of solar treatment to reach the detection limit where the initial concentration was $2\cdot 3\cdot 10^3$ CFU/mL. *F. solani* showed a very high resistance to this treatment compared with *E. coli*. This strong difference is due to the higher resistance of spores. Contrary to *E. coli* cells, fungal cell walls are rigid structures composed mainly of polymeric sugars -like glucans, mannans, chitin-, proteins and glycoproteins. The hyphal wall of *Fusarium* consist of three layers, an outer microfibrillar layer composed of chitin, an electron dense central non-fibrillar layer, and an inner electron transparent microfibrillar layer. Nevertheless, the conidial wall contained in addition a fourth mucilaginous outer layer composed of xylan [38]. This structure not only confers shape to the cell but it is also involved in mating, adherence to substrates and protection of the cell. The high resistance of *F. solani* to photocatalytic treatment compare to *E. coli* cells was investigated by Sichel *et al.* [5] using the photocatalytic process of suspended TiO₂ under solar light. They observed that inactivation of 3-log of *Fusarium* required 4-5 times more solar-UVA dose than a 6-log drop of *E. coli* under similar experimental conditions. They also concluded that the UV dose needed to inactivate each microorganism depends on its own spore or cell structure. Our results with Fe³⁺/solar light have shown similar behaviour although we used a different treatment. This demonstrates that the resistance to the treatment depends on the kind of microorganism. In the case of heterogeneous photocatalysis (TiO₂), the main oxidative stress is produced by OH[•], which are produced outside the cells [18]. In Fe³⁺/solar light the diffusion of the iron inside the spore plays an important role. Furthermore, the damage is generated once the spore has initiated its germination process which includes the water uptake to hydrate the spore core, initiating the activation of metabolic activity. This phase of the germination is called “swelling”. During swelling the iron can diffuse inside the spore generating ROS and damaging internal structures [39].

The photo-induced process H₂O₂/solar light has demonstrated very good inactivation results in *E. coli* and in *F. solani* spores. The effect of near UV- light and H₂O₂ on *E. coli* has been discussed in literature. Imlay et al., 1986 reported that exogenous millimolar of 1–3 mM of H₂O₂ concentrations kill logarithmically growing *E. coli* cells and attributed the main effect to DNA damage in Fenton-like reactions [40]. This process is favored by the freely diffuse of H₂O₂ trough membranes. Once H₂O₂ concentration increases inside the cell, different

reactions may occur like the oxidation of protein cysteinyl and methionine residues as well as lipids. Furthermore, the iron naturally present in cells increases stress over bacteria cells due to OH^\bullet generated by the Fenton/Haber-Weiss cycle. Iron occurs naturally in cells in two main forms, “free iron” and iron incorporated into enzymes or storage in proteins. The susceptible target to generated OH^\bullet is the free iron linked to metabolites or biomolecules surfaces. Also, iron-sulphur clusters are release as a source of internal iron and also, nucleic acids bind iron [41]. It has been also reported that growing *E. coli* cells contain approximately 20 μM of chelatable Fenton-active ferrous ions [42]. The broad evidence of a free iron pool inside cells support the hypothesis of internal Fenton reaction which derives in the *E. coli* inactivation kinetics obtained in this experimental work. More recently, Spuhler et al., 2010 reported a good inactivation rate of *E. coli* with 10 mg/L of H_2O_2 using an artificial sunlight source (UVA range from 330 to 390 nm). They showed a complete bacterial inactivation in 180 minutes [27]. Our results obtained under natural sunlight shows that *E. coli* was inactivated with 10 mg/L of H_2O_2 within 15 minutes of solar exposure (Fig. 3).

Our results obtained with H_2O_2 /solar light for *F. solani* microconidia inactivation (Fig. 4) were also much slower than those found for *E. coli*. The detection limit was reached in all cases (2.5, 5 and 10 mg/L of added H_2O_2) after 3 h of solar treatment. The inactivation mechanism of spores are similar to those describe in *E. coli*. Nevertheless, the higher time and energy accumulated in the system needed to achieve the complete inactivation is due to the time required by the spore to initiate their germination process (swelling) as it was explained before. When swelling, the H_2O_2 diffuses into the cell generating OH^\bullet via Haber-Weiss due to presence of iron labile pool [4].

The main advantage of H_2O_2 /solar light system is the low cost of the reagent (H_2O_2) and also the very low amounts needed for disinfection. It does not require a post-treatment because the auto-decomposition of hydrogen peroxide in water and oxygen avoids concerns about secondary pollution due to the disinfectant itself. This is not the case of other advanced oxidation processes like titanium dioxide which require a post-treatment to remove the catalyst from the water, or photo-Fenton process which requires pH neutralization and iron removal.

Photo-Fenton results (Fig. 5 and 6) showed the highest inactivation efficiency in both pathogens under study. Its high efficiency lies in the great amount of extracellular OH^\bullet generated during the process (Eq. 1-9). These radicals attack mainly the pathogens membranes [27], they may reach DNA, producing strands breaks, nucleic base modifications and lethal damages [16,35] and they may form O_2^- [17]. Rincón *et al.* [43], reported that the system $\text{Fe}^{3+}/\text{H}_2\text{O}_2/\text{solar light}$ was more efficient than $\text{Fe}^{3+}/\text{solar light}$ alone because the high production of oxidative species inside and outside the cell is the responsible for this higher efficiency. The authors used real water from Lemans Lake in Switzerland, adding 10 mg/L of iron from iron-sulphur and 10 mg/L of H_2O_2 at neutral pH. Spuhler *et al.* [27] showed similar results with *E. coli* using a solar simulator and very low added iron concentration under UVA light in the range of 330 - 390 nm, 0.6 mg/L of Fe^{2+} or Fe^{3+} and 10 mg/L of H_2O_2 . They found inactivation times of 180 minutes. Our results show a significant reduction on treatment time to 20 minutes. We may attribute our shorter inactivation times of *E. coli* to the different irradiance source used (natural solar radiation instead of UV-lamp). Results shown in Fig. 6 show *F. solani* inactivation with solar photo-Fenton for the first time. We observed a very good inactivation efficiency if compared with the other solar treatments under study.

A very marked difference between results obtained for *E. coli* and *Fusarium* is observed (Fig. 7 and 8). *E. coli* is more sensitive to all treatments but it is not a good model to observe differences among them, while *Fusarium*, being more resistant, shows high differences between the use of solar light alone, or with addition of Fe^{3+} , or H_2O_2 or photo-Fenton process (Fig. 8). In the case of *Fusarium* we observed that all reactions are delayed in time is compared to the *E. coli* results. An initial shoulder phase is observed (Fig. 8) when the spores are not being lethally damaged. Then, after approximately 2 hours, the inactivation kinetics are log-linear until the detection limit is achieved, which occurs when spores begin to uptake water and start the germination process, increasing their susceptibility to ROS attack. The tail at the end of the experimental time was not observed. This behavior is especially important in the photo-induced process of $\text{H}_2\text{O}_2/\text{solar light}$ and $\text{Fe}^{3+}/\text{solar light}$ which can injure spores only when they have initiated their germination process. While in photo-Fenton case, the shoulder length is wider and the slope of log-linear is higher because the OH^\bullet are generated outside the spore, therefore, the attack to external walls is initiated from the beginning of the solar treatment.

The photo-Fenton for *Fusarium* inactivation was carried out at pH 4; it had higher efficiency than the other treatments. This was not the case for *E. coli*, where the near neutral pH used in photo-Fenton made the photo-Fenton to be less efficient than at acidic conditions. However, due to the great sensibility of *E. coli*, no differences were observed.

In previous studies done under sunlight at the PSA with different photoreactors, an optimum concentration of 0.2–0.5 mM of iron (III) was found after many experiments to remove chemical compounds from water [18]. We found the best disinfection results of this work for concentrations 4 times lower than those previously observed, although the optimal concentration might be different for disinfection processes in solar reactors due to different optical properties and mechanisms involved.

CONCLUSIONS

- Solar/ Fe^{3+} , solar/ H_2O_2 , and solar photo-Fenton process have been demonstrated to have detrimental effects over the vegetative cells of *E. coli* and *F. solani* microconidia in distilled water.
- *E. coli* inactivation with solar light was enhanced by adding Fe^{3+} , H_2O_2 , and photo-Fenton process. The best bacterial disinfection results were obtained with photo-Fenton for 5 mg/L of Fe^{3+} -10 mg/L of H_2O_2 , for which complete inactivation was attained with 0.96 kJ/L.
- *F. solani* spores were also inactivated with solar light and Fe^{3+} , H_2O_2 and photo-Fenton process. We found the best disinfection results with photo-Fenton at 2.5 mg/L of Fe^{3+} -5 mg/L of H_2O_2 , when 14.47 kJ/L of solar UVA accumulated energy was received in the system. This work shows for the first time the successful treatment of water contaminated with *Fusarium solani* using solar photo-Fenton treatment.
- *E. coli* is more sensitive to all solar treatments than *Fusarium solani*. Therefore, *E. coli* is not a good model to observe differences between different solar technologies, while the higher resistance of *Fusarium* permits one to observe marked differences between the treatments evaluated.
- H_2O_2 and Fe^{3+} are good additives to be used with solar light with a synergistic effect on disinfection performance. Solar irradiation with added H_2O_2 , Fe^{3+} and photo-Fenton process are a low-cost alternative to standard water disinfection technologies.

- The use of these technologies require the deep study of experimental conditions to determine appropriate reagents concentrations to achieve good disinfection results. These results will also depend on different water types and microorganisms present.

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TABLES

Table 1. Dissolved iron for each case at pH 5.5-6 (Fig. 1).

	Fe ³⁺ added	Initial dissolved Fe ³⁺
—◆—	0 mg/L	0.0
—■—	1 mg/L	0.94 mg/L
—●—	5 mg/L	0.36 mg/L
—▲—	10 mg/L	1.42 mg/L
—▼—	50 mg/L	0.24 mg/L

Table 2. Dissolved iron (initial) and pH (initial and final) for each case (Fig. 2).

	Fe ³⁺ added	Initial dissolved Fe ³⁺	pH _i / pH _f
—◆—	0 mg/L	0.0 mg/L	5.5 / 5.5
—■—	1 mg/L	0.6 mg/L	4.4 / 4.7
—◀—	2.5 mg/L	2.5 mg/L	3.7 / 3.7
—●—	5 mg/L	4.0 mg/L	3.7 / 3.7
—▲—	10 mg/L	9.3 mg/L	3.3 / 3.3
—▼—	50 mg/L	54.2 mg/L	3.0 / 2.6

Table 3. Hydrogen peroxide concentration measured (initial and final) for each case at pH 5.5-6 (Fig. 3).

	H ₂ O ₂ added	Initial H ₂ O ₂	Final H ₂ O ₂
—◆—	0 mg/L	0.0 mg/L	0.0 mg/L
—▲—	2.5mg/L	1.5 mg/L	0 mg/L
—●—	5 mg/L	4.0 mg/L	3.7 mg/L
—■—	10 mg/L	12.9 mg/L	10.3 mg/L

Table 4. Measured hydrogen peroxide (initial and final) for each case at pH 6.4-6.2 (Fig. 4).

	H ₂ O ₂ added	Initial H ₂ O ₂	Final H ₂ O ₂
—◆—	0 mg/L	0.0 mg/L	0.0 mg/L
—▲—	2.5mg/L	2.61 mg/L	1.99 mg/L
—●—	5 mg/L	5.15 mg/L	4.32 mg/L
—■—	10 mg/L	10.27 g/L	7.58 mg/L

Table 5. Initial and final measured concentrations of dissolved iron, H₂O₂ and pH (Fig. 5).

	Fe _i /H ₂ O _{2i} (mg/L)	Fe _f /H ₂ O _{2f} (mg/L)	pH _i /pH _f
—◆—	0/0	0/0	6.3/6.4
—●—	0/0	0.1/0	5.1/5.4
—■—	0.5/14.2	0.1/4.7	5.5/6.6
—◀—	0.4/7.2	0.2/2.1	5.6/6.1
—▼—	0.3/5.8	1.3/0.9	5.7/5.6

Table 6. Initial and final measured concentrations of dissolved iron, H₂O₂ and pH (Fig. 6).

	Fe _i /H ₂ O _{2i} (mg/L)	Fe _f /H ₂ O _{2f} (mg/L)	pH _i /pH _f
—◆—	0/0	0/0	6.1/6.2
—●—	1.0/1.9	0.6/0	4.0/4.1
—▲—	1.9/4.4	0.7/0	3.9/3.9
—■—	3.4/13.6	0.9/1.1	3.7/3.7
—◀—	7.7/15.6	5.1/7.4	3.3/3.3
—★—	37.1/11.9	19.2/11.5	3.0/3.0
—▼—	57.6/12.0	40.5/12.6	2.9/2.6

FIGURE CAPTIONS

Figure 1. *E. coli* concentration versus Q_{UV} under natural sunlight with 1 (–■–), 5 (–●–), 10 (–▲–) and 50 mg/L (–▼–) of Fe^{3+} and without iron (solar disinfection, –◆–). Dark controls are represented with the corresponding empty symbol. DL = 4 CFU/mL.

Figure 2. *F. solani* spore concentration versus Q_{UV} under natural sunlight with 1 (–■–), 2.5 (–◄–), 5 (–●–), 10 (–▲–) and 50 mg/L (–▼–) of Fe^{3+} and without iron (solar disinfection, –◆–). Dark controls are represented with the corresponding empty symbol. DL = 2 CFU/mL.

Figure 3. *E. coli* concentration versus Q_{UV} under natural sunlight in the presence of 2.5 (–▲–), 5 (–●–) and 10 mg/L (–■–) of H_2O_2 and solar disinfection (–◆–). Dark controls are represented with the corresponding empty symbol. DL = 2 CFU/mL.

Figure 4. *F. solani* concentration versus Q_{UV} under natural sunlight in the presence of 2.5 (–▲–), 5 (–●–) and 10 mg/L (–■–) of H_2O_2 and solar disinfection (–◆–). Dark controls are represented with the corresponding empty symbol. DL = 2 CFU/mL.

Figure 5. *E. coli* concentration versus Q_{UV} under natural sunlight in the presence of Fe^{3+}/H_2O_2 at the following concentration ratios: 1/2.5 (–●–), 5/10 (–■–), 10/10 (–◄–), 50/10 mg/L (–▼–) and solar disinfection (–◆–). Dark controls are represented with the corresponding empty symbol.

Figure 6. *F. solani* concentration versus Q_{UV} under natural sunlight in the presence of Fe^{3+}/H_2O_2 at the following concentration ratios: 1/2.5 (–●–), 2.5/5 (–▲–), 5/10 (–■–), 10/10 (–◄–), 35/10 (–★–), 50/10 mg/L (–▼–) and solar disinfection (–◆–). Dark controls are represented with the corresponding empty symbol.

Figure 7. *E. coli* inactivation for 4 solar treatments: 5 mg/L of Fe^{3+} and 10 mg/L of H_2O_2 (–▲–); 10 mg/L of H_2O_2 (–●–); 10 mg/L of Fe^{3+} (–■–); and solar disinfection test (–◆–). DL = 4 CFU/mL.

Figure 8. *F. solani* spore inactivation for 4 solar treatments: 2.5 mg/L of Fe³⁺ and 5 mg/L of H₂O₂ (-▲-); 5 mg/L of H₂O₂ (-●-); 2.5 mg/L of Fe³⁺(-■-); and solar disinfection tests (-◆-). DL = 2 CFU/mL.

Figure 1

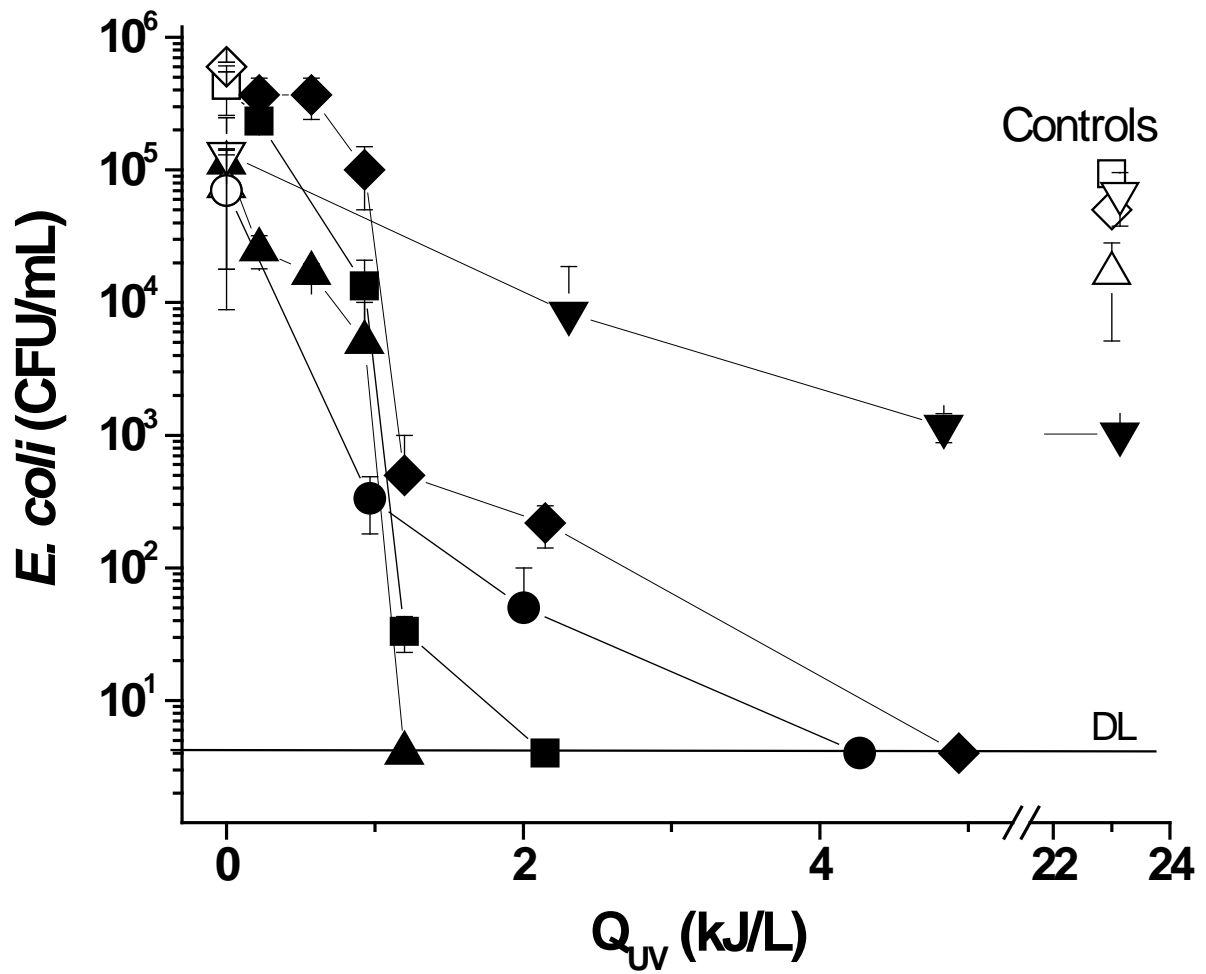


Figure 2

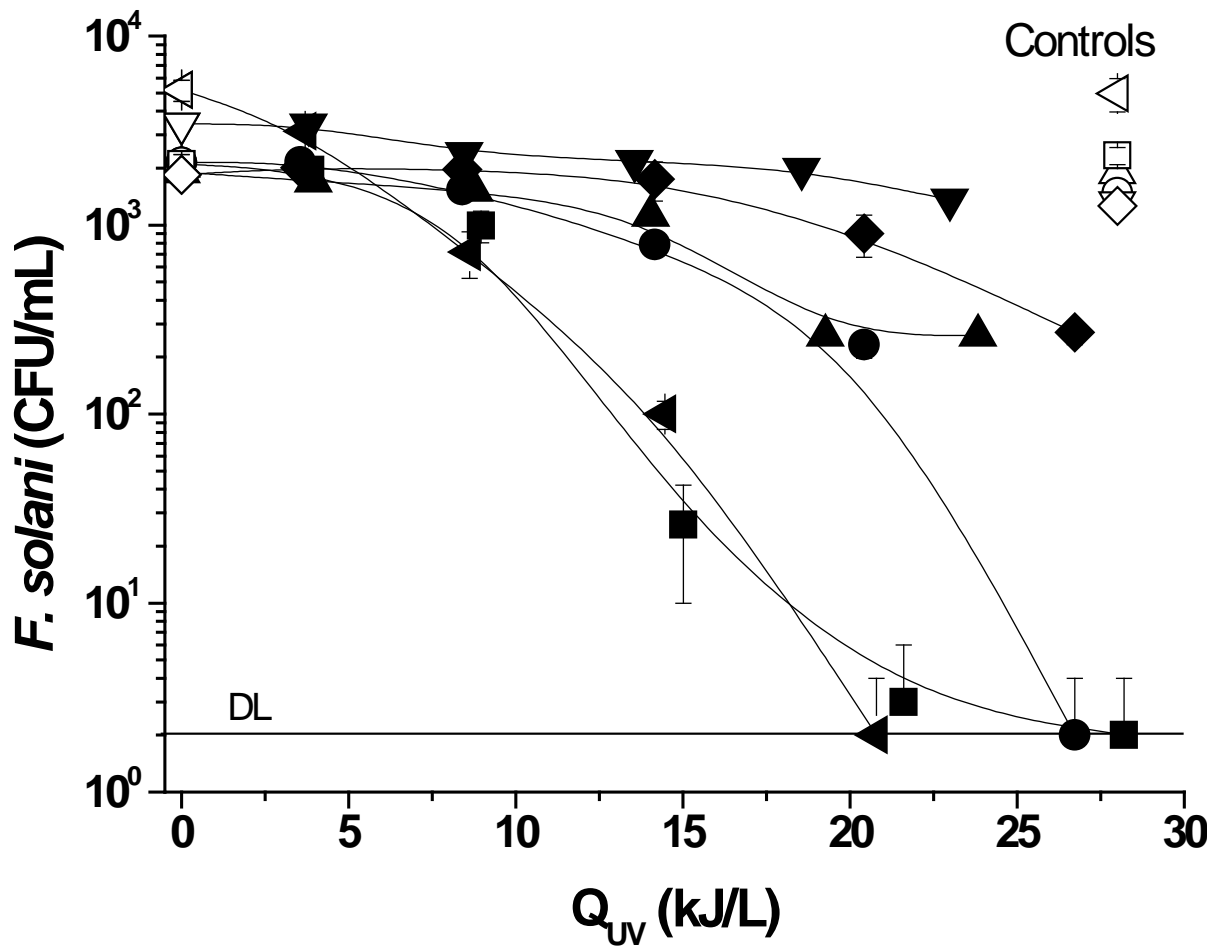


Figure 3

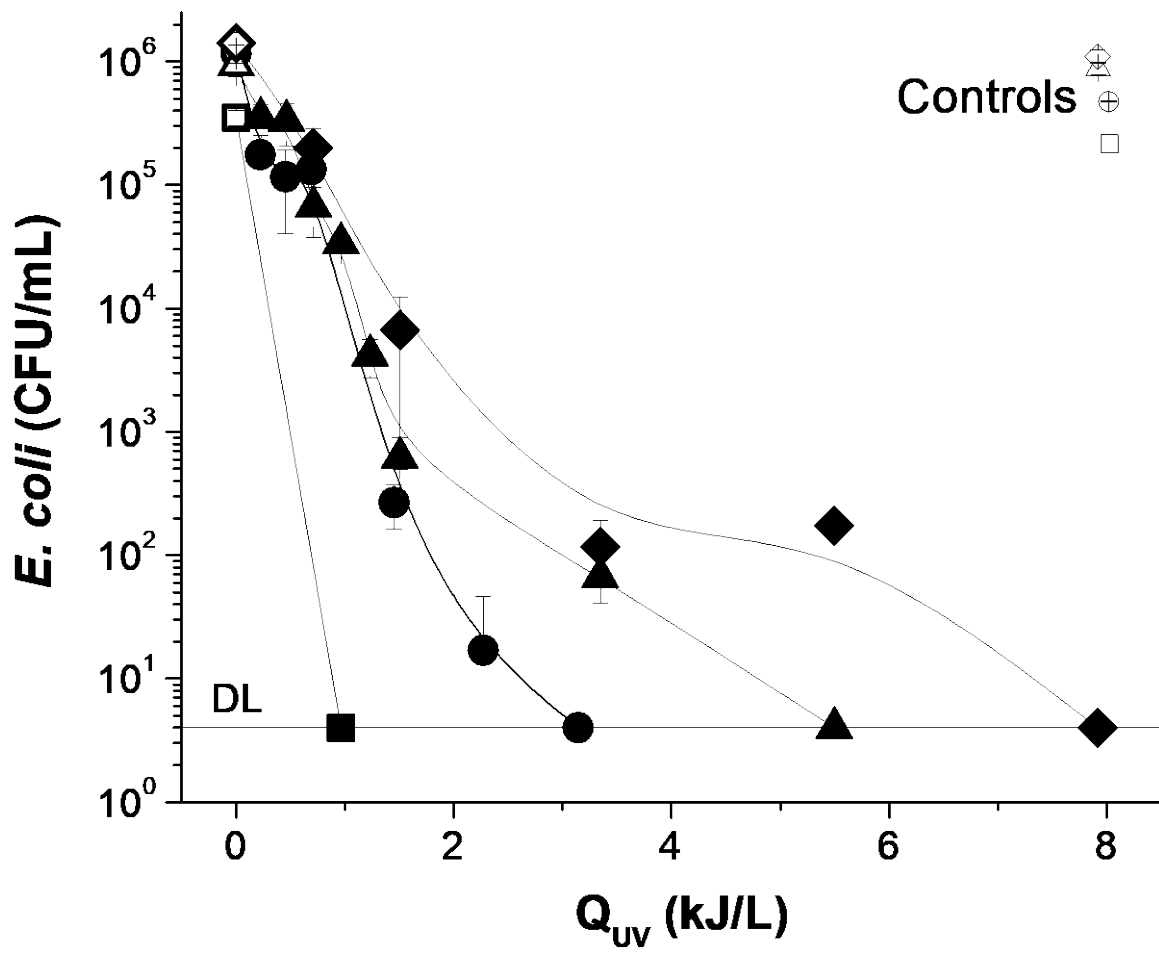


Figure 4

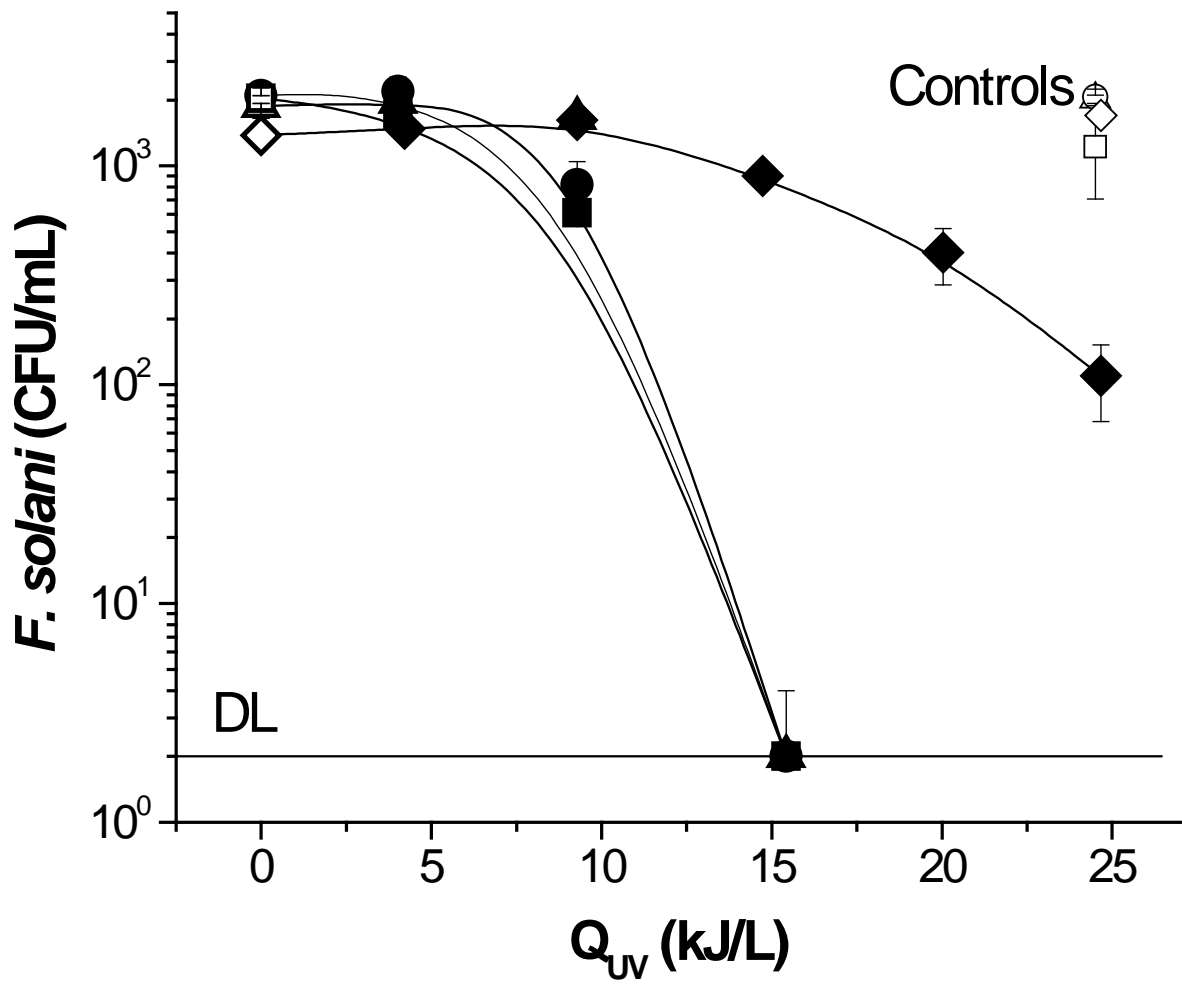


Figure 5

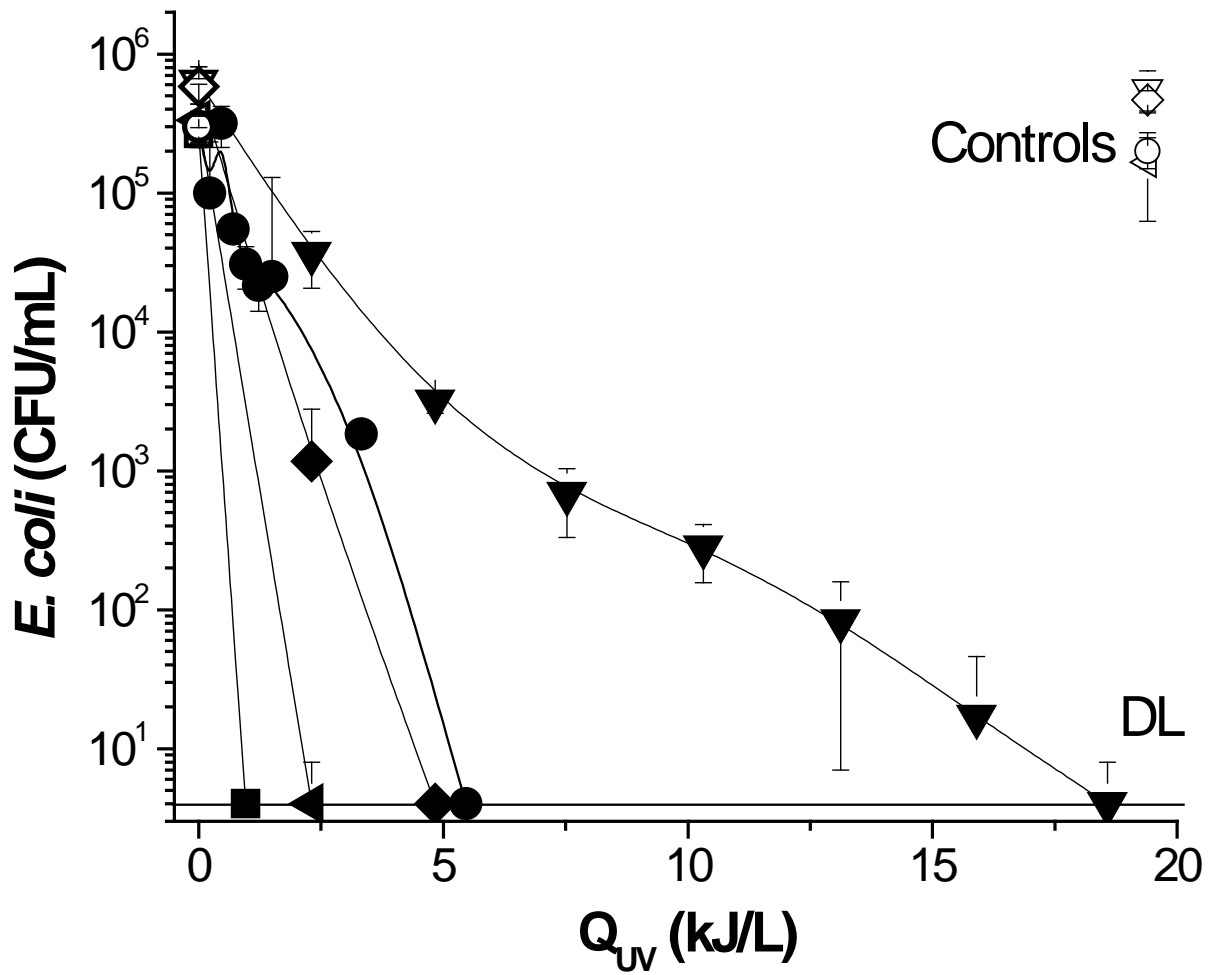


Figure 6.

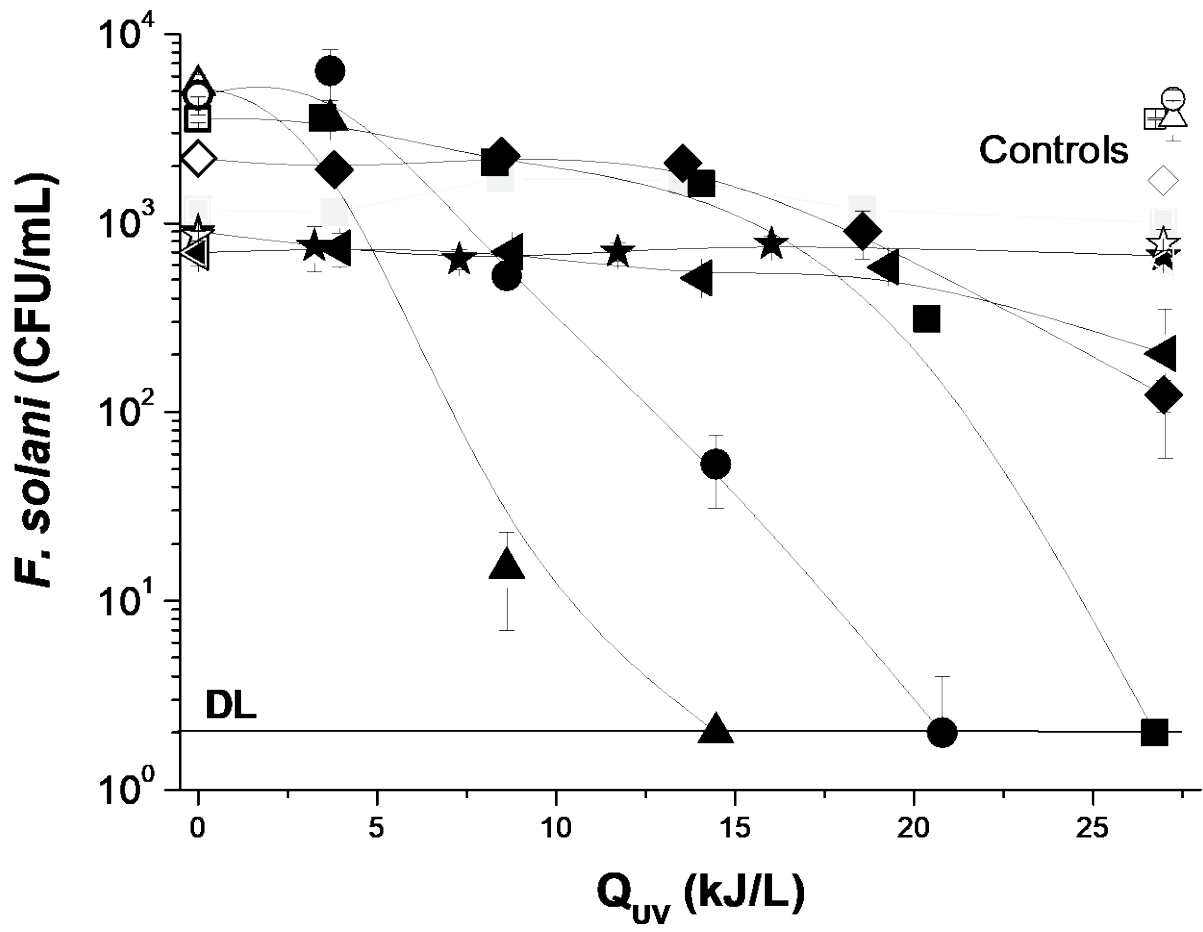


Figure 7

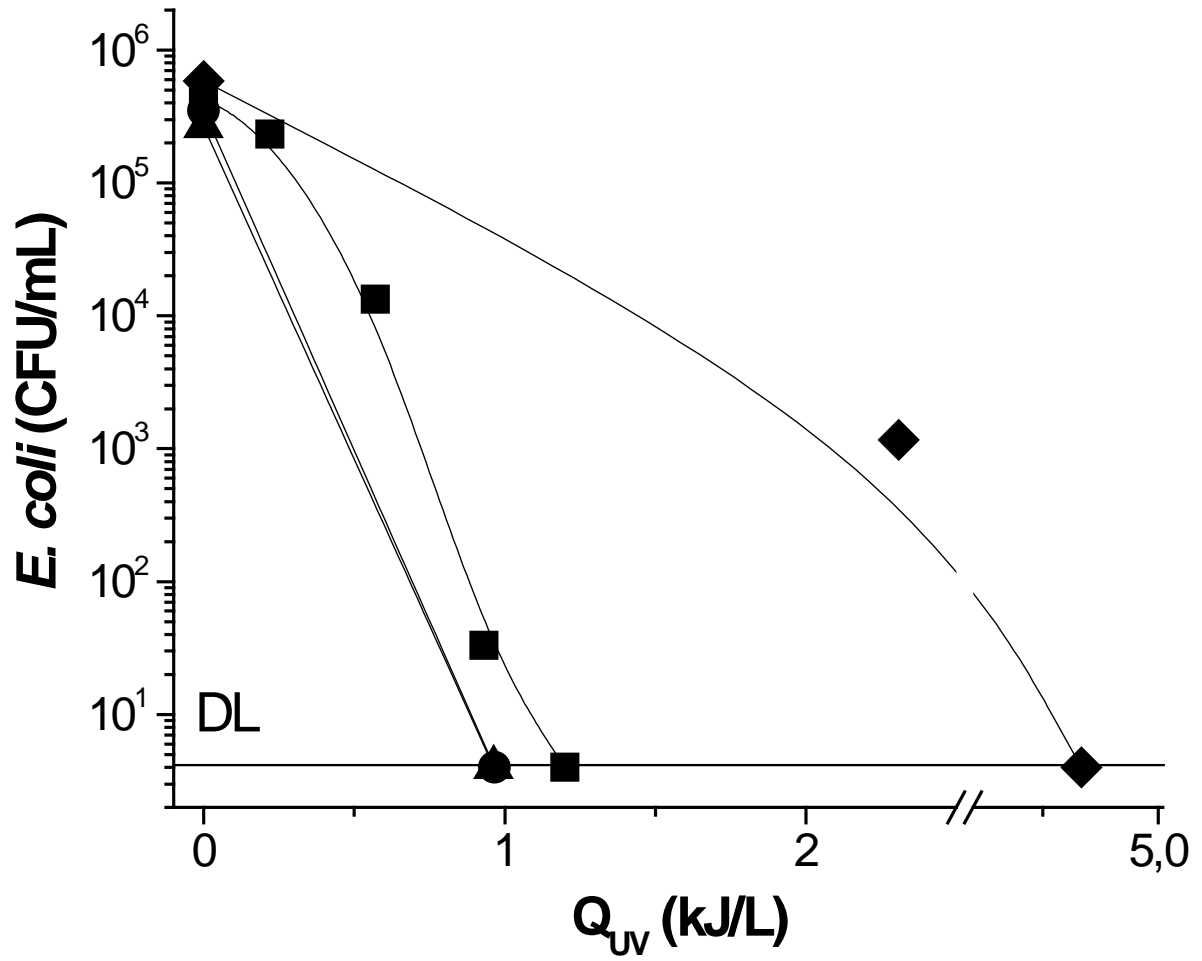


Figure 8

