1 **Title:** Assessment of solar photo-Fenton, photocatalysis, and H_2O_2 for removal of

2 phytopathogen fungi spores in synthetic and real effluents of urban wastewater.

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1   **Abstract**

2   Scarcity of fresh water is a major environmental problem, and properly treated

3   wastewater could be an alternative renewable water resource, especially for agriculture as

4   the final point-of-use. But before wastewater can be reused, it must be treated to meet

5   chemical and biological quality standards, which depend on the final use and legislation.

6   Advanced Oxidation Processes (AOPs) have been demonstrated to be very efficient in

7   decreasing the pathogen load in contaminated water. This study presents the experimental

8   evaluation of several solar-driven AOPs, i.e., photo-Fenton (Fe^{2+}, Fe^{3+}) at low reagent

9   concentration, heterogeneous photocatalysis (TiO_2), and solar photoassisted H_2O_2

10   treatment for removal of the spores of *Fusarium* sp, a worldwide phytopathogen. The

11   experimental work was done in a pilot solar photoreactor with compound parabolic

12   collectors (CPC). Disinfection of *Fusarium solani* spores by all treatments was excellent

13   in distilled water and in simulated municipal wastewater effluent (SMWWE).

14   Degradation of dissolved organic carbon (DOC) was also evaluated. The inactivation

15   rates varied depending on the water matrix, and disinfection was fastest in distilled water

16   followed by SMWWE. The best *F. solani* inactivation rate was with photo-Fenton

17   treatment (10/20 mg/L of Fe^{2+}/H_2O_2) at pH 3, followed by H_2O_2/Solar (10 mg/L) and

18   finally TiO_2/Solar was the slowest. These results underline the importance of solar AOPs

19   and the CPC reactor technology as a good option for waterborne pathogen removal.

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30   **Keywords:** *Fusarium* sp, Compound Parabolic Collector, Photo-Fenton, Solar radiation,

31   Titanium dioxide, Wastewater reuse.

1. Introduction
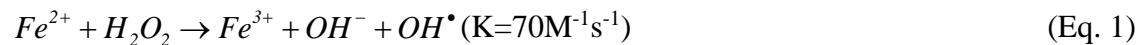
Reuse of wastewater is currently one of the strongest alternative solutions for water scarcity [1], and the need to make use of this resource is constantly increasing due to urban, industrial and agricultural water pollution [1]. Reuse for agriculture is of special interest, because according to the FAO, it is the largest fresh water consumer [2]. Scarcity of fresh water sources, biological contamination of water, and soil salinization affect food production worldwide. To save water, especially where there is a salinity problem and/or water sources are scarce, hydroponic agriculture could be a solution, as plants are grown in a recirculating nutrient solution instead of soil, thereby reducing the water requirement and making use of land lost to salinization. In addition, it can also make use of water from properly treated wastewater effluents [3]. However, before waste water can be used, any pathogens must be removed, because this is one of the most common ways that diseases are spread in hydroponic crops. The largest group of pathogens is the fungi, which also produce the most diseases in plants. The pathogen load in water reclaimed for irrigation must therefore be reduced to decrease the incidence of crop diseases and health risk to agricultural products [4].

Traditional techniques used to eliminate pathogens from water do not always ensure complete disinfection. Chemical pesticides, fungicides, etc., increase pollution of soil and fresh water and are often ineffective. The use of chlorine has been restricted because of its generation of hazardous trihalomethane by-products in the presence of organic matter, and other traditional chemical fungicides, such as etridiazol, are known to become phytotoxic [5]. On the other hand, interest of researchers in new water treatment techniques like the Advanced Oxidation Processes (AOPs) has increased in the last few decades. These processes have been widely studied since the 60s [6]. AOPs generate oxidizing species (especially OH$^\bullet$) which attack organic chemical compounds, often completely mineralizing them into CO_2 and H_2O [7]. Research on the degradation of hazardous chemical compounds in water by AOPs, and in particular, photo-Fenton and titanium dioxide (TiO_2) using natural solar radiation, should be highlighted [6]. The effectiveness of this technology is especially enhanced by the solar reactors used. AOPs in Compound Parabolic Collector (CPC) reactors have been demonstrated to be highly efficient for removing chemical compounds and pathogens from water [6,8]. The

1 effectiveness of solar photo-Fenton and TiO_2 has been shown for several kinds of

2 microorganisms, spores of fungi like *Fusarium* spp (a large genus of filamentous soil

3 fungi found in water systems worldwide, including reservoirs, rivers, coastal seawater,

4 wastewater effluents, and even hospital water distribution systems [9,10,11]), and

5 bacteria like *E. coli* [12,13].

6   Titanium dioxide is one of the most widely studied photocatalysts. It is a

7 semiconductor particle, which when photoexcited by photons at $\lambda < 385$ nm in water in

8 the presence of oxygen, generates superoxide ions ($O_2^{\bullet-}$) and hydroxyl radicals ($OH^{\bullet}$)

9 [14].

10   More recently, research on photo-Fenton for water disinfection has increased due

11 to its high potential for producing $OH^{\bullet}$ radicals at acid pH (optimum for Fenton reaction

12 2.8). This system consists of generating $OH^{\bullet}$ through the catalytic cycle of iron ions

13 (Fe^{2+}) combined with H_2O_2 irradiated by UV-Vis up to a wavelengths of 600 nm [15]:

14

15 $$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\bullet} \, (\text{K=70M}^{-1}\text{s}^{-1}) \qquad \qquad \text{(Eq. 1)}$$

16 $$Fe(OH)^{2+} + h\nu \rightarrow Fe^{2+} + OH^{\bullet} \qquad \qquad \text{(Eq. 2)}$$

17

18   An alternative to the above mentioned solar AOPs is solar photo-assisted H_2O_2

19 (H_2O_2/Solar). This process has recently shown good inactivation efficiencies using less

20 than toxic H_2O_2 concentrations to remove microorganisms like bacteria and fungi in

21 water [11,12,16,17].

22   This article reports on the efficiency of solar-driven photo-Fenton with Fe^{2+} and

23 Fe^{3+}, TiO_2 and H_2O_2/sunlight in a 60-L CPC reactor for inactivating spores of *Fusarium*

24 *solani* in distilled water (DW) and simulated municipal wastewater effluent (SMWWE).

25

26 **2. Materials and methods**

27 **2.1 Compound Parabolic Collector (CPC) solar reactor**

28   The CPC solar photo-reactor (Figure 1a) used for all experiments is described

29 elsewhere [10,11]. It consists of two CPC mirror modules, with 20 borosilicate-glass

30 tubes. The total irradiated collector surface is 4.5 m^2 and the illuminated water volume is

31 45 L over a total volume of 60 L. The flow rate is set at 30 L/min for turbulent flow

1 (Reynolds = 16,600). pH, dissolved oxygen, and temperature were continuously

2 monitored and recorded by software.

3

4 **2.2 Enumeration and quantification of *Fusarium solani* spores**

5   *F. solani* was isolated from samples taken in the Andarax River in Almería,

6 Spain, as described elsewhere [9,10,11]. Spores were counted using the pour plate

7 technique by spreading 50, 250, 500 µL in acidified malt agar (Panreac, Spain). Spore

8 concentration is expressed as colony forming units per milliliter (CFU/mL). The

9 quantification method detection limit (DL) is $2(\pm 2)$ CFU/mL. Three replicates were done

10 of all samples and treatments. For real wastewater experiments, all naturally occurring

11 fungi (so-named 'other fungi' in Figure 7b) present in the effluent were detected and

12 enumerated using same growing media and culture protocol as that used for *Fusarium.*

13 These water samples were plated without any dilution and the DL was the same.

14

15 **2.3 Solar treatments**

16   All experiments were conducted in triplicate at the PSA under natural solar

17 radiation on completely sunny days and lasted 5 h. The results of the three replicates were

18 highly reproducible. The average of the results is reported along with an error equal to the

19 standard deviation. Reactor tank was filled with 60L of either distilled water, or synthetic

20 WW effluent, or real WW effluent, for each type of experiment. In all cases, *Fusarium*

21 suspension and reagents were added to the CPC reactor water and recirculated in the dark

22 for 15 min (homogenization time). After that, the reactor was uncovered and 10-mL

23 samples were taken during the experiment for spore and reagent quantification. The first

24 sample was used to determine the starting spore concentration and as a control, and

25 therefore, it was kept in the dark at 25°C and re-plated again at the end of the experiment.

26 Fungal regrowth (48 h) was also evaluated at the end of the experiment, and no regrowth

27 was found in water samples when the DL was reached.

28   For the case of synthetic effluents disinfection, the following solar runs were

29 done: (i) H_2O_2/sunlight (10 mg/L); (ii) TiO_2/sunlight (100 mg/L); (iii) photo-Fenton at pH

30 8, with Fe^{2+}/H_2O_2: 5/10 mg/L; (iv) photo-Fenton at pH 3 with Fe^{2+}/H_2O_2: 5/10 mg/L and

31 10/20 mg/L, same photo-Fenton tests with additional H_2O_2 dosing to avoid limitation of

1   photo-Fenton reactions by lack of H_2O_2, therefore the minimum H_2O_2 concentration was

2   above 2 mg/L during the treatment in all cases; (v) photo-Fenton at pH3 with Fe^{3+}/H_2O_2:

3   5/10 mg/L and 10/20 mg/L, and same photo-Fenton tests with similar H_2O_2 adding.

4   For the case of real effluents disinfection, the following solar runs were done: (i)

5   H_2O_2/sunlight (10 mg/L) with adding of 60 mg/L (6 times 10 mg/L to maintain the level

6   of H_2O_2 in the water); (ii) TiO_2/sunlight (100 mg/L); (iii) photo-Fenton at pH 3, with

7   Fe^{2+}/H_2O_2: 5/10 mg/L with additional H_2O_2 dosing of 50 mg/L.

8

9   **2.4 Types of water**

10   Distilled water was used as a model water to study inactivation behavior in

11   absence of organic and inorganic salts. The simulated municipal wastewater effluent

12   (SMWWE), which avoided fluctuation in Dissolved Organic Matter (DOC) generated by

13   the microbiological load and the chemical compound variability of real MWWE, was the

14   complex water. SMWWE with 25 mg/L of DOC was used as the wastewater effluent

15   model. The same kind of simulated wastewater effluent has been used elsewhere [11,17].

16   Real effluents from a municipal wastewater treatment plant in Almería were

17   freshly collected for the experiments (RMWWE). This effluent proceeds from a

18   secondary treatment, i.e. activated sludge treatment followed by sedimentation in settling

19   ponds. RWWE had an average DOC of 16.5 mg/L, dissolved inorganic carbon of 72

20   mg/L, turbidity of 8.5 NTU, pH 7.6, and conductivity 1790 μS/cm.

21

22   **2.5 Reagents**

23   H_2O_2 (30 wt%, Riedel-de-Haën, Germany) was used as received and diluted

24   directly in the reactor water. The H_2O_2 concentration was measured during the solar

25   experiment by a colorimetric method with Titanium (IV) oxysulphate (Riedel-de-Haën,

26   Germany) [11,16].

27   Ferrous sulphate heptahydrate ($FeSO_4·7H_2O$, Panreac, Spain) and ferric nitrate

28   ($Fe(NO_3)_3·9H_2O$, Panreac, Spain) were used as the sources of Fe^{2+} and Fe^{3+}, respectively.

29   Iron concentration was determined with 1.10 phenanthroline according to ISO 6332. The

30   mg/L iron to H_2O_2 concentration ratio was 1:2.

1   TiO$_2$ Aeroxide P25 particles (Evonick, Degussa Corp., Germany) were used as

2   received from the manufacturer. The powder was diluted in a small volume of water and

3   then added to the reactor to form a homogeneous suspension.

4

5   **2.6 Solar radiation**

6   A global UVA pyranometer (295–385 nm, Model CUV4, Kipp & Zonen,

7   Netherlands) tilted 37 degrees was used to measure UV irradiance (in W/m^2) during the

8   experiments. As the experiments were conducted on different days, to compare results,

9   the inactivation kinetics was evaluated as a function of cumulative energy per unit of

10   volume (Q_{UV}, kJ/L) received in the photo-reactor [8]. Q_{UV} is commonly used to compare

11   results under different conditions as calculated by Equation 3:

12

13   $Q_{uv,n} = Q_{uv,n-1} + \Delta t_n UV_{G,n} A_r / V_t; \Delta t_n = t_n - t_{n-1}$ (Eq. 3)

14

15   where $Q_{uv,n}$ and $Q_{uv,n-1}$ is the cumulative UV energy per liter (kJ/L) at times *n* and *n-1*;

16   $UV_{G,n}$ is the average incident radiation on the irradiated area (W/m^2), Δt_n is the

17   experimental time of sample (s), A_r is the illuminated area of collector (m^2), and V_t is

18   the total volume of water treated (L).

19   All experiments started at the same local time and lasted 5 hours in consecutive

20   days, so that water temperature and solar UVA irradiance was similar between different

21   solar tests. The average solar UVA irradiance during all the experiments was 35.3($\pm$1.2)

22   W/m^2, with maximum and minimum values of 28.2($\pm$1.2) W/m^2 and 49.1($\pm$2.0) W/m^2,

23   respectively (Figure 1b). The typical solar spectrum recorded in the location of the

24   experiments during one of these days is also presented (inset of Figure 1b).

25

26   **2.7 Kinetics reaction**

27   The inactivation kinetics of the different treatments was calculated using the Q_{UV}

28   parameter instead of the duration of the experiment. For this purpose Chick's law was

29   adjusted to the photo-chemical results using the solar UVA energy received in the photo-

30   reactor (Q_{UV}) which also takes time into account (see Eq. 3)[8]:

1

$$Log\left(\frac{N}{N_0}\right) = -k'\cdot Q_{UV}$$

(Eq. 4)

2   where N/N_0 is the reduction in spore concentration, k' is the disinfection kinetic rate, and

3   Q_{UV} is the solar UVA energy received in the photo-reactor.

4

5   **3. Results and Discussion**

6   **3.1 Spore inactivation by H_2O_2/Solar**

7   Figure 2 shows the inactivation kinetics of *F. solani* spores in DW and SMWWE

8   with 10 mg/L of H_2O_2 added. The detection limit was reached in DW with a cumulative

9   solar UV irradiance of 13.3 kJ/L. In SMWWE, a similar result required 18.9 kJ/L of Q_{UV}.

10   pH did not show change significantly and remained at 6.7 and 8 in DW and SMWWE,

11   respectively. DOC in SMWWE is shown in Figure 2, where no significant reduction

12   (2 %) is observed.

13   Previous findings have shown *Fusarium* spp spore susceptibility to the synergistic

14   killing effect of H_2O_2/Solar. Sichel et al. demonstrated that addition of H_2O_2 at low

15   concentrations (5-500 mg/L) could enhance the inactivation effect of solar-only

16   disinfection, while similar concentrations in the dark did not have any negative effect on

17   spore viability [16]. *F. equiseti* chlamydospores were also found to be susceptible to

18   inactivation with 10 mg/L H_2O_2 and solar radiation, achieving higher inactivation kinetics

19   in DW than in SMWWE [11]. Results of our study were similar for *F. solani*

20   microconidia, where inactivation efficiency was higher in DW than SMMWE. This could

21   be because, the diffusion of H_2O_2 inside cells in absence of any chemical compound may

22   be faster than in presence of organic matter, which under certain conditions, is known to

23   react with H_2O_2 to form disinfection by-products (DBPs) [18]. Therefore, the H_2O_2

24   reaction with organic matter in SMWWE could cause lower fungi inactivation kinetics

25   than in DW.

26   The inactivation mechanism of this treatment has also been described in the

27   literature for bacteria like *E. coli* [12], *Salmonella* and coliforms [19], and for fungi

28   spores [11]. This mechanism is based on the H_2O_2 diffusion inside cells, reacting with the

1 free iron (labile iron pool) present inside them, and generating $OH^\bullet$ by reactions similar to

2 photo-Fenton (Eq. 1-2), which damage internal structures and finally lead to cell death.

3

4 **3.2 Spore inactivation by TiO₂/Solar**

5   Solar photocatalytic experiments were conducted using 100 mg/L of TiO_2

6 Aeroxide P25 slurry catalyst. The best conditions for TiO_2 suspensions in the 60-L CPC

7 photo-reactor for similar fungi strains have previously been reported to be 100 mg/L [10].

8 To achieve complete spore inactivation, 31.8 kJ/L of Q_{UV} were required (Figure 3), and

9 DOC was reduced 56% at the end of the experimental time with 55.42 kJ/L of Q_{UV}. The

10 highest temperature was 44.1ºC and pH was almost constant at 7.8.

11   The microorganism inactivation efficiency by TiO_2 with solar energy has been

12 widely studied during the last decades for bacteria to cancer cells [6,19], including

13 *Fusarium* sp spores in a 200-mL solar bottle reactor [9], a 14-L CPC photoreactor [8] and

14 60-L CPC photoreactor [10].

15   Inactivation strongly depends on the $OH^\bullet$ radicals generated during photocatalysis.

16 First the cell wall is attacked and then cell integrity is disrupted, leading to cell death

17 [20]. Irradiated TiO_2 surfaces react with intermediate hydroperoxide, initiating cascades

18 of autoxidation reactions [21]. The formation of peroxidation products such as aldehydes,

19 ketones, and carboxylic acids at the same time as cell wall membrane constituents

20 disappear has been reported in the literature [21]. Fungi inactivation kinetics could be

21 enhanced by the formation of aggregates of catalyst and microorganisms, allowing attack

22 by $OH^\bullet$ generated directly on the microorganism cell wall. This effect has been well

23 documented in *Fusarium* sp spores by direct observation of the aggregates formed during

24 the experiments [10]. On the other hand, chemicals like carbonates and bicarbonates in

25 water limit the photocatalytic reaction, as they act as $OH^\bullet$ scavengers [22]. SMWWE

26 contains approximately 17 mg/L of Inorganic Carbon (IC) at pH8, which could diminish

27 the efficiency of the photocatalytic treatment. However, these results showed good spore

28 inactivation and DOC degradation even in their presence.

29

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31 **3.3 Spore inactivation by photo-Fenton (Fe^{2+} or Fe^{3+}) in distilled water.**

Figure 4a shows spore inactivation by photo-Fenton with different Fe^{2+}/H_2O_2 concentrations, (5/10 and 10/20 mg/L, respectively). H_2O_2 dosing during the solar treatment was also evaluated. pH was not adjusted to optimum (pH 2.8) to avoid any chemical modification in the water matrix. Table 1 shows H_2O_2 consumption, pH, Fe^{2+}, Fe^{3+} and total iron (Fe^T) measured during the experiments. Dissolved iron measured during the experiment was almost the total starting amount, and high iron loss due to precipitation at neutral pH was not observed. The detection limit (DL) was reached in all cases. No significant differences were observed among the different test conditions. The energy required to reach the DL varied from 26 to 29.5 kJ/L of Q_{UV}, with best results with 5 and 10 mg/L of Fe^{2+}/H_2O_2 (Figure 4a). Therefore, in DW, neither the addition of H_2O_2 nor increasing iron concentration from 5 to 10 mg/L led to better microconidia inactivation kinetics.

Similar conditions were evaluated using Fe^{3+} with inactivation results as shown in Figure 4b. In all cases, the DL was met, and a slight enhancement of disinfection efficiency was observed for 5/10 mg/L of Fe^{3+}/H_2O_2. On the other hand, no significant improvement was achieved with higher iron concentrations (10/20 mg/L of Fe^{3+}/H_2O_2).

Microorganism inactivation by solar photo-Fenton is the result of accumulated damage done by the following simultaneous or sequential processes: (i) Reactive Oxygen Species (ROS) generated by the direct action of sunlight, which mainly attack the DNA molecules, generating cross links; (ii) the $OH^•$ generated by photo-Fenton reactions (Eq. 1-2), affecting mainly the integrity of the external cell wall of the spores.

Nevertheless, when inactivation results with 5 and 10 mg/L of $Fe^{2+ \text{ or } 3+}$ are compared, photo-Fenton inactivation with Fe^{2+} (26.5 kJ/L, in 3 hours) required less solar UV-energy than Fe^{3+} (37.9 kJ/L, in 4 hours) (Fig. 4a and 4b). This difference can be attributed to the different biological behavior of the iron depending on its speciation in the water. In the literature, Fe^{2+} could reportedly cross biological membranes freely, while Fe^{3+} could form exciplexes with organic compounds in the cell wall [12,23]. Therefore, Fe^{2+} and Fe^{3+} inactivation mechanisms may behave different. Fe^{2+} diffusion inside cells may increase the inactivation efficiency by generating $OH^•$ which reacts with metabolic H_2O_2 according to Eqs. 1-2, while Fe^{3+} may form complexes in the cell wall where it causes damage. It should be remarked that the starting total dissolved iron (Fe^T)

10

1   concentration of 5 mg/L remained nearly constant during the experiment for Fe^{2+}, while it

2   decreased from 4.73 to 1.89 mg/L for Fe^{3+}. This difference could also account for lower

3    $OH^{\bullet}$ generation by Fe^{3+} than Fe^{2+}.

4   Inactivation of *F. solani* spores was not enhanced by increasing Fe^{2+} or Fe^{3+}

5   concentration from 5 to 10 mg/L, which is in agreement with previous findings [17]. This

6   is explained by precipitation of the iron in water. In both cases, high losses of the initial

7   iron added were observed. This is probably the reason why increasing iron over 5 mg/L

8   does not lead to significant improvement of spore inactivation efficacy. These results

9   show that regardless of how much iron is added at the beginning, it is the amount actually

10   dissolved that is important to photo-Fenton efficiency.

11   On the other hand, the tests with dosed H_2O_2 showed the same efficiency as those

12   without it. H_2O_2 dosing depended on consumption during solar treatment according to

13   Eq. 1. In this case, only was required the addition of a 10 mg/L dose of H_2O_2 during the

14   experiment which did not result in any enhancement in process efficiency (Fig. 4a).

15

16   **3.4 Spore inactivation by photo-Fenton (Fe^{2+} and Fe^{3+}) in SMWWE.**

17   Spore inactivation was evaluated in SMWWE to study the effect of this water on

18   photo-Fenton efficiency. 5 and 10 mg/L of Fe^{2+} or Fe^{3+} combined with 10 and 20 mg/L of

19   H_2O_2, respectively, and the effect of dosing H_2O_2 during the experiment were studied.

20   The DOC concentration was also measured during all tests. Data on pH, H_2O_2 and iron

21   concentration are shown in Table 2.

22   Figure 5a shows that all the photo-Fenton reagent combinations at pH 3 and

23   concentrations tested with Fe^{2+} reached the DL, except for natural pH (pH ~ 8). In that

24   case both spores and DOC concentrations remained almost constant during 5 hours of

25   solar exposure, and dissolved Fe^{T} concentration measured was 0 (Table 2), i.e., all the

26   iron added was precipitated and there was no iron available to produce oxidative damage

27   to spores.

28   Moreover, chemical species like carbonates/bicarbonates present in SMWWE

29   decrease process efficiency, as they scavenge radicals like $OH^{\bullet}$, and compete with other

30   organic contaminants for $OH^{\bullet}$. This also occurs with phosphate, sulphate, fluoride,

31   bromide and chloride, etc. [6]. Therefore, for the photo-Fenton assays, the pH of the

1   water was adjusted to ~3 (optimum for photo-Fenton [15]) for several reasons: i) spore

2   inactivation at pH8 was very poor; ii) the iron concentration was entirely precipitated; iii)

3   the viability of *Fusarium* sp spores was not affected at acid pH [17]; and iv) the

4   acidification of the solution also allows carbonates/bicarbonates to be eliminated as CO_2.

5   The detection limit was met with 5/10 and 10/20 mg/L of Fe2+/H2O2, with no

6   significant differences observed between those conditions, which required 20 and

7   18.9 kJ/L of Q_{UV}, respectively (Fig. 5a). Therefore, increased iron concentration did not

8   enhance spore inactivation efficiency. However, DOC was lowered more with 10 mg/L

9   of Fe^{2+} (75%) than with 5 mg/L (65%) (Fig. 5b).

10   The enhancement of inactivation kinetics by dosing H_2O_2, observed in all

11   SMWWE tests (Fig. 5b), was not observed in DW. In these experiments, H_2O_2 demand

12   was high, and therefore, the 10 mg/L doses had to be added every 30 minutes. Both spore

13   inactivation and DOC reduction were enhanced for 5 and 10 mg/L of Fe^{2+} with

14   continuous addition of H_2O_2. The best inactivation rate was achieved with 10 mg/L of

15   Fe^{2+}, which required 8.4 kJ/L of Q_{UV}. At the end of the experiment, 83% DOC reduction

16   was obtained with a Q_{UV} of 44.8 kJ/L.

17   The pattern of spore inactivation kinetics with Fe^{3+} (Fig. 6a) was similar to Fe^{2+},

18   i.e., dosing of H_2O_2 during the experiment led to better spore inactivation results, and

19   increasing iron concentration from 5 to 10 mg/L did not significantly enhance them. DOC

20   degradation (Fig. 6b) was slightly better for 10 mg/L of iron, although no significant

21   difference was observed between the two cases.

22   Of the two iron salts, Fe^{2+} inactivation kinetics were better, requiring 8.4 kJ/L and

23   12.1 kJ/L of Q_{UV} for 10 and 5 mg/L of iron, respectively, and dosed H_2O_2, while the same

24   concentrations of Fe^{3+} needed 12.3 kJ/L and 13.9 kJ/L of Q_{UV} for the same inactivation.

25   On the contrary, DOC reduction was very similar in all cases. This means that

26   degradation of chemical compounds is not affected by the starting amount of iron salt

27   added to the photo-Fenton cycle.

28   Spore inactivation mechanisms are similar to those described in the previous

29   section. The direct action of sunlight, OH• generated by Eqs. 1-2 and OH• generated by

30   internal photo-Fenton reactions are mainly responsible for inactivation with Fe^{2+}. In

31   SMWWE, the Fe^{3+} reaction with inorganic compounds like sulphates and chlorides may

1   reduce process efficiency. These inorganic compounds have been shown to reduce the

2   H_2O_2 decomposition rate for Fe^{3+}/H_2O_2 in the dark. This effect is due to the formation of

3   Fe^{3+} complexes with the generation of less reactive ($SO_4^{\bullet-}$) or much less reactive ($Cl_2^{\bullet-}$)

4   species [24]. Thus the presence of inorganic anions may affect iron reactivity, and can

5   also scavenge $OH^{\bullet}$ radicals, producing less reactive inorganic radicals [24].

6   Water temperature can be also an important factor affecting inactivation

7   efficiency in SMWWE. Mild temperatures (from 25 to 45°C) may affect process

8   efficiency in different ways. Raising the temperature in this range increases the

9   disinfection rate, favoring spore germination, affecting distribution of iron and the H_2O_2

10 consumption rate in the photo-Fenton reaction. Very few studies have reported on the

11 effect of high temperatures (over 50°C) in the photo-Fenton reaction. Gernjak *et al.*

12 (2006) found that under certain conditions (maximal iron concentration 2.6 mM and

13 maximal temperature 70°C) the reaction rate increased about 5 times in terms of H_2O_2

14 consumption by increasing temperature from 20°C to 50°C, which did not affect the iron

15 concentration [25]. In view of the above considerations, the effect of increasing the

16 temperature required more H_2O_2 to be added during the dosing experiments, i.e., the

17 increased temperature determined that 10 mg/L doses of H_2O_2 had to be added every 30

18 minutes to maintain H_2O_2 concentration. The effect of the water matrix composition on

19 H_2O_2 demands was discarded because H_2O_2 demands in experiments performed with DW

20 at higher temperatures (data not shown) were also higher.

21   Total consumption of H_2O_2 for 5 and 10 mg/L of Fe^{2+} was 50 and 100 mg/L,

22 respectively, while 10 and 20 mg/L of H_2O_2 were consumed when no H_2O_2 was added. In

23 the experiments done with Fe^{3+}, 70 and 140 mg/L of H_2O_2 were consumed during the

24 dosage experiments. Spore inactivation and DOC degradation were slightly enhanced

25 when H_2O_2 was added. DOC degradation became stationary where the optimized

26 photoreaction conditions did not lead directly to organic matter degradation

27 enhancement. This is because the organic matter present in the SMWWE consists mainly

28 of aliphatic compounds like carboxylic acids which are very difficult to degrade past a

29 certain percentage.

30   Table 3 shows the inactivation kinetics for SMWWE treatments. The highest

31 inactivation rate was found for photo-Fenton with Fe^{2+} (10 mg/L + dosed H_2O_2).

Nevertheless, there are no significant differences in inactivation rates among the treatments.

Moreover, for solar treatment applications in the field, it should be taken into account that the solar treatments studied require post-treatment (pH neutralization and catalyst removal) before the treated wastewater can be reused. TiO_2 catalyst should be removed from the treated water prior to reuse, for which sedimentation/flocculation and filtration are necessary. For H_2O_2/solar, post-treatment is unnecessary if the reagent concentration is below 50 mg/L (10 mg/L), as it is non-toxic for plants [16]. Moreover, Bichai *et al.* demonstrated the ability of H_2O_2 (10 mg/L)/solar to disinfect real and simulated MWWE contaminated by *E. coli*, and the treated WW was reused for lettuce irrigating [26]. Small amounts of iron (~10 mg/L) should be used for photo-Fenton treatments to meet water pollution control regulations (e.g., Spanish Royal Decree 849/1986, on Regulation of Water Pollution Control), and these amounts could also be used as fertilizer. On the other hand, H_2O_2 concentrations added for photo-Fenton could be neglected as it is consumed by the reactions. The major post-treatment of photo-Fenton is neutralization of the pH, as low pH could damage plants.

3.5 Spore inactivation by three solar AOPs in real MWWE

For experimental validation of the capabilities of these solar AOPs for WW disinfection, the following evaluation has been done in real MWWE. Figure 7 shows the inactivation efficiency of solar/H_2O_2, TiO_2 at 100 mg/L, and photo-Fenton with ferrous sulphate at Fe^{2+}/H_2O_2: 5/10 mg/L at pH3. These experiments were done at natural pH (8) except photo-Fenton, which was done at pH 3 to avoid iron precipitation. This acidification induced carbonates/bicarbonates content removal from this WW (dissolved inorganic carbon = 72 mg/L), which avoids any scavenging effect of these species for hydroxyl radicals. *Fusarium* inactivation in RWWE (Fig. 7a) was very similar for the three AOPs, reaching the DL at 27 kJ/L of Q_{UV}. Solar H_2O_2 and photo-Fenton in SMME required shorter treatment times and lower Q_{UV} values to achieve similar results, while no differences were observed when TiO_2 photocatalysis was employed.

Similarly to the results obtained with SMWWE, DOC was no reduced using solar/H_2O_2. Regarding solar photocatalysis (TiO_2), DOC reduction in real effluents was

around 2% (data not shown), while in synthetic effluents this reduction was 55% (Figure 3). This may be attributed to the high complexity (chemical and biological) of real WW effluents compared with synthetic ones. Photo-Fenton led to a 34% reduction in the organic matter (data not shown). This DOC decrease in not very high because the effluent proceeds from an activated sludge treatment and the chemical composition is based in highly degraded/oxidized compounds.

The role of organic matter in water disinfection by AOPs remains still uncertain. There are controversial studies regarding this matter. According recent contributions, the presence of natural organic matter [12, 27] in real WW favor the disinfection performance of photo-Fenton, which was explained by the authors as an accelerating effect of natural photosensitizers. On the other hand, other articles show a clear detrimental effect of organic matter content on the disinfection efficiency [17, 28], attributed to a competence phenomenon between organic compounds and microorganisms. This study adds more experimental evidences of the capability of solar AOPs for real wastewater disinfection, highlighting also the unfavorable effect of organic content.

4. Conclusions

- Our results demonstrate that the application of solar AOPs for water disinfection and further reuse in agriculture may be an efficient practice.
- Different disinfection efficiency results observed for ferrous sulphate and ferric nitrate clearly show that iron speciation in water is a key factor in photo-Fenton; moreover Fe^{2+} was found to be the best option for *Fusarium* removal although, DOC reduction was unaffected by this.
- Some differences in disinfection efficiency were observed between iron (5 and 10 mg/L) and H_2O_2 (10 and 20 mg/L) concentrations. The best disinfection result was observed at 10/20 mg/L of Fe^{2+}/H_2O_2 with periodic adding H_2O_2.
- Experimental testing of these solar AOPs in synthetic and real urban WW effluents led to similar inactivation times to attain the detection limit. In all cases (synthetic

and real), the treatments lasted less than three hours to reduce the *Fusarium* load to the detection limit.

- The 60-L CPC solar reactor used in this study showed that this technology is a promising option for wastewater disinfection in the presence of resistant water microorganisms.

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1   **TABLE CAPTIONS**

2

3   **Table 1.** Concentration of Fe^{2+}, Fe^{3+} and total iron (Fe^T), initial and final, and pH for

4   different photo-Fenton reactions in distilled water (Figure 3).

5

6   **Table 2.** Concentration of Fe^{2+}, Fe^{3+} and total iron (Fe^T), initial and final, and pH for

7   different photo-Fenton reactions in distilled water (Figures 4 and 5).

8

9   **Table 3.** *F. solani* inactivation kinetics constants.

10

FIGURE CAPTIONS

Figure 1. Photograph of the CPC photo-reactor used in this work (a). Solar UVA and spectral irradiance distribution at PSA (Spain) during one of the days of experiment (b).

Figure 2. *F. solani* inactivation in CPC reactor under sunlight and 10 mg/L H_2O_2 in DW and SMWWE.

Figure 3. *F. solani* inactivation in CPC reactor with solar photocatalysis (100 mg/L of TiO_2) in SMWWE.

Figure 4. *F. solani* inactivation kinetics in CPC reactor using photo-Fenton at different ratios of Fe^{2+}/H_2O_2 (a) and Fe^{3+}/H_2O_2 (b) in distilled water.

Figure 5. *F. solani* inactivation in CPC reactor using photo-Fenton with ferrous sulphate in SMWWE at several concentrations (a), DOC reduction during same experiments (b).

Figure 6. *F. solani* inactivation in CPC reactor using photo-Fenton with ferric nitrate in SMWWE at pH3 and several reagent concentrations (a), DOC removal during same experiments (b).

Figure 7. Inactivation profile of *F. solani* (a) and other naturally present fungi (b) in CPC reactor using solar photo-Fenton (ferrous sulphate, pH3), TiO_2, and H_2O_2 in real effluents of MWWE.

1 **Table 1**

2

	Fe/H_2O_2	Fe^{2+}_i/Fe^{2+}_f	Fe^{3+}_i/Fe^{3+}_f	Fe^T_i/Fe^T_f	pH
	Fe^{2+}-Photo-Fenton *(Fig. 3a)*				
(-■-)	5/10	0.8/1.2	2.1/1.1	2.9/2.4	4.8
(-●-)	5/10(+10)*	0.8/1.0	2.8/1.6	3.7/2.6	5.4
(-▲-)	10/20	0.0/1.2	8.4/0.4	8.4/1.6	6.4
	Fe^{3+}-Photo-Fenton *(Fig. 3b)*				
(-■-)	5/10	0.2/1.1	4.5/0.7	4.7/1.9	5.2
(-▲-)	10/20	0.4/1.8	6.9/0.8	7.2/2.7	5.0

3   *Experiment with H_2O_2 dosage

Table 2

	Fe/H_2O_2	addition of H_2O_2 during experim.	Fe^{2+}_i/Fe^{2+}_f	Fe^{3+}_i/Fe^{3+}_f	Fe^T_i/Fe^T_f	pH
			Fe^{2+}**-Photo-Fenton** (*Fig. 4*)			
(-■-)	5/10	-	-	-	-	8.0
(-●-)	5/10	-	0.7/0.7	2.9/2.1	3.6/2.8	3.0
(-▲-)	5/50*	4 x 10 mg/L	1.0/0.5	2.9/0.6	3.9/1.2	3.3
(-♦-)	10/20	-	1.7/2.2	6.6/4.3	8.4/6.6	3.0
(-▼-)	10/20*	4 x 20 mg/L	1.6/1.9	6.2/3.7	7.8/5.7	3.0
			Fe^{3+}**-Photo-Fenton** (*Fig. 5*)			
(-■-)	5/10	-	0.5/1.5	4.6/3.5	5.1/4.9	3.1
(-●-)	5/10*	6 x 10 mg/L	0.4/1.1	3.6/2.3	4.0/3.4	3.1
(-▲-)	10/20	-	0.6/2.5	7.7/4.9	8.4/7.5	3.0
(-▼-)	10/20*	6 x 20 mg/L	0.2/1.1	6.1/6.3	8.2/7.4	3.0

*Experiments with H_2O_2 dosage

1   **Table 3**

Solar treatment	Fe (mg/L)	H_2O_2 (mg/L)	k (L/kJ)	R^2	Kinetic model
H_2O_2 *(Fig. 1)*	-	10	0.10±0.02	0.95	Log-linear
TiO_2 *(Fig. 2)*	-	-	0.07±0.01	0.95	Log-linear
Fe^{2+}-Photo-Fenton *(Fig. 4a)*					
	5 (pH8)	10	0.01±0.00	0.99	Log-linear
	5	10	0.10±0.03	0.95	Log-linear
	5	10+40*	0.17±0.02	0.98	Log-linear
	10	20	0.10±0.01	0.98	Log-linear
	10	20+80*	**0.30±0.10**	0.93	Log-linear
Fe^{3+}-Photo-Fenton *(Fig. 5a)*					
	5	10	0.10±0.01	0.98	Log-linear
	5	10+60*	0.14±0.01	0.99	Log-linear
	10	20	0.12±0.01	0.99	Log-linear
	10	20+120*	0.14±0.03	0.95	Log-linear

2   *Experiments with H_2O_2 dosage

1   **Figure 1**
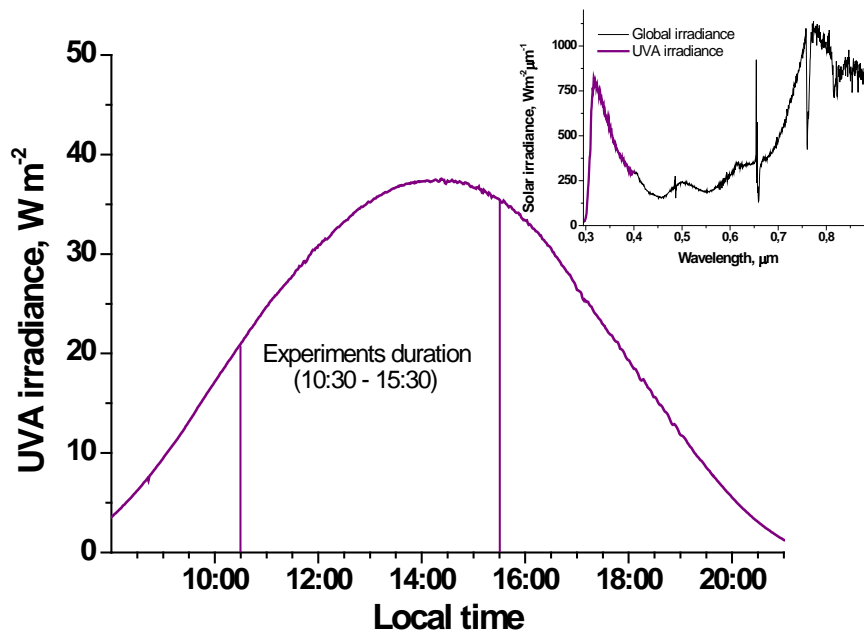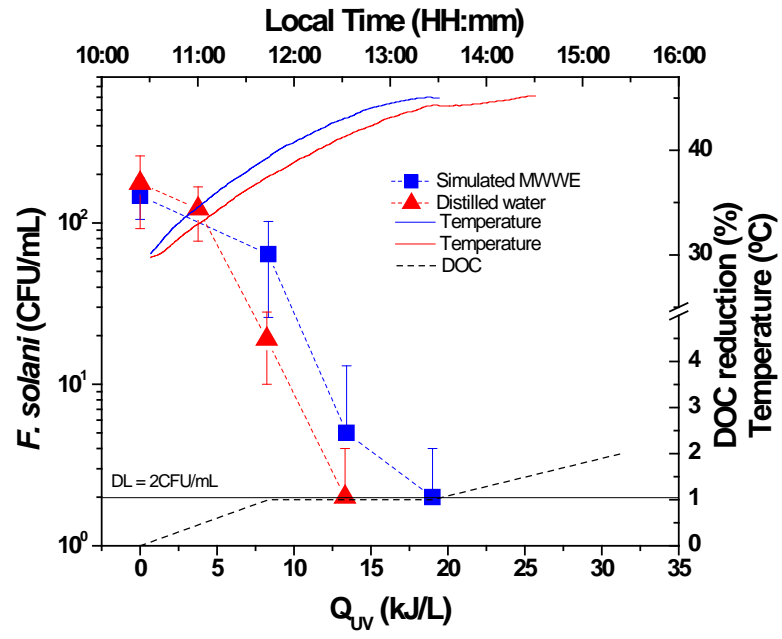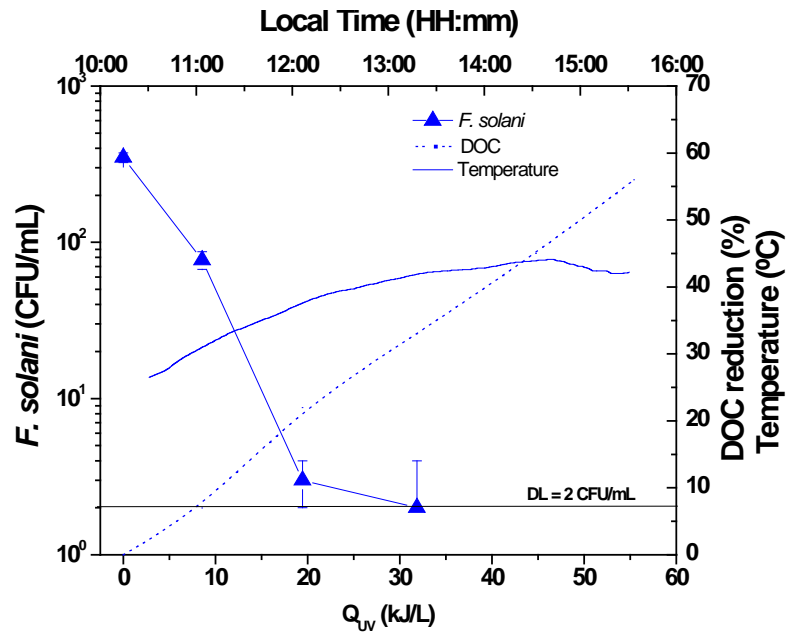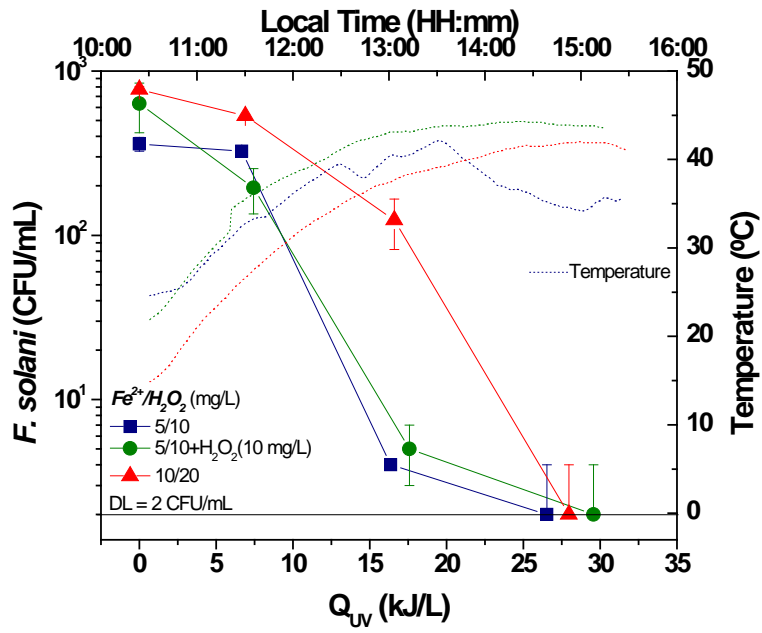
2

3   **(a)**

4

5   **(b)**

1   **Figure 2**

2

3

1 **Figure 3**

2

3

1   **Figure 4**

2

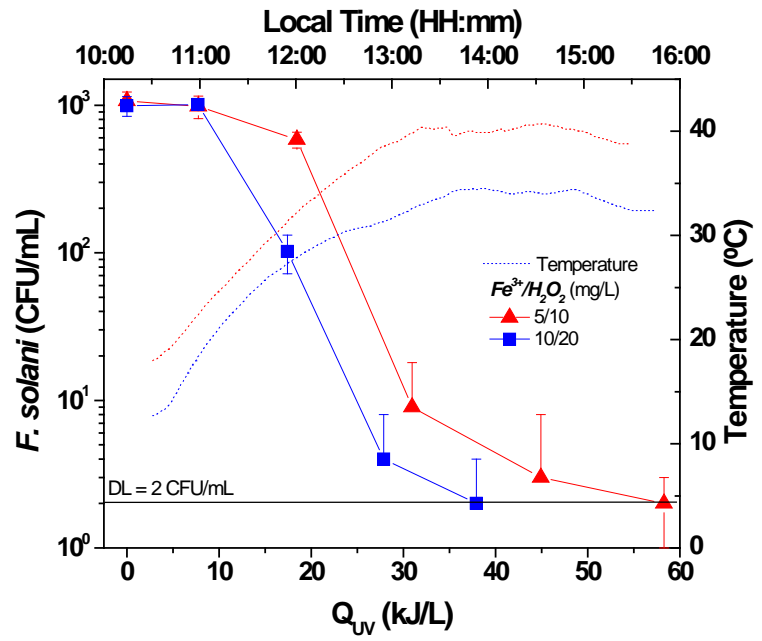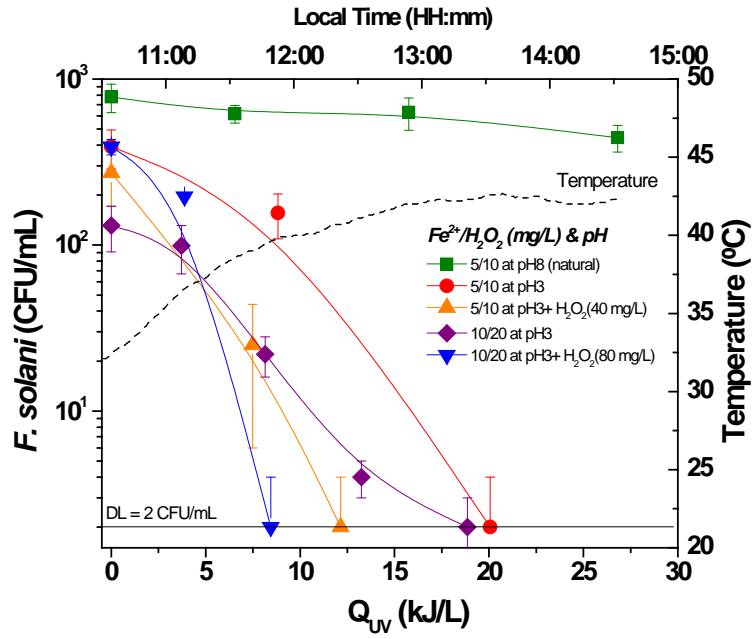3   **a)**

4   **b)**

1   **Figure 5**

2

3   **a)**

4   **b)**

1   **Figure 6**
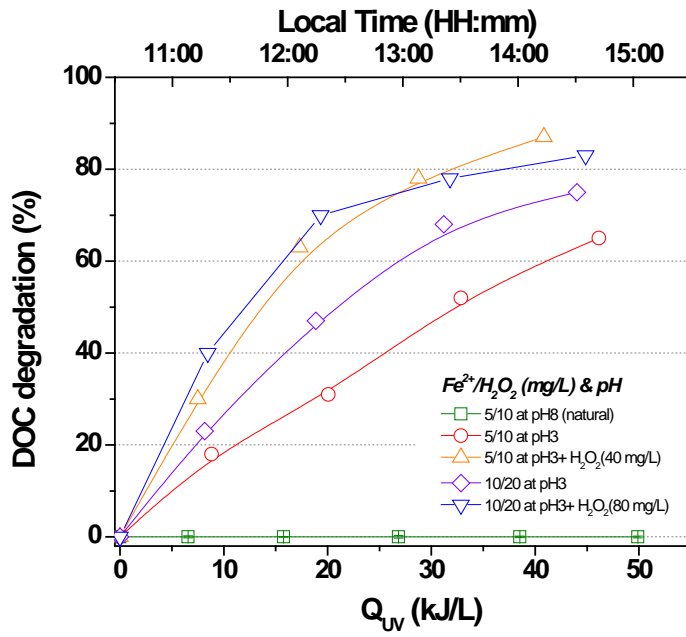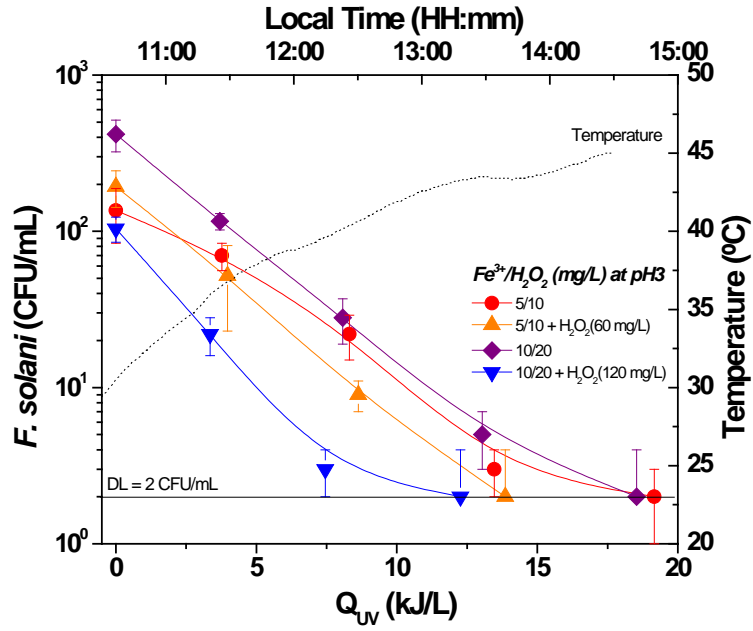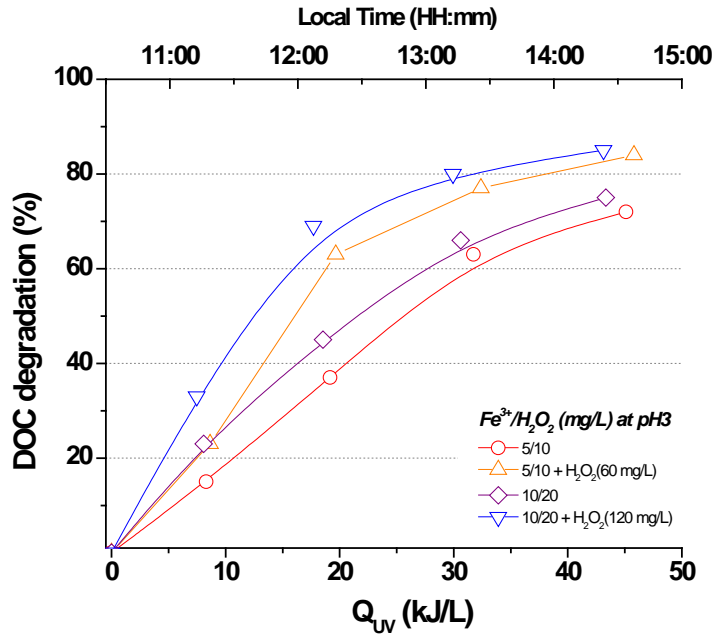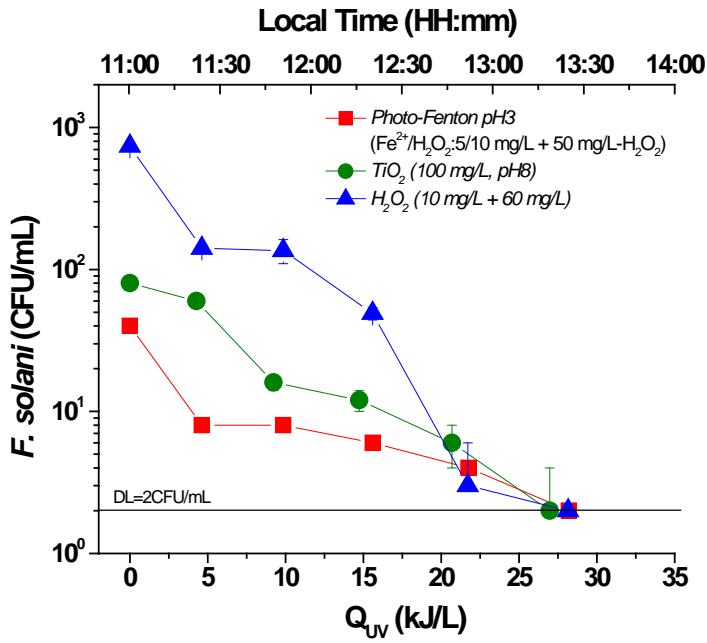
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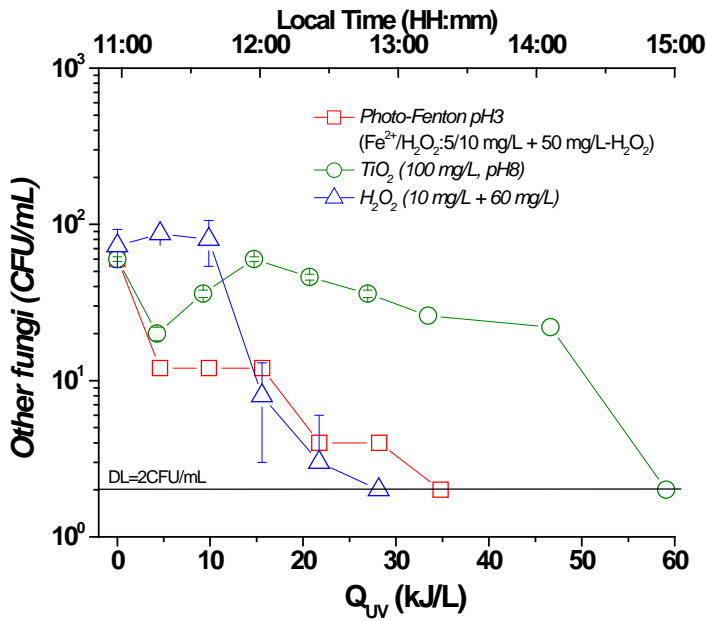3   **a)**

4   **b)**

5

1   **Figure 7**

2   **a)**

3   **b)**