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Application of azo dyes as dosimetric indicators for enhanced photocatalytic solar disinfection (ENPHOSODIS)

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ABSTRACT

The use of azo dyes as dosimetric indicators to measure the efficiency of enhanced photocatalytic solar disinfection has been developed based upon the solar dose required to inactivate helminth ova, a highly resistant waterborne pathogen frequently found in surface water sources in developing countries. A range of treatment conditions were examined to determine the optimal inactivation conditions required for a range of pathogens. The inactivation data were fitted using a modification of the Chick–Watson kinetic model. It was determined that the radiation dose required for >5-log helminth egg inactivation was approximately 140 kJ L^{-1} (using photo-Fenton reaction at [Fe(II)] = 10 mM and initial [H_2O_2] = 280 mM). In order to develop a dosimetric indicator providing a visual color change corresponding to this dose, a range of reaction conditions were examined to achieve removal of a dye, Acid Orange 24 (AO24). For experiments performed at [Fe(II)] = 0.7 mM and initial [H_2O_2] = 5 mM, complete color removal was achieved following receipt of a dose equal to 155 kJ L^{-1}. 6-log inactivation of *Escherichia coli* and *Pseudomonas aeruginosa* was achieved following receipt of less than 10 kJ L^{-1}. No significant increase in the inactivation dose was required when up to 5 mg L^{-1} natural organic matter (NOM) was added to the bacterial suspension. These results confirm that helminth eggs are an appropriate index for microbiologically safe water following enhanced photocatalytic solar disinfection. AO24 dye degradation was determined to serve as an accurate dosimetric indicator. The indicator employed is easy to use in the laboratory and field conditions, where the dye solutions may be prepared on-site and submitted to solar radiation in a glass vial in close proximity with water being disinfected in the solar collector. The user can easily and quickly monitor the treatment efficiency and be confident that the water disinfection process is complete when complete discoloration has been reached.

1. Introduction

The need to supply safe drinking water in developing countries is a major critical necessity. In Africa, Latin America and the Caribbean, nearly one billion people have no access to safe water supplies [1]. The resultant burden of waterborne diseases has serious human health effects and results in the death of 1.5 million children every year [2]. In addition to health concerns, the lack of access to safe drinking water is commonly associated with poverty [3,4] and presents considerable limitations for sustainable development [5,6]. In Mexico diseases caused by waterborne microorganisms, and other water contaminants, affect 6.4% of the total population [7]. Small rural communities, with population of less than 2500 inhabitants are usually the most affected. This sector represents around 25.3% of the Mexican population, of whom only about 65% have access to piped water supply systems [8].

Upon release into the environment, human pathogens become sensitive to the environmental conditions. Therefore, certain environmental parameters such as temperature and ultraviolet (UV) radiation can be used to inactivate the pathogens present in polluted water [9]. Solar water disinfection (SODIS) is a simple, environmentally friendly and low cost point-of-use treatment technology for drinking water purification [10]. SODIS uses the bacteriostatic effect of the UV-A part (wavelength 320–400 nm) of the solar radiation and the presence of dissolved oxygen to inactivate pathogens in water by production of reactive forms of oxygen. These reactive oxygen species (ROS) contribute to the inactivation of pathogenic microorganisms. SODIS typically uses UV-A-transparent polyethylene terephthalate (PET) bottles of

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volumes ranging from 0.3 to 2.1 L that are filled to ¾ of their capacity with water and agitated to increase dissolved oxygen content in water. The bottles are exposed to sunlight for a minimum period of 6 h. According to the department of water and sanitation in developing countries (SANDEC) of the Swiss Federal Institute of Aquatic Science and Technology [9,11], the best bacteria inactivation effect is reached on sunny days when heat and UV radiation combine synergistically [12,13]. However, in a cloudy day (more than 50% covered sky), it is necessary to use longer exposure times (two days or even longer) to disinfect water [14,15]. Several different systematic studies have been undertaken to determine energy requirements and doses to achieve pathogen inactivation to a certain level [16–22]. Some studies [10,20] have shown that thermal inactivation of *Escherichia coli* is important only when water reaches temperatures over 45 °C, when a strong synergy with the effect of radiation is observed. These studies concluded that in places with high ambient temperatures, disinfection using solar energy is a low cost and effective method to improve the microbiological quality of water. However, bacterial re-growth after short storage (24 h) of SODIS treated water has been observed [14,19]. In more recent reports, seeking improvements in SODIS performance, research has focused on a reduction in irradiation time and prevention of bacterial re-growth. Advanced oxidation processes (AOPs) could play a critical role in SODIS enhancement. AOPs generate hydroxyl radicals (•OH) via titanium dioxide (TiO_2) photocatalysis, Fenton reagent (ferrous iron and hydrogen peroxide), UV/hydrogen peroxide, UV/ozone, electron beam excitation, sonolysis, and gamma irradiation. Among various AOPs, photocatalytic processes are very attractive for the mineralization (conversion to carbon dioxide, water, and other mineral species) of aqueous pollutants [23–25,53] and inactivation of pathogenic microorganisms [26–32,52]. Application of these technologies to water disinfection using solar radiation, coined as enhanced photocatalytic solar disinfection (ENPHOSODIS), has allowed the efficient inactivation of highly resistant microorganisms [30,33–35,55]. However, the problem concerning the determination of the amount of radiation required for complete inactivation of the microorganisms is just starting to be reported [36,54]. To date, despite that several methodologies for radiation dose measurements have been reported, research on inexpensive and easy way to determine the end point of the inactivation process in poor and isolated rural zones of developing countries, is necessary. The aim of this work is to explore the use of an azo dye as dosimetric indicator in enhanced photocatalytic solar disinfection processes for the inactivation of *Ascaris* ova, a highly resistant waterborne pathogen. In addition, the validity of the indicator system is evaluated for the inactivation of two strains of pathogenic bacteria: *E. coli* and *Pseudomonas aeruginosa*.

2. Methodology

2.1. Reagents

Chemicals used in the experiments, $FeSO_4{\cdot}7H_2O$ (Baker), H_2O_2 (50% stabilized) industrial grade and sodium hydroxide (Merck) were used as received. Acid orange 24 (AO24) industrial grade was supplied by Orion Co. (Cuernavaca, Mexico). *Ascaris suum* eggs were purchased from Excelsior Sentinel Inc. (Ithaca, NY) as a concentrate with 100,000 eggs (viability of 90%).

2.2. Photoreactor

Photo-assisted experiments were carried out using solar radiation and conducted in a bench-scale solar collector. Tests for enhanced photocatalytic solar disinfection using helminth ova and for dye degradation were carried out in individual 50 mL pyrex glass vials transparent to solar UV and visible radiation. The 50 mL glass vials were placed in the focus of a compound parabolic concentrator (CPC) detailed elsewhere [25,37,56]. The system was fixed at 19° (local latitude) and had a total collection surface of 0.1 m^2. Global radiation from 280 to 2800 nm was measured during the experiments using a Li-Cor pyranometer (LI-200SA) which was placed at the same angle as that of the solar collector. Since the photo-Fenton reaction allows the use of wavelengths from 300 to 650 nm for solar driven processes, the actual incoming irradiation was estimated using as reference an AM1.5 standard, from which a 0.35 factor was obtained for the radiation included in this wavelength range, as proposed in previous works [37,38]. Accumulated energy, defined as the total amount of irradiative energy reaching the reactor since the beginning of the experiment up to a given time per unit volume, was determined using the relation previously reported by Goslich et al. [39] and previously used as a measurement of solar radiation dose on the photocatalytic disinfection of bacteria and fungi [19]:

$$Q_n = Q_{n-1} + \Delta t\, G_n \left(\frac{A}{V}\right), \quad \Delta t = t_n - t_{n-1} \tag{1}$$

where Q_n is the accumulated energy (kJ L^{-1}), Δt is the time between radiation measurements, G_n is the adjusted global radiation (W/m^2) measured, in the 300–1200 nm range, in the radiometer in each experiment, A is the module area (m^2) and V is the total system volume (L).

2.3. Culture preparation

From the *A. suum* concentrate, dilutions with approximately 6250 ova were prepared in 20 L of sterilized, distilled, de-ionized water. All the experimental runs were carried out using this *A. suum* egg concentration. To determine egg viability during the disinfection process, the methodology proposed by the Mexican legislation (NOM-004-SEMARNAT-2002) was used. Briefly, samples obtained at different exposure times were diluted to 30 mL with distilled-deionized water and incubated at 26 °C during 4 weeks, while mixing once per week by hand. After incubation, each sample was concentrated by centrifugation (1000 × *g* for 5 min) and the pellet was observed using a microscope. Presence of larvae in the eggs was considered as a positive viability test. The percentage of viable eggs was calculated by dividing the number of viable eggs by the total number of eggs observed and multiplying by 100.

2.4. Photo-assisted pathogen inactivation

All the inactivation experiments were performed in sterilized deionized water. *A. suum* eggs were placed in silanized 50-mL glass vials. Once the eggs were transferred to the glass vial, Fe(II) was added until desired concentration (0, 5 or 10 mM) was reached. The vial was shaken in a vortex mixer and a sample (100 μL) removed. This was considered as the initial time point ($t = 0$). The sample was filtered, rinsed and stored in the freezer at 2 °C until incubation. At, $t = 0$ H_2O_2 (0, 140 or 280 mM) was added to photoreactor; the addition of hydrogen peroxide was considered as the start of the inactivation process. Samples were collected at $t = 30$, 60, 90 and 120 min for *A. suum* viability analysis. Samples were immediately filtered and rinsed with sterilized deionized water, to remove the remaining reagents and avoid further oxidation reaction, and stored at 2 °C until incubation. Upon completion of the experimental run, all samples were submitted to the viability test described in Section 2.4. All experiments were carried out in triplicate, and the error was estimated to be less than 15%.

2.5. Design of visual dosimeter

AO24 dye samples were prepared by dissolving 200 mg L^{-1} in reagent grade water. The effects of Fe(II) (0, 0.7, 1.0 and 1.5 mM) and H_2O_2 (0, 5.0 and 10 mM) concentrations were examined in the experiments, considering previous results reported by Chacon et al. [37]. The initial pH in the synthetic samples was adjusted to c.a. 3.0 using H_2SO_4 0.1 M. The dye samples were added to 50-mL glass vials (as described in Section 2.3). For every experiment, an initial sample (1 mL) was taken following preparation of the AO24 solution. Ferrous iron was then added to the dye solution and the vial content was mixed using a vortex mixer. Following addition of H_2O_2, the vial was caped, and immediately placed in the focus of the solar collector. This point was considered as the beginning of the photo-assisted degradation process ($t = 0$). Samples were removed every 5 min and the AO24 concentration was determined immediately using a diode array HP-8452 UV–vis at 430 nm.

2.6. Inactivation of E. coli and P. aeruginosa

E. coli (ATCC-25922) was obtained from ATCC and *P. aeruginosa*, isolated from a clinic sample (wound) and identified using the 32GN gallery of mini-API system, were used in this work.

For the ENPHOSODIS experimental runs, each strain (stored at −20 °C) were first inoculated in soy tripticasein agar (STA) (Bioxon, México) and incubated at 37 °C overnight. Two colonies were then inoculated in trypticasein soy broth and incubated under constant agitation (orbit shaker at 250 rpm) overnight. Suspensions were prepared using 300 mL of sterile de-ionized water in a glass flask and inoculated with the bacterial cells from a logarithmic phase bacterial culture. The initial concentration (C_0) of bacteria ranged from 10^6 to 10^7 CFU mL^{-1}. For every experimental run, two flasks were used, one for the ENPHOSODIS treatment and the other as blank, to assess the effect on inactivation by solar disinfection. In both cases, magnetic stirring was used to mix the solution. No pH adjustment was carried out for the reaction mixture in any of the additional experiments described here. For the photo-Fenton reaction, the same Fe(II)/H_2O_2 concentration determined as the most effective for helminth eggs inactivation was used. After preparation of the culture suspension, $FeSO_4 \cdot 7H_2O$ was added to reach the desired Fe(II) concentration and the mixture stirred in the dark for 1 min. After this time, the required quantity of H_2O_2 was added and the flasks exposed to sunlight. No significant variation in the pH value of the mixture was observed.

In all tests, 100 μL samples were taken at 0, 15, 45, 75 and 105 min. Once taken, samples were diluted up to 10^8 times using 0.85% sodium chloride solution at pH 7.0. Between dilution steps, the bacterial suspensions were mixed using a vortex mixer to ensure the homogeneity and 10 μL of every dilution were inoculated in STA. Colonies were visually identified and counted following 24 h incubation at 37 °C in a microbiological incubator. In all the experiments, solar radiation was measured as described in Section 2.2. Experiments were carried out in triplicate.

2.7. Influence of natural organic matter on disinfection rate

In order to test the effect of the presence of natural organic matter (NOM) on the inactivation reaction rate, experiments using different NOM concentrations (2.5, 5 and 10 mg L^{-1}) were carried out. To perform these experimental runs, Suwannee River Natural Organic Matter (SR-NOM, obtained from IHSS) was used. A SR-NOM stock solution was prepared in distilled water and the required amount of the stock solution transferred to the reaction mixture in the vials to obtain the desired NOM concentration. For experiments using SR-NOM, this reagent was added before addition of Fenton reagents to the mixture and the inactivation experiments were performed as previously described.

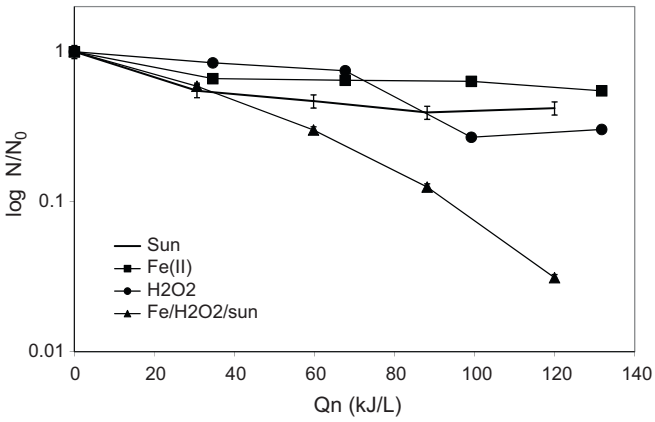

Fig. 1. Solar inactivation of helminth eggs using mild ENPHOSODIS reagents ([Fe(II)] = 5 mmol L^{-1}; [H_2O_2] = 140 mmol L^{-1}).

3. Results and discussion

3.1. Photo-assisted pathogen inactivation

Low helminth ova inactivation was obtained by the use of Fenton reaction under solar radiation using mild reagents concentrations (Fig. 1). For these conditions ([Fe(II)] = 5 mM, [H_2O_2] = 140 mM), 97% inactivation (slightly over 1.5-log inactivation) after 120 kJ L^{-1} of accumulated energy was achieved. The effects of solar radiation alone (solar disinfection) and those of hydrogen peroxide and Fe(II) alone did not result in marked disinfection.

Ova inactivation via solar disinfection process showed better results than when Fe(II) was used without addition of any other reagent to the photoreactor. This result could be due to the ability of Fe(III), resulting from the oxidation of Fe(II) in water and in the presence of oxygen, to absorb solar radiation in the UV–vis region (about 300 and 550 nm) as proposed earlier [40] which could compete with helminth eggs for photon absorption in this wavelength range. A more pronounced effect was shown by the use of solar radiation without addition of any reagent (solar disinfection) where 58% inactivation was achieved using 120 kJ L^{-1} of accumulated energy. SODIS has been widely reported to inactivate a range of pathogens producing microbiologically safe drinking water, however, it is normally considered ineffective against resistant microorganisms [14,19,41]. If solar radiation is able to inactivate up to 50% kill of a resistant organism like the helminth ova, it is anticipated that, at this dose, solar radiation will be capable of inactivation of less resistant microorganisms. When hydrogen peroxide was used, ova inactivation slightly improved (70% inactivation under approximately 130 kJ L^{-1} of accumulated energy), probably as a result of the photo-assisted cleavage of the hydrogen peroxide by the solar radiation. While this phenomenon was reported to occur at shorter wavelengths (i.e., 254 nm), previous studies report that it can also occur even at UVA wavelengths, ~5% of the solar UV radiation available at ground level [42].

It is worthy to note that, in experiments using Fe(II) alone, increasing Fe(II) concentration in the reaction mixture did not show significant improvements in the inactivation of helminth ova. On the other hand, increase in hydrogen peroxide under solar radiation shows a considerable improvement.

Results for the effect of high Fenton reagent concentrations on pathogen inactivation are presented in Fig. 2. Under these conditions ([Fe(II)] = 10 mM, [H_2O_2] = 280 mM, solar radiation) a

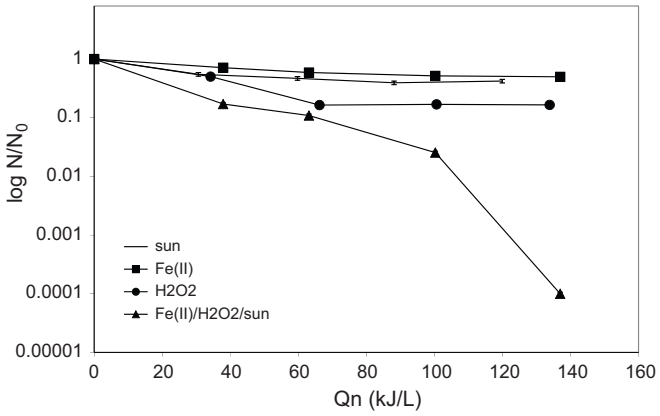

Fig. 2. Solar inactivation of helminth eggs using strong Fenton reagent conditions ([Fe(II)] = 10 mmol L^{-1}; [H_2O_2] = 280 mmol L^{-1}).

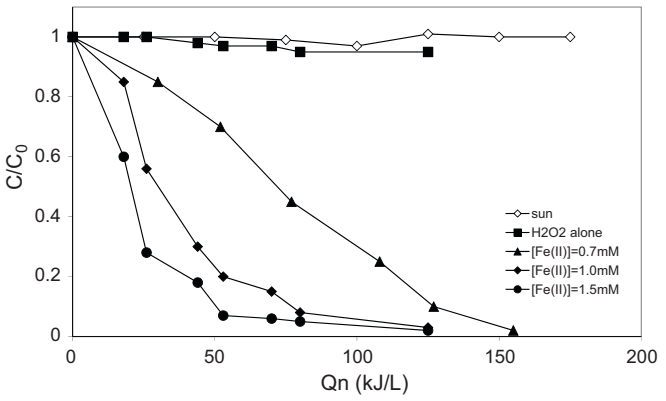

Fig. 3. Effect of Fe(II) concentration on the solar photo-Fenton degradation of AO24 (5 mmol L^{-1} initial H_2O_2 concentration).

4-log reduction in viable helminth ova was achieved using about 140 kJ L^{-1} of accumulated energy. By increasing H_2O_2 concentration from 140 to 280 mM, the helminth egg inactivation increased from 70 to 84%, when using about 140 kJ L^{-1} of accumulated energy.

Further increases in Fenton reagent concentrations are not shown because further improvement in the disinfection process was not observed. This is most probably due to the role of competitive reactions between hydroxyl radicals and excess Fe(II) or hydrogen peroxide taking place in the photo-Fenton process at high reagent concentrations exceeding certain determined levels [43,44].

For comparative purposes, results in Figs. 1 and 2 were fitted using a modification of the widely known Chick–Watson kinetics [45–47]. The modification made in the Chick–Watson expression in this study was to replace the $C \times t$ factor (the product of disinfectant concentration and reaction time) with the accumulated energy (dose). The use of Q_n as an estimation of the radiation dose has been proposed in the past [19,26] (shown in Eq. (1)). The first order reaction kinetic model used for fitting the experimental results is shown in Eq. (2):

$$\ln\left(\frac{N}{N_0}\right) = -kQ_n \tag{2}$$

where N_0 is the ova concentration at $t = 0$, N is the ova concentration at any process time, k is the inactivation rate constant (L kJ^{-1}) and Q_n is the accumulated energy (kJ L^{-1}). Data obtained applying Eq. (2) to experimental results are shown in Table 1.

From Table 1, although not all the kinetics follow a true 1st order model, results obtained using the model are useful to compare among the different conditions. The rate constant obtained for the lower concentration of Fe(II) alone is higher than those determined for the highest Fe(II) concentration. The reaction rate constant increased from 2.7×10^{-3} to 3.4×10^{-3} min^{-1} when the initial hydrogen peroxide concentration was increased from 140 to 280 mmol L^{-1}. Nevertheless, the most important improvement observed is when both, Fe(II) and H_2O_2 were used. For this process, the rate constant doubled (from 5.9×10^{-3} to 11.9×10^{-3} min^{-1}) due to increasing reagent concentration.

Table 1
Modified Chick–Watson kinetic values obtained for the different experimental conditions carried out for helminth ova inactivation.

Conditions	k ($/10^3$ L kJ^{-1})	R^2
Fe(II) (5 mmol L^{-1})	2.5	0.81
Fe(II) (10 mmol L^{-1})	2.0	0.6
H_2O_2 (140 mmol L^{-1})	2.7	0.85
H_2O_2 (280 mmol L^{-1})	3.4	0.93
Fe(II)/H_2O_2 (5 and 140 mmol L^{-1})	5.9	0.82
Fe(II)/H_2O_2 (10 and 280 mmol L^{-1})	11.6	0.94

3.2. Photo-assisted dye degradation

Fig. 3 shows the results of dye degradation using mild photo-Fenton conditions under solar radiation for the range of Fe(II) concentrations showed in Section 2.4 and low initial hydrogen peroxide concentration (5.0 mmol L^{-1}). As shown, no color removal was observed when the dye was subjected to solar radiation without addition of any reagent (photolysis) after about 175 kJ L^{-1} of accumulated energy. Similarly, no significant dye degradation was observed by using hydrogen peroxide alone during 125 kJ L^{-1}. As observed in the disinfection process, a significant increase in dye degradation rate occurred when Fenton reagent was used in combination with solar radiation (photo-Fenton), as shown in Fig. 3.

For the lowest Fe(II) concentration tested (0.7 mmol L^{-1}) using an initial hydrogen peroxide concentration of 5 mmol L^{-1}, complete dye removal was reached following absorption of 155 kJ L^{-1}. Increasing the Fe(II) concentration to 1.0 and 1.5 mmol L^{-1}, at the same initial concentration of hydrogen peroxide, resulted in increase in the reaction rate and complete dye degradation was reached within about 70 kJ L^{-1}. Further increases in accumulated energy caused further reduction in dye concentration but at a much slower rate, resulting in almost complete degradation of AO24 in 125 kJ L^{-1}.

Increasing the initial concentration of hydrogen peroxide to 10 mM, resulted in a significant increase in the reaction rate for dye degradation as shown in Fig. 4. Significant dye concentration depletion can be observed at the different Fe(II) concentrations

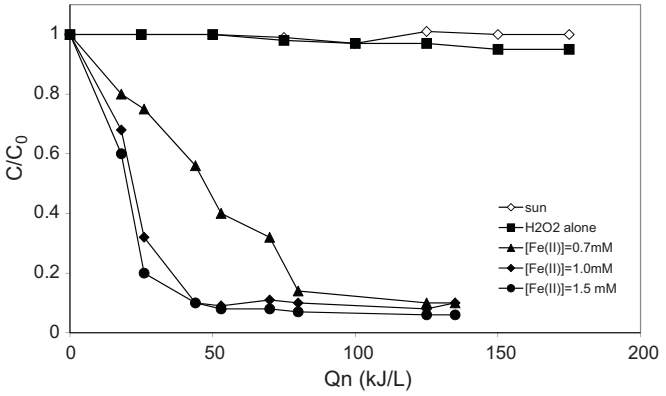

Fig. 4. Effect of Fe (II) concentration on the solar photo-Fenton degradation of AO24 (10 mmol L^{-1} initial H_2O_2 concentration).

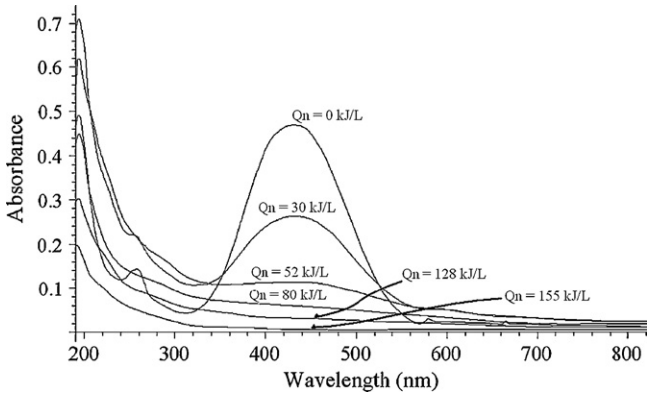

Fig. 5. Removal of AO24 color as a result of solar photo-Fenton degradation using different Q_n values ([Fe(II)] = 0.7 mmol L^{-1}; [H_2O_2] = 5 mmol L^{-1}).

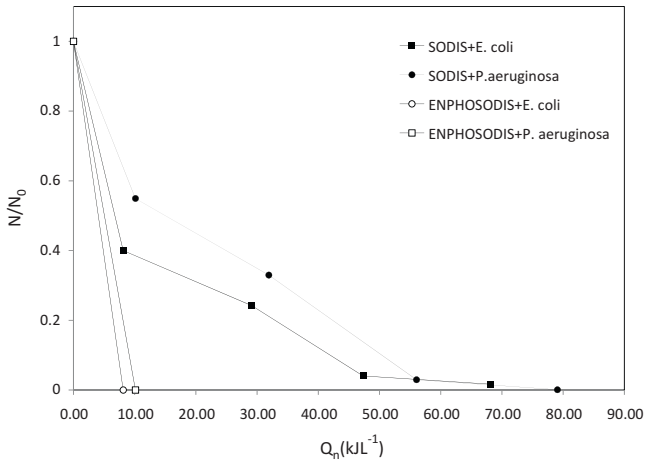

Fig. 6. Inactivation of *E. coli* and *P. aeruginosa* using solar photo-Fenton ([Fe(II)] = 5 mmol L^{-1}; [H_2O_2] = 140 mmol L^{-1}).

tested. In the case of the lowest Fe(II) concentration assessed (0.7 mmol L^{-1}), about 90% dye degradation was reached within 65 kJ L^{-1} of accumulated energy whereas for Fe(II) concentration of 1.0 and 1.5 mmol L^{-1}, dye degradation as high as 95% was achieved for a Q_n of 50 kJ L^{-1}.

3.3. Inactivation of E. coli and P. aeruginosa

In our research, we are interested not only in the fast dye degradation but also to determine the experimental conditions where both processes, water disinfection and dye degradation, match in the energy dose required for achieving the desired results. From Fig. 2, it can be observed that the highest helminth egg inactivation (4-log inactivation) was achieved using over 140 kJ L^{-1} and a H_2O_2/Fe(II) molar ratio of 28. By using these conditions, no viable *A. suum* egg was observed during the viability tests. These results suggest that, for the described experimental conditions, 140 kJ L^{-1} is a sufficient solar radiation dose to completely kill the helminth eggs in the synthetic water samples used in this work.

Because field measurements of global solar radiation require appropriate instruments not readily available where SODIS is practiced, a visual indicator which exhibits a color change equivalent to the solar dose is required. To determine the effectiveness of photo-assisted degradation of dye AO24 as an indicator, a solution containing 200 mg L^{-1} of AO24, [Fe(II)] = 0.7 mM and initial [H_2O_2] = 5 mM was exposed to solar radiation. Under such experimental conditions, dye degradation was achieved after absorption of a radiation dose of 155 kJ L^{-1} (Fig. 3). Fig. 5 shows the UV–vis absorption spectrum of AO24 for a range of different Q_n values (0, 30, 52, 89, 128 and 155 kJ L^{-1}). From the figure, it can be observed that AO24 has an important absorption band at 430 nm which is mainly associated with the characteristic orange color of the dye. As the process proceeds and the energy dose is increased, the absorbance value decreases until complete disappearance (after 155 kJ L^{-1}).

As suggested from Fig. 5, the time when the color in the vial containing the AO24 disappears could be considered equivalent to that when water subjected to ENPHOSODIS treatment, under the proposed conditions, will receive enough energy to inactivate 4-log of viable helminth ova. Complete color removal in the dosimeter, which must be irradiated separately and simultaneously to the water sample, would be a simple process use thereby demonstrating when ENPHOSODIS is complete.

We have chosen helminth eggs as index pathogen because it is well known that this kind of microorganism is highly resistant to adverse conditions [28,48–51]. Nevertheless, other pathogenic microorganisms such as *E. coli*, *P. aeruginosa*, *Candida albicans* and *Fusarium solani*, are expected to require lower energy doses to become inactivated. Sichel et al. [19] reported that *E. coli* can be inactivated up to 5-log after 13.2 kJ L^{-1} of UVA accumulated energy when immobilized titanium dioxide is used as photocatalyst in a CPC type photoreactor. The energy dose we have determined for this work includes, as described in Section 2.2, the solar UV radiation and also a portion of visible light. The common value for solar UV radiation is in the range from 5 to 9% of global incoming of solar spectrum at ground level [29]. Considering the lowest value, 5% of the total impinging radiation, the Q_{UV} value determined for our Q_n value is 22 kJ L^{-1}. Based upon the above, this would be high enough to ensure 99.999% inactivation of the microorganism and still remain a conservative dosimetric index. Another study carried out by Lonen et al. [41] reports up to inactivation of *P. aeruginosa* was achieved with less than 500 J of UV radiation. Over 5-log inactivation of *C. albicans* and *F. solani* was also achieved following receipt of 1500 and 2000 J with immobilized titanium dioxide used as a photocatalyst (lamp output 200 W/m^2, 300–400 nm).

In order to evaluate the accuracy of helminth eggs as conservative microbiological index for ENPHOSODIS and test the proposed solar radiation dose for the inactivation of pathogenic bacterial strains, additional experiments were carried out using two common waterborne pathogens: *E. coli* and *P. aeruginosa*. Both are widely recognized as human pathogens and involved in several previous solar water disinfection studies [9,14,19,22].

Fig. 6 shows the behavior observed for SODIS and ENPHOSODIS processes carried out using up to 10^7 CFU mL^{-1} as initial concentration of *E. coli* and *P. aeruginosa*. In both cases, the effect of solar radiation on the final bacteria count is significant: complete pathogen inactivation was achieved by the use of solar radiation without any additional reagent in about 80 kJ L^{-1}, approximately 75% of the accumulated energy required for the ENPHOSODIS inactivation of helminth ova described in Section 3.1. A slight difference is noticed when comparing data from the disinfection curves obtained with the two additional microorganisms tested: *E. coli* seems to be more sensitive than *P. aeruginosa* to solar radiation at the beginning of the experimental run. Nevertheless, this difference disappears as the radiation dose reached a value close to 60 kJ L^{-1} and the final inactivation achieved is almost the same for the two microorganisms.

The application of ENPHOSODIS showed a significant improvement on the bacteria inactivation. We were not able to determine any viable bacteria after the first 10 kJ L^{-1} of accumulated energy. These results were obtained without pH adjustment of the samples. The pH value of the treated water was about 6 and, under these pH conditions, only a fraction of Fe(II) will be dissolved and able to

carry out the photo-Fenton reaction as proposed by Orozco et al. [40]. Despite these unfavorable reaction conditions, it is worthy to note that both bacterial strains were completely inactivated using less than 10 kJ L^{-1}, about one order of magnitude less than the value determined for helminth ova and no evidence of microorganisms adsorbed on precipitated Fe was obtained. Presence of NOM in the water samples did not produce different results when compared with those previously described at NOM concentration in water, up to 25 mg L^{-1}. For the experiments including SR-NOM (data not shown) no change in the rate constant was observed in experiments without NOM, and with 2.5 and 5 mg L^{-1} of NOM. However, when NOM concentrations were increased to 10 mg L^{-1}, microorganism inactivation was decreased by up to 50%. These results may lead one to consider application of the proposed technology even in raw water containing low levels of NOM [46].

In the light of these findings, we believe that it is reasonable to suggest that using the experimental conditions described, for the helminth egg inactivation, other less resistant waterborne pathogens should be inactivated following ~120 min exposure to average solar radiation levels. The radiation dose proposed is high enough to ensure complete inactivation of *E. coli*. Using the dosimetric indicator proposed in this work, the end point for the disinfection process can easily be determined, even under field conditions or in isolated rural areas where there is lack of radiation measurement equipment. This approach may also serve as an easy way to train local individuals assuring good microbiological quality drinking water.

4. Conclusions

The results demonstrate that enhanced photo-assisted solar disinfection processes can achieve complete inactivation of highly resistant waterborne pathogens. Helminth eggs inactivation occurred using the photo-Fenton process at low reagent concentrations of Fe(II) from 5 to 10 mmol L^{-1} and H_2O_2 from 140 to 280 mmol L^{-1}.

Practical application of AO24 dye solution as a dosimetric indicator appears to be as very useful to determine the time necessary for to acquire the necessary solar dose required to assure pathogen inactivation. A solar radiation dose of 155 kJ L^{-1} was required for complete dye degradation ([AO24] = 200 mg L^{-1}), with the dosimetric indicator changing from red to colorless. This is very comparable with that necessary for complete helminth ova inactivation (140 kJ L^{-1}).

The use of the proposed methodology for solar radiation dose measurement could be a low-cost way to assess and measure solar water disinfection processes carried out in isolated rural zones in developing countries where solar radiation measurements are not readily available, or weather conditions can be very variable (i.e., during rainy season).

Further research is necessary to test the effect of parameters, such ionic strength, alkalinity, and turbidity, on the overall disinfection process to determine if the proposed dosimetric indicator is applicable under these conditions.

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