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EFFECT OF THE PHOTO-FENTON PROCESS ON ASCARIS HELMINTH EGGS AND BACTERIAL GROWTH IN THE WASTEWATER TREATMENT PLANT IN HILLA-BABYLON PROVINCE, IRAQ

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Abstract
In this study, one of the photo-oxidation techniques (Fenton Photo) was used to inactivate Ascaris helminth eggs and bacterial growth sampled in a wastewater treatment plant in Al-Hilla City, which is considered an indicator of pollution and restricts water use. Fenton Photo technology relies on the formation of free radicals, such as hydroxyl radicals, through the use of ultraviolet radiation from sunlight, as well as the use of peroxide as a catalyst and iron oxides as an oxidizing agent. The effect of treatment time on the percentage of removal efficiency (R.E.%) for eggs and bacterial growth rate was studied. The average of R.E% was 69.40% at treatment time 15 minute ,89.73% at treatment time 30 minute, and 100% at treatment time 40 minute. The removal efficiency of bacteria was 100% at all treatment times.

Keywords: Wastewater, treatment, bacteria, helminthes, photooxidation.

Introduction

Water is an essential component of life on the planet. The rise of industry and modernity had a severe impact on the availability of clean water supplies (Alkurdy and Ebrahim, 2020). Population growth and economic development lead to an increase in the demand for clean water. It is expected that the demand for water will increase in the year 2025 by 50% in developing countries and by 18% in developed countries (UNEP, 2007). The World Health Organization (estimates that about 1 billion people are infected with *Ascaris* sp. in developing countries (WHO,1987). These cases are linked to contaminated water and food (Jimenez.,2007). Therefore, protecting water from pollution and treating pollution is important to maintain health Humans and the environment (Wick ,2011).

Parasitic infections are a health and environmental problem that occurs throughout the world and affects the poorest strata of society (Morales *et al*., 2003). The high incidence of helminths is closely linked to the scarcity of water supplies, and infection with these worms does not only have a health impact, but there are intellectual and cognitive effects through affecting growth and development in people under the age of 15 years (Silva *et al*., 2003). Ascaris eggs are the most widespread helminth eggs around the world and cause ascaris. Among the helminth diseases, ascaris is the most widespread and common, and it is considered an endemic disease in Latin America, Africa, and the Far East.

Traditional methods such as: Chlorine, Ultraviolet Radiation, and Ozone use in the disinfection process is not sufficient to eliminate disease causes (Jimenez.,2007) . On the

contrary, it has been found that some disinfection processes are not only unable to inactivate Ascaris eggs, but also sometimes accelerate the development of their larvae (Aladawi *et al*., 2006). In recent years, advanced photo-oxidation (APO) has been used to inactivate helminth eggs as an alternative technology that can inactivate these eggs and is economically feasible(Velásquez *et al*., 2004, Zamora *et al*.,2005). It has been found that advanced photo-oxidation technology is useful in sterilizing water from bacterial pathogens (Rincon, & Pulgarin .,2003)).also viruses and fungi (Lonnen *et al*., 2005). Rennecker *et al*., (2000) and Corona *et al*., (2002) stated that the use of ozone, free chlorine, hypochlorous acid, or chloramines has a high efficiency in inactivating parasitic worm eggs through the production of active hydroxyl radicals, So that advanced oxidation is one of the important physical and chemical processes in treating polluted water. It produces strong oxidizing groups such as the hydroxyl group which has a higher oxidation strength of 2.8E°/V, which is the second strongest group after fluorine (Cuerda *et al*., 2019), so that It is very effective in oxidizing organic materials and can also inactivate a wide range of harmful microorganisms and used in several fields including environmental health, biomedical applications, the industrial food industry, and the treatment of polluted water (Ortega *et al*., 2012). Also there are many literature about the inactivate effect of phenton photo oxidation on bacterial growth (Wang et al,2024).

2- Materials and Methods:

The treatment unit is designed and consists of a basin with dimensions of 100 cm, length and width of 50 cm, and height of 50 cm, made of glass. The basin contains a mixer for the purpose of homogenizing the water with the additives. It contains two openings for the inlet and outlet, a thermometer to measure the temperature, and a pH meter to adjust the acidity.

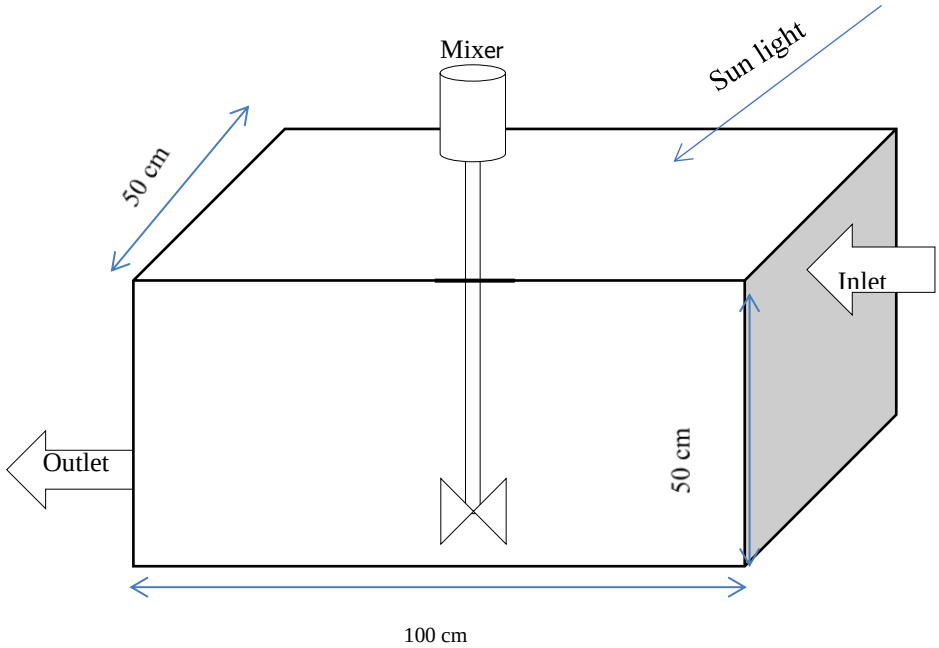

Fig.1. Reactor of Fenton Photo method shows the dimensions of it, the reactor made from cleared matter to permit the sunlight to penetrate it reaching to wastewater and the reaction was began

The oxidizing substance used was iron as ferric sulphate (FeSO4.7H2O) (Ebrahiem et al., 2017), with a concentration of 10 mg per liter. The catalyst was hydrogen peroxide, which is an eco-friendly material (Carra et al., 2014).

In treatment, the variable time (15, 30, 40) minutes was used to find out the best time for processing. The removal efficiency (RE%) was calculated according to the following equation (Lu et al., 2021).

$$R.E.\% = \frac{C1 - C2}{C1} * 100$$

C1: Initial concentration

C2: Final concentration

2-1-Detection of helminthes eggs by Bailenger mothed:

1- Collect a sample of wastewater of known volume (V liter), usually 1 liter for raw or partially treated wastewaters and 10 liters for final treated effluents.

2- Allow the sample to sediment for I-2 hours, depending on the size of the container.

3- Remove 90% of the supernatant using a suction pump or siphon.

4- Carefully transfer the sediment to one or more centrifuge tubes, depending on the volume, and centrifuge at 1000 g for 15 min. Remember to rinse the container well with detergent solution, and add the rinsing's to the sediment.

5- Remove the supernatant. If more than one centrifuge tube has been used in step 4, transfer all the sediments to one tube (remember to rinse thoroughly with detergent solution to ensure that no sediment is discarded), and recentrifuge at 1000g for 15 min.

6- Suspend the pellet in an equal volume of acetoacetic buffer, pH 4.5 (i.e. if the volume of the pellet is 2 ml, add 2 ml of buffer) If the pellet is less than 2 ml, add buffer up to 4 ml to ensure that, after extraction with ethyl acetate (steps 7 and 8), there is sufficient volume of buffer above the pellet to allow the ethyl acetate layer to be poured off without resuspension of the pellet.

7- Add two volumes of ethyl acetate or ether and mix the solution thoroughly in a vortex mixer.

8- Centrifuge the sample at 1000g for 15 min . The sample will now have separated into three distinct phases. All the non-fatty, heavier debris, including helminth eggs, larvae and protozoa, will be in the bottom layer. Above this will be the buffer, which should be clear. The fatty and other material moves into the ethyl acetate or ether and forms a thick dark plug at the top of the sample.

9- Record the volume of the pellet containing the eggs, and then pour off the rest of the supernatant in one smooth action. It may be necessary to loosen the fatty plug first by running a fine needle around the side of the centrifuge tube.

10- Resuspend the pellet in five volumes of zinc sulfate solution.(i.e. if the volume of the pellet is 1 ml, add 5 ml of ZnSOn). Record the volume of the final product (X ml). Mix the sample thoroughly, preferably using a vortex mixer. Note that a minimum of 1.5 ml is required to fill a two-chambered Sedgewick rafter slide.

11- Quickly remove an aliquot with a Pasteur pipette and transfer to a Sedgewick rafter slide for final examination.

12- Leave the full Sedgewick rafter slide to stand on a flat surface for 5 min before examination. This allows all the eggs to float to the surface.
13-Place the Sedgewick rafter slide on the microscope stage and examine under 10x or 40x magnification. Count all the eggs seen within the grid in both chambers of the Sedgewick rafter slide For greater accuracy, the mean of two slides, or preferably three, should be recorded.
14- Calculate the number of eggs per liter from the equation:
N = AX/PV
where: N : number of eggs per liter of sample
A : number of eggs counted in the Sedgewick rafter slide or the mean of counts from two or three slides
X : volume of the final product (ml)
P : volume of the Sedgewick rafter slide (1 ml)
V : original sample volume (liters) .

2-2-Total bacteria count (TBC) :

It is a laboratory method used to determine bacterial contamination in water**.** Total bacteria count is considered an internationally accepted method as an indicator of bacterial contamination (Wang *et al.*, 2023).It is a quantitative estimate of the number of bacteria present in a sample The examination was carried out by taking samples before and after treatment with a change in the treatment time and culture them in Petri dishes containing neutron agar to see the effect of treatment on bacteria.

3- Results and Discussion:

Table 1 shows the number of helminth eggs (Ascaris) before treatment and after 15 min of treatment.

date		before. treatment	AVERG	after .15 min of treatment	AVERG	RE%
22/1/2023	N.of eggs1	21	19.3	5	6.3	67.35
	N.of eggs 2	19		7		
	N.of eggs 3	18		7		
12/3/2023	N.of eggs 1	19.0	18.6	6	5.3	71.50
	N.of eggs2	21		4		
	N.of eggs 3	16		6		
14/5/2023	N.of eggs 1	16	17.3	7	5.3	69.36
	N.of eggs 2	18		4		
	N.of eggs 3	18		5		

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Table 2 shows the number of helminth eggs (Ascaris) before treatment and after 30 min of treatment.

date		before. treatment	AVERG	after . 30min of treatment	AVERG	RE%
22/1/2023	N.of eggs1	21	19.3	2	1.66	91.39
	N.of eggs 2	19		1		
	N.of eggs 3	18		2		
12/3/2023	N.of eggs 1	19.0	18.66	1	1.66	91.10
	N.of eggs2	21		1		
	N.of eggs 3	16		3		
14/5/2023	N.of eggs 1	16	17.3	2	2.3	86.70
	N.of eggs 2	18		3		
	N.of eggs 3	18		2		

Table 3 shows the number of helminth eggs (Ascaris) before treatment and after 40 min of treatment.

date		before. treatment	AVERG	after 40 min of treatment	AVERG	RE%
22/1/2023	N.of eggs1	21	19.3	0	0	100
	N.of eggs 2	19		0		
	N.of eggs 3	18		0		
12/3/2023	N.of eggs 1	19.0	18.6	0	0	100
	N.of eggs2	21		0		
	N.of eggs 3	16		0		
14/5/2023	N.of eggs 1	16	17.3	0	0	100
	N.of eggs 2	18		0		
	N.of eggs 3	18		0		

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Table 4 shows total count of bacteria before and after treatment with different treatment time.

date	dolution	total count of bacteria before treatment	total count of bacteria after.15 min of treatment	total count of bacteria after.30 min of treatment	total count of bacteria after.40 min of treatment
22/1/2023	0.1	>100	5	3	0
	0.01	>100	2	1	0
	0-001	>100	0	0	0
12/3/2023	0.1	>100	5	2	0
	0.01	>100	3	1	0
	0-001	>100	0	0	0
14/5/2023	0.1	>100	7	2	0
	0.01	>100	3	0	0
	0-001	>100	0	0	0

Through Table No. 1,2,3, we notice that the treatment efficiency using the photo oxidation technique (Fenton Photo), where the removal efficiency of helminth eggs (Ascaris eggs) was (67.3 ,71.5, 69.3) % when the treatment time was 15 minutes and at a time of 30 minutes the removal efficiency was (93.2,91.3, 86.7) % and the removal efficiency reached 100% at a treatment time of 40 minutes. also notice that there is a relationship between the treatment time and the efficiency of removal, as the percentage increases with time. Bandala *et al.*, (2012) found that the Fenton Photo technique can destroy the eggs of Ascaris worms by more than 99% .

Rennecker et al., (2000) and Corona et al., (2002) stated that the use of ozone, free chlorine, hypochlorous acid, or chloramines has a high efficiency in inactivating parasitic worm eggs through the production of active hydroxyl radicals. From what was mentioned, the Fenton Photo process is effective because it produces hydroxyl radicals that generated by the interaction of iron with peroxide. The concentration of iron decreases as a result of the oxidation and reduction process.

The oxidation process occurs in a rapid reaction, as in equation (1) and the process can occur in the presence of light, as in equation (2) or in darkness, as in equation (3), Note that the presence of ultraviolet radiation increases the reaction rate (Carra *et al.*,2014).

$Fe^{2+} + H2O2 \rightarrow Fe^{3+} + HO^{-} + HO\bullet$ (1)

$Fe^{3+} + H2O + hv \rightarrow Fe^{2+} + HO\bullet + H^{+}$ (2)

$Fe^{3+} + H2O2 \rightarrow Fe^{2+} + HO2\bullet + H^{+}$ (3)

Table 4: We note that the total number of bacteria in the wastewater before treatment and for all dilutions was above the specified limit. As for after treatment, we note that there is a significant decrease in bacteria and that this decrease increases as the treatment time increases

until it reaches a percentage of 100% removal for all dilutions when the treatment time was 40 minutes. The disinfection process using the Fenton Photo technique occurs as a result of damage to the cell wall of microorganisms and the liberation of their contents by hydroxyl radical (Li *et al*.,2019) and Damage can occur through the passage of Fe_2 through the cell membrane and causing changes inside the cell (Shwetharani & Balakrishna 2014). Recent research indicates that damage occurring inside the cell is more effective than damage outside the cell (Giannakis *et al*.,2016).

The mechanism of destroying living cells using the Fenton photo technique is due to the fact that free radicals (hydroxyl) react with organic compounds and begin a series of chemical reactions that are due to the fact that these radicals contain an unpaired electron, which raises a high chemical instability and thus takes an electron from the stable molecules to reach stability. Electrochemical Thus, a stable electron-donating molecule is transformed into a free radical, initiating a chain reaction that leads to cell destruction(Escobar et al 2014).

In studying photo-oxidation of a group of bacterial strains, researchers noticed that the survival rate for all strains became zero after six hours of the operation (Liu *et al*.,2023) . We note that there is a difference in time because our study relied on the use of Fenton Photo, which is one of the photo-oxidation process techniques in which peroxide and iron oxide are used. And sunlight to produce effective hydroxyl groups, and the time to produce these groups is faster, considering the use of factors to help in the production of these radicals.

4-Conclusions:

1- Advanced photo-oxidation (Fenton photo) is important in water purification and inactivation of some pathogens.
2- Increasing the treatment time increases the removal efficiency of contaminants.

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