Inactivation of *Ascaris* eggs in water using sequential solar driven photo-Fenton and free chlorine

Erick R. Bandala, Liliana González, Jose Luis Sanchez-Salas and Jordana H. Castillo

ABSTRACT

Sequential helminth egg inactivation using a solar driven advanced oxidation process (AOP) followed by chlorine was achieved. The photo-assisted Fenton process was tested alone under different H_2O_2 and/or Fe(II) concentrations to assess its ability to inactivate *Ascaris suum* eggs. The effect of free chlorine alone was also tested. The lowest egg inactivation results were found using Fe(II) or H_2O_2 separately (5 and 140 mmol L^{-1}, respectively) in dark conditions, which showed about 28% inactivation of helminth eggs. By combining Fe(II) and H_2O_2 at the same concentrations described earlier, 55% of helminth egg inactivation was achieved. By increasing the reagent's concentration two-fold, 83% egg inactivation was achieved after 120 min of reaction time. Process efficiency was enhanced by solar excitation. Using solar disinfection only, the *A. suum* eggs inactivation reached was the lowest observed (58% egg inactivation after 120 min (120 kJ L^{-1})), compared with tests using the photo-Fenton process. The use of the photo-Fenton reaction enhanced the process up to over 99% of egg inactivation after 120 kJ L^{-1} when the highest Fe(II) and H_2O_2 concentration was tested. Practically no effect on the helminth eggs was observed with free chlorine alone after 550 mg min L^{-1} was used. Egg inactivation in the range of 25–30% was obtained for sequential processes (AOP then chlorine) using about 150 mg min L^{-1}.

Key words | advanced oxidation processes, free chlorine, helminth eggs, sequential inactivation, solar radiation, water disinfection

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INTRODUCTION

Lack of access to water and sanitation services is a current problem in communities of developing countries (WHO/UNICEF 2004). About one-sixth of the world's population suffers without access to proper water and sanitation services related to poverty (Gelover *et al.* 2006; Montgomery & Elimelech 2007). Among the human costs associated are deaths of children under 5 years of age related to waterborne diarrheal diseases (WHO/UNICEF 2004). Waterborne transmitted diseases cause over 2 million deaths worldwide each year (Mintz *et al.* 2001; Morales *et al.* 2003). Of these, parasitic infections are a very important environment-related health issue occurring worldwide and affecting the poorest sectors of society (Morales *et al.* 2003). For example, the World Health Organization has estimated that about 1 billion people in developing countries are infected by *Ascaris* (WHO 1987) and that 25–33% of these populations are affected by helminthiasis alone. Many of these cases are related to contaminated water and food (Jemaneh 1998; Morales *et al.* 2003; Jimenez 2007). The implications of intestinal parasitic infections go further than just the health effects. These diseases are related to poor physical growth and development, as well as retardation of intellectual and cognitive development in children less than 15 years of age (Callender *et al.* 1992; Nokes *et al.* 1992; Silva *et al.* 2003; SSa 2003). This high incidence of helminthiasis has been related to a scarce water supply and the deficient

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quality of the supplied water (Sánchez-Pérez *et al.* 1995; SEMARNAP 2001; CNA 2010).

Inactivation of helminth eggs in water is very difficult because of their resistance to external agents as a result of their basic structure. Removal of helminth eggs from water, mainly wastewater, has been attempted using many different sanitary engineering processes such as stabilization ponds (Jimenez 2007), constructed wetlands (Rivera *et al.* 1995; Stott *et al.* 1999), coagulation–flocculation (Jimenez *et al.* 2001; Jimenez 2003; Mara 2003), filtration (Landa *et al.* 1997; Jimenez *et al.* 2001; Riahi *et al.* 2009), and UASB (Diaz *et al.* 1991; Von Sperling *et al.* 2002) which have demonstrated the achievement of a helminth egg removal rate of 80–100% within 20–35 h of treatment. Although the main source, wastewater is not the only matrix where helminth eggs can be present, several studies have reported dealing with the presence of these pathogens in surface and even ground water (Diaz *et al.* 1991; Geldreich 1998; Esrey *et al.* 2000; Blumenthal *et al.* 2001; Ashbolt 2004; Cifuentes *et al.* 2004), and it is well documented that they possess high resistance to disinfection, resisting treatments and emerging live from domestic taps (Bertolucci *et al.* 1998; Campos 2008). *Ascaris* eggs can survive for long periods and are particularly resistant to disinfection and the ingestion of just a few *Ascaris* eggs may be enough to cause infection (Carr 2000). These pathogens cannot be inactivated using chlorine, UV radiation or economic doses of ozone (Jimenez 2007). Contrarily, it has been demonstrated that some non-conventional disinfection methods are not only unable to deactivate *Ascaris* egg, but, in some cases, accelerate larval development (Aladawi *et al.* 2006).

In recent years, the application of advanced oxidation processes (AOPs) for helminth egg inactivation has emerged as an alternative for reducing the risk of consumption of water contaminated with these pathogens. Destruction of helminth eggs using ozone (Orta *et al.* 2002, 2004), UV radiation with the use of photosensitizers (Alouini & Jemli 2004) and photo-Fenton reaction (Ramírez *et al.* 2005, 2006) have demonstrated the viability of using these technologies for deactivating those pathogens in water. Application of AOPs to accelerate inactivation of pathogens has been widely described in the past for bacteria (Block *et al.* 1997; Blake *et al.* 1999; Herrera *et al.* 2000; Otaki *et al.* 2000; Morita *et al.* 2002; Rincon & Pulgarin 2003), fungi, viruses (Lonnen *et al.* 2005) and other resistant microorganisms (Guisar *et al.* 2007; Bandala *et al.* 2009, 2011).

Despite the interesting results of the application of AOPs to pathogens in water, for helminth egg inactivation, poor results have been obtained (Orta *et al.* 2004; Ramírez *et al.* 2006; Jimenez 2007). An interesting strategy proposed relatively recently to improve chemical disinfection is the use of sequential disinfection (Corona-Vasquez *et al.* 2002). It has been suggested that the application of ozone followed by free chlorine, hypochlorous acid, or chloramines, significantly increases the inactivation rate of *Cryptosporidium parvum* oocysts (Driedger *et al.* 2000, 2001; Rennecker *et al.* 2000). The high efficiency demonstrated by sequential disinfection using the ozone–chlorine pair could be related to the generation of different reactive oxygen species (i.e. hydroxyl radicals) by the use of ozone which could synergically enhance the oxidative properties of chlorine improving the overall inactivation rate. If this hypothesis is true, it could mean that other methods producing hydroxyl radicals would be able to produce a similar synergic effect in sequential processes. The purpose of this study is to show our results in the application of a sequential disinfection process using the photo-Fenton reaction, a hydroxyl radical-producing process, coupled with free chlorine in the inactivation of *Ascaris suum* eggs in water.

EXPERIMENTAL

Reagents

The chemicals used in the experiments, $FeSO_4 \cdot 7H_2O$ (Baker), H_2O_2 (50% stabilized, Baker) and $NaClO_4$ (available chlorine 10–13%) were reagent grade and were used as received. *A. suum* eggs were purchased from Excelsior Sentinel Inc. (Ithaca, NY) as a concentrate with 100,000 eggs (90% of viability).

Preparation of microorganisms

From the *A. suum* concentrate, dilutions with approximately 6,250 eggs were prepared in 20 mL of distilled-deionized water. All the experimental runs were carried

out using the same egg concentration. To determine actual egg viability after the different experimental runs, the methodology proposed by the Mexican legislation (NOM-004-SEMARNAT-2002) was used for viable *A. suum* egg enumeration. Briefly, samples obtained at different exposure times to the experimental conditions were diluted to 30 mL with distilled-deionized water and incubated at 26 °C for 4 weeks mixing once per week by hand. After incubation, the samples were concentrated by centrifugation (1,000 *g* for 5 min) and the pellets were observed under a microscope. The presence of larvae in the eggs was taken as a sign of viable eggs. 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.

Experimental procedures

Dark oxidation process

For Fenton reaction under dark conditions, three different Fe(II) concentrations (0, 5 and 10 mmol L^{-1}) and three H_2O_2 concentrations (0, 140, 280 mmol L^{-1}) were tested at room temperature. All the inactivation experiments were performed in sterilized deionized water without further pH adjustment. *A. suum* eggs were put in silanized 50-mL glass vials as batch reactors with 35 mL of sterile, deionized water. Once the eggs were transferred to the glass vial, Fe(II) was added until reaching the desired concentration and then mixed by vortex 5 min. After that, at what was taken as initial time (t_0), a sample of 1,000 μL was collected (ca. 312 eggs). The sample was filtered, rinsed and stored in the freezer at 2 °C until incubation. Then, enough H_2O_2 reagent was added to the vial until reaching the desired hydrogen peroxide concentration. The moment the hydrogen peroxide was added was taken as the start of the oxidation experiment; subsequent samples were collected after 30, 60, 90 and 120 min. As stated earlier, all samples were immediately filtered and rinsed several times with sterilized deionized water to remove the remaining reagents and to avoid further oxidation reaction (Ramírez *et al.* 2006) and then stored at 2 °C until incubation. After the end of the experimental run, all samples collected were diluted as described earlier and incubated to start the viability analysis. All the experimental assessments were carried out in triplicate and are shown as the average value with error bars showing the variation coefficient for every experiment.

Photo-assisted oxidation process

For the photo-Fenton process, the same reaction conditions described in section 'Dark oxidation process' were used. In this case, the experiments were carried out using solar radiation and conducted in a bench-scale solar collector. The 50-mL glass vials were put in the focus of a compound parabolic concentrator (CPC) detailed elsewhere (Chacon *et al.* 2006; Bandala *et al.* 2007; Bandala & Estrada 2007). The system was bent 19° (local latitude) and had a total collection surface of 0.1 m^2. Global radiation from 280 to 2,800 nm was measured during the experiments using a Li-Cor pyranometer (LI-200SA) placed at the same sloping as a solar collector in order to avoid angle adjustments. 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 (Chacon *et al.* 2006; Bandala *et al.* 2008). Accumulated energy, defined as the total amount of irradiative energy reaching the reactor since the beginning of the experiment up to a given time by unit volume, was determined using the relationship previously reported by Goslich *et al.* (1997):

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

where Δt is the time between radiation measurements, Q_n the accumulated energy (kJ L^{-1}), G_n the adjusted global radiation (W m^{-2}) measured in the radiometer in each experiment, A the module area (m^2), and V the total system volume (L). Accumulated energy has been used as a measurement of solar radiation dosage on the photocatalytic disinfection of bacteria and fungi (Sichel *et al.* 2007), so we will use it in order to carry out comparative solar photocatalytic disinfection results.

During the application of the photocatalytic process, different samples were obtained under the same conditions as described earlier for dark experiments and handled in

the same way to obtain the viability determination. In this case, as in dark experiments, all the experimental runs were also carried out in triplicate.

Sequential inactivation

Experimental runs to test the sequential inactivation of *A. suum* eggs were carried out as follows: After the application of photo-Fenton process (60 min of irradiation), the helminth eggs were filtered, rinsed several times with sterilized de-ionized water in order to remove the remaining reagent in the matrix as proposed by Ramírez *et al.* (2006). Once the samples were free of Fe(II) and hydrogen peroxide, helminth eggs were re-suspended in a phosphate buffer solution (pH = 7.0) and its viability was determined before chlorine application. The viability value determined at this stage was considered initial (t_0) time for chlorine inactivation. After the viability sample was taken, a few mL of $NaClO_4$ solution were added to the suspension until reaching the desired chlorine concentration (7 mg L^{-1}). Samples were obtained at different times (10, 20, 50 and 80 min) and residual chlorine was quenched in the samples by using a 0.1-mM sodium thiosulfate solution and transferred to the incubator to start the viability assessment. Free chlorine concentration in the sample after the completion of the disinfection experiment was determined by the DPD colorimetric method (Sirikanchana *et al.* 2008). For comparative purposes, a set of experimental runs were carried out by using chlorine alone without previous application of the photo-assisted process in order to test the ability of the oxidant agent alone on *A. suum* inactivation. In this case, as in the rest of the experiments, all the experimental runs were also carried out in triplicate.

RESULTS AND DISCUSSION

Dark oxidation process

Despite differences identified between adult worms of *A. suum*, and *A. lumbricoides* – the former infects pigs whereas the latter infects humans (Cutter *et al.* 2006) – it is not completely clear if they represent distinct species (Anderson & Jaenike 1997; Zhu *et al.* 1999). Agreeing with our literature review, several studies have reported no morphological or physiological difference between the eggs from the two parasites. For this reason, *A. suum* eggs are usually used as a model for *A. lumbricoides* anytime they are easier to obtain in large quantities. If considering the source of the eggs, an egg obtained by dissection of mature female worms and those isolated from feces are similar in terms of their infectivity and inactivation (Oksanen *et al.* 1990; Ghiglietti *et al.* 1995); nevertheless, we have found important differences in the resistance of the eggs obtained from these two sources. Eggs obtained from dissection are not as resistant as eggs obtained from feces (Garcia *et al.* 2007). This behavior may be due to the eggshell becoming more resistant to environmental conditions after exposure to intestinal contents due to a 'tanning' process (Nelson & Darby 2001).

Figure 1 shows the behavior of *A. suum* eggs viability as a function of reaction time for experiments under dark conditions. It is possible to see that, as expected, the helminth eggs viability did not change under dark conditions without reagent addition. Nevertheless, in the experiments using Fe(II) (5 mmol L^{-1}), an important inactivation was reached (28% of viability reduction, after 120 min). This result can be rationalized considering that Fe(II) has been reported to be able to generate oxidant species, such as $HO_2^{\bullet}$ or $^{\bullet}O_2^{-}$ radicals, in the presence of dissolved oxygen under conditions following the stated sequence of Fe(II)–Fe(III) oxidation proposed for Fenton reaction as mentioned in Reaction (1) from Table 1 (Hug & Leupin 2003) or by the generation of oxo-iron species (Fe^{2+}–O_2; Fe^{2+}–O) reported as potent

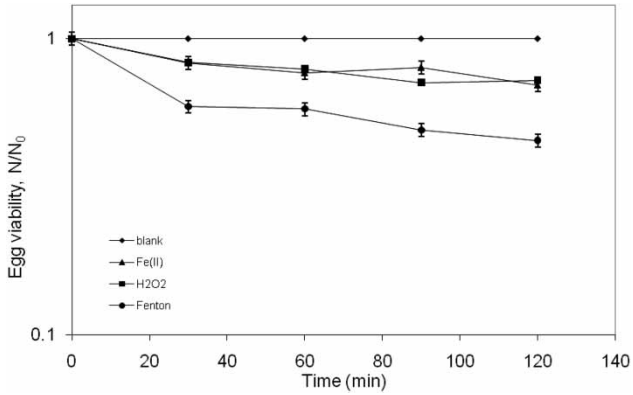

Figure 1 | Helminth egg inactivation under low reagent concentration for the Fenton process ([Fe(II)] = 5 mmol L^{-1}; [H_2O_2] = 140 mmol L^{-1}) under dark conditions.

Table 1 | Constant rate value for the different reactions involved in the photo-Fenton process

Reaction	Constant rates
(1) $Fe^{2+} + O_2 \rightarrow O_2^{\bullet -} + Fe^{3+}$	$k = 1.15\ M^{-1}\ s^{-1}$
(2) $H_2O_2 \leftrightarrow HO_2^- + H^+$	$k = 1.26 \times 10^{-2}\ M^{-1}\ s^{-1}$
(3) $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{\bullet} + HO^-$	$k = 55\ M^{-1}\ s^{-1}$
(4) $Fe(OH)^{2+} + \hbar\nu \rightarrow Fe^{2+} + HO^{\bullet}$	$k = 2 \times 10^{-3}\ M^{-1}\ s^{-1}$
(5) $H_2O_2 + \hbar\nu \rightarrow 2HO^{\bullet}$	$\phi_{HO^{\bullet}} = 0, 98, 254$ nm
(6) $HO^{\bullet} + H_2O_2 \rightarrow HO_2^{\bullet} + H_2O$	$k = 2.7 \times 10^7\ M^{-1}\ s^{-1}$
(7) $Fe^{2+} + HO^{\bullet} \rightarrow Fe^{3+} + HO^-$	$k = 2.7 \times 10^8\ M^{-1}\ s^{-1}$

initiators of lipid peroxidation (Qian & Buettner 1999; Cha'on *et al.* 2007). Application of hydrogen peroxide alone under dark conditions generated a similar viability reduction as obtained with Fe(II) alone in the dark (28% helminth egg inactivation). In this case, it is known that H_2O_2 oxidative properties may play an important role in egg inactivation, probably following the superoxide production mechanism proposed in Equation (2) (De Laat *et al.* 2004; De Laat & Le 2006). Nevertheless, a clear effect in the inactivation process was observed when Fe(II) and H_2O_2 were used together for Fenton reaction (Reaction (3) in Table 1, Balmer & Sulzberger 1999). For this case, as shown in Figure 1, *A. suum* inactivation reached 55% after 30 min of reaction.

Interesting results were obtained when the reagents' concentration was increased. Figure 2 depicts egg inactivation using Fe(II) and H_2O_2 by doubling the reagent' concentration (10 and 280 mmol L^{-1}, respectively). As

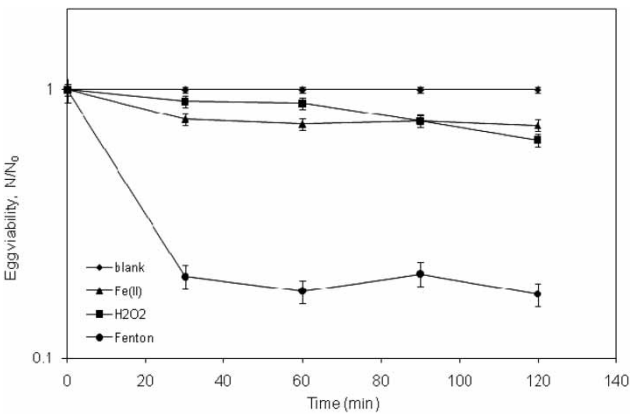

Figure 2 | Helminth egg inactivation under Fenton reaction at high reagent concentration ($[Fe(II)] = 10$ mmol L^{-1}; $[H_2O_2] = 280$ mmol L^{-1}) under dark condition.

observed, the performance of Fe(II) and hydrogen peroxide under dark conditions is slightly different from those shown in Figure 1.

Whereas maximum egg inactivation using Fe(II) under dark conditions remained equal (28% egg inactivation) using any concentration, egg inactivation using hydrogen peroxide at this value is slightly increased (35% inactivation). In the former case, the result supports the proposed hypothesis concerning the generation of oxidant species from the reaction of Fe(II) with dissolved oxygen (DO). Since DO was the same for both iron doses, further addition of Fe(II) did not produce the expected increase in egg inactivation considering that Fe(II) reacted with DO in a Fenton-like process until complete DO depletion. Following this hypothesis, increasing Fe(II) concentration would cause the consumption of oxygen to occur faster, as can be observed from Figure 2, where practically no changes in N/N_0 value can be determined after the first 30 min of reaction.

On the other hand, the increase of H_2O_2 concentration produced the expected increase in the helminth egg inactivation reaching up to 35% of egg inactivation as shown also in Figure 2. The combination of Fe(II) and H_2O_2, the Fenton reaction, showed an important increase in the *Ascaris* egg inactivation from 55% reached by $[Fe(II)] = 5$ and $[H_2O_2] = 140$ mmol L^{-1} to 83% obtained for $[Fe(II)] = 10$ and $[H_2O_2] = 280$ mmol L^{-1}. It is worth noting that, in the latter case, 30 min of reaction gave almost the maximum inactivation (%) comparable with the results previously reported (Ramirez *et al.* 2006) for the photo-Fenton inactivation process and little effect was seen thereafter. This could be due to the complete iron oxidation to Fe(III) that occurs during dark Fenton processes. After complete oxidation of ferrous iron, the reaction rate decreases because further hydroxyl radical generation slows for reactions with Fe(III). For comparative purposes, data in Figures 1 and 2 were used to estimate the decimal reduction time (D), the time required at every reaction condition for the viable egg count to decrease by 90% (Katzin *et al.* 1943; Iversen *et al.* 2004). Decimal reduction time is considered to be defined by (Katzin *et al.* 1943):

$$D = \frac{(t_2 - t_1)}{\log (C_1/C_2)} \tag{2}$$

where C_1 and C_2 are the initial and final count of viable eggs,

respectively, subjected to the same inactivation process for $(t_2 - t_1)$ minutes.

Results for the application of Equation (2) to the experimental data included in Figures 1 and 2 are depicted in Table 2. As shown, the largest D values were obtained for experiments using Fenton reagents separately. In the case of, for example, Fe(II) alone in the dark D values as high as 879.8 min were determined. Application of hydrogen peroxide generated slightly high D values reaching up to 641.7 min for the case of the highest H_2O_2 concentration in the dark. From data in Table 2 it is noticeable that the best D values were found for the Fenton reaction: 346.8 min were determined for D when using 5 mmol L^{-1} of Fe(II) combined with 140 mmol L^{-1} of H_2O_2, whereas 158.9 min value was determined for the reaction assessing the highest Fenton reaction conditions ([Fe(II)] = 10 mmol L^{-1}; [H_2O_2] = 280 mmol L^{-1}). These results suggest that using Fenton reaction may improve the egg inactivation process more than five times when compared with the use of iron or hydrogen peroxide separately.

Photo-assisted oxidation process

Using solar radiation conditions as the energy source for driving the photo-Fenton process, as shown in Figure 3, we found that solar radiation alone was able to produce 58% egg inactivation after 120 min of irradiation (120 kJ L^{-1} of accumulated energy). As shown, over half of the initial eggs were inactivated by using solar radiation without addition of any reagent. These results are reasonable considering that solar disinfection (SODIS) has been reported as a very powerful tool to produce safe drinking water. It is clear

Table 2 | Decimal reduction time (D) obtained for the different experimental conditions tested in dark and irradiated experiments

Conditions	D (min) Dark	Irradiated
Fe(II) (5 mmol L^{-1})	874.2	449.3
Fe(II) (10 mmol L^{-1})	879.8	387.5
H_2O_2 (140 mmol L^{-1})	863.3	229.9
H_2O_2 (280 mmol L^{-1})	641.7	150.0
Fe(II)/H_2O_2 (5 and 140 mmol L^{-1})	346.8	79.6
Fe(II)/H_2O_2 (10 and 280 mmol L^{-1})	158.9	30.0

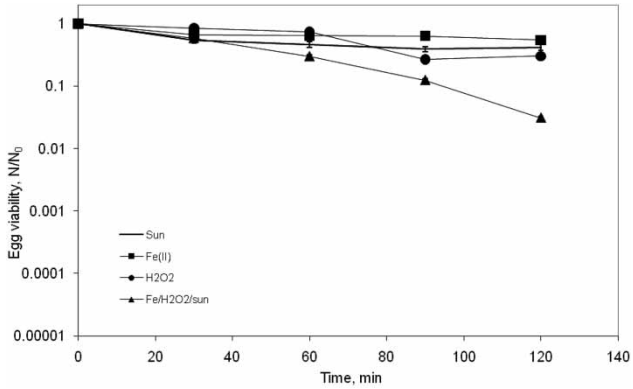

Figure 3 | Helminth egg inactivation in irradiated condition. Photo-Fenton process experimental conditions were 5 mmol L^{-1} Fe(II) and/or 140 mmol L^{-1} H_2O_2.

that, if solar radiation is able to inactivate up to 50% of microorganisms as resistant as the *Ascaris* eggs, it will surely be able to completely inactivate other less resistant pathogens such as protozoan, bacteria and fungi, as previously reported (Otaki *et al.* 2000; Sichel *et al.* 2007). As in the case of dark experiments, adding iron and/or hydrogen peroxide to the system allowed improvement in egg inactivation. In the former case, using 5 mmol L^{-1} of Fe(II), 46% egg inactivation was obtained, which was lower than solar radiation alone (58% inactivation). This can be rationalized considering that Fe(II) and helminth eggs could compete for solar radiation. Nevertheless, it is important to note that total egg inactivation reached for Fe(II) alone under solar radiation was considerably higher than the results for the dark process. Whereas, under dark conditions, the best egg inactivation obtained using Fe(II) alone was around 30%; when solar radiation was included in the experiments, egg inactivation as high as 46% was reached for the lowest Fe(II) conditions. In this case, the synergic effect of solar disinfection and the Fenton-like process described earlier between Fe(II) and dissolved oxygen could improve the reaction rate of the process generating the observed improvement in the overall egg inactivation.

The same trend can be observed from Figure 3, when hydrogen peroxide was added under solar radiation conditions. In this case, the use of solar radiation increased egg inactivation probably by hydroxyl radical generation according to Reaction (5), (Table 1). The process could be, at least, partially responsible for the improvement of egg inactivation of up to 70% after 120 min of irradiation

(about 1,131 kJ L^{-1} of accumulated energy) compared to 28% egg inactivation under the dark condition. The photo-assisted cleavage of hydrogen peroxide proposed in Reaction (5), (Table 1) is suggested to take place at 254 nm; nevertheless, Pignatello *et al.* (1999) have demonstrated that this reaction is able to occur even at wavelengths of up to 360 nm but at lower quantum yields than those included in Table 1. Once formed, hydroxyl radicals can react fast with organic matter with a second order rate constant of 10^9–10^{10} M^{-1} s^{-1} (Mabury & Crosby 1996) probably generating the observed egg inactivation.

More interesting results were achieved for the photo-Fenton reaction (Reactions (3) and (4), Table 1) where 97% of *Ascaris* eggs inactivation was reached after 120 min of treatment (ca 120 kJ L^{-1} of accumulated energy). Table 2 shows also the results of the application of Equation (2) to the data shown in Figure 3.

It is easy to note the important improvement of D values obtained for photo-assisted processes when compared with equal conditions in experiments in the dark. In particular, comparison between Fenton and photo-Fenton processes is very interesting considering that the decimal reduction time value was improved almost five times by using solar radiation. Although a synergic effect of temperature and UV–Visible radiation, as proposed in other works for solar disinfection (Gelover *et al.* 2006), cannot be neglected, it needs to be considered that the average temperature of the experiments was 38 °C and it is well known that helminth eggs are resistant to higher temperatures when exposed over several days (Jimenez 2007). However, we should not completely discard the thermal effect of solar infrared radiation because we did not attempt to eliminate this energy at all.

Results for the application of solar radiation at a higher concentration of Fe(II) and/or H_2O_2 (10 and 280 mmol L^{-1}, respectively) as a function of radiation time are depicted in Figure 4. From this figure, it is noticeable again that helminth egg inactivation using 10 mmol L^{-1} Fe(II) alone was not very different from results shown in Figure 3. In contrast, by using H_2O_2, egg inactivation was improved when hydrogen peroxide concentration was increased, reaching 84% egg inactivation with 280 mmol L^{-1} of H_2O_2 compared to 70% egg inactivation when 140 mmol L^{-1} of H_2O_2 was used.

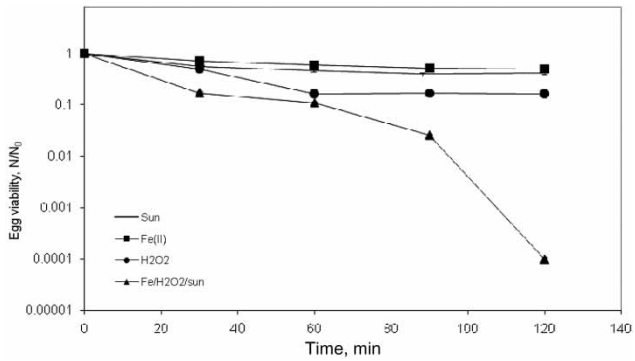

Figure 4 | Helminth egg inactivation. Photo-Fenton process at 10 mmol L^{-1} of Fe(II) and/or 280 mmol L^{-1} of H_2O_2.

The same trend is observed when comparing rate constant values in Table 2. For low H_2O_2 concentration, D = 229.9 min whereas by increasing hydrogen peroxide to 280 mmol L^{-1}, the decimal reduction time decreased almost 50% to give D = 150.0 min. Nevertheless, the trend to an increase in the inactivation rate will surely be limited by the scavenging effect of the excess of hydrogen peroxide in the reaction mixture as has been widely reported, agreeing with Reaction (6) (Table 1) (Pignatello *et al.* 1999).

In the same way, the photo-Fenton process is improved by the increase of reagent concentration. Using the highest reagent concentrations ($[Fe^{2+}]$ = 10 mM; $[H_2O_2]$ = 280 mM), similar egg inactivation is achieved by using a lower radiation dose than in an experiment with low reagent concentration (97% egg inactivation in both cases using 100 and 120 min, 100 and 120 kJ L^{-1}, respectively) and over 4-log inactivation (>99.99% inactivation) was reached after 120 min (less than 140 kJ/L of accumulated energy), almost two log units higher than the results obtained for the application of ozone for eggs disinfection (Orta *et al.* 2004). Reaction rate is also improved as expected by the increase in the amount of the reagents; D value went from 79.6 to 30 min (2.65 times lower) by using H_2O_2 and Fe(II) doubly concentrated. Sequential disinfection processes with chlorine, however, were carried out at the lower reagent concentrations reported in this paper ($[Fe^{2+}]$ = 5 mM; $[H_2O_2]$ = 140 mM).

Sequential inactivation assessments

In order to determine the actual effect of chlorine alone on egg inactivation, experimental runs using this oxidant as the

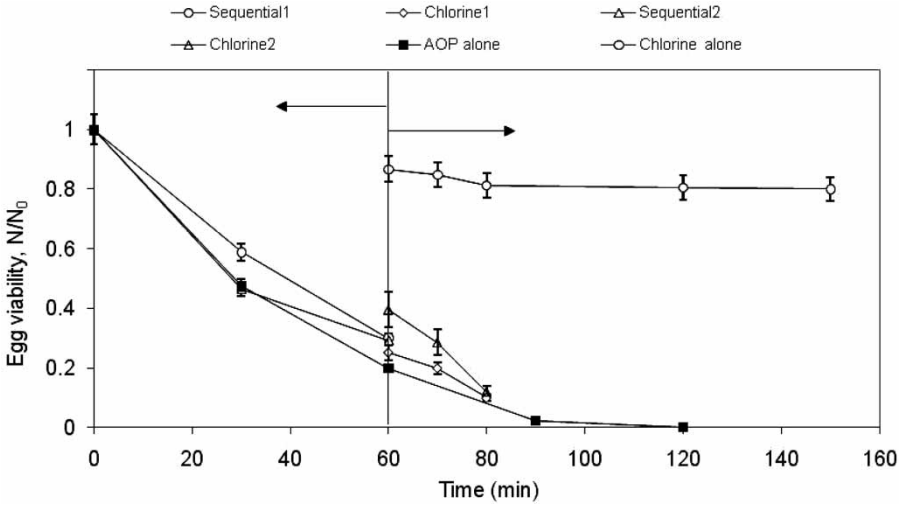

Figure 5 | Helminth eggs inactivation using complete sequential (solar AOPs followed by free chlorine) process compared to the application of the two procedures by separate as a function of reaction time.

only disinfectant agent were carried out. In Figure 5, results of the use of chlorine alone for *A. suum* egg inactivation as a function of $C \times t$ value is shown. In these results, $C \times t$ values are based on the concentration of chlorine that was initially added since no significant variation of initial chlorine concentration was observed during all the experimental trials. For comparison, Figure 5 also shows the behavior of viable helminth eggs remaining after sequential inactivation using photo-Fenton and chlorine. As shown, practically no effect is observed when chlorine alone was added even with $C \times t$ values up to 550 mg min L^{-1}. On the other hand, egg inactivation in the range of 25–30% was obtained for sequential processes using about 150 mg min L^{-1}. Sequential 1 and 2 shown in Figure 5 are two experiments carried out separately under the same reaction conditions ($[Fe(II)] = 5$ mM; $[H_2O_2] = 140$ mM). After the first 60 min of photo assisted treatment, the eggs were submitted to chlorine treatment (chlorine 1 and 2, respectively), so the line at 60 min labels the start of chlorine treatment. In Figure 5, for comparative purposes, data with the behavior of chlorine alone and the photo-Fenton process alone are also displayed. As mentioned, the effect of chlorine by itself is almost negligible. By comparing with the photo-assisted process alone, the sequential process shows a very close trend to those observed for the AOP. Nevertheless, after the initial 20 min of application of sequential chlorine inactivation, the eggs' viability was observed, remaining unchanged at 10%, whereas in the case of the photo-Fenton process, the helminth egg inactivation continues until reaching almost 2-log inactivation.

The use of highly-resistant pathogens, such as helminth eggs, as a conservative index for water disinfection is also a very interesting issue because the inactivation of the helminth eggs is a complex task. Nevertheless, in agreement with previous results from our research group (Bandala *et al.* 2011), by achieving almost complete helminth egg inactivation, it is possible to assume that any other less resistant pathogen microorganisms (i.e. bacteria) present in the raw water will be inactivated under the same reaction conditions and after providing the same solar radiation dose. It is reasonable then that the disinfection reached using AOPs may be, as demonstrated here, improved by the further adding of free chlorine in the sequential process.

CONCLUSIONS

Helminth eggs have been inactivated >99% in water by the use of sequential advanced oxidation processes (photo-Fenton reactions) coupled with free chlorine. The sequential process assessed has demonstrated high efficiency in the viability depletion of high resistant pathogen microorganisms usually present in surface water in Mexico and provides an interesting alternative as a water disinfection process prior

to any further drinking water procedure, mainly when surface water is used as the main drinking water source.

Inactivation data estimated from the experiments (decimal reduction time, *D*) performed showed that the use of solar radiation as the driving force to carry out the AOP included in the proposed sequential process allowed the reaction to improve several times and also led to an interesting use of alternative energy sources to potentially improve water quality and, consequently, the quality of life for vulnerable segments of the population in developing countries.

Despite solar activated water purification technologies being demonstrated to be suitable for use in developing countries, more work is required in order to generate a reliable, cost-efficient technology to be applied in, for example, point-of-use drinking water treatment devices in isolated, marginal rural areas of developing countries. Results shown in this work are a very interesting possibility for the use of non-conventional energy sources for application in the generation of safe drinking water for poor areas with problems related to water scarcity.

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