Disinfection of real and simulated urban wastewater effluents using mild solar photo-Fenton

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ABSTRACT

This work aims to assess the effectiveness of mild solar photo-Fenton (low reagents concentrations and near neutral pH) for the removal of faecal bacterial in urban wastewater effluents. *E. coli* and *E. faecalis* were simultaneously evaluated in real and simulated effluents at initial concentrations of 10^3 and 10^6 CFU/mL simultaneously. Several concentrations of ferrous sulfate (2.5 – 10 mg-Fe^{2+}/L) and hydrogen peroxide (5 – 50 mg/L) were tested in solar CPC reactors (total volume: 20 L) under natural sunlight. Photo-Fenton results were compared with the bactericidal effect of solar exposure and H_2O_2 under the same experimental conditions. Solar photo-Fenton at pH 5 and pH 3 were compared. The results showed complete bacterial inactivation in almost all conditions; although the solar UVA energy dose required to achieve similar results at pH 5 (24–30 kJ/L) was higher than at pH 3 (2 – 20 kJ/L). This work also demonstrates experimentally that the presence of precipitated iron at near neutral pH has not benefits in the disinfection efficacy; even it provokes a slight decrease in the effectiveness under these experimental conditions. *E. feacalis* clearly showed higher resistance than *E. coli* to all treatments (Photo-Fenton and H_2O_2/solar) for both cases, naturally occurring and seeded bacteria. The disinfection tests in real effluents showed very promising results in spite of the complexity and variability of the organic and inorganic matter of the effluents. A 3-log decrease of *E. coli* and *E. faecalis* was attained in real effluents, while in simulated wastewater a 6-log abatement was observed when solar photo-Fenton at pH 5 was applied, which has important implications for reclaimed wastewater.

Keywords: *E. coli*, *E. faecalis*, solar photo-Fenton, Compound Parabolic Collector, pilot plant.

1. Introduction

The growth of world population brings an increase in industrial, agricultural and recreational activities, and therefore an increase of the fresh water demand. For this reason, in the next decades the access to clean fresh water sources is becoming a serious global problem due to either the water scarcity or the health risks associated to polluted water resources.

The primary purpose of reclamation and reuse of water is to catch water directly from non-traditional sources such as industrial or municipal wastewaters and restore it to a higher quality effluent [1]. Wastewater contains a large diversity of chemical pollutants and pathogens, and has a high amount of organic matter, all of which must be removed or transformed to harmless compounds.

Wastewater reuse may become new and stable source of water supply for agriculture, industrial processes, and some domestic uses, which do not require potable water. Even, the potential benefits accrued for agriculture, environmental preservation, and enhancement and energy conservation through the reclaimed water may be more important.

Agriculture is the largest fresh water activity consumer, being responsible for the consumption of the 70-95% for irrigation. Wastewater reuse for agriculture will reduce the water pressure in semi-arid and very contaminated areas [2]. Guidelines and specific national policies for reclaimed water quality and reuse limit the load of several water pathogens, like faecal coliforms and *E. coli* [3-7]. Depending on the final uses of the reclaimed water, the maximum allowed concentration of microbial agents vary; being more restrictive for urban and agricultural uses, and less limiting for industrial, recreational, and environmental uses. In particular, the guidelines for water recycling established by different water authorities for unrestricted irrigation regarding *E. coli* or coliforms in terms of CFU per 100 mL is: <1 by the USEPA [3], <1000 by the WHO [4], <10 by the Italian rule [5], <1 by Australian guidelines [6] and <100 by Spanish regulations [7].

Urban wastewaters are commonly treated by activated sludge followed by sedimentation systems (secondary treatment). Depending on the regulations on each area or country, these wastewater effluents would be discharged to surface waters or restricted irrigation and some industrial applications. According to the microbial quality

requirements established by the regulations, it is clear that an efficient tertiary treatment of effluents from secondary is required.

Mostly, urban wastewater effluents present, among others, a high load of faecal bacteria, which is commonly reported in terms of *E. coli*, total coliforms (TC) and faecal coliforms (FC) concentration. *E. coli* normally account for the majority of the faecal coliform group [8]. The typical quality of these wastewater effluents is around 10^3 –10^5 TC /100 mL [9-11]. The limits of faecal load established by the WHO recommendations for unrestricted irrigation uses are ≤1000 CFU of FC/100 mL [4].

Different physicochemical water treatments are currently in use, like chlorine, UVC, and ozone. Although chlorine is very strong oxidant and has a residual effect, it may react with natural organic matter (NOM) present in natural waters forming carcinogenic halogenated disinfection by-products (DBP), like trihalomethanes (THMs) and haloacetic acids (HAA) [12-14]. The use of UVC has limited efficiency against very resistant pathogens [15], non-residual effect, and it requires high investment, operation and maintenance costs. Therefore, alternative technologies are under study for the removal of water pathogens to overcome these limitations. Some Advanced Oxidation Processes (AOPs) like H_2O_2/UV-C, photocatalysis with titanium dioxide, photo-Fenton and H_2O_2/O_3 are being proposed as new approaches for water disinfection [16-22]. The efficacy of AOPs lies in the generation of hydroxyl radicals ($OH^{\bullet}$). These highly oxidant species can oxidize almost all organic compounds and inactivate a wide range of microorganisms. Furthermore, the use of solar radiation to promote some AOPs has been demonstrated to be very efficient for water purification with the advantage of using an environmental friendly source of photons [23].

Recently, research is being done on mild photo-Fenton and solar radiation with low concentrations of H_2O_2 for water disinfection [21, 24]. Photo-Fenton produces hydroxyl radicals via a series of catalytic cycle reactions with iron (Fe^{2+} and Fe^{3+}), H_2O_2 and UV-vis radiation (≤ 600 nm). These reactions are summarised as follows [25]:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\bullet} \ (K = 70\ M^{-1}s^{-1}) \quad (1)$$

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

The highest photo-Fenton efficacy is found at pH 2.8 [25], since iron salts precipitate far from this pH value. Nevertheless, photo-Fenton at near neutral pH would be desirable to reduce operational costs associated to acidification and neutralization

prior- and post- treatment of the large volumes of wastewater. Only few articles report on this subject [21, 26, 27]. These papers report successful inactivation of a single bacterium (*E. coli* or *E. faecalis*) in different water matrixes under very different conditions. There is still scarce information about the applicability of this process for the disinfection of real wastewaters under real solar conditions.

The solar photo-assisted treatment of H_2O_2/solar radiation induces accelerated inactivation of several types of microorganisms in water due to photo-chemical and photo-biological processes occurring when solar photons and non-toxic amounts of hydrogen peroxide interact with living cells [20, 28, 29]. This phenomenon cannot be considered as any of the well-known AOPs, since it does not generate hydroxyl radicals by photo-chemical reaction of H_2O_2 with sunlight (wavelengths > 300 nm) [30]. Our previous research on H_2O_2/sunlight processes for water decontamination, demonstrated there is no significant degradation of organic matter neither on disinfection [24]. It is believed that the acting mechanism for microorganisms of this method is based on the stressing effect produced by H_2O_2 and solar photons due to internal photo-Fenton reactions with available iron inside the cells [31].

The efficiency of water disinfection strongly depends on water composition, and bacteria consortium. The controversial role of the presence of the organic matter has been shown in the literature, as some articles report on its beneficial effect and others on the detrimental [21, 24, 27, 32]. There are scarce contributions on the removal of bacteria consortia and naturally occurring bacteria in real wastewaters [29, 33] using photo-Fenton and H_2O_2/solar treatment.

The aim of this work was to evaluate the efficiency of solar photo-Fenton at near neutral pH and H_2O_2/solar to remove *E. coli* K-12 and *E. faecalis* simultaneously spiked in simulated municipal wastewater effluents and natural occurring *E. coli* and *E. faecalis* in real municipal wastewater effluents. Several concentrations of ferrous sulphate (2.5 – 10 mg-Fe^{2+}/L) and hydrogen peroxide (5 – 50 mg/L) were evaluated in two solar CPC reactors under natural solar conditions. The effect of pH in the solar treatment efficiency was evaluated at pH 3 and 5. Furthermore, the influence of precipitated and dissolved iron on the efficiency of photo-Fenton at near-neutral pH was also investigated. pH 7 was not experimentally evaluated as our previous publications [24, 33] showed that the inactivation of *E. coli* and *Fusarium solani* spores using photo-

Fenton at pH 7 were very similar to those observed for H_2O_2/solar process. This was attributed to the zero amount of dissolved iron measured in the samples at this pH.

2. Materials and methods

2.1. Solar experiments

All experiments were conducted at Plataforma Solar de Almeria (PSA) under natural solar radiation on completely sunny days from April to July 2012 (summer conditions) for 4 h (10:30–14:30, local time) of solar exposure.

Three types of solar experiments were done in this work: i) H_2O_2/solar and ii) solar photo-Fenton in simulated (SE) and real effluents (RE) of urban wastewater treatment plant using CPC pilot reactors; iii) Solar photo-Fenton experiments to study the effect of the presence and absence of precipitated iron were done in small stirred vessel reactors in distilled water (DW).

Solar CPC pilot reactors

Most of the experiments were done in two pilot plant compound parabolic collectors (CPC) reactors (Figure 1). Both reactors are recirculated batch systems with total volume of 20 L, illuminated volume in CPC photo-reactor of 14 L. The ratio of illuminated volume to total volume was 0.7. The CPC mirrors (total surface area of 1 m^2) are titled 37º to the horizontal plane which enhances the solar radiation collection [34]. Flow rate was 10 L/min in both reactors.

The experiments were done in SE and RE. Water was acidified using sulphuric acid (Merk, Germany, analytical grade) after adding iron salts in photo-Fenton experiments. Then, the bacteria suspensions were added to the SE, and finally the hydrogen peroxide. Same procedure was followed in RE samples without spiking bacteria, as the naturally occurring *E. coli* and *E. faecalis* were evaluated.

Samples were taken at predetermined times for a whole period of 4h. The ‘dark control sample’ was the first sample of each experiment kept in the dark at room temperature, which was analysed at the end of the experiment to examine the effects of the process in the dark on the bacteria viability. Temperature (T) (Checktemp, Hanna instruments, Spain), dissolved oxygen (DO) (Crison Oxi 45+) and pH (multi720, WTW, Germany) were measured directly in the CPC reactor during the experiments. All experiments were performed in duplicate at least. Reproducibility of the results was

high; to check it, the data obtained in the measurements were analysed using the one-way ANOVA analysis tool P<0.05, Confidence > 95% (Origin v7.0300, OriginLab Corp., Northampton, USA). The results shown in the graphs were obtained as the average of the replicates, and the error bar is the standard deviation.

Stirred vessel reactors

Special tests were done to evaluate the effect of the presence and absence of precipitated iron, at near neutral pH, on the photo-Fenton efficiency for bacterial inactivation. For this purpose, DW was used as water matrix to avoid any interference of ions and molecules. These experiments were done in 250 mL DURAN-glass (Schott, Germany) stirred vessel reactors, with 200 mL of DW, 10 mg/L of Fe^{2+} and pH adjusted to pH 5 using NaOH.

Two reactors (replicates) were prepared under these conditions, other two reactors (replicates) were prepared similar, but the water was filtered using 0.2 µm filters CHROMAFIL Xtra PET-20/25 (PANREAC, Spain) after adding the iron salt, pH 5 adjustment and stirring for 5 min; this was done to remove the precipitated iron form water samples. After this, 20 mg/L of H_2O_2 was added to all reactors at the same time, as well as the bacterial suspension so that the initial concentration was 10^6 CFU/mL. Then, the reactors were exposed to natural sunlight for 4 h. Samples were taken and evaluated as described in the bacterial quantification section.

Solar radiation measurement

Proper evaluation of solar promoted processes should take into account two variables: i) the accumulated solar UVA energy received into the solar reactor per unit of treated water volume (Q_{UV}, Eq. 3; [35]), and ii) the experimental time (t), which also plays an important role not only in the equation of Q_{UV} but also in the kinetics of the solar mechanisms occurring under exposure. For example, two case studies (in two different days) with the same accumulated Q_{UV}, one case reached within 2 h, and the other in 5 h. They will lead to very different inactivation kinetics and final disinfection results, as the experimental time is very different in both cases. For this reason, all the experiments were done at same local times, similar environmental temperatures, and with similar variations on solar UVA irradiance. The maximal and minimum UVA irradiances were 27.1 (±1.4) W/m^2 and 48.2 (±2.4) W/m^2, respectively, in all the

experiments presented in this work. The average solar incident UVA irradiance registered during all the tests through the experimental time is presented in Figure 2, showing same irradiation pattern in all cases.

The solar UVA irradiance was measured using a global UVA pyranometer (295–385 nm, Model CUV4, Kipp & Zonen, Netherlands), with typical sensitivity of 264 mV/(W/m^2), which provides data in terms of incident W/m^2. This is used to calculate the total UV energy received per unit volume according to Eq. (3).

$$Q_{UV,n} = Q_{UV,n-1} + \frac{\Delta t_n \overline{UV}_{G,n} A_r}{V_t} \qquad \Delta t_n = t_n - t_{n-1} \tag{3}$$

where $Q_{UV,n}$, $Q_{UV,n-1}$ is the UV energy accumulated per unit volume (kJ/L) at times n and $n-1$, respectively, $UV_{G,n}$ is the average incident irradiation on the irradiated area, Δt_n is the experimental time of sample, A_r is the illuminated area of collector (m^2), and V_t is the total volume of treated water (L).

2.2. Water types

Simulated effluent of urban wastewater treatment plant (SE) containing 25 mg/L of Dissolved Organic Carbon (DOC) was used as model of wastewater in order to investigated the inactivation efficiency of solar treatments avoiding chemical and microbiological fluctuations often observed in sewage effluents. The SE composition is as follows: $NaHCO_3$ (96 mg/L), NaCl (7 mg/L), $CaSO_4 \cdot 2H_2O$ (60 mg/L), urea (6 mg/L), $MgSO_4$ (60 mg/L), KCl (4 mg/L), $CaCl_2 \cdot 2H_2O$ (4 mg/L), peptone (32 mg/L), $MgSO_4 \cdot 7H_2O$ (2 mg/L) and meat extract (22 mg/L) [24].

Real urban wastewater treatment plant effluent (RE) from El Bobar (Almería, Spain) was used as real urban sewage effluent. The first stage in the plant consists of a pre-grinding to remove coarse solids, before lifting the wastewater. Then the water is subjected to a pre-treatment consisting of grinding, sanding and degreasing. Water from the pre-treatment is directed to a primary settling tank where the solids decanted from passing below the secondary treatment. Secondary treatment consists of a biological treatment by activated sludge and subsequent decantation. The same wastewater source effluent has been investigated elsewhere [29, 33]. The main physicochemical characteristics of the SE and RE are shown in table 1.

Ions concentrations were measured by ion chromatography (IC) using a DX-600 model (Dionex Corporation, Sunnyvale, California) for anions and a DX-120 model for cations. DOC and dissolved inorganic carbon (DIC) were measured by direct injection 2of samples filtered with 0.2 µm syringe-driven filters into a Shimadzu – 5050A TOC analyzer (Shimadzu Corporation, Kyoto, Japan). Turbidity was measured with a turbidimeter Model 2100N, Hach (USA). The natural presence of dissolved iron in RE was analyzed by spectrophotometric technique with phenanthroline/acetic acid (UV-VIS measurements, detection limit of 0.05 mg/L). No iron was detected in any of the RE water samples with this method.

2.3. *Escherichia coli* and *Enterococcus faecalis* enumeration

Escherichia coli K12 strain (CECT 4624) and *Enterococcus faecalis* strain (CECT 5143) were obtained from the Spanish Culture Collection (CECT). They were used to prepare the bacterial suspensions spiked for SE assays. Fresh liquid cultures were prepared in Luria-Bertani nutrient medium (LB Broth, Panreac) and incubated at 37 ºC under rotary shaking for 20 h. Bacteria stationary phase concentration was 10^9 CFU/mL. Bacterial suspensions were harvested by centrifugation at 900 x g for 10 min. Bacterial pellet was re-suspended in Phosphate Buffer Saline (PBS) and diluted in the reactor to have 10^6 CFU/mL as initial concentration. The samples taken during the experiments were enumerated using the standard plate counting method through a serial 10-fold dilution in PBS; diluted samples of 60 µL were plated on ChromoCult® Coliform Agar (Merck KGaA, Darmstadt, Germany) for *E. coli* and Slanetz&Bartley agar (Scharlau®, Spain) for *E. faecalis*. Colonies were counted after incubation of 24 h at 37 ºC. The detection limit (DL) of this experimental method was found to be 2 CFU/mL. For RE experiments, the naturally occurring *E. coli* and *E. faecalis* concentrations were enumerated following the same methodology.

2.4. Reagents and analysis

Ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$, PANREAC, Spain) was used to obtain Fe^{2+} concentrations of 2.5, 5 and 10 mg/L. Water samples were filtered with NY 0.2 µm CHROMAFIL® Xtra PET-20/25 (PANREAC, Spain) to remove precipitated iron. Then each sample was mixed with 1 mL of 1,10-fenantroline (1 g/L) and 1 mL of buffer solution according to ISO 6332 to measure the dissolved Fe^{2+} and total iron (Fe^T)

concentrations, i.e. concentration of Fe^{2+} and Fe^{3+}. The coloured complex formed was measured with a spectrophotometer (PG Instruments Ltd T-60-U) at 510 nm in glass cuvettes (1 cm path length). Fe^{2+} and Fe^{T} concentrations were determined using corresponding calibration curves. The concentration ratios of Fe^{2+}:H_2O_2 used were 1:2 and 1:5; with Fe^{2+} of 2.5, 5 and 10 mg/L and H_2O_2 of 5, 10, 20, 25 and 50 mg/L.

Hydrogen peroxide (35%, w/v aqueous solution) was provided from Merk and diluted directly into the waters samples from 5 to 50 mg/L to do the H_2O_2/solar, photo-Fenton and Fenton experiments. H_2O_2 concentration was measured in a spectrophotometer (PG Instruments Ltd T-60-U) at 410 nm in glass cuvettes (1 cm path length) according to DIN 38409 H15 based on the formation of a yellow complex from the reaction of titanium (IV) oxysulfate with H_2O_2. The titanium (IV) oxysulfate method has a 0.1 mg/L detection limit. The signal was read after 5 min incubation time against a H_2O_2 standard curve linear in the 0.1–100 mg/L H_2O_2 concentration range. The titanium (IV) oxysulfate solution (Riedel-de Haën, Germany) was used as received. Catalase was added to water samples to eliminate residual hydrogen peroxide: 1-mL samples were mixed with 100 mL of 2300 U/mg bovine liver catalase at 0.1 g/L (Sigma Aldrich, USA).

3. Results

3.1. H_2O_2/solar radiation

Figure 3 (a and b) shows the simultaneous inactivation of *E. coli* and *E. faecalis* in SE with H_2O_2 (5, 10, 20, 25 and 50 mg/L) and solar radiation at pH 5 for 4 hours. In the case of *E. coli,* the detection limit was achieved with all H_2O_2 concentrations except for 5 mg/L. The highest inactivation was found for 50 mg/L of H_2O_2 with an accumulated solar UV dose of 21.7 kJ/L. For *E. faecalis*, the inactivation was slower than for *E. coli*, and only with 50 mg/L of H_2O_2 the detection limit was reached when a higher amount of solar energy dose was received: 36.7 kJ/L of Q_{UV}. The mere oxidative effect of H_2O_2 (the highest concentration of H_2O_2 tested: 50 mg/L) over both bacteria viability was very low compared with the synergistic effect of H_2O_2 and solar radiation (dark controls in figure 3), and the inactivation curve showed a very different shape to that found when H_2O_2 and solar radiation were applied simultaneously.

Parameters as pH, DO and DOC were measured during the solar test (Table 2). pH 5 remained nearly constant during the experiment. No significant DOC reduction was

observed in any case. H_2O_2 was monitored throughout the experiment; a slight reduction in H_2O_2 (<10 %) was observed at the end of the experiments.

Other control experiments in the dark in the CPC reactor under re-circulation without H_2O_2, showed no significant decrease of *E. coli* and *E. faecalis* (data not shown). Thermal inactivation of E. coli during the experiments was discarded by control tests in the dark at pH 3 and 5 with temperature increasing from 25 to 44 ºC like in a solar photo-Fenton experiment but in the absence of radiation. As expected, the results demonstrated that there is no detrimental effect over bacterial survival of these pH and temperature values (data not shown). This permitted to discard any thermal and mechanical detrimental effects on the inactivation curves observed in figure 3.

3.2. Photo-Fenton at pH 3 and 5.

3.2.1. Photo-Fenton at pH 5

This part of the study is focused on photo-Fenton at pH 5, because this value is a compromise between the optimal pH value for photo-Fenton process, i.e. pH 2.8 [25] and neutral pH (7-8) of natural waters and wastewaters discharges to the environment.

Figure 4a shows the inactivation of *E. coli* with photo-Fenton at pH 5 using several Fe^{2+} and H_2O_2 concentrations: 2.5/5, 5/10, 5/25, 10/20 and 10/50 mg/L, respectively. 6-log reduction (until DL) was observed for the cases of 10/50, 10/20 and 5/25 mg/L of Fe^{2+}/H_2O_2 with 24.71, 30.35 and 34.77 kJ/L of Q_{UV}, respectively. Lower reagent concentrations (2.5/5, 5/10) showed a 4.5 log decrease after 4 hours of solar treatment. Fenton tests were done in the same reactor in the dark for the two highest concentrations tested in this work, i.e. 10/20 and 10/50 mg/L of Fe^{2+}/H_2O_2; 1- and 5-log reduction of *E. coli* were observed, respectively. The highest inactivation for 10/50 mg/L of Fe^{2+}/H_2O_2 is not only due to the Fenton reaction (Eq. 1) under this condition, but also to the germicidal effect of 50 mg/L of H_2O_2 already shown in Figure 3a.

The inactivation of *E. faecalis* under similar photo-Fenton conditions is shown in figure 4b. Lower inactivation rates were observed for *E. faecalis* than for *E. coli*, in all cases. The DL was only reached with the highest tested concentrations, i.e. 10 mg/L of Fe^{2+} and 50 mg/L of H_2O_2, requiring 29.77 kJ/L of Q_{UV}. Lower photo-Fenton reagents

conditions yielded from 2.5- to 4-log decrease. Fenton (dark) inactivation results with 10/20 and 10/50 mg/L of Fe^{2+}/H_2O_2 lead to 0.5- and 2.7-log reduction, respectively.

The total dissolved iron concentration (Fe^T) in the reactor was measured every hour through all the experiments. Added iron were 2.5, 5 and 10 mg/L, while initial Fe^T values measured were in the range to 0.1-1 mg/L (Table 2); that means that most of the added iron precipitated as ferric hydroxide (pH 5) in all cases. The H_2O_2 consumption after 4 h of photo-Fenton treatment was in the range of 75-98% of the initial. No significant DOC reduction was observed for the three lower concentrations, while around a 20% DOC decrease was measured for 10/20 and 10/50 mg/L of Fe^{2+}/H_2O_2.

Solar photo-inactivation of both bacteria in SE in the same CPC reactors conditions in the presence and absence of Fe^{2+} (10 mg/L) was evaluated for comparison purposes, as a moderate detrimental effect was also expected due to the action of solar UVA photons and UVA/Fe [21, 36]. The results (Figure 5) showed a 3-log and 5-log decrease induced by the solar radiation after 4h for *E. faecalis* and *E. coli*, respectively. As expected, residual bacteria concentrations remain in the reactor regardless the treatment time. On the other hand, the addition of 10 mg/L of Fe^{2+} produced a clear enhancement, mainly in *E. faecalis*, although the DL was not reached in any bacteria.

3.2.2. Solar photo-Fenton at pH 3

Complete inactivation of *E. coli* and *E. faecalis* was obtained with solar photo-Fenton at pH 3 at all tested concentrations of Fe^{2+}/H_2O_2, 2.5/5, 5/10, 10/20 and 10/50 mg/L (Figure 6a and 6b). Viability of both bacteria at pH 3 was tested in the reactor in the dark and no significant decrease (<1-log) was observed during 3h. For proper homogenisation of reagents and bacterial suspensions in the reactor, the first 10 minutes of the experiment were conducted in the dark. Then, Fenton reaction occurred in these 10 min (Eq. 1); $OH^{\bullet}$ generated by this reaction are responsible for the losses of viability observed at this point. A very small bacterial decrease was observed in *E. faecalis* in all cases; nevertheless, *E. coli* showed to be more sensitive to Fenton as 0.5-log to 3-log decrease were observed as the reagents concentrations increased. Furthermore, Fenton process was also evaluated with 10/20 mg/L of Fe^{2+}/H_2O_2 at pH 3. For *E. coli*, Fenton results confirm our findings for photo-Fenton at the same reagents conditions in the dark for the first 10 min, i.e. 2-log drop. Then, Fenton leads to a gradual bacterial decrease of 3 log in 4 hours, which is much slower than solar photo-Fenton inactivation

curve. *E. coli* concentration after Fenton treatment is quite over the DL (Figure 6a). For *E. faecalis*, the effect of Fenton was almost negligible in the first 10 min; these bacteria showed a resistance to the treatment for the first 2h followed by a sharp decrease of nearly 5 log at the end (4h) of the experiment (Figure 6b). The *E. faecalis* Fenton kinetics shows a very different shape compared with solar photo-Fenton, which also leads to worse final disinfection performance.

For both bacteria, the fastest inactivation curve was found at 10/50 mg/L of Fe^{2+}/H_2O_2 with 1.65 kJ/L of Q_{UV} (Fig. 6a) for *E. coli*, and 20.68 kJ/L of Q_{UV} for *E. faecalis* (Fig. 6b). For lower reagents concentrations, higher Q_{UV} values were required to achieve the DL. Moreover, in the case of *E. faecalis* no significant differences among all tested concentrations were observed (Fig. 6b). It is very clear the enhancement produced by photo-Fenton process at pH 3 compared with pH 5, as the bacterial inactivation observed was much faster. Following the same tendency, the DOC reduction observed at pH 3 photo-Fenton was substantially higher than at pH 5, with a maximum reduction of 70% at 10/50 mg/L of Fe^{+2}/H_2O_2 (Table 2). In terms of DOC, we observed the higher reagents concentration the higher DOC reduction.

Fe^T and H_2O_2 concentrations were measured every hour and every 30 min, respectively. Fe^T decreased during the first hour of the experiment around 30-50%, remaining constant until the end of the experiment. H_2O_2 was totally consumed during the first 2 hours of the experiment (Table 2).

3.3. Evaluation of the precipitated iron in photo-Fenton efficiency

Two kinds of experiments were done to evaluate the effect of precipitated iron in comparison with dissolved iron, at near neutral pH, on the photo-Fenton efficiency. One type had DW with iron salts (10 mg/L of added Fe^{2+}) and H_2O_2 (20 mg/L) at pH 5 in the presence of *E. coli* and *E. faecalis* spiked together (so called 'non-filtered' tests). This experiment was run simultaneously to the other type, which consisted in the same photo-Fenton condition but with the water filtered previously to both bacteria adding ('filtered' test). Both experiments (200 mL total volume) were exposed in stirred vessel reactors to non-concentrated solar radiation.

The influence of precipitated iron (Fe^P) and dissolved iron (Fe^D) on the photo-Fenton efficiency to inactivate *E. coli* and *E. faecalis* is shown in Figure 7. Added reagent concentration was 10 mg/L of Fe^{2+} with 20 mg/L of H_2O_2 and pH was adjusted

to 5 to be near the mild photo-Fenton conditions (close to neutral pH) for final applications of this process for water treatment. This condition favoured the precipitation of iron in a great per cent of the initially iron added. The dissolved iron measured was 0.05 mg/L, while the rest of the iron might be precipitated as relatively inactive hydrous oxyhydroxides giving an orange-brown shade to the water solution [25]. On the contrary, the samples with filtered showed no coloured water as precipitated iron was removed by filtration. No significant differences in the inactivation of *E. coli* were observed, while the inactivation of *E. faecalis* with filtered samples was faster than in unfiltered one (Fig. 7).

Results with *E. faecalis* demonstrate that Fe^{D} is more efficient as bottles with filtered water achieved the detection limit faster than in bottles with unfiltered water. These results evidence that the presence of precipitated iron in the water samples does not provide extra hydroxyl radicals via photo-Fenton reactions. On the contrary, the precipitated iron would shade the light entering the reactor so that the inactivation efficiency is decreased, as it is shown in *E. faecalis* inactivation result in the unfiltered water sample.

3.4. Disinfection of real municipal wastewater effluents (RE)

Figures 8a and b show the inactivation of naturally occurring *E. coli* and *E. faecalis* within 4 hours of solar photo-Fenton at pH 5. Initially added reagents were 10 mg/L of Fe^{2+} with 20 and 50 mg/L of H_2O_2, respectively. In addition, blank experiments regarding to H_2O_2/solar radiation (10 and 50 mg/L) and Fe^{2+}/solar radiation (10 mg/L) treatments in RE were done.

All solar treatments lead a complete *E. coli* removal (DL = 2 CFU/mL) (Fig. 8a). Nevertheless, small differences in the required solar UV energy dose (between 7 and 15 kJ/L of Q_{UV}) were observed between all the solar treatments except for Fe^{2+}/solar radiation, which required 31.29 kJ/L of Q_{UV}. The kinetics inactivation order found was: 50 mg/L-H_2O_2/solar radiation (7.4 kJ/L of Q_{UV}) > solar photo-Fenton with 10/50 mg/L of Fe^{2+}/H_2O_2 (8 kJ/L of Q_{UV}) > 20 mg/L-H_2O_2/solar radiation (12 kJ/L of Q_{UV}) > solar photo-Fenton at 10/20 mg/L of Fe^{2+}/H_2O_2 (13.1 kJ/L of Q_{UV}) > 10mg/L-Fe^{2+}/solar radiation (31.3 kJ/L of Q_{UV}). The *E. coli* inactivation rate was faster in both H_2O_2/solar radiation treatments than solar photo-Fenton treatments probably due to the amount of iron dissolved was very low in the photo-Fenton. Table 3 summarized the Fe^{T} and H_2O_2

concentration measured trough the solar experiments. The initial dissolved concentration of Fe^T decreased during the first hour of the experiment around 80-90% in photo-Fenton treatments, remaining constant until the end of the process, while in the treatment Fe^{2+}/solar radiation decreased during the first three hours around 90%. In addition, H_2O_2 was totally consumed during the first 2 hours of photo-Fenton process, and remained constant in H_2O_2/solar radiation treatments.

The inactivation results for *E. faecalis* were similar to those observed for *E. coli* (Fig. 8b), although higher differences according to solar UV accumulated were found for *E. faecalis* compared to *E. coli*. The inactivation order found was: 50 mg/L-H_2O_2/solar radiation (15.9 kJ/L of Q_{UV}) > solar photo-Fenton at 10/50 mg/L of Fe^{2+}/H_2O_2 (22.2 kJ/L of Q_{UV}) > 20 mg/L-H_2O_2/solar radiation (25.9 kJ/L of Q_{UV}) > solar photo-Fenton at 10/20 mg/L of Fe^{2+}/H_2O_2 (27.9 kJ/L of Q_{UV}) > 10 mg/L-Fe^{2+}/solar radiation (31.3 kJ/L of Q_{UV}).

On the other hand, the reduction of DOC was remarkable in photo-Fenton experiments reaching 59.29% using 10/50 mg/L of Fe^{2+}/H_2O_2, and 32.56% with 10/20 mg/L of Fe^{2+}/H_2O_2 (Table 3).

4. Discussion

The presence of organic and inorganic chemical compound in the water matrices was investigated, i.e. SE and RE~~, should be taken into account to a proper evaluation of the bacterial inactivation results~~. Table 1 shows the anions and cations presents in the water matrixes ~~used in this experimental work. It is well known that some particular chemical compounds have a strongly negative effect in the photocatalytic efficiency.~~ Carbonate (CO_3^{2-})/bicarbonate (HCO_3^-) present in water may be a limiting factor during photocatalytic process due to they can react with $OH^\bullet$ resulting in $OH^\bullet$ scavenging, and hence limiting the oxidative attack [37, 38]. Other anions as sulfates, nitrates, chlorides and phosphates present in the water may react with iron, H_2O_2 as well as other Reactive Oxygen Species (ROS), and therefore limiting the amount of available $OH^\bullet$ (Eq. 1) for oxidizing bacteria and organic matter during the photo-Fenton treatment.

Moreover, the presence of dissolve organic matter (DOM) also affects the photocatalytic efficiency. ~~In the literature,~~ The positive effect of natural organic matter (NOM) on photo-Fenton at near neutral pH for inactivation of water pathogens has been

reported [26, 39-42]. Spuhler *et al.* investigated the influence of resorcinol for *E. coli* inactivation in photo-Fenton. They found that the presence of resorcinol enhances the inactivation kinetics compared with results without this organic compound. They suggested that the formation of photo-active Fe^{3+} or Fe^{2+}-resorcinol complexes could favors the inactivation process [21].

DOM can be a highly complex mixture of organic compounds generated by the decomposition and bio-process of macro-cellular structures. The influence of the DOM could be different depending on the diversity of chemical organic compounds generated and naturally present in different water resources, therefore the effect of it on the photocatalytic efficiency could vary substantially. Although a deep and complete organic chemical characterization of real wastewater effluents is very difficult, it is well known that some organic acids like oxalic, carboxylic, humic and fulvic acids and other intermediates have an important effect on the photodegradation of a variety of pharmaceuticals through a number of processes [43-45]. DOM can absorb solar radiation promoting the singlet-excited state, ^{1}DOM*, which then undergoes to the ground state or crosses to the longer-lived excited triplet state ^{3}DOM* producing ROS like excited singlet oxygen ($^1O_2$), superoxide/hydroperoxyl radicals ($O_2^{-\bullet}/HO_2^{\bullet}$) and $OH^{\bullet}$. The excited triplet state can also act as a photosensitizer, transferring energy directly to the molecules enhancing the chemical degradation [43-45]. Nevertheless, the presence of DOM has been also observed to decrease the rate of photodegradation by acting as a sunlight filter [46].

Furthermore, in photo-Fenton case, iron and H_2O_2 react also with organic matter (quenchers, scavengers or other molecules). As result, carboxylic and dicarboxylic acids could be generated and react with iron to form ligand radicals [21, 23, 25]:

$$Fe^{3+} - (L)_n + h \rightarrow Fe^{2+} - (L)_{n-1} + L_{ox}^{\bullet} \qquad \text{(Eq. 4)}$$

Fe^{2+} and radicals generated may react also with O_2 leading to the formation of new ROS. This way could be specially interesting at pH above 3, where Fe^{3+} tends to precipitate, and Fe^{3+} organo complexes play an important role for the efficiency of photo-Fenton systems at near neutral pH [21]. Therefore, it is very important to know the nature and chemical composition of the DOM~~ to do a proper interpretation of the results~~.

~~Under the experimental conditions of this work,~~ The inactivation results of *E. coli* and *E. faecalis* (Fig. 8ab) in RE at pH 5 were significantly better than those obtained in

SE (Fig. 4ab). These results evidence that the DOM of the RE benefits the solar photo-chemical process efficiency in all the treatment. ~~and conditions evaluated in this experimental work~~.

The inactivation results show that *E. faecalis* is more resistant than *E. coli* to the effects of the solar treatments evaluated. This difference may be attributed to the different architecture of the cytoplasmatic membrane of Gram-negative (*E. coli*) and Gram-positive (*E. faecalis*) bacteria. Gram-negative bacteria have a cytoplasmic membrane, a thin peptidoglycan layer and an outer membrane containing lipopolysaccharide. Meanwhile, Gram-positive bacteria have only a cytoplasmic lipid membrane. The peptidoglycan layer is thicker than that of Gram-negative bacteria. Some contributions have been reported that complete bacterial inactivation by photocatalysis require a high number of oxidative attacks by $OH^{\bullet}$ [47-51].

Van Grieken et al., reported that the osmotic stress is a factor affecting highly the inactivation of *E. coli* compare to *E. faecalis* in distilled water and simulated municipal wastewater treatment plant [52]. Despite the more complex external structure of Gram-negative bacteria, osmotic stress may induce a higher weakening of *E. coli* cell wall, enhancing the permeability to the oxidant species, and doing *E. coli* cells highly susceptible. The osmotic stress provoked by distilled water was also proven to be a critical factor on *E. coli* photocatalytic disinfection by Sichel et al. [19].

Nevertheless, there are a number of papers demonstrating also that *E. coli* is more resistant than *E. faecalis* to the TiO_2 photocatalytic treatment. They attribute this resistance to: i) the presence of the outer membrane, which adds an extra protecting wall against oxidative agents [53-56]; ii) the absence of the outer membrane in Gram-positive bacteria makes easier for hydroxyl radicals to damage the bacterial DNA; and iii) the differences in the chemical composition of the cell wall and protection mechanisms of both bacteria [57].

However, our results demonstrated clearly that *E. faecalis* is more resistant than *E. coli* to all solar treatments evaluated. Probably this is due to the thicker cell wall of *E. faecalis* together with different internal defence mechanisms of this bacterium. Nevertheless, as only two strains were experimentally evaluated, we cannot attribute the different sensitivity of both strains to the structural difference between membranes; therefore further research should be done to make clear this point. For the first time,

these results report a higher resistance of naturally occurring *E. faecalis* compared with natural *E. coli* present in RE against solar photo-Fenton and H_2O_2/solar treatment.

The bacterial inactivation mechanisms during photo-Fenton can be attributed to three mechanisms: i) the generation of external $OH^{\bullet}$ radicals (Eq. 1-2); ii) the diffusion of Fe^{2+} inside the cells where the increased internal iron concentration provoke the generation of $OH^{\bullet}$ via internal Fenton reactions between iron and metabolic H_2O_2; and iii) the direct or indirect oxidation of lipids, proteins, sugars, DNA and site-specific oxidation by the iron [58]. The type of iron salt (Fe^{2+} or Fe^{3+}) used for photo-Fenton may affect the inactivation results, as proven by Polo-López *et al.* [59]. However, in this case both bacteria were evaluated under the same conditions, and therefore, their different inactivation should be due to the different internal mechanisms defence due to iron could diffuse inside both kind of cells.

~~The chemistry of iron inside living cells is very complex, but still unclear and under investigation.~~ The role of iron is very important in the cell homeostasis. In *E. coli*, a number of chelating compounds are involved in the transport of this metal through the outer membrane like citrate, ferrichrome, enterobactin, aerobactin, yersiniabactin, and heme, which are catalysed by highly specific proteins across the cytoplasmic membrane by ABC transport systems. Similar transport mechanisms happen in the cell wall of *E. faecalis*. In both bacteria, transcription of the transport protein genes is regulated by the so called *Fur* protein. Some works reports that *Fur* mutant cells permit a permanent influx of iron, which overwhelms the iron storage capacity of the cells leading to an intracellular overload of iron leading to oxidative stress sensitivity [60]. Iron metabolism deregulation in *Fur* mutant cells produced a 2.5-fold iron overload in *E. coli* [61]. When an oxidative treatment (like photo-Fenton) affects the defence and regulatory metabolic systems of bacteria cells, an overload of iron inside cells will eventually occur. The observed higher resistance of *E. faecalis* to photo-Fenton and solar treatments may be due to a better capacity to respond to this iron overload. López *et al*., reported that cultures of *E. faecalis* exposed to 6 h in excess of iron (0.5 mM $FeCl_3$-NTA) show a significant decrease in the amount of total glutathione, which is associated to an increase in the transcripts encoding superoxide dismutase (sodA), catalase (katA), thioredoxin (trx), hydroperoxide resistance protein (ohrA and ohrB) and peptide methionine S-sulfoxide reductase (msrA). Therefore, under an excess of iron, the transcriptional response of *E. faecalis* to iron excess includes a general oxidative

stress response [62]. Bronstein *et al*. reported that cultures of *E. faecalis* grown under excess of iron (0.5 and 1 mM of $FeCl_3$-NTA) increase the intracellular iron content without changes in the cell viability, which suggest that *E. faecalis* gets adapted to these conditions and can regulate its iron needs with a proper control of the associated effects of iron overload [63]. These contributions explain why *E. faecalis* can be more resistant to the photo-Fenton oxidative stress than *E. coli*.

Few works have investigated the influence of pH on the disinfection efficiency with photo-Fenton [24, 27, 59]. Recently, research is focused on the application of photo-Fenton for real wastewater treatment at near neutral pH with the aim of avoiding acidification (pH 2.8) before photo-Fenton and neutralisation (pH 6-8) following photo-Fenton and before discharge of treated water; this would make the treatment more environmentally friendly and reduce reagents costs and treatment steps [21, 64].

Moreover, ~~from the research point of view,~~ when photo-Fenton for disinfection is carried out at the optimal pH (2.8), a very negative effect of the acidic pH on the viability of some pathogens (like *E. coli* and related) is observed, so that the inactivation of the microorganism is due to the mere action of the low pH instead the solar treatment.

If we compare the results at pH 3 (Fig. 6) and pH 5 (Fig. 4) in SE, at pH 3 was observed a 3-log, and 0.5-log reduction in *E. coli* and *E. faecalis* respectively before starting the solar experiment, in only 10 min. As expected, photo-Fenton at pH 3 led to a fast decrease of both types of bacteria in all conditions studied to reach the detection limit (Fig. 6) with UVA energy dosages (Q_{UV}) lower than those required at pH 5 (Fig. 4). pH 3 is very close to the optimal photo-Fenton pH and at pH5, most of the active iron is lost from the solution due to precipitation at that pH (Table 2). In spite that the photo-Fenton conditions are not the best at pH 5, the promising results at this pH, evidence that the presence of only a little amount of dissolved iron can produce enough oxidative damages to get complete (until DL) inactivation of bacteria, opening new perspectives in the treatment of real wastewaters at near neutral pH.

The influence of the precipitated iron at pH 5 was also experimentally evaluated (Fig. 7). The results clearly showed that the precipitated iron negatively affect the inactivation results, especially in *E. faecalis*. For *E. coli* results showed no significant differences between both, the presence and the absence of precipitated iron. The precipitated iron screens sunlight, therefore the generation of hydroxyl radicals could be

limited, so that the inactivation efficacy decrease. This more clear in the case of *E. faecalis* because it is a more resistant bacterium against photo-Fenton than *E. coli*, which is very sensitive. In the case of *E. coli*, although the precipitated iron may limit the generation of radicals, the limited oxidant action of the process still produces lethal damages in the *E. coli* to complete inactivation.

The accelerated photo-inactivation of microorganism in water by the presence of low amounts of hydrogen peroxide has a remarkable importance as it has been demonstrated in several works during last years [20, 21, 26, 28, 65]. Most of these works have been carried out in simple waters like DW, and DW with added chemical organic or inorganic compounds, well water or even simulated municipal wastewater effluents. Also, this process has been studied with bacteria like *E. coli* [21, 26], *Bacillus* spores [65] and fungi *Fusarium* spp [28, 66]. Bichai *et al.* tested the efficiency of H_2O_2-aided solar disinfection processes with 5 and 10 mg/L in 1.5-L PET bottles and 20-L batch borosilicate glass reactors equipped with CPC. They demonstrated inactivation of naturally occurring *E. coli* in RE during 5 h of solar exposure [29]. Agulló-Barcelo *et al.* also demonstrated very good inactivation results of several human waterborne pathogens (*E. coli*, spores of sulphite-reducing clostridia, somatic coliphages and F-specific RNA bacteriophages) with 20 and 50 mg/L of H_2O_2 in flow solar CPC-reactors. These authors did not find a marked difference in the *E. coli* inactivation between the use of both concentrations of H_2O_2 [33]. Our results in RE for *E. coli* and *E. faecalis* agree with these findings, as inactivation results at 20 and 50 mg/L were very similar. The main hypothesis to explain this is based on the mere oxidative effect of H_2O_2 and derived ROS on internal organelles and cellular membrane, and the generation of hydroxyl radicals via internal Fenton reactions (Eqs. 1-2) with intracellular free or loosely bound iron [28]. According to this, the amount of oxidative species and oxidative attacks responsible for bacterial destruction or inhibition would be limited by the iron available inside cells. Therefore, an extra amount of H_2O_2 will not necessarily produce better disinfection results. This is observed in our results on H_2O_2/solar radiation treatment (Fig. 3), where the adding of 50 mg/L of H_2O_2 did not improve the results with lower concentrations of H_2O_2. On the other hand, the addition of an appropriate concentration of hydrogen peroxide plays an important role in the inactivation because of the presence of DOM in water, which can compete with the inactivation of microorganisms [22].

All solar treatments evaluated in this contribution for disinfection of RE lead to very promising results (Fig. 8a-b). Furthermore, it is worth mentioned that the treatment of Fe^{2+}/solar radiation with 10 mg/L at pH 5 achieved the DL in *E. coli* (5.44-log) and *E. faecalis* (5.26-log) after 4 hours of treatment at 32.28 kJ/L. Different solar UV-A dosages accumulated in the sample (Q_{UV}) were needed to inactivate both bacteria; *E. faecalis* required higher Q_{UV} than *E. coli*. In both cases, the inactivation order was: H_2O_2/Solar (50 mg/L) > photo-Fenton (10/50 mg/L of Fe^{2+}/H_2O_2) > H_2O_2/Solar (20 mg/L) > Photo-Fenton (10/20 mg/L of Fe^{2+}/H_2O_2) > Fe^{2+}/Solar (10 mg/L).

The best inactivation result in RE was obtained with 50 mg/L of H_2O_2/solar (Fig. 8). The worse inactivation performance for photo-Fenton compared to H_2O_2/solar treatment may be due to the following factors acting together: i) the effect of precipitated iron at pH 5 (Table 3), since the initially added iron quickly precipitated so that the concentration of dissolved iron was 0.3-0.2 mg/L at the end of the experiment; this lead to iron loose and light screening of precipitated iron during the experiment; ii) the competition of DOM and bacteria, as well as other microorganisms present in the RE for the $OH^{\bullet}$ radical generated during the photo-Fenton. Nevertheless, when DOC reduction is considered, photo-Fenton is much more effective than H_2O_2/solar treatment, as a 59.3 % and 10.6 % of DOC reduction was obtained, respectively. SE contains simple organic matter, i.e. linear chain organic compounds, aliphatic type like carboxylic acids (acetic, formic, propionic, pyruvate and maleic), which are difficult to degrade by photo-Fenton. These are more difficult to mineralise than the organic matter present in RE, although this matrix has more ions and some suspended matter. This explain a better DOC degradation for RE than for SE (tables 2 and 3). Moreover, at near neutral pH in RE, the presence of humic acids and other natural photosensitizers help the iron to be active for photo-Fenton reactions. This may not happen in artificial water matrix, which doesn't contain any of such organic matter [21].

In conclusion, very promising results with solar photo-Fenton at pH 5 in RE were obtained. The complexity of the application of these solar treatments to real effluents and wastewater lies in the chemical and microbiological composition of this kind of effluents, high organic and faecal load and presence of inorganic scavengers of hydroxyl radicals.

5. Conclusions

The main conclusions drawn from this study are summarized as follows:

- Solar photo-Fenton at pH 3 achieved complete inactivation of bacteria in SE with low dosages of solar UV energy. However at pH 5, due to precipitated iron, the reaction rate decreases and higher dosages of UV energy accumulated are needed to achieve a good inactivation result.
- Precipitated iron at pH 5 does not increase the generation of hydroxyl radicals via Fenton reaction. The presence of precipitated iron provides an orange-brown shade to the water and decelerates the photo-Fenton treatment.
- The combination of hydrogen peroxide and solar irradiation provides an important synergetic effect in the inactivation of bacteria present in SE and RE. Diffusion of hydrogen peroxide into the cells permits the generation of hydroxyl radical via Fenton reaction with intracellular iron. Proper hydrogen peroxide dosage will provoke complete bacterial inactivation in water containing both organic matter and inorganic scavengers.
- ~~Structural differences between Gram-negative and Gram-positive bacteria imply a different behaviour in their inactivation.~~ *Escherichia coli* (Gram-negative bacteria) is more sensitive that *Enterococcus faecalis* (Gram-positive bacteria) to the disinfection treatments. Therefore, the use of *Escherichia coli* as indicator for water disinfection studies should be reconsidered, because their high sensitivity to all solar treatments does not imply the absence of other faecal bacteria.
- The use of solar disinfection treatments like, solar photo-Fenton and H_2O_2/solar at pH 5 for real wastewater effluent (RE) lead to promising results. Complete bacterial (*E. coli* and *E. faecalis*) inactivation was reached in spite of the complex real wastewater matrix, with its inherent sample chemical composition variability and the presence of a high amount of organic and inorganic matter.

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Table captions

Table 1. Physicochemical characteristics of the simulated (SE) and real municipal wastewater treatment plant effluent (RE) used in this experimental work.

Table 2. Initial (i) and final (f) values of pH, DO and DOC for the experiments in SE samples. The last column shows if the detection limit (DL = 2 CFU/mL) was reached at any time of the experiment. Q_{UV} shows the accumulated solar UV irradiation per unit volume after 4 hours of experiment.

Table 3. Initial (i) and final (f) values of pH, DO and DOC for the experiments in RE samples. The last column shows if the detection limit (DL = 2 CFU/mL) was reached at any time of the experiment. Q_{UV} shows the accumulated solar UV irradiation per unit volume after 4 hours of experiment.

Figure captions

Figure 1. Solar CPC reactors at Plataforma Solar de Almería facilities.

Figure 2. Average solar UVA irradiance and temperature of water samples over all experiments in the CPC pilot reactors (April-July 2012, 10:30-14:30 local time). Error bars correspond to standard deviation.

Figure 3. Inactivation of (a) *E. coli* and (b) *E. faecalis* in SE with H_2O_2/solar radiation at pH 5 and different concentrations of H_2O_2: 5 mg/L (■); 10 mg/L (●); 20 mg/L (▲); 25 mg/L (▼), 50 mg/L (♦) and dark control with 50 mg/L (◇). Open squared symbols (□) were used to indicate that the detection limit (2 CFU/mL) was reached in the experiment.

Figure 4. Inactivation of (a) *E. coli* and (b) *E. faecalis* in SE using solar photo-Fenton at pH 5 at several Fe^{2+}/H_2O_2 concentrations: 2.5/5 mg/L (■); 5/10 mg/L (●); 5/25 mg/L (▲); 10/20 mg/L (▼); 10/50 mg/L (◆). Dark Fenton reactions are sown at Fe^{2+}/H_2O_2 concentrations: 10/20 mg/L (▽) and 10/50 mg/L (◇). Open squared symbols (□) were used to indicate that the detection limit (2 CFU/mL) was reached in the experiment.

Figure 5. *E. coli* and *E. faecalis* in SE viability evolution in the CPC reactor under solar light alone (□,○), and with added 10mg/L- Fe^{2+} (■, ●).

Figure 6. Inactivation curves of (a) *E. coli* and (b) *E. faecalis* in SE using solar photo-Fenton at pH 3 (prior 10 min Fenton in the dark) at Fe^{2+}/H_2O_2 concentrations of 2.5/5 mg/L (■), 5/10 mg/L (▼), 10/20 mg/L (●), and 10/50 mg/L (♦). Dark Fenton reactions are sown at Fe^{2+}/H_2O_2 concentration of 10/20 mg/L (▽). Open squared symbols (□) were used to indicate that the detection limit (2 CFU/mL) was reached in the experiment.

Figure 7. *E. coli* (■, □) and *E. faecalis* (●, ○) evolution under solar photo-Fenton at pH5, with 10/20 mg/L of Fe^{2+}/H_2O_2 in bottle reactors: unfiltered (full symbols) and filtered water (open symbols).

Figure 8. Comparison of inactivation levels on a) *Escherichia coli* and b) *Enterococcus faecalis* in RE after the application of different treatments: 20 mg/L H_2O_2, pH 5 (■);Solar photo-Fenton 10 mg/L Fe^{2+}, 20 mg/L H_2O_2, pH 5 (●);10 mg/L Fe^{2+}, pH 5 (▲);50 mg/L H_2O_2, pH 5 (▼);Solar photo-Fenton 10 mg/L Fe^{2+}, 50 mg/L H_2O_2, pH 5 (♦). Open squared symbols (□) were used to indicate that the detection limit (2CFU/mL) was reached in the experiment.

Table 1

	SE	RE
Na^+ (mg/L)	35.80 ± 1.10	211.40 ± 22.80
NH_4^+ (mg/L)	2.70 ± 1.00	32.00 ± 11.70
K^+ (mg/L)	3.40 ± 0.60	33.10 ± 5.80
Mg^{2+} (mg/L)	17.20 ± 0.30	48.00 ± 5.90
Ca^{2+} (mg/L)	21.63 ± 2.30	117.00 ± 10.30
SO_4^{2-} (mg/L)	9.00 ± 1.40	102.60 ± 28.80
Cl^- (mg/L)	11.50 ± 2.10	337.50 ± 10.80
NO_3^- (mg/L)	130.40 ± 7.60	23.50 ± 16.00
PO_4^{3-} (mg/L)	12.10 ± 3.00	17.10 ± 29.80
pH	8.15 ± 0.30	7.53 ± 0.10
Conductivity (µS/cm)	362 ± 12	1458 ± 89.80
Turbidity (NTU)	1.50 ± 0.10	14.60 ± 6.62
DOC (mg/L)	20 - 30	17.00 ± 3.00
IC (mg/L)	0.5 - 4	56.50 ± 6.60
***E. coli* (CFU/mL)**	-	10^3
***E. faecalis* (CFU/mL)**	-	10^3

DOC = Dissolved Organic Carbon
DIC = Dissolved Inorganic Carbon

Table 2

Conc. (mg/L)	Fig.	pH	DOC reduction (%)	Dissolved Fe^{T}_{i}/Fe^{T}_{f} (mg/L)	Q_{UV}, (kJ/L)	DL
(H_2O_2)			***H_2O_2/Solar at pH 5***			
5	3	5.1	-	-	32.4	NO
10	3	4.9	-	-	32.3	*E. coli*
20	3	5.3	-	-	35.0	*E. coli*
25	3	4.9	-	-	32.4	*E. coli*
50	3	5.1	-	-	36.7	YES
(Fe-H_2O_2)			***Solar photo-Fenton at pH 5***			
2.5-5	4	5.1	-	0.2/0.1	35.1	NO
5-10	4	5	-	0.2/0.0	36.9	NO
5-25	4	5	-	1.0/0.1	34.8	*E. coli*
10-20	4	4.9	19	0.5/0.2	35.6	*E. coli*
10-50	4	4.9	22	0.14/0	34.8	YES
(Fe-H_2O_2)			***Solar photo-Fenton at pH 3***			
2.5-5	6	2.9	28	0.9/0.6	32.6	YES
5-10	6	3	51	2.9/1.2	34.1	YES
10-20	6	3.1	69	6.5/2.6	37.1	YES
10-50	6	3	70	7.5/1.0	35.2	YES
(Fe)			***Fe^{2+}/Solar***			
10	5	5.2	8	6.0/4.5	33.8	NO

DOC: Dissolved Organic Carbon, no data are shown when reduction was below the detection limit of DOC measurement.
Dissolved Fe^{T}_{i}/Fe^{T}_{f} : total (Fe^{2+} and Fe^{3+}) dissolved iron (mg/L) in the initial and final samples.
Q_{UV}: solar UV-A radiation accumulated in the sample after the treatment.
DL: Detection limit

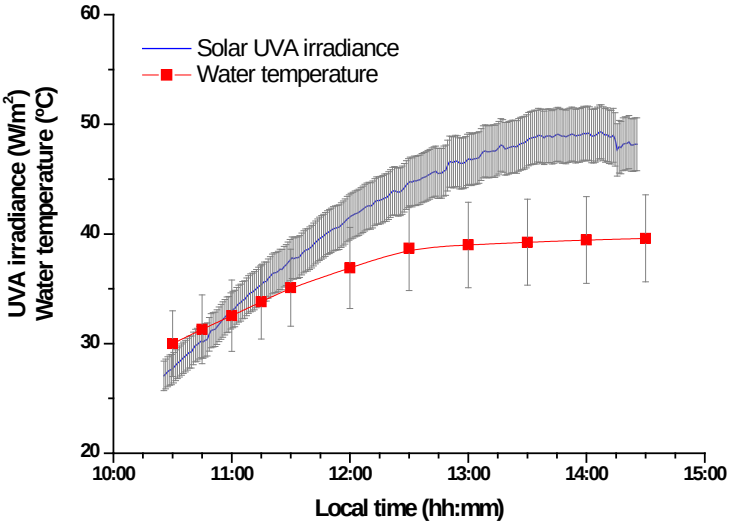
Table 3

Conc. (mg/L)	Fig.	pH	DOC reduction (%)	Dissolved Fe^{T}_{i}/Fe^{T}_{f} (mg/L)	Q_{UV}, (kJ/L)	DL
(H_2O_2)			***H_2O_2/Solar at pH 5***			
20	8	5.2	8.3	-	34.9	*YES*
50	8	4.9	10.6	-	34.1	*YES*
(Fe-H_2O_2)			***Solar photo-Fenton at pH 5***			
10-20	8	5.2	32.5	12/0.3	37.4	*YES*
10-50	8	4.7	59.3	15/0.2	37.0	*YES*
(Fe)			***Fe^{2+}/Solar***			
10	8	5	1	6.7/0.5	36.0	*YES*

DOC: Dissolved Organic Carbon, no data are shown when reduction was below the detection limit of DOC measurement.
Dissolved Fe^{T}_{i}/Fe^{T}_{f} : total (Fe^{2+} and Fe^{3+}) dissolved iron (mg/L) in the initial and final samples.
Q_{UV}: solar UV-A radiation accumulated in the sample to achieve the detection limit after the treatment.
DL: Detection limit.

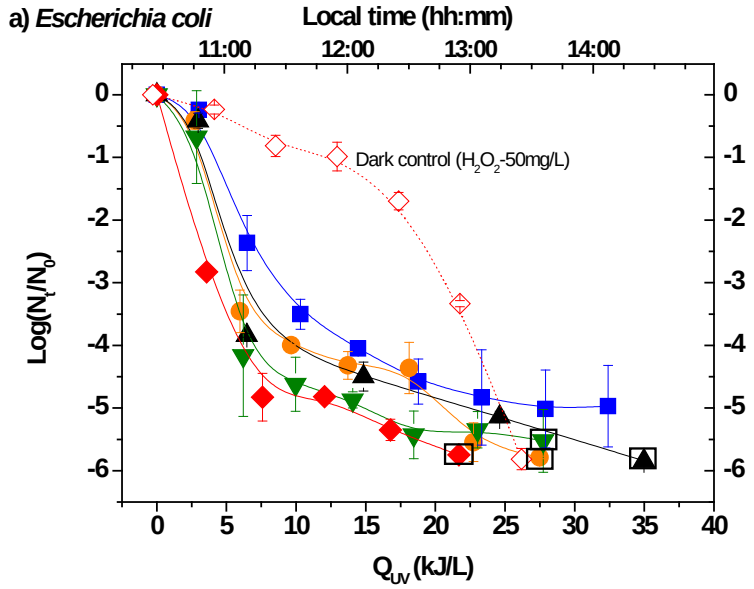
Figure 1

1 **Figure 2**


Solar UVA irradiance
Water temperature
UVA irradiance (W/m²)
Water temperature (°C)
60
50
40
30
20
10:00
11:00
12:00
13:00
14:00
15:00
Local time (hh:mm)

2

Figure 3


a) Escherichia coli
Local time (hh:mm)
11:00
12:00
13:00
14:00
Dark control (H_2O_2-50mg/L)
Log(N_t/N_0)
Q_{UV} (kJ/L)

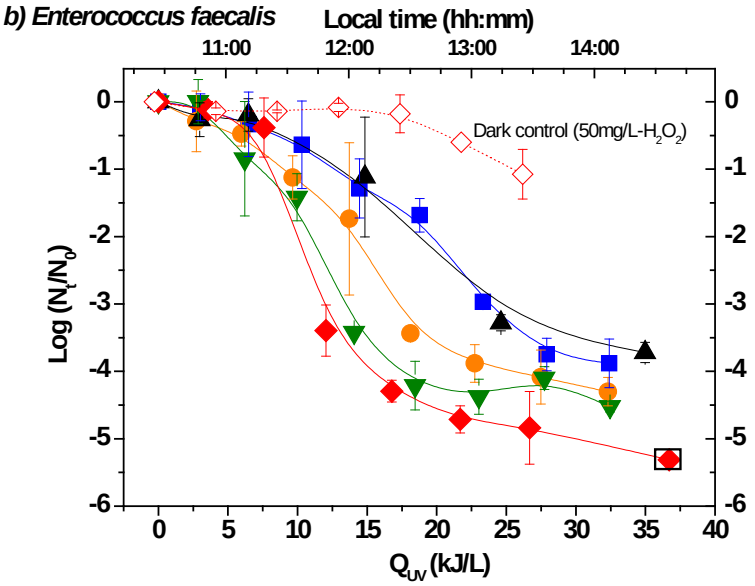
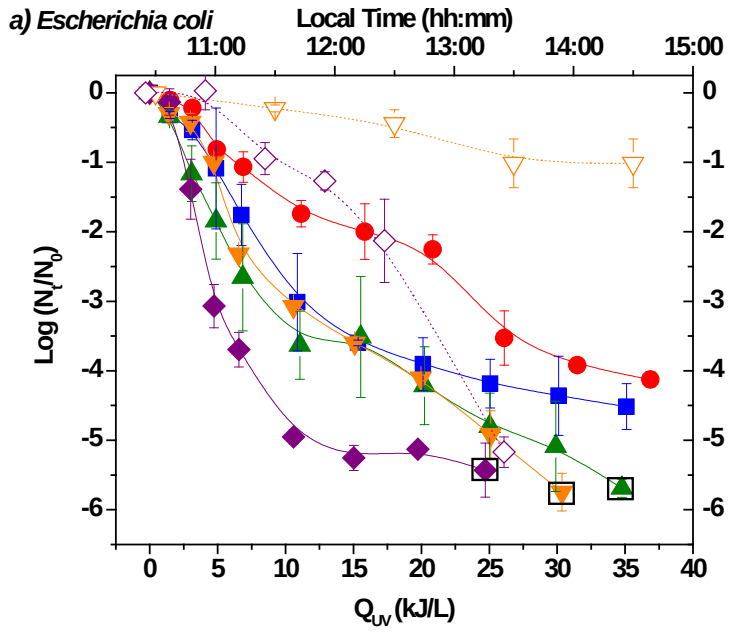

b) Enterococcus faecalis
Local time (hh:mm)
11:00
12:00
13:00
14:00
Dark control (50mg/L-H_2O_2)
Log (N_t/N_0)
Q_{UV} (kJ/L)

Figure 4


a) Escherichia coli
Local Time (hh:mm)
11:00
12:00
13:00
14:00
15:00
Log (N/N0)
QUV (kJ/L)

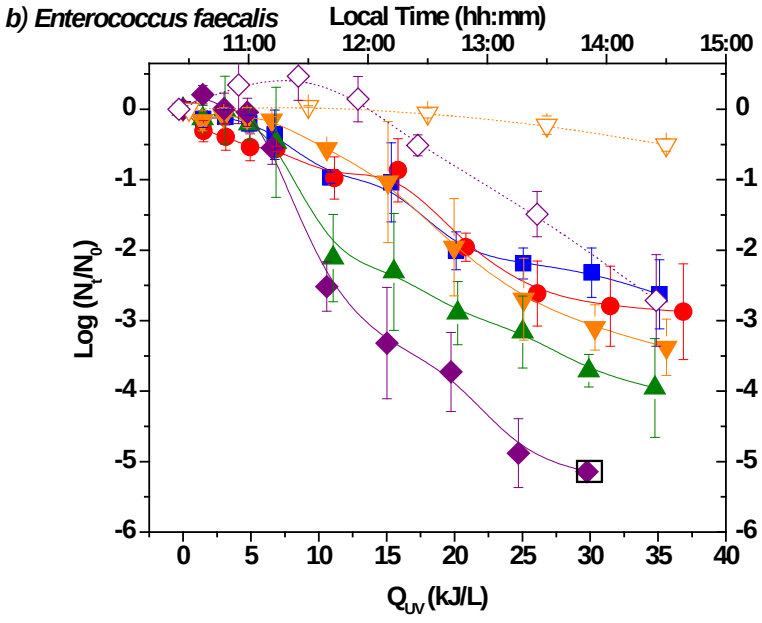

b) Enterococcus faecalis
Local Time (hh:mm)
11:00
12:00
13:00
14:00
15:00
Log (N/N0)
QUV (kJ/L)

Figure 5

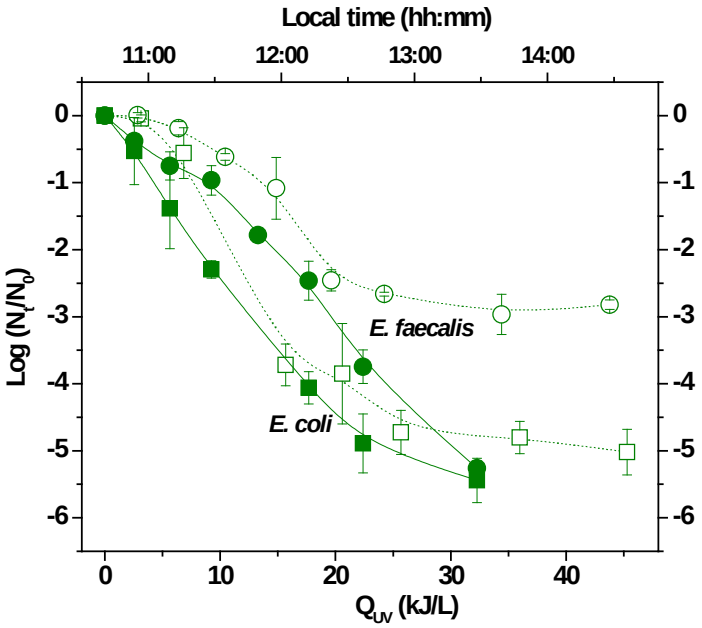
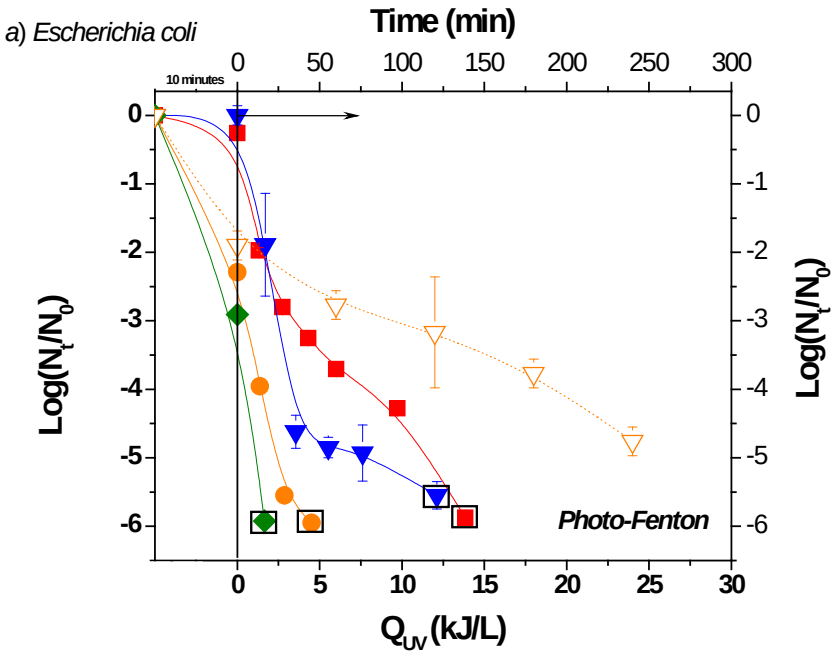

Local time (hh:mm)
11:00
12:00
13:00
14:00
0
-1
-2
-3
-4
-5
-6
Log (N/N0)
E. faecalis
E. coli
0
10
20
30
40
QUV (kJ/L)

Figure 6


a) Escherichia coli
Time (min)
0 50 100 150 200 250 300
10 minutes
0 -1 -2 -3 -4 -5 -6
Log(N_t/N_0)
Log(N_t/N_0)
Photo-Fenton
0 5 10 15 20 25 30
Q_UV (kJ/L)

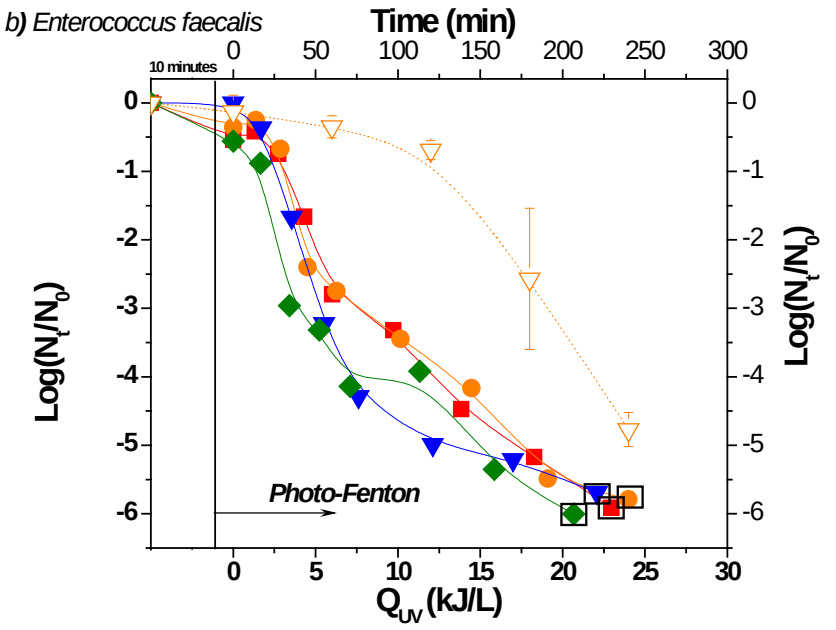

b) Enterococcus faecalis
Time (min)
0 50 100 150 200 250 300
10 minutes
0 -1 -2 -3 -4 -5 -6
Log(N_t/N_0)
Log(N_t/N_0)
Photo-Fenton
0 5 10 15 20 25 30
Q_UV (kJ/L)

Figure 7

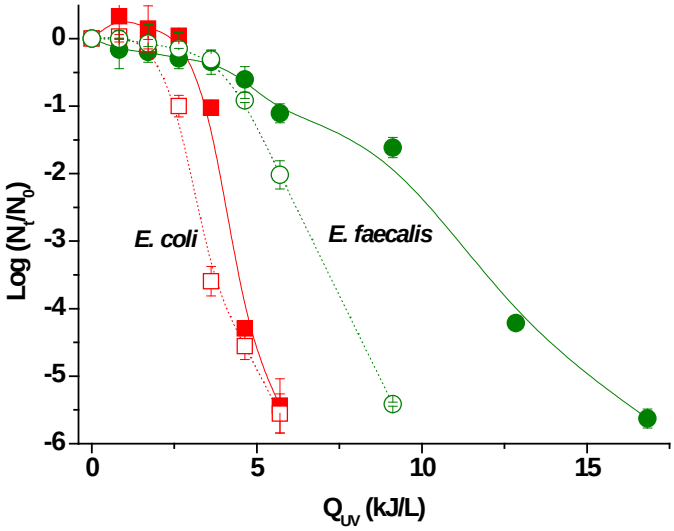
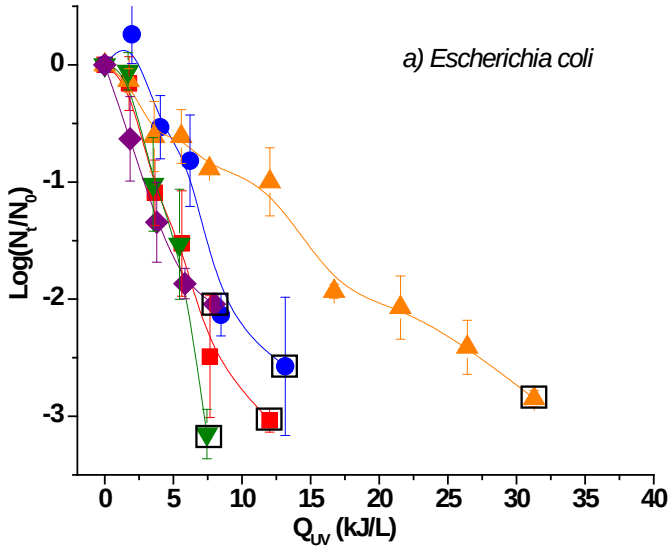
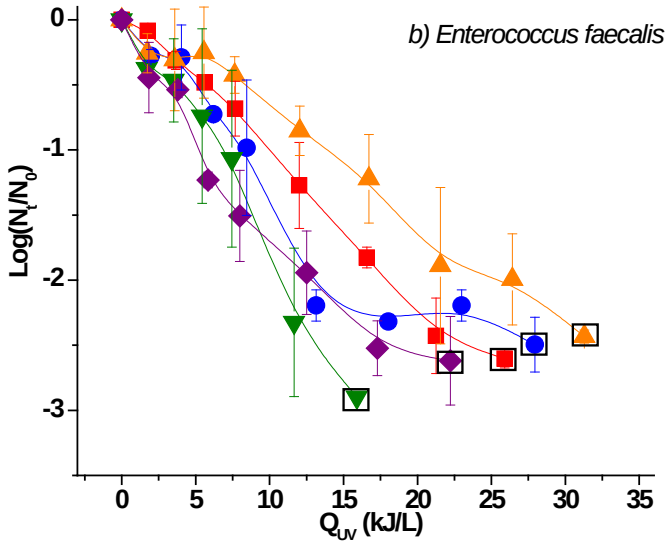


Figure 8


a) Escherichia coli
Log(N_t/N_0)
Q_UV (kJ/L)
0
-1
-2
-3
0
5
10
15
20
25
30
35
40


b) Enterococcus faecalis
Log(N_t/N_0)
Q_UV (kJ/L)
0
-1
-2
-3
0
5
10
15
20
25
30
35