

Application of the Fenton process in the elimination of helminth eggs

Sandra Isabel Escobar-Megchún, Hugo Alejandro Nájera-Aguilar, Magaly González-Hilerio, Javier Gutiérrez-Jiménez, Rubén Fernando Gutiérrez-Hernández and María Neftalí Rojas-Valencia

ABSTRACT

This study relates to a method for evaluating the degradation efficiency of *Ascaris lumbricoides* eggs through a Fenton reaction, using hydrogen peroxide (H_2O_2) at 100, 250, and 500 mg/L, ferrous sulfate ($FeSO_4$) at 458 mg/L Fe^{2+} and pH values of 3, 4.5, and 6. The experiments were conducted according to a 3^2 experimental design, with 1:1, 3:1, and 5:1 (H_2O_2/Fe^{2+}) molar ratios. The oxidation and flocculation stages were performed at 130 rpm during 2 hours and at 25–30 rpm during 20 min, respectively. As a result of the Fenton reaction, an average of 91.2% *Ascaris lumbricoides* egg degradation was achieved at pH 6 using a 500 mg/L dose of H_2O_2 and a 3:1 (H_2O_2/Fe^{2+}) molar ratio. Thus, this process is an alternative for eliminating parasites that are resistant to conventional disinfection processes and significantly reduces the human health hazard they represent.

Key words | action mechanism, *Ascaris lumbricoides*, Fenton reaction, lipidic peroxidation, oxidizing agent, parasite elimination

Sandra Isabel Escobar-Megchún
Hugo Alejandro Nájera-Aguilar (corresponding author)

Magaly González-Hilerio
Escuela de Ingeniería Ambiental,
Facultad de Ingeniería, Edificio 3,
Universidad de Ciencias y Artes de Chiapas,
Libramiento Norte Poniente No. 1150,
Colonia Lajas Maciel, C.P. 29039,
Tuxtla Gutiérrez, Chiapas, México
E-mail: hnajera72@hotmail.com

Javier Gutiérrez-Jiménez
Facultad de Ciencias Biológicas,
Universidad de Ciencias y Artes de Chiapas,
Libramiento Norte Poniente No. 1150,
Colonia Lajas Maciel, C.P. 29039,
Tuxtla Gutiérrez, Chiapas, México

Rubén Fernando Gutiérrez-Hernández
Departamento de Ingeniería Química y Bioquímica,
Instituto Tecnológico de Tapachula,
Km 2 Carretera Puerto Madero, C.P. 30700,
Tapachula, Chiapas, México

María Neftalí Rojas-Valencia
Universidad Nacional Autónoma de México,
Instituto de Ingeniería,
Cd. de México, C.P. 04510, México

INTRODUCTION

Residual waters and biosolids are used in agriculture because of the nutrients they bring to the soil. However, they carry a large number of pathogenic microorganisms, among them helminth eggs (HE) (Lannacone 2002). HE constitute an important human health hazard because of their persistence in water and their capacity to survive in adverse conditions. Among the diseases they may cause, intestinal diseases induced by *Trichuris trichiura* and *Ascaris lumbricoides* are especially significant (De Souza *et al.* 2011).

The eggs of the parasites are released in the feces of infected people and can reach wastewater treatment plants (García *et al.* 2008). Helminth egg concentration is a parameter that has to be constantly monitored before recycling

treated wastewater or sludge generated in biological treatment processes. Moreover, pathogens' concentration must be controlled with treatment processes in order to meet the applicable standards (Jiménez *et al.* 2006).

Due to the high resistance of these parasites, traditional water disinfection methods are not able to inactivate them (Leal *et al.* 2006). Thus, in order to carry out an efficient elimination, thermal treatments or acidic conditions have to be included (López 2009). In this study, an alternative for inactivation of *A. lumbricoides* through an advanced oxidation process called the Fenton reaction is proposed. It consists of a system based on the generation of hydroxyl radicals ($\bullet OH$) produced by catalyzing hydrogen peroxide

(H₂O₂) with iron (Fe²⁺) in acidic medium. The Fenton process basic mechanism consists of the oxidation and chemical coagulation of organic compounds (Telles *et al.* 2006) taking advantage of the fact that the reagents used are easy to handle and environmentally friendly.

The Fenton process has been tested in the removal of various recalcitrant contaminants such as surfactants (De la Cabada *et al.* 2000; Naldoni *et al.* 2010), chlorinated biphenyls (Vione *et al.* 2004; Forero *et al.* 2005) and mature landfill leachate products (Zhang *et al.* 2005; Deng & Englehardt 2006; Nájera *et al.* 2011). However, its application to inactivation of HE (*A. lumbricoides*) has not been reported. The objective of this study is to evaluate the efficiency of helminth egg degradation through the Fenton reaction at various pH values and using various oxidizing agents.

MATERIALS AND METHODS

HE (*A. lumbricoides*) were obtained from feces samples taken from 250 infected children whose average age, weight, and stature were 2.4 years, 11.2 kilos, and 0.81 meters, respectively. The presence of the parasites was evidenced through coproparasitoscopic analysis.

To evaluate the effect of the Fenton reaction on the HE, in a plastic container, 3 L of distilled water were mixed with 3 g of feces containing *A. lumbricoides* eggs. An average of 115 eggs/L was quantified in the preparation and the result expressed as HE/L according to Mexican standard NMX-AA-113-SCFI-1999. The viability of said eggs was determined by 0.1% trypan blue vital staining. On a slide, 0.1 mL of the sample was mixed with 0.1 mL of dye, a cover slip was placed on it and it was examined under an optical microscope with 10× and 40× objectives looking for the presence of viable and non-viable eggs. With this staining, non-viable eggs are permeable to the dye and are stained blue while viable eggs are not stained (Rojas 1998; De Victorica & Galván 2003; Morales *et al.* 2013). A test was performed with a non-treated sample in order to confirm the viability technique. Dye was added to the sample that was observed under the microscope to identify viable and non-viable eggs. Viable eggs were not blue stained. To confirm the egg non-viability, dye was added to a fecal sample containing the eggs and conserved in 10% formol. Blue stained

non-viable eggs were observed. Formol is a protein- and nucleic acid-denaturing aldehyde. Non-viable eggs are stained as a consequence of permeability changes in the outer layer caused by the modification of the chemical structures of the layer components. Alkalinity, pH, and H₂O₂ reaction time were also determined. For this purpose, peroxide was added to the water sample and samples were taken at different times (10, 25, 40, 60, 75, 90, 105, and 120 min), determining the chemical oxygen demand (COD) for each of them.

The Fenton reaction was performed in a jar test equipment JarTester (Phipps & Bird PB-700), adapting three 250 mL beakers with flat stirring paddles. The sample volume per beaker was 150 mL and the pH was adjusted at 3, 4.5, and 6 with 10% H₂SO₄. All the treatments were developed at 1:1, 3:1, and 5:1 (H₂O₂/Fe²⁺) molar ratios (Deng 2007; Primo *et al.* 2008; Nájera *et al.* 2011). One hundred, 250, and 500 mg/L peroxide doses were used, while the catalyzer dose was 458 mg/L Fe²⁺ for all the treatments.

In this way, a 3² experimental design was established, with nine treatments of three repetitions for each one. In the oxidation stage, the jar equipment was adjusted to 130 rpm during 2 hours. Then, the pH was adjusted between 7 and 8 with 2 M NaOH and the stirring speed was lowered to 25–30 rpm during 20 min in order to facilitate flocculation. The sample was left to settle for 1 hour, and a sediment sample was taken for quantifying viable and non-viable eggs, the result being expressed as the number of viable eggs per liter. The mean was determined as well as the Student's *t*-test for paired samples, 95% confidence interval, using the software SPSS version 15 for this purpose.

RESULTS AND DISCUSSION

Determination of the reaction time

Figure 1 shows the COD behavior profile throughout the considered reaction time, with a significant concentration reduction during the first 90 min and then stabilization is observed. This justifies the decision to establish a maximum oxidizing time of 2 hours for all the tests. García *et al.* (2008) also determined the same reaction time for the inactivation of *Ascaris suum* eggs using homogeneous photocatalysis.

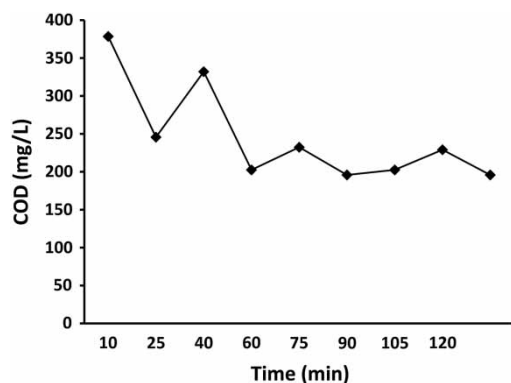


Figure 1 | Fenton reaction time.

Fenton reaction

The efficacy of the Fenton reaction was evidenced in all the treatments. The average number of viable *A. lumbricoides* eggs/L before treatment was 115 (total eggs/L, 143), and after treatment was 16 eggs/L. This reduction was statistically significant ($p = 0.001$) with a Student's *t*-test value of 5.005 (degrees of freedom = 8), above the critical value ($t = 2.31$) at a 95% confidence level.

In all the treatments, the degradation of *A. lumbricoides* eggs ranged between 60.4 and 91.2%, the highest degradation percentages being observed at pH 6 (Table 1). 80.4% of a total of 92 *A. lumbricoides* eggs used in this study were viable, while 19.6% were not. This was confirmed by means of the dye technique before and after treatments. In some cases, larval eggs were observed after the treatments.

In the literature, lower pH is mentioned, such as in Orta *et al.* (2004) and García *et al.* (2008), who reported pH 5 and 3 for *Ascaris suum* egg destruction and inactivation using ozone and homogeneous photocatalysis, respectively.

In other studies, other techniques are also reported for removing, destroying, or inactivating HE; for example, Rojas *et al.* (2004) eliminated 96.7% of *Ascaris suum* eggs using ozone with a 1-hour contact time, while Leal *et al.* (2006) found that their viability was reduced to zero with a 6-hour treatment using solar photocatalysis with TiO₂. García *et al.* (2008) reached 79% inactivation rate with a 2-hour exposure using homogeneous photocatalysis. On the other hand, Mun *et al.* (2009) compared three treatments and obtained 2.5 log inactivation rate of *A. lumbricoides*

Table 1 | Effect of the Fenton reaction on the degradation of viable *A. lumbricoides* eggs

Treatment	H ₂ O ₂ mg/L	pH	<i>A. lumbricoides</i> viable eggs (HE/L) ^a		<i>A. lumbricoides</i> eggs degradation (%)
			Before the treatment	After the treatment	
T1	100	3.0	59	14 (±0.99)	76.3
T2	250	3.0	80	18 (±1.63)	77.6
T3	500	3.0	53	21 (±1.79)	60.4
T4	100	4.5	64	14 (±1.23)	78.1
T5	250	4.5	102	14 (±1.51)	86.3
T6	500	4.5	107	17 (±1.16)	84.1
T7	100	6.0	187	20 (±3.31)	89.3
T8	250	6.0	193	17 (±1.58)	91.2
T9	500	6.0	193	17 (±1.26)	91.2
Mean			115	16	

^aHE/L: helminth eggs/liter.

eggs with a 60-second contact time using microwave treatment; they obtained 0.13 and 0.22 log inactivation rates in soil and water, respectively, with a 30-minute treatment time using the ozone treatment; with regard to the UV treatment, approximately 0.01 and 0.32 log eggs in soil were inactivated with a 60-minute treatment time. De Souza *et al.* (2011) inactivated 100% of *A. lumbricoides* eggs exposing them to a 5 kGy dose of gamma radiation and Bandala *et al.* (2012) obtained up to over 99% inactivation rate of *Ascaris suum* eggs after 120 min kJ L using photo Fenton, when the highest Fe(II) and H₂O₂ concentrations were tested.

The degradation of *A. lumbricoides* eggs observed in this work can be attributed to the action of hydroxyl radical, that is the base of the Fenton reaction (Corona *et al.* 2002). On the other hand, our results indicate that this treatment may help remove a high percentage of *A. lumbricoides* present in wastewater and biosolids; it is possibly due to the fact that the free radical (\bullet OH) reacts with organic compounds and initiates a chain reaction. Because these radicals contain an unpaired electron provoking high chemical instability, they tend to subtract an electron from stable molecules in order to reach electrochemical stability. Thus, the stable molecule donating the electron turns into a free radical, initiating a chain reaction that destroys the cells (Avello & Suwalsky 2006). The free radicals generated through the Fenton reaction can affect the lipid bilayer

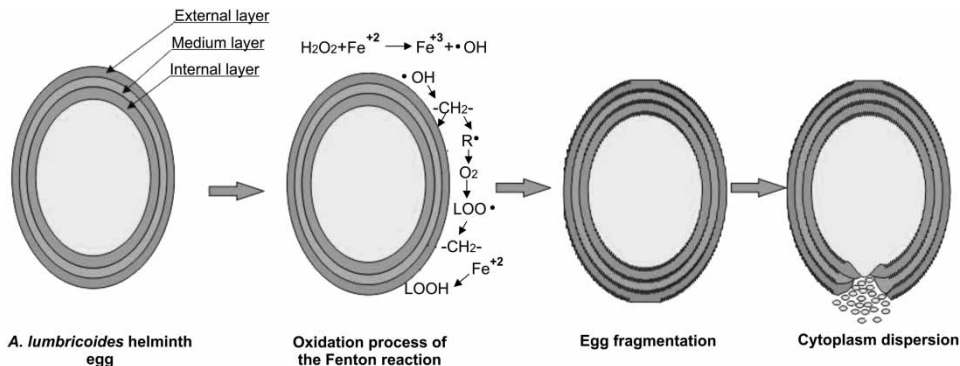


Figure 2 | Proposed action mechanism of Fenton reaction on *A. lumbricoides* eggs.

present in the membrane of *A. lumbricoides* eggs thus generating lipidic peroxidation reactions (Sarria 2005).

The possible proposed action mechanism (Figure 2) is initiated by a hydroxyl radical ($\cdot\text{OH}$) abstracting a hydrogen from the lateral chain of a fatty acid ($-\text{CH}_2-$) forming a carbonated radical ($\text{R}\cdot$) (Avello & Suwalsky 2006) that reacts with oxygen to form cyclic peroxides and hydroperoxide radicals ($\text{ROO}\cdot$). Thus, a hydrogen can be abstracted from another polyunsaturated fatty acid; when the reactions end and secondary metabolites that cannot be used by the cell have been produced, lipidic hydroperoxide is formed, which is a stable peroxidation product.

CONCLUSIONS

The Fenton reaction showed its efficacy in the degradation of HE, especially *A. lumbricoides*, reaching a 91.2% inactivation/removal at pH 6 and dose of 500 mg/L H_2O_2 , with a 3:1 ($\text{H}_2\text{O}_2/\text{Fe}^{2+}$) molar ratio and 2 hours' contact time.

The Fenton process can be used not only for the degradation of recalcitrant contaminants but also as an alternative for inactivation and removal of parasites that are resistant to conventional disinfection processes, thus significantly reducing the human health hazard they represent.

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