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RESEARCH ARTICLE

Electro-Fenton technology as an advanced oxidation process for eliminating antibiotic-resistant bacteria from Euphrates river water in Iraq

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ABSTRACT

Background and Aim: The global rise of antibiotic-resistant bacteria (ARB) poses a major threat to environmental and public health, particularly in regions with inadequate wastewater treatment. Iraq's Euphrates River is heavily contaminated with pharmaceuticals and resistant pathogens due to poor disposal practices and untreated effluents. This study evaluated the antibacterial efficacy of the electro-Fenton (EF) process, an advanced oxidation method that generates hydroxyl radicals (•OH), in reducing ARB from Euphrates river water.

Materials and Methods: Water samples were collected during May–August 2024 from three sites in Al-Muthanna Governorate: Al-Samawah, Al-Mahdi, and Al-Khidhir. Samples were subjected to eight EF treatments (5–40 V, 20 min each) using a locally fabricated EF unit with hydrogen peroxide, potassium chloride, and nitric acid to optimize hydroxyl radical generation. Bacterial contamination was assessed using multiple tube fermentation, thermotolerant detection of fecal coliforms, total plate counts, biochemical identification, and analytical profile index (API) 20E tests. Antibiotic susceptibility was determined through disk diffusion against eight antibiotics.

Results: Before EF treatment, high bacterial loads were recorded across all sites, with counts exceeding >1600 most probable number (MPN)/100 mL for Escherichia coli, fecal coliforms, Klebsiella pneumoniae, Enterobacter cloacae, and *Pseudomonas aeruginosa*. Many isolates exhibited multidrug resistance, including resistance to vancomycin, ampicillin, and tetracycline. After EF treatment, bacterial counts markedly declined to <1.8 MPN/100 mL for most species, aligning with World Health Organization and Environmental Protection Agency water quality standards. Thermotolerant fecal coliforms were reduced to 6.8 MPN/100 mL. A slight resurgence of bacterial growth occurred at 40 V (treatment 8), likely due to competing side reactions at higher voltages.

Conclusion: This is the first study in Iraq to demonstrate the application of EF for ARB removal from river water. The results confirm EF as a highly effective, environmentally sustainable, and scalable approach for degrading pharmaceutical residues and reducing ARB contamination in surface water. Future research should include molecular profiling of resistance genes and broader geographic evaluation.

Keywords: advanced oxidation process, antibiotic resistance, electro-Fenton, Euphrates river, public health, water pollution.

INTRODUCTION

The contamination of water with toxic, chemical, and pharmaceutical substances, combined with the growing prevalence of antibiotic-resistant bacteria (ARB), represents a significant threat to human health, particularly among vulnerable populations. Surface and groundwater often contain a wide range of pollutants, influenced by factors such as geology, hydrology, oceanographic processes, and human activities [1]. In Iraq, water resources face heavy contamination from multiple sources, including waste from government and healthcare facilities. Improper disposal of pharmaceuticals, especially antibiotics, facilitates the introduction of harmful bacteria into water supplies. Industrial effluents from petrochemicals, manufacturing, food processing, and heavy metal industries exacerbate this problem, while chemical pollutants further disrupt aquatic ecosystems.

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Agricultural runoff containing pesticides and herbicides is another major contributor to water contamination [2]. In addition, widespread oil production and transportation activities in Iraq have resulted in frequent oil pollution [3, 4]. Household chemical waste and expired or unused pharmaceuticals, when improperly discarded, may also infiltrate aquatic systems [2, 5].

Chronic exposure to such chemical toxins poses a significant public health challenge, as symptoms may not emerge until after a prolonged latency period. Consumption of contaminated water has been linked to serious health conditions, including cancers, neurological disorders, and reproductive complications [6, 7]. Antibiotics, widely used in human and veterinary medicine, are frequently detected in wastewater. Their persistence in the environment fosters the emergence and dissemination of antibiotic-resistant microorganisms, making infections increasingly difficult to treat [8–10]. Aquatic environments are now recognized as reservoirs for ARB, raising global concerns about waterborne resistance [11–13]. Untreated or inadequately treated wastewater often harbors high loads of pathogenic bacteria [14–16], many of which display antibiotic resistance. Municipal wastewater treatment facilities often fail to completely eliminate these contaminants, resulting in their release into natural water bodies and thereby exacerbating public health risks [17].

To monitor and assess water safety, bacteriological, biochemical, and antibiotic susceptibility tests are routinely applied. These tests are crucial for detecting pollutants and pathogenic bacteria, ensuring that water is free from infectious agents. Indicator organisms, such as *Escherichia coli* and coliforms, are frequently used to assess fecal contamination [18].

The implementation of effective water treatment systems is critical for enhancing chemical reactions, controlling microbial growth, reducing corrosion, and improving the performance of treatment chemicals [19]. A variety of physical, chemical, biological, and mechanical treatment processes have been developed to remove toxic pollutants from water [20, 21]. Among these, the electro-Fenton (EF) technique, an advanced oxidation process (AOP), has emerged as a particularly effective method for eliminating refractory organic contaminants, ARB, and antibiotic resistance genes (ARGs). The EF process generates hydroxyl radicals (•OH), highly reactive oxidants that degrade pollutants and destroy microbial cells. The classical Fenton process, which uses hydrogen peroxide (H_2O_2) and ferrous ions, is noted for its simplicity, cost-effectiveness, and high efficiency. The EF modification enhances this reaction by producing •OH electrochemically, typically through the reaction of H_2O_2 with ferrous iron (Fe^{2+}) under acidic conditions. These radicals effectively degrade organic pollutants and disrupt microbial cells, including resistant strains. Due to its strong oxidative potential and well-characterized chemical mechanisms, EF technology has been widely applied in wastewater treatment, biogeochemical cycling, atmospheric chemistry, and biomedical fields [3, 22, 23]. Compared with the traditional Fenton method, EF requires fewer added chemicals, making it a more environmentally sustainable alternative [24]. A previous study by Chen *et al*. [25] has demonstrated its efficacy in removing resistant bacterial species, including *E. coli* and *Pseudomonas aeruginosa*.

Despite growing global concern over the spread of ARB in aquatic environments, effective strategies for their removal remain limited, particularly in regions with poor wastewater management. Conventional treatment systems often fail to completely eliminate ARB and pharmaceutical residues, resulting in the continuous discharge of resistant strains into rivers and other surface waters. Although AOPs such as Fenton and photocatalytic methods have been explored, their application in real-world contaminated river systems remains under-investigated. In Iraq, the Euphrates river is a critical water source for domestic, agricultural, and industrial purposes, yet it suffers from heavy contamination due to untreated hospital effluents, industrial discharges, pharmaceutical waste, and agricultural runoff. Previous research in Iraq has primarily focused on the detection of bacterial contamination and antibiotic resistance, but there is a notable lack of studies evaluating innovative water treatment technologies for controlling ARB in this river. Importantly, the potential of the EF process, a cost-effective and environmentally friendly AOP that generates •OH to degrade organic pollutants and destroy bacterial cells, has not been systematically assessed for ARB elimination in Iraqi water systems. This gap leaves uncertainty regarding the practical applicability of EF technology in high-burden settings such as the Euphrates river.

This study was designed to address this critical knowledge gap by evaluating the antibacterial efficacy of the EF process in eliminating ARB from the Euphrates river in Iraq. Specifically, the research aimed to (i) identify and characterize bacterial species contaminating the river at different sites, (ii) determine their antibiotic resistance profiles using standard susceptibility testing, and (iii) assess the ability of the EF process, under varying voltage

conditions, to reduce bacterial loads and bring contamination levels within acceptable public health limits. To the best of our knowledge, this represents the first study in Iraq to apply EF technology for the treatment of surface water contaminated with ARB. By demonstrating the effectiveness of EF against resistant bacteria, this study provides valuable evidence supporting its potential as a sustainable and scalable water treatment strategy for regions facing similar environmental and public health challenges.

MATERIALS AND METHODS

Ethical approval

Ethical approval was not needed in this study because there were no animals or human participants included.

Study period and location

The study was conducted from May to August 2024 in Al-Muthanna Governorate, Iraq (Latitude: 29.91331710; Longitude: 45.29938620) [26]. Figure 1 illustrates the administrative borders of Al-Muthanna city on the map of Iraq [27]. Figure 2 shows the course of the Euphrates river as it flows through the city of Al-Muthanna [28].

Figure 1: The administrative borders of Al-Muthanna city [27].

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Figure 2: The course of the Euphrates river as it passes through Al-Muthanna [28].

Sampling design

Water samples were collected from the Euphrates river from three locations: The first is from the center of Al-Samawah city (A1), the second is from the Al-Mahdi district (west of A1 city) (A2), and the third is from Al-Khidhir province (southeast of A1 city) (A3), as shown in Figure 2. Water samples were collected over 4 months in 2024: May, June, July, and August. During these months, the temperatures rise and the river level drops. We used sterile glass containers provided by the Public Health Laboratory in Al-Muthanna Governorate for sampling. The samples were transferred directly to the Public Health Laboratory and the Environment Directorate in Al-Muthanna for biochemical and microbiological tests and antibiotic susceptibility testing.

Sample collection protocol

In this study, water samples (500 mL) of both experimental groups (water samples before and after treatment) and control group (untreated water samples) were collected from three areas of the Euphrates river (A1, A2, and A3) in sterile bottles (500 mL) from each site during the fourth summer months in 2024 (May, June, July, and August), when the temperature is elevated and the weather is dry. The samples were collected from a depth of 30 ± 2 cm below the water surface to avoid contamination from surface debris such as suspended matter floats, settles, other contaminates, or where algae thrive. The intake points of the samples were approximately 500 m away from potential pollution sources. The water bottles were tightly closed and kept at 4°C to conduct the required test as soon as possible. Water samples collected from the three Euphrates river sites (A1, A2, and A3) were each subjected to eight treatments, with 5 V increments of voltage from 5 to 40 V. To prevent microbial shifts, all tests were performed promptly at the Public Health and Environment Directorate Laboratories in Al-Muthanna.

EF system setup

A locally fabricated EF unit (Figure 3) was used in this study. The device is composed of a plastic basin for electrical insulation, connected to a valve that draws treated water and a lower valve for draining sediments. Carbon steel electrodes are placed in the plastic basin connected to the voltmeter by electrical wires.

Operational parameters and experimental procedure

Each 500 mL sample was treated with a standardized dose of 0.5 mM H_2O_2, 0.05 M potassium chloride (KCl), and 1 M nitric acid (HNO_3). The system voltmeter was turned on, and the voltage was set to 5 V. The time was set to 20 min. The EF process generates •OH *in situ* to effectively eliminate bacteria. Several factors, including the amount of applied voltages, could influence the performance of the EF mechanism [29]. Previous studies by used Omar *et al*. [30] and Minnalkodi Senguttuvan *et al*. [31] different ranges of electric voltages, including 0.5–4 V as well as 5–15 V. In addition, Chen *et al*. [25] indicated that using a current density of about 21.42 mA/cm^2 effectively limited bacterial growth. In the present study, the voltage used ranged from 5 to 40 V, which could be effective for the generation and continuation of the chemical reaction to produce •OH, which are crucial for disinfecting water samples and eradicating various bacteria present in water samples.

A variety of examination time intervals (0, 20, 30, 60, 90, and 120 min) were used in a previous study by Minnalkodi *et al*. [31], which showed that the EF technique may be best performed after a 30-min treatment

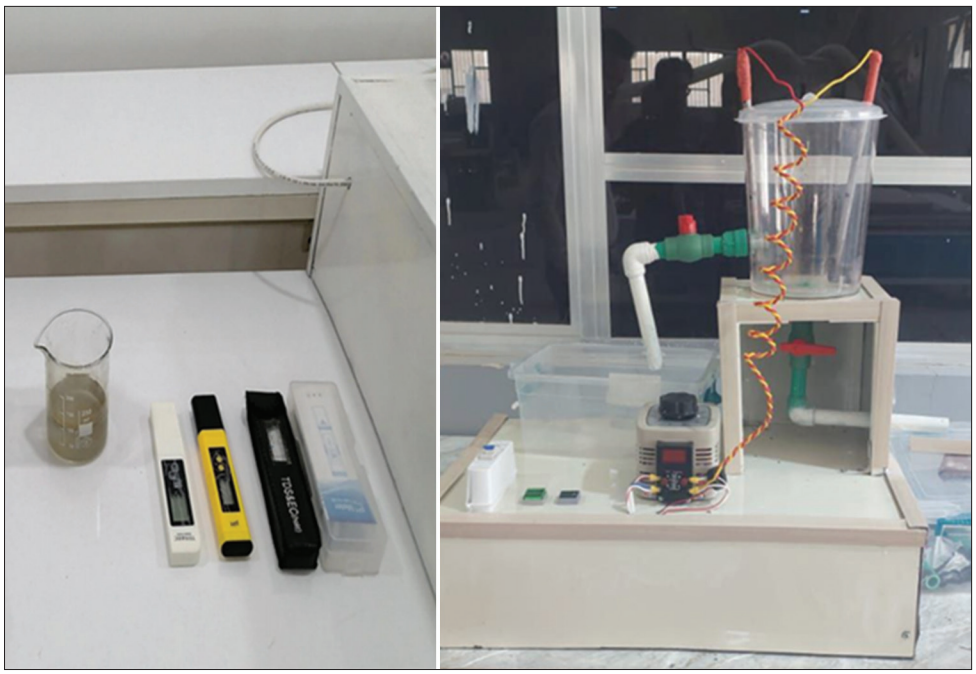

Figure 3: Locally manufactured electro-Fenton device for the experiment.

session. Similarly, another study by Chen *et al*. [25] evaluated ARB inactivation and ARG destruction using the EF technique, which indicated that bacterial growth was interrupted after 30 min of EF treatment until the maximum range reached 120 min. A 20-mine session length may be sufficient in the present study to guarantee that there is enough •OH formation to enter bacterial cells, destroy cell membranes, DNA, and proteins, and cause bacterial death. The selected voltage and treatment duration may conserve energy and reduce the formation of unwanted byproducts.

After that, the device was stopped, and water was drained through the drain tap. Biochemical and microbiological tests were then performed on the treated water. The abovementioned steps were repeated 8 times (eight treatments). During each treatment, the voltage was gradually increased by 5 V (from 5 V to 40 V), while the duration of the experiment was fixed at 20 min. For each treatment, one drop (0.05 mL) of H_2O_2, KCl, and HNO_3 was consistently added. These steps were chosen based on a previous study by Meijide *et al*. [32], which showed that these criteria are suitable for performing the required treatment process and removing pollutants.

Reagents and their rationale

H_2O_2 is essential for initiating the reaction that generates •OH, which plays an important role in the degradation of organic contaminants in wastewater [33, 34]. The addition of KCl promotes the EF process by increasing the conductivity of the solution and accelerating the breakdown of pollutants by generating more oxidant radicals [35]. HNO_3 is added to maintain an acidic environment that is optimal for the Fenton reaction and halogen oxidation [36].

EF reaction

The mechanism of the EF reactions would be as following: [32].

$$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2 \quad (1)$$

$$Fe^{2+} + H_2O_2 + H^+ \rightarrow Fe^{3+} + H_2O + {}^{\bullet}OH \quad (2)$$

$$Fe^{3+} + e^- \rightarrow Fe^{2+} \quad (3)$$

$$Fe^{2+} + {}^{\bullet}OH \rightarrow Fe^{3+} + OH^- \quad (4)$$

Bacteriological analyses

Bacteriological testing is essential for detecting bacterial contaminants and assessing water quality. They were performed to ensure the absence of enteric pathogens, which can cause infectious diseases in individuals

who consume the water. Fecal contamination was assessed using indicator organisms, primarily *E. coli* and other coliforms. Gram-negative, non-spore-forming coliforms are facultative anaerobes that ferment lactose to produce gas and acid. To understand the potential bacteriological risks associated with receiving water, it is often necessary to employ multiple approaches. A 10 mL or appropriately measured sample volume was used to initiate the primary bacterial culture in each tube that contained Lauryl tryptose broth (LTB), which was then spread out on Brilliant Green Lactose Bile Broth, *E. coli* broth, plate count agar, MacConkey agar, and blood agar to create bacterial subculture (all the above mentioned broths and agars were obtained from HiMedia- India). The plates were then incubated at 35°C ± 0.5°C for 24 h to read the results [37]. Three methods are used to examine the water samples:

Multiple tube fermentation

This is a popular technique for identifying and counting particular bacterial species, such as *E. coli* and coliforms, in soil, food, and water samples. The presumptive test, confirmed test, and completed test are the three phases that make up this approach [37].

Presumptive test

The sample was prepared in a series of 10-fold serial dilutions (e.g., 1:10, 1:100, and 1:1000) to guarantee a variety of bacterial proportions. LTB, a selective medium that promotes the growth of coliform bacteria, was placed in a series of fermentation tubes (10 mL for each tube) along with a measured portion of each dilution. If any lactose-fermenting bacteria proliferate and release gas, the medium will become yellow and the Durham tube (HiMedia) will contain bubbles. Coliforms are, therefore, presumed to be present. The samples were incubated at 35°C for 48 h. A confirmed test is conducted if the test results are positive.

Confirmed test

This refers to a subsequent verification step used to confirm the identity of the microorganisms initially detected in a sample. This process ensures that the identified organisms are indeed those of interest and not false positives or other contaminants. It involves taking a sample from a positive lactose broth tube and streaking it onto Brilliant Green Lactose Bile Broth or EC broth. The appearance of turbidity and gas in the broth indicates a coliform and bacterial presentation.

Completed test

In this test, EMB agar (HiMedia) or MacConkey agar was used, both of which are specific agars for coliforms and are distinguished by their ability to ferment lactose. The inoculated plates were incubated for 24 h at 35°C.

Thermotolerant detection of fecal coliforms

They belong to a subgroup of fecal coliform bacteria that have evolved to survive at high temperatures, usually (44.5°C ± 0.2°C). Their presence was detected using EC broth (10 mL per tube); the tested samples were incubated in a water bath at 44.5°C for 24 h. The presence of these bacteria indicates the presence of fecal material and contamination from animals in the water, which can carry dangerous pathogens, even though they are not pathogens themselves. The presence of thermotolerant fecal coliforms aids in determining possible sources of contamination, evaluating the safety of drinking and recreational water in environmental monitoring and water quality testing. Elevated concentrations of these bacteria may indicate increased contamination and risk of waterborne illness [38].

Total plate count

It serves as a gauge for the aerobic bacteria in a sample. For these bacteria to grow, oxygen is required. This test provides an estimate of the total microbial load in a sample, regardless of whether it is soil, food, water, or another type of sample. A known volume of the sample (1 mL) was spread onto a plate of count agar (10–12 mL), which promotes the growth of aerobic bacteria after dilution. For a predetermined time, usually 48 h, the plate was incubated at a particular temperature, typically between 35°C. The number of bacterial colonies that developed on the plate following incubation was determined. The count provides an estimate of the quantity of viable aerobic bacteria in the original sample, as each colony originates from a single bacterial cell or a group of cells [39].

Biochemical identification of bacterial isolates

Biochemical tests were conducted as described by Winn Washington *et al*. [40], MacFaddin [41], and Leboffe and Pierce [42]. The tests were as follows:

Kligler's iron agar test

Gram-negative enteric bacteria, particularly those belonging to the *Enterobacteriaceae* family, were identified using Kligler's Iron Agar (Microxpress-India), which distinguishes between bacteria according to their capacity to produce gas, hydrogen sulfide, and lactose and glucose fermentation.

Urease activity test

Urease hydrolyzes urea into ammonia (NH_3) and carbon dioxide (CO_2). Thus, this test is used to identify bacteria that can produce this enzyme. The potential of hydrogen (pH) indicator (phenol red) changes from orange-yellow to pink when the pH is raised by ammonia. This test used urea broth or urea agar base (Christensen).

Indole production test

Sulfur, indole, motility medium, or peptone water broth (HiMedia) was used in this test. The tryptophanase enzyme was used in this test to determine whether a bacterium can convert the amino acid tryptophan into indole. Indole is detected by Kovac's reagent (HiMedia), which forms a reddish-pink ring at the surface.

Simmon's citrate utilization test

The purpose of this test is to determine whether bacteria can use inorganic ammonium salts as their sole source of nitrogen and sodium citrate as their sole source of carbon after the preparation of the test medium Simmons Citrate Agar (HiMedia).

Rapid identification using the analytical profile index 20E system

The API-20E system (BioMérieux, France) is used clinically for the rapid identification of the isolates. This test was performed according to Bauer *et al*. [43]; primarily, this method serves to identify *Enterobacteriaceae* and differentiate between its different species.

The system consists of a plastic strip containing 20 microtubules, each of which houses a dehydrated biochemical substrate. A pure bacterial colony was suspended in either sterile saline or distilled water, and this suspension was then inoculated into the wells of the API 20E strip. After incubation at 35°C for 24 h, the strip underwent color changes, which served as indicators of positive or negative results for each biochemical test.

Antibiotic susceptibility testing

This test is crucial because it determines the sensitivity of various bacterial isolates to antibiotics. According to Bauer *et al*. [43], a range of antibiotic disks was employed in the disk diffusion technique.

Disk diffusion method

Two types of bacterial isolation were tested (*E. cloacae* and *K. Pneumonia* as an example of ARB). The test bacterium suspension (typically matching the 0.5 McFarland standard) was inoculated onto an agar plate (Mueller-Hinton agar; Titan Biotech, India) with antibiotic disks on the surface. The antibiotics will then diffuse outward from the disks into the agar during the 24 h that the plate is incubated at 35°C. A distinct inhibition zone surrounds the disk, preventing bacterial growth if the bacteria are susceptible.

Antibiotic disk selection and concentrations

Different antibiotic types and concentrations were used in this study, including Amikacin-30 μg, Cefatazidime-30 μg, Nitrofurantoin-300 μg, Vancomycin-30 μg, Gentamycin-10 μg, Ampicillin-10 μg, Rifampin-5 μg, and Tetracycline-30 μg) (Bioanalyse, Turkey).

Interpretation criteria for susceptibility results

If the bacterial strain is suppressed by the antibiotic at standard dosages, the antibiotic could prove beneficial in managing the infection (susceptible [S]). However, if the bacterial strain partially responds to the antibiotic at standard dosages, it may not be completely effective, necessitating higher dosages, longer treatment periods, or alternative treatments (intermediate [I]). In the most unfavorable situation, if the bacterial strain is unresponsive to the antibiotic at standard dosages, the antibiotic is ineffective against the infection, necessitating alternative treatment options (resistant [R]). The larger the inhibition zone, the more potent the antibiotic is against the microbe [43]. The results were recorded and interpreted according to the guidelines of the Clinical and Laboratory Standards Institute [44].

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MULTIPLE-TUBE FERMENTATION TECHNIQUE (9221)/Estimation of Bacterial Density

TABLE 9221:IV. MPN INDEX AND 95% CONFIDENCE LIMITS FOR VARIOUS COMBINATIONS OF POSITIVE RESULTS WHEN FIVE TUBES ARE USED PER DILUTION (10 mL, 1.0 mL, 0.1 mL)*

Combination of Positives	MPN Index/100 mL	Confidence Limits		Combination of Positives	MPN Index/100 mL	Confidence Limits	
		Low	High			Low	High
0-0-0	<1.8	—	6.8	4-0-3	25	9.8	70
0-0-1	1.8	0.090	6.8	4-1-0	17	6.0	40
0-1-0	1.8	0.090	6.9	4-1-1	21	6.8	42
0-1-1	3.6	0.70	10	4-1-2	26	9.8	70
0-2-0	3.7	0.70	10	4-1-3	31	10	70
0-2-1	5.5	1.8	15	4-2-0	22	6.8	50
0-3-0	5.6	1.8	15	4-2-1	26	9.8	70
1-0-0	2.0	0.10	10	4-2-2	32	10	70
1-0-1	4.0	0.70	10	4-2-3	38	14	100
1-0-2	6.0	1.8	15	4-3-0	27	9.9	70
1-1-0	4.0	0.71	12	4-3-1	33	10	70
1-1-1	6.1	1.8	15	4-3-2	39	14	100
1-1-2	8.1	3.4	22	4-4-0	34	14	100
1-2-0	6.1	1.8	15	4-4-1	40	14	100
1-2-1	8.2	3.4	22	4-4-2	47	15	120
1-3-0	8.3	3.4	22	4-5-0	41	14	100
1-3-1	10	3.5	22	4-5-1	48	15	120
1-4-0	11	3.5	22	5-0-0	23	6.8	70
2-0-0	4.5	0.79	15	5-0-1	31	10	70
2-0-1	6.8	1.8	15	5-0-2	43	14	100
2-0-2	9.1	3.4	22	5-0-3	58	22	150
2-1-0	6.8	1.8	17	5-1-0	33	10	100
2-1-1	9.2	3.4	22	5-1-1	46	14	120
2-1-2	12	4.1	26	5-1-2	63	22	150
2-2-0	9.3	3.4	22	5-1-3	84	34	220
2-2-1	12	4.1	26	5-2-0	49	15	150
2-2-2	14	5.9	36	5-2-1	70	22	170
2-3-0	12	4.1	26	5-2-2	94	34	230
2-3-1	14	5.9	36	5-2-3	120	36	250
2-4-0	15	5.9	36	5-2-4	150	58	400
3-0-0	7.8	2.1	22	5-3-0	79	22	220
3-0-1	11	3.5	23	5-3-1	110	34	250
3-0-2	13	5.6	35	5-3-2	140	52	400
3-1-0	11	3.5	26	5-3-3	170	70	400
3-1-1	14	5.6	36	5-3-4	210	70	400
3-1-2	17	6.0	36	5-4-0	130	36	400
3-2-0	14	5.7	36	5-4-1	170	58	400
3-2-1	17	6.8	40	5-4-2	220	70	440
3-2-2	20	6.8	40	5-4-3	280	100	710
3-3-0	17	6.8	40	5-4-4	350	100	710
3-3-1	21	6.8	40	5-4-5	430	150	1100
3-3-2	24	9.8	70	5-5-0	240	70	710
3-4-0	21	6.8	40	5-5-1	350	100	1100
3-4-1	24	9.8	70	5-5-2	540	150	1700
3-5-0	25	9.8	70	5-5-3	920	220	2600
4-0-0	13	4.1	35	5-5-4	1600	400	4600
4-0-1	17	5.9	36	5-5-5	>1600	700	—
4-0-2	21	6.8	40				

* Results to two significant figures.

Figure 4: MPN index/100 mL and 95% confidence limits for various combinations of positive results when five tubes were used per dilution (10, 1.0, and 0.1 mL) [37]. MPN=Most probable number.

Statistical analysis

Calculation of the most probable number (MPN)

The MPN, which calculates the number of coliforms in the water sample, is frequently used to express the results of the multiple tube fermentation (MTF) method. A statistical table (Figure 4) that links the number of positive tubes in each dilution set to a likely coliform concentration was used to calculate the MPN [37]. The presence of total coliforms was indicated by the production of gas in all tubes (positive result). On the other hand, a negative result is recorded if there is no gas or color change in the tubes, and there are either no or very few coliforms present [37].

Statistical significance criteria

The MPN (MPN index/100 mL) and 95% confidence limits for different combinations of positive results were calculated using a statistical table (Figure 4), which correlates the number of positive tubes in each dilution set to a likely coliform concentration. This is done for five tubes per dilution (10, 1.0, and 0.1 mL) [37]. Public health and regulatory agencies (e.g., Environmental Protection Agency, Food and Drug Administration) have accepted the MPN for testing food safety and water quality. To produce results and estimate bacterial populations, MPN applies statistical probability theory and principles.

RESULTS

Bacterial species and counts before treatment

Initial detection of bacteria

For the primary detection of bacteria, the MTF technique was conducted. The data showed yellowing of the medium and gas formation in the Durham tube, indicating lactose-fermenting bacteria (Figure 5) [45]. High bacterial counts were also observed on plate agar (Figure 6).

Subsequently, confirmed and completed tests were performed, and the data were recorded as follows.

E. coli–MPN/100 mL

E. coli and fecal coliforms are key water contaminants that serve as strong indicators of fecal pollution, and surface water should be routinely tested for water pollution [12, 15]. *E. coli* was detected in three sites of the Euphrates river: (A1, A2, and A3), during the 4 months of 2024 (May, June, July, and August) (Table 1 and Figure 7).

Table 1: Preliminary bacteriological test results (before treatment) of water samples taken from three areas of the Euphrates river (A1, A2, and A3) during 4 months of the year (May, June, July, and August).

Parameters	Areas											
	A1				A2				A3			
Months	May	June	July	August	May	June	July	August	May	June	July	August
Escherichia coli (MPN/100 mL)	120	240	540	920	110	>1600	>1600	430	47	8.2	540	1600
Fecal *coliforms* (MPN/100 mL)	120	240	540	920	110	>1600	>1600	430	47	8.2	540	1600
Thermotolerant fecal *coliforms* (MPN/100 mL)	120	240	540	920	110	>1600	>1600	430	47	8.2	540	1600
Total plate count (MPN/1 mL)	300	S	S	S	S	S	S	300	200	20	S	S
Pseudomonas aeruginosa (MPN/100 mL)	120	240	540	920	110	>1600	>1600	430	47	8.2	540	1600
Klebsiella pneumoniae (MPN/100 mL)	120	240	540	920	110	>1600	>1600	430	47	8.2	540	1600

MPN = Most probable number, S = Spreader, A1 = Al-Samawah, A2 = Al-Mahdi, A3 = Al-Khidhir site

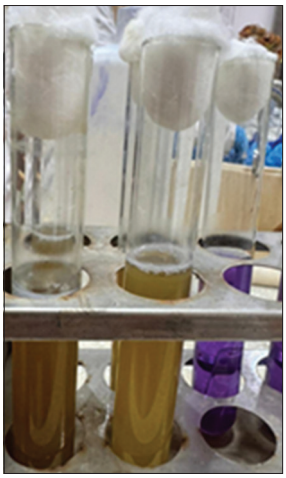

Figure 5: Bubble formation in the Durham tube, indicating the proliferation of lactose-fermenting bacteria and the release of gas.

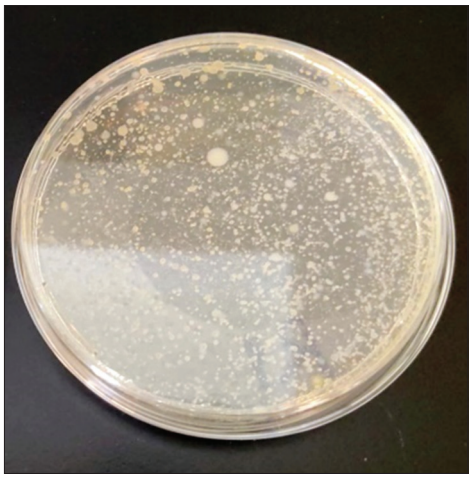

Figure 6: Number of bacterial colonies on the plate count agar plate before treatment.

Overall, *E. coli* levels across all sites were markedly above the acceptable range during the testing period. Counts ranged from 8.2 MPN/100 mL at A3 in June to >1600 MPN/100 mL at A2 in June and July.

Fecal coliform bacteria–MPN/100 mL

Aquatic coliform bacteria (e.g., Escherichia, Enterobacter [Figure 8], and Klebsiella [Figure 9]) enter waterways through sewage and excrement of living organisms. Their harmful impact on drinking water necessitates removal during treatment. Elevated levels indicate contamination from organic matter and potential fecal input [12, 15, 37, 46].

Coliform bacterial counts were consistently elevated across all three sampling sites (A1, A2, and A3) during the 4 months, as shown in Table 1. Levels exceeded the limits of [37] (<1.8 MPN/100 mL) (Figure 4), [38], and World Health Organization (WHO) [7] (0 coliform/100 mL). The lowest value was 8.2 MPN/100 mL (A3) in June, while the highest was >1600 MPN/100 mL (A2) in June and July.

Total plate count–MPN/1 mL

Fecal contamination is implied by the presence of these microorganisms. For every milliliter of aquatic bacteria entering water through sewage and excreta, the total plate count was calculated by the components of the plate [15].

Counts exceeded permissible limits [37] (Figure 4), suggesting widespread bacterial contamination across all sampled locations and months. Table 1 shows that the lowest value was 20 MPN/1 mL in A3 during June, followed by 200 MPN/1 mL in A3 during May, and 300 MPN/1 mL in A1 during May and A2 during August. Bacterial growth was also widespread across all sites in other months (Figure 6 and Table 1).

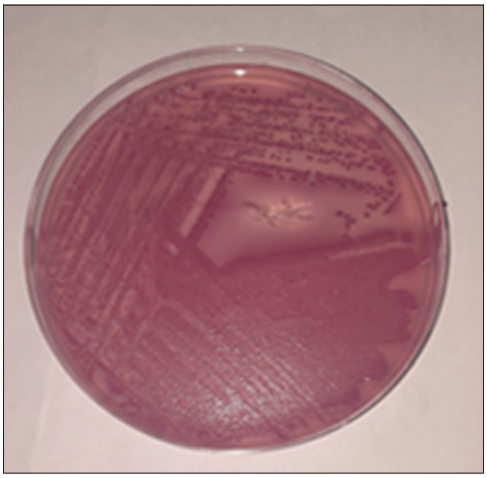

Figure 7: *Escherichia coli* growth on MacConkey agar and lactose fermentation.

Figure 8: Growth of *Enterobacter cloacae* on MacConkey agar and lactose fermentation.

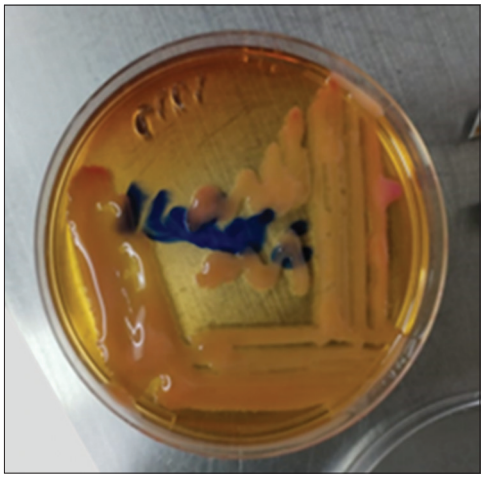

Figure 9: Growth of Klebsiella *pneumonia* (visible mucoid colonies) on MacConkey agar.

Figure 10: *Pseudomonas aeruginosa* growth on blood agar.

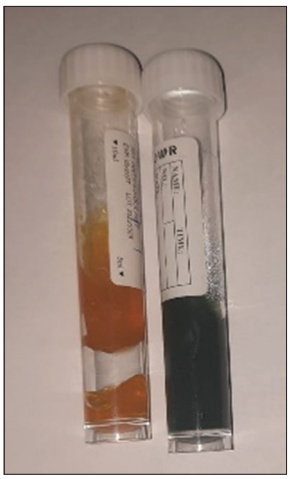

Figure 11: Escherichia coli bacteria show a negative result to Simmon's citrate and on Kligler's iron agar was acid/acid with gas without H_2S.

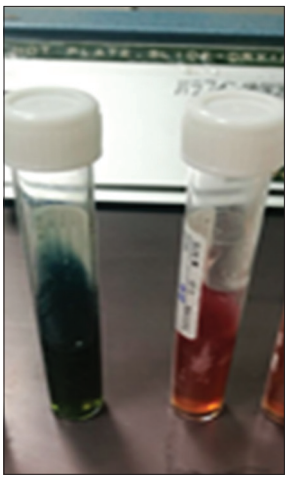

Figure 13: *Pseudomonas aeruginosa* bacteria show a positive result on Simmon's citrate and on Kligler's iron agar was alkaline/alkaline without gas and H_2S.

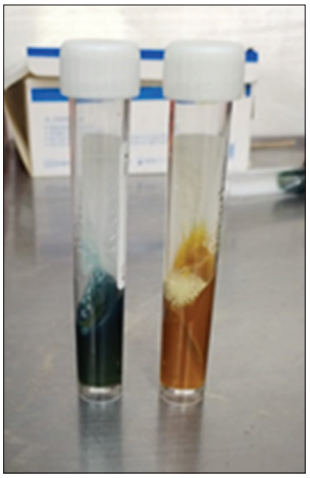

Figure 12: Both *Enterobacter* cloacae and *Klebsiella pneumoniae* show a positive result to Simmon's citrate and on Kligler's iron agar was acid/acid with gas without H_2S.

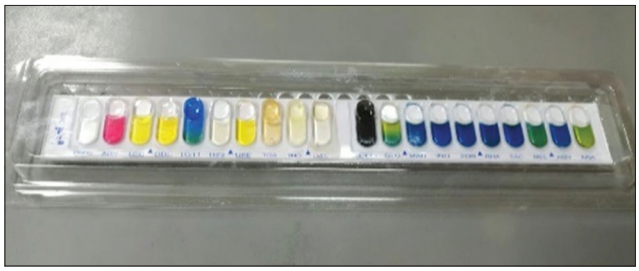

Figure 14: Identification of *Pseudomonas aeruginosa* by analytical profile index 20E.

P. aeruginosa–MPN/100 mL

P. aeruginosa was detected at all three sites (A1, A2, and A3) throughout the 4 months of 2024 (May–August), as shown in Table 1 and Figure 10. Counts varied across sites and months, with several readings exceeding standard safety thresholds.

Biochemical tests

Biochemical tests showed that:

- *E. coli* was indole-positive, motile, produced acid/acid with gas on Kligler's iron agar without H_2S, and was negative for Simmon's citrate (Figure 11).
- *E. cloacae* and *K. pneumoniae* were indole-negative, citrate-positive, and acid/acid with gas (no H_2S) on Kligler's agar (Figure 12). *E. cloacae* was urease-negative and motile, while *K. pneumoniae* was urease-positive and non-motile.
- *P. aeruginosa* was indole- and urease-negative, showed an alkaline/alkaline reaction on Kligler's agar without gas or H_2S, and tested positive for Simmon's citrate (Figure 13).

API 20E system

The API 20E biochemical test distinguishes *Enterobacteriaceae* (Shigella, Proteus, Salmonella, Citrobacter, Klebsiella, Serratia). Identified species included *E. coli*, *E. cloacae*, *K. pneumoniae*, and *P. aeruginosa* (Figure 14).

Table 2: Antibiotic susceptibility test results of water samples collected from two areas (A1 and A2) of the Euphrates river, as examples of contaminated water.

Bacterial species	Antibiotic name	Abbreviation	Dose	Response (degree of susceptibility)		
				Sensitive (S)	Intermediate (I)	Resistant (R)
Enterobacter cloacae	Amikacin	AK	30 µg	√		
	Cefatazidime	CAZ	30 µg	√		
	Nitrofurantoin	F	300 µg		√	
	Vancomycin	VA	30 µg			√
	Gentamicin	CN	10 µg	√		
	Ampicillin	AMP	10 µg			√
	Rifampin	RD	5 µg		√	
	Tetracycline	TE	30 µg			√
Klebsiella pneumonia	Amikacin	AK	30 µg	√		
	Cefatazidime	CAZ	30 µg	√		
	Nitrofurantoin	F	300 µg	√		
	Vancomycin	VA	30 µg			√
	Gentamicin	CN	10 µg		√	
	Ampicillin	AMP	10 µg			√
	Rifampin	RD	5 µg		√	
	Tetracycline	TE	30 µg		√	

A1 = Al-Samawah, A2 = Al-Mahdi

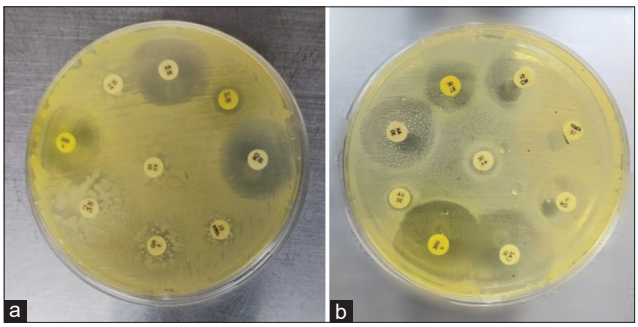

Figure 15: Antibiotic susceptibility test: (a) Sensitivity of *Enterobacter cloacae* isolated from A1; (b) sensitivity of *Klebsiella pneumoniae* isolated from A2 on Mueller-Hinton agar.

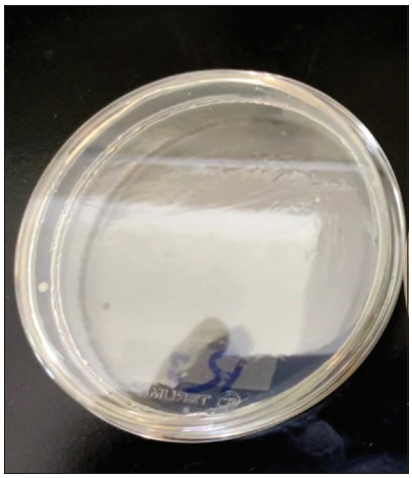

Figure 16: Post-treatment polyacrylamide plate showing no visible bacterial colony formation, indicating effective disinfection.

Antibiotic susceptibility testing

Antibiotic susceptibility testing was conducted to evaluate the resistance profiles of isolated microbes. Water samples were collected from A1 to A2 (highly contaminated sites) to assess antibiotic efficacy (Table 2, Figures 15a and b).

The results indicated the presence and proliferation of ARB. For example:

- *E. cloacae* was identified as an ARB species in A1.
- *K. pneumoniae* was identified as an ARB species in A2.

Bacteria in A1 exhibited higher resistance to multiple antibiotics compared with those in A2.

Bacterial species and counts after treatment

Post-treatment analysis using EF technique

Representative water samples from A1 in August were selected to assess EF treatment efficacy. This site was chosen due to high exposure to biological pollutants from hospitals, industrial facilities, and dense human activity [2], as well as potential ARB strains introduced through improper pharmaceutical disposal [16, 46, 47].

Post-treatment bacteriological test results are presented in Table 3 and Figure 16.

Table 3: Bacteriological test results of post-treatment water samples from A1, Euphrates river, collected in August. Treat = Treatment (in treatment 1, the voltage was 5 V, the duration was 20 min, and 0.05 mL) of H_2O_2, KCl, and HNO_3 was consistently added); for other treatments, the voltage was increased by 5 V in each treatment, while the duration and the amount of reagents were fixed).

Parameter	A1							
	Treat 1	Treat 2	Treat 3	Treat 4	Treat 5	Treat 6	Treat 7	Treat 8
Escherichia coli (MPN/100 mL)	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8	<3.6
Fecal *coliforms* (MPN/100 mL)	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8	<3.6
Thermotolerant fecal *coliforms* (MPN/100 mL)	6.8	6.8	6.8	6.8	6.8	6.8	6.8	10
Total plate count (MPN/1 mL)	60	1	8	8	8	1	8	S
Pseudomonas aeruginosa (MPN/100 mL)	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8	<3.6
Klebsiella pneumoniae (MPN/100 mL)	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8	<3.6

MPN = Most probable number, S = Spreader, A1 = Al-Samawah

Reduction of bacterial counts

The results showed significant reductions in *E. coli*, fecal coliforms, *P. aeruginosa*, and *K. pneumoniae*, with counts dropping to within normal limits (<1.8 MPN/100 mL) as per [37, 38], and WHO guidelines [7] (0 coliforms/100 mL).

Thermotolerant fecal coliforms declined to 6.8 MPN/100 mL. Total plate count also showed a strong reduction in colonies. However, a slight resurgence was observed in treatment 8 (40 V, 20 min), where bacterial regrowth was indicated by total plate counts.

Despite this, the overall data suggested that the 5–40 V EF voltage range effectively reduced ARB counts in treated water.

DISCUSSION

Environmental and public health risks

The presence of toxic, chemical, and pharmaceutical substances, along with the proliferation of pathogenic and ARB, poses significant environmental and public health risks. Aquatic environments increasingly act as reservoirs for ARB, worsening the public health burden of antimicrobial resistance (AMR) [11, 18].

Detection of bacteria in water

Several examinations, such as bacteriological, biochemical, and antibiotic susceptibility tests, are used to detect the presence of bacteria in water. This study aimed to identify bacterial species, assess their antibiotic resistance profiles, and evaluate the antibacterial efficacy of the EF process. Water samples were collected from three areas of the Euphrates river (A1, Al-Mahdi [A2, and Al-Khidhir site [A3]) during the four summer months of 2024 (May, June, July, and August).

Bacterial proliferation and spread

After performing the bacteriological and biochemical examinations, the data indicated the proliferation and spread of different types of bacteria in water, such as *E. coli*, fecal coliforms, thermotolerant fecal coliforms, *K. pneumoniae*, *E. cloacae*, and *P. aeruginosa*.

Indicators of fecal contamination

E. coli and fecal coliform levels were consistently elevated across sites and exceeded the recommended thresholds throughout the study period. Detection of *E. coli* and coliforms is a strong indicator of fecal contamination in water. Therefore, the presence of these bacteria in water should be routinely tested to measure water pollution [5, 15].

Total plate count and microbial balance

In addition, the total plate count (TPC) MPN/1 mL values were highly increased in all A1, A2, and A3 during the four summer months when the experiment was conducted. As reported by Papaioannou *et al*. [5], Baird *et al*. [37], high total plate counts in water bodies negatively affect aquatic microbial balance.

Role of wastewater and temperature in bacterial proliferation

Wastewater flowing directly into the river without treatment increases the likelihood that the water in these areas may contain large amounts of harmful bacteria. Temperature significantly influences bacterial

proliferation; thus, higher ambient temperatures and human activity are expected to elevate downstream bacterial loads. A previous study by Kadir *et al*. [48] investigated the prevalence of *Cryptosporidium* spp. in calves and water samples, supporting the seasonal impact on microbial contamination. In addition, higher temperatures can indirectly increase bacterial growth and activation by speeding up the decomposition of organic matter (e.g., dead plants and sewage), which provides bacteria with additional nutrients, thereby boosting bacterial populations [49, 50].

Antibiotic resistance in water samples

Antibiotic susceptibility results confirmed the presence and spread of several ARB species in the water samples. The data also suggest that the growing ARB differs in their susceptibility to various antibiotics, which could be the result of several factors, such as the bacterial species and the type of antibiotics. Numerous sources, including pharmaceutical waste, hospital effluents, and inappropriate medication disposal, can contaminate water with antibiotics. These factors facilitate the survival and dissemination of ARB in aquatic ecosystems.

Persistence of antibiotics in aquatic environments

These antibiotics may persist in the environment for an extended period if they are not completely broken down or eliminated during wastewater treatment processes. Furthermore, sublethal levels of antibiotics in water can still selectively affect microbial populations even though they are not potent enough to eradicate bacteria. Bacteria subjected to these sub-inhibitory concentrations may develop resistance mechanisms that enable their survival over time [46, 51, 52].

Methods for removing toxic contaminants

Several physical, chemical, biological, and mechanical methods associated with organic treatment are commonly used to remove toxic contaminants from water [19, 20]. The EF technique was employed to eliminate pathogenic and ARB from contaminated water samples.

Efficacy of the EF technique

The data after treating water with the EF technique indicated a substantial reduction in the number of specific bacterial taxa, such as *E. coli*, fecal coliforms, *P. aeruginosa*, *K. pneumoniae*, and thermotolerant fecal coliforms, to reach normal levels, as suggested by World Health Organization [7], Baird *et al*. [37], and Blodgett [38].

Mechanism of EF action

The EF technique is a promising advanced oxidation method that creates highly reactive •OH by combining electrochemical techniques with Fenton's reagent (Fe^{2+} and H_2O_2), Fe^{2+} is generated by the electrodes during the electro-Fenton reactions, while the H_2O_2 is provided from (Sigma-Aldrich Chemie GmbH-Germany). Due to their high oxidative potential, EF-generated •OH can degrade organic pollutants and disrupt bacterial cell walls and nucleic acids [2, 23, 53]. Proteins and lipids found in bacterial cells can also be oxidized by •OH, harming the bacteria, including strains resistant to antibiotics, in ways that cannot be reversed. In addition, EF treatment may generate secondary oxidants, such as hydroperoxides or peracetic acid, enhancing bactericidal effects [25].

Optimization of EF treatment conditions

In addition, the results of treatment 8 (40 V for a 20 min session) showed a slight increase in the spectrum of various isolated bacterial species. Despite the slight resurgence in treatment 8, the applied voltage range (5–40 V) was effective in substantially reducing the bacterial load. It has been proposed that optimizing the voltages used in the EF technique is crucial. The killing effect on ARB is reduced when too low voltages are used because fewer reactive oxygen species are formed. However, excessively high voltages can result in undesirable side reactions, such as the breakdown of H_2O_2 or the generation of chlorine (from chloride ions), which may decrease the effectiveness of bacterial cell destruction [54]. The data also indicated that selecting a 20-min session length provides enough time for •OH formation to disinfect the water from ARB with minimum energy usage and by-product formation.

Broader potential of EF in water treatment

EF has also demonstrated efficacy in degrading pharmaceutical compounds present in contaminated water, including residual antibiotics. This breakdown is significant because bacteria may become resistant to antibiotics if sublethal levels of the antibiotics are present in the water [55].

CONCLUSION

This study demonstrated that water samples from three sites of the Euphrates river (A1, Al-Mahdi, and Al-Khidhir) harbored diverse bacterial contaminants, including *E. coli*, fecal coliforms, thermotolerant fecal coliforms, *K. pneumoniae*, *E. cloacae*, and *P. aeruginosa*. Elevated *E. coli* and coliform levels consistently exceeded recommended thresholds at all sites, indicating strong fecal contamination. The TPC values were also markedly increased during the summer months, highlighting the seasonal influence of temperature and untreated wastewater inflow on microbial proliferation. Antibiotic susceptibility testing further confirmed the presence of multiple ARB, reflecting the impact of pharmaceutical effluents, hospital discharges, and improper drug disposal on aquatic ecosystems.

The EF process effectively reduced the bacterial load across all sampling sites, with significant declines in *E. coli*, fecal coliforms, *P. aeruginosa*, and *K. pneumoniae* after treatment. •OH generated during EF treatment played a pivotal role in disrupting bacterial cell integrity and degrading residual antibiotics. Optimization experiments suggested that applying 20 min of treatment at 20–40 V provided substantial bacterial inactivation with minimal energy consumption and limited by-product formation. These results confirm the EF process as a promising advanced oxidation method for mitigating ARB contamination in water.

The findings underscore the potential of EF as a scalable, eco-friendly technology for enhancing water quality in regions affected by untreated wastewater discharge and antibiotic pollution. Its ability to simultaneously degrade pharmaceuticals and eradicate resistant bacteria makes it particularly valuable for integration into municipal wastewater treatment facilities and environmental health interventions. A major strength of the study lies in its combined use of bacteriological, biochemical, and antibiotic susceptibility tests, multi-site sampling during the peak summer season, and experimental validation of EF's effectiveness under varying operational conditions. Nevertheless, the research was limited by the absence of molecular profiling of ARGs, the relatively narrow geographic coverage, and the lack of field-scale operational assessment.

Future work should expand sampling to additional locations and seasons, integrate molecular methods for resistance confirmation, and evaluate EF under continuous-flow pilot applications to assess long-term efficiency, cost-effectiveness, and scalability. Combining EF with other AOPs may also enhance disinfection capacity while minimizing energy demand.

The study provides strong evidence that the EF process is a viable and efficient method for reducing pathogenic and ARB in river water. While further molecular and field-scale investigations are needed, the approach holds significant promise as part of an integrated strategy to combat AMR and safeguard public health through improved water sanitation.

AUTHOR'S CONTRIBUTIONS

RAMJ: Designed and conducted the study, collected and analyzed the data, and drafted and revised the manuscript. The author has read and approved the final manuscript.

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COMPETING INTERESTS

The author declares that she has no competing interests.

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