Review Article

Effect of pH on *Escherichia coli* Removal by Electrocoagulation and Elimination Kinetics after Treatment

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Received 23 August 2021; Revised 22 December 2021; Accepted 20 January 2022; Published 8 February 2022

Academic Editor: Mohamed Azaroual

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There are different techniques for removing microorganisms in wastewater, each with its own advantages and disadvantages. Electrocoagulation because of its simplicity has gained great attention and is used for the removal of various ions, organic matters, and microorganisms. In this study, the effectiveness and mechanism of *Escherichia coli* (*E. coli*) removal by electrocoagulation process using aluminum and ordinary steel electrodes at different initial-pH and the kinetics of elimination of *E. coli* in solution after treatment were investigated. Artificial wastewater contaminated by *E. coli* culture was used in the experiments. The results show that the initial-pH influences significantly the effectiveness of *E. coli* removal. Under the experimental conditions used, more than 5 log removal of *E. coli* is obtained, irrespective of the nature of the electrode (ordinary steel or aluminum) and the value of the initial pH. On the one hand, the best rates of elimination are obtained for solutions that are slightly acidic (pH 5.5) and for an alkaline pH (8.5 and 10). On the other hand, the elimination decreases for a neutral solution and for a very acidic solution (pH 2.9) because of the strong resistance developed by *E. coli* at those pH values. For optimal treatment, the choice of electrode material depends on the initial pH. Furthermore, the study of the kinetics of elimination of *E. coli* after treatment shows the remanent power of the electrocoagulation process. It allows reducing treatment time and energy consumption, thus reducing the cost of treatment.

1. Introduction

Waterborne diseases caused by bacterial contamination are a serious concern in the world health community. To address this issue, there is a continuous need to develop efficient and cost-effective disinfection technologies. Several disinfection technologies, such as ozonation, ultraviolet disinfection, and chlorination, are currently in use for water treatment. However, even though these technologies are effective, their utilization is limited by operational safety, the formation of unwanted by-products, and a high cost for operation and maintenance [1–4].

One of the emerging technologies that proved its efficiency against a broad spectrum of microorganisms is electrochemical disinfection. It has gained increasing attention as an alternative to the conventional methods of

disinfection since it is environmentally friendly and is known to inactivate a wide variety of microorganisms, from bacteria to viruses and algae [3–10].

Recently, it has been demonstrated that electrocoagulation as an electrochemical disinfection technology offers an attractive alternative compared to conventional technologies for the treatment of microorganisms in water, with relatively low energy consumption [6, 7, 10, 11]. Electrocoagulation has been combined with an ultrasonic source [12] and with electro-Fenton treatment [13] to improve the removal of bacteria, even if, like any other treatment method, the electrocoagulation technology still has some drawbacks that could limit its applications [14]. Among microorganisms, the removal of *E. coli* in water is of great interest because of its importance as an indicator of fecal pollution [15, 16]. Moreover,

E. coli is one of the bacteria generally responsible for intestinal infections [17].

The efficiency of electrocoagulation depends on various parameters, among which the initial-pH is known as one of the main factors that controls its performance since it plays an important role in the rate of metal hydroxides formation, and the majority of species in the solution are pH-dependent [18]. Moreover, pH plays a crucial role in the removal of microorganisms in an aqueous solution. In fact, alkaline pH results in a significant decrease in the survival time of *E. coli* in wastewater [19, 20], and at low pH environments, *E. coli* develops tolerance to acidity [21]. In addition, most of the microorganisms grow better at pH values between 6.6 and 7.5 [22, 23].

To the best of our knowledge, very few studies investigated the pH effect on the removal of microorganisms while using the electrocoagulation process [3, 10], and the kinetics of elimination of microorganisms after treatment have never been investigated. Therefore, the main purpose of this study was the assessment of the pH effect on the removal of *E. coli* while using electrocoagulation and the investigation of the kinetics of elimination of *E. coli* in solution after treatment. Elsewhere, the mechanism involved during the treatment will be suggested.

2. Material and Methods

2.1. Experimental Apparatus. The experiments were conducted in an electrochemical cell under the conditions of a batch system. The electrocoagulation reactor consists of a 100 cm³ glass beaker mounted with two electrodes plate of 10 cm × 2 cm × 0.2 cm each. The electrodes were positioned vertically and parallel to each other, with an interelectrode distance of 1 cm. The effective area of the electrode used during a given experiment depends on the electrocoagulation parameters. The electrode substrate material was aluminum and ordinary steel. Prior to each experiment, the electrodes were (1) sanded with a finely graded sandpaper to remove corrosion products from the electrode's surface, (2) The electrodes were washed with distilled water, (3) immersed in alcohol (90%) to be sterilized, (4) rinsed with distilled water, and dried in the incubator at 110°C. A magnetic stirrer (Stuart heat-stir SB 162) was used to homogenize the solution during the experiment. The current was provided by a laboratory DC power supply (ERMES M10-SP-303, 30 V-3 A). After each treatment, conductivity and pH were determined using a multiparameter apparatus (Multi 340i/SET Weight Watchers International pH-meter).

2.2. Treatment Procedures and Counting of *E. coli*. *E. coli* (strain ATCC 833), provided by Centre Pasteur of Yaounde—Cameroon, was used as an indicator bacterium in all experiments. The pure strain of *E. coli* as provided was used to prepare an artificial solution of *E. coli* at a concentration of 10⁸ UFC/100 mL. For each group of experiments, 5 cm³ of this contaminated solution was diluted into deionized and sterilized water to give an initial cell density

between 5 × 10⁵ UFC/100 mL and 4 × 10⁷ UFC/100 mL. To increase the conductivity of the artificially polluted water, sodium nitrate (1 g/L) (NaNO₃, 99% from Prolabo) was used as a supporting electrolyte.

To study the effect of the initial-pH, the charge loading ranging from 0 to 40 F/m³ was applied while keeping the current density constant at 12.5 mA/cm². These experimental conditions are motivated by previous works, which have established that charge loading influenced significantly the inactivation efficiencies than current density [7, 24]. The initial pH was adjusted with 0.1 M nitric acid (HNO₃, 96% from Aldrich) and 0.1 M sodium hydroxide (NaOH, 99.9% from Prolabo) solutions.

To study the elimination kinetics after treatment, a highly concentrated solution of *E. coli* (4 × 10⁷ CFU/100 mL) was treated, using four different charge loadings values (1.24 F/m³, 3.73 F/m³, 6.21 F/m³, and 12.43 F/m³), with a current density of 12.5 mA/cm², an initial pH of 7.02, and the iron electrodes and NaNO₃ (1 g/L) as supporting electrolytes. The treated solutions by electrocoagulation were analyzed just after the treatment and after 2 h, 6 h, 18 h, and 24 h.

For each treatment, 50 cm³ of the contaminated water was spiked in the electrocoagulation reactor, where either aluminum or ordinary steel electrode was used, and after the electrochemical process was conducted at room temperature (≈ 22°C), an aliquot sample was collected and filtered using the Whatman filter paper no. 40 (2.5 μm) before counting the residual *E. coli*.

The concentration of *E. coli* was determined using the membrane filtration method with appropriate culture media [Lactose TTC (2, 3, 5-triphenyltetrazolium chloride) agar with tergitol 7 base]. An incubation time of 24–48 h at 44°C allowed the colonies to grow [25]. All experiments were performed in triplicate for statistical analyses, which were conducted using Microsoft Excel 2013. The inactivation efficiency of *E. coli* was determined for each treatment taking into account the number of *E. coli* in the solution and expressed as log units.

3. Results and Discussion

3.1. Effect of Initial pH on *E. coli* Removal by Electrocoagulation. To illustrate the effect of pH on *E. coli* removal by electrocoagulation using ordinary steel and aluminum electrodes, experiments were performed at different initial pH values (2.9, 5.5, 7.1, 8.5, and 10.0). For each pH value, the charge loading was varied from 0 to 37.3 F/m³.

Figure 1 depicts the effect of charge loading on *E. coli* inactivation using ordinary steel electrodes.

It appears that *E. coli* elimination rates increase significantly with the charge loading at any initial-pH value. The log (CFU/100 mL) decreases strongly with the charge loading. However, it can be noticed that there is a considerable difference between the curves. For a charge density of 6.21 F/m³, the removal rates are 2.36 log, 3.33 log, 4.31 log, 4 log, and 6.14 log for the initial pH values of 2.92, 7.1, 8.5, 10, and 5.5, respectively. The elimination rates increase with pH from 2.92 to 5.5 and then decrease around the neutral pH

FIGURE 1: Effect of charge loading and initial pH on inactivation of *E. coli*: current density: 12.5 mA/cm², supporting electrolyte: NaNO₃ 1 g/L, and ordinary steel electrode.

and finally increase with a subsequent pH raise. Elsewhere, the total removal of *E. coli* is obtained for the charge loading of 6.21 F/m³ at pH 5.5, 12.43 F/m³ at pH 8.5 and 10, and 24.87 F/m³ at pH 2.92 and 7.1. The change in initial pH, therefore, has a significant effect on the removal efficiency of *E. coli* using the ordinary steel electrodes. The best removal rates are obtained at pH 5.5. Similar results were obtained by Aronsson et al. following the exposure of *E. coli* to an electric field and varying the pH of the medium [26], as well as by Lynn et al. while using electrocoagulation as a pretreatment for the electrooxidation of *E. coli* [3].

From the analysis of the curves in Figure 2, it appears that the elimination rates of *E. coli* increase strongly with the charge loading, irrespective of the initial pH while using aluminum as electrode material. The curves decrease strongly with the charge loading, irrespective of the initial pH. They are similar for the initial pH of 5.5 and 10, whereas there is a considerable difference between the other curves. For example, for a charge loading of 6.21 F/m³, there is a reduction in the bacterial load of 1.92 log, 2.7 log, 4.2 log, 6.3 log, and 6.3 log, respectively, at the initial pH of 7.1, 2.92, 8.5, 10, and 5.5. The bacterial load reduction increases from 2.9 to 5.5, reaching a maximum, and then, it decreases from 5.5 to 7.1, and it increases thereafter for alkaline pH. The total elimination of *E. coli* in solution is obtained for a charge loading of 6.21 F/m³ for pH 5.5 and pH 10, 12.43 F/m³ for pH 8.5, 24.87 F/m³ for pH 2.9, and finally, 37.30 F/m³ for pH 7.1 (Figure 2).

From this result, it appears that the initial pH has a significant effect on the removal efficiency of microorganisms in solution by electrocoagulation with aluminum electrodes. The best removal rates are obtained at pH 5.5 and 10.

On the aluminum electrode, the difference between the elimination rates of *E. coli* in water following the initial pH can be explained by the fact that the main chemical reactions during electrocoagulation are as follows:

FIGURE 2: Effect of charge loading and initial pH on inactivation of *E. coli*: current density: 12.5 mA/cm², supporting electrolyte: NaNO₃ 1 g/L, and aluminum electrode.

Depending on the concentration of Al³⁺ electro-generated and the initial pH range of the solution, different species of aluminum (monomers and polymers) can be formed in the solution.

The dependence of the elimination rate on the initial pH using ordinary steel and aluminum electrodes can be explained by the following facts:

- (i) For a low initial pH of about 2 to 4, the hydroxide ions generated at the cathode can be neutralized by the hydronium ions present in the solution [27], thus limiting the formation of the metal complexes responsible for the neutralization of the bacterial charge and their elimination. Indeed, in the case of ordinary steel, the equation of the reaction likely to be realized in the very acidic medium is given by,

Although the oxygen produced is likely to form disinfectants in solution, it does not compensate for the absence of Fe(OH)₃ precipitates, which are more adsorbent than Fe(OH)₂ precipitates [10].

In the case of aluminum electrodes, monomeric cationic species, such as Al₃⁺, Al(OH)²⁺, and Al(OH)₂⁺, are predominant [28, 29]. These are very poorly adsorbent compared to neutral Al(OH)₃ species and polymeric species [30].

Moreover, *E. coli* can survive and resist in a very acidic medium (pH <4), developing a tolerance to acidity. In these extreme physical conditions, strong acids dissociate at the outer membrane of *E. coli*, however, they are unable to penetrate the cell membrane [31]. To counteract this adverse effect, *E. coli* synthesizes enzymes like carboxylases that neutralize excess protons [21]. To adapt and tolerate

low pH environments, bacterial cells tend to change the value of their external pH. Brown and Booth have demonstrated in 1991 that *E. coli* secreted carboxylase enzymes preferentially at very acidic pH [32]. The role of such enzymes is to increase the value of the external pH, inducing thus acid tolerance in certain situations [33]. *E. coli* has several acid resistance systems that can be classified into 2 categories, depending on whether the system can be induced and functions in minimal medium without acid-tolerance responses (ATR) or requires some supplement forms (acid-resistance [AR] mechanisms) [21].

All these effects justify the low elimination rates observed at pH 2.92. Similar results were obtained by Gernaout et al. in 2008 during the electrocoagulation treatment of surface water containing *E. coli*. They found that the elimination rates were lower in a very acidic medium [10].

Moreover, inactivation efficiencies are almost similar with ordinary steel and with aluminum as electrode material using an initial pH between 2 and 4. It can be explained by the fact that, on the one hand, whatever the type of electrode material used, the metal hydroxides responsible for the neutralization of the bacterial charge and their elimination are weakly produced, and on the other hand, *E. coli* can survive and resist in a very acidic medium.

- (ii) At pH 5.5, the removal efficiency of *E. coli* in solution is higher, following the combination of several effects: most parts of the hydroxide complexes responsible for coagulation and precipitation of pollutants in solution are formed in the pH range between 5 and 9. It is the optimum pH range for performing electrocoagulation [27, 28]. The adhesion of those precipitates to the cell walls results in bacteria encapsulation in flocs because of the interactions between electrocoagulation precipitates and phosphate functional groups on bacteria surfaces [34].

In the case of aluminum electrodes, the consecutive reactions that occur on the surface of the electrodes and in the solution can lead to the formation of monomeric hydroxides, such as $\text{Al}(\text{OH})_{2+}$, $\text{Al}(\text{OH})^{2+}$, $\text{Al}(\text{OH})_3$, and $\text{Al}(\text{OH})_4^-$, and polymeric species, such as $\text{Al}_6(\text{OH})_{15^{3+}}$, $\text{Al}(\text{OH})_{17^{4+}}$, $\text{Al}_8(\text{OH})_{20^{4+}}$, $\text{Al}_{13}\text{O}_4(\text{OH})_{24^{7+}}$, and $\text{Al}_{13}(\text{OH})_{34^{5+}}$ [28–30]. All these oligometric species are finally transformed into a water-insoluble amorphous compound, $\text{Al}(\text{OH})_{3(\text{s})}$, via complex polymerization/precipitation kinetics. The chemical species responsible for the effectiveness of electrocoagulation in the pH range of 5 to 9 are the hydroxoaluminum polymer ions and the $\text{Al}(\text{OH})_3$ aluminum hydroxide precipitate. These two coagulating agents have a complementary action. Polymeric cations can adsorb to the negative charges

of *E. coli*, thereby promoting aggregation by charge neutralization and/or bridging for flocculation.

Moreover, at low acidic pH, the undissociated weak acids can diffuse freely through the cytoplasmic membrane. Once inside the cytoplasm, they dissociate and increase the internal pH of *E. coli* [21, 35]. The ability to maintain the pH inside the cell close to neutrality (pH homeostasis) is then disrupted, leading to the death of the cell. In addition, the membrane permeability increases because of the formation of pores in the cell wall (during the passage of electric current), and the H^+ -transport rate also increases because of the osmotic imbalance around the cell [36]. It results in a reduction in cytoplasmic pH because a higher number of protons is available compared to a neutral pH. The change in pH in the cell may induce chemical changes in the basic compounds, such as DNA or adenosine triphosphate, as explained by Wiggins [37]. In addition, oxidation and reduction reactions may occur within the cell structure [38]. All these effects are bactericidal, and the high inactivation efficiency of *E. coli* observed at pH 5.5 is mainly attributed to them. Lynn et al., using electrocoagulation as a pretreatment for the electrooxidation of *E. coli*, also found that lower initial pH (pH 5–6) improved *E. coli* mitigation during electrocoagulation [3].

Inactivation efficiencies at this pH are similar upon using ordinary steel or aluminum as an electrode material because the high elimination rate observed at a weakly acidic pH is mainly attributed to the free diffusion of chemical species using the cell wall, which causes a malfunction and destroys the bacterial cell [36].

- (iii) At pH 7.1, the efficiency of *E. coli* elimination decreases, although within the optimal pH range of electrocoagulation [28]. This decrease can be attributed to the fact that this pH is also in the optimal pH range of *E. coli* development [39]. Indeed, most microorganisms grow best at pH values between 6.6 and 7.5 [22, 23]. When microorganisms grow outside their optimal pH range, it results in increased latency [40]. The role of pH in the survival of microorganisms is related to the ability of organisms to maintain the cytoplasmic pH near neutrality. To adapt and tolerate pH environments different from their optimum pH range, bacterial cells attempt to change the value of their external pH by engaging in complex physiological and genetic mechanisms [21]. In the case of pH 7.1, the cells do not need any particular arrangement, and therefore, they are more resistant to environmental conditions. It leads to a decrease in the effectiveness of the treatment. Delaire et al., using Fe electrocoagulation to attenuate *E. coli* in synthetic Bengal

groundwater, also found that *E. coli* reduction was significantly enhanced at lower initial pH compared to neutral pH [41].

Furthermore, as Ndjomgoue-Yossa et al. recently demonstrated during the treatment of water containing *E. coli* by electrocoagulation, inactivation efficiency is higher with ordinary steel as electrode material than with aluminum using an initial-pH 7.1, because of the fact that iron hydroxides $\text{Fe}(\text{OH})_3$ lead to higher enmeshment and adsorption than aluminum hydroxides $\text{Al}(\text{OH})_3$ [7].

- (iv) At alkaline pH (8.5 and 10), the elimination of *E. coli* in solution increases because the kinetics of production of the metal hydroxides responsible for the coagulation and the adsorption of the pollutants in the solution is high [28].

In the case of aluminum electrodes, the concentration of the monomeric anionic species $\text{Al}(\text{OH})_4^-$ increases at the expense of the $\text{Al}(\text{OH})_{3(s)}$ formation [28]. Moreover, the cathode could be chemically attacked by the OH^- ions generated at the same time as the hydrogen gas H_2 at high pH values [42] according to the following equation:

These aluminum complexes, $(\text{Al}(\text{OH})_4^-)$, polymerize to form microsuspensions, and by the agglomeration of destabilized colloids, they turn into microflocs and then into larger flocs. These flocs are thus formed at the origin of the coagulation and precipitation of pollutants in the solution [43].

In addition, the alkaline pH has a harmful effect on *E. coli*. Mezrioui and Baleux showed in 1992 that the survival time of *E. coli* in wastewater purified by lagooning and protected from all solar radiation is much shorter in an alkaline medium in comparison with acidic and neutral environments [19]. This bactericidal effect of the alkaline pH can be attributed to the fact that the internal pH of *E. coli* is around neutrality, [44] and unlike the highly acidic environments where the bacterium adapts, the ability to maintain this internal pH of the cell near neutrality is low when the external medium is alkaline. It inflicts stress on the body, which can lead to the death of the cell. The devastating damage of a strong base also occurs because phospholipids, which form the cell membrane and the nucleic membrane, are broken down by hydroxyl ions. Base hydrolysis causes the saponification reaction of lipids, leading to the breakdown and lysis of membrane structure [35]. All this justifies the increased effectiveness of the treatment observed in an alkaline medium. Similar results were obtained by Gernaout et al. in 2008, who found that the efficiency of the elimination of *E. coli* by electrocoagulation using iron electrodes is high in an alkaline medium [10].

Moreover, the effectiveness of the treatment is higher with aluminum as electrode material than with ordinary steel in alkaline pH because of the high production of aluminum complexes $\text{Al}(\text{OH})_4^-$ in the solution following the simultaneous degradation of the aluminum anode and

cathode at alkaline pH, which is not the case for iron electrodes.

3.2. Elimination Kinetics of *E. coli* after Treatment.

Electrocoagulation is a very efficient process for the removal of *E. coli* from water, which can be optimized by changing the electrical and physicochemical parameters, such as charge density, current density, electrode material, supporting electrolyte, initial pH, and others. [3, 7–10]. In most cases, it takes quite a long treatment time to achieve the total removal of *E. coli* from water, which results in greater energy consumption, and therefore, a high cost of treatment.

To reduce treatment time for total removal, the elimination kinetics of *E. coli* after electrocoagulation treatment have been investigated. In fact, the idea of this study comes from the fact that the analyses carried out a few days after the treatment on solutions, which still contained *E. coli* even at very high concentrations after treatment, showed that *E. coli* were completely inactivated.

To study the elimination kinetics of *E. coli* after electrocoagulation treatment, four different charge loadings values (1.24 F/m^3 , 3.73 F/m^3 , 6.21 F/m^3 , and 12.43 F/m^3) have been used. The results obtained are shown in Figure 3.

As seen in Figure 3, it appears that the concentration of *E. coli* in solution continues to decrease after treatment. The kinetics of elimination increase with the charge loading used. A 1.5 log, 1.9 log, 2 log, and 2.2 log reductions in *E. coli* were observed for a charge loading of 1.24 F/m^2 , 3.73 F/m^2 , 6.21 F/m^2 , and 12.43 F/m^2 , respectively, 2 h after the treatment. Total inactivation was achieved after 6 h, 18 h, and 24 h for a charge loading of 12.43 F/m^3 , 6.21 F/m^3 , and 3.73 F/m^3 , respectively. This result clearly shows that *E. coli* continue to be destroyed after treatment. It can be explained by two combined effects, which are as follows:

- (1) The action of oxidants produced in situ during electrolysis [7]: those strong oxidants may continue to react with the material of the cell wall by oxidizing, for example, the N-terminal amino groups of the membrane proteins. It weakens the resistance of the cells, thus causing massive lyses of the cells, which can lead to the death of the bacteria. The action of those oxidants was confirmed using sodium chloride as a supporting electrolyte. In fact, the analysis of the solution immediately after treatment with a charge loading of 6.21 F/m^3 gave a concentration of $6 \times 10^2 \text{ CFU/100 mL}$, and the total removal of *E. coli* was achieved after 5 h. This rapid inactivation of *E. coli* in the solution containing chloride ions is because of the formation in the treated solution of active chlorine species (such as ClO^- and ClO_2) electrogenerated during the treatment [5, 7, 45]. It accelerates the inactivation of bacteria after treatment. In addition, the reactive iron species formed during electrocoagulation [46] capable of inactivating *E. coli* and viruses [41, 47] can continue to inactivate them after treatment.

FIGURE 3: Elimination kinetics after treatment.

- (2) The diffusion of chemical species through pores formed in the cell wall of *E. coli* during the passage of electric current [48] leads to their inactivation. Indeed, the electric field causes the permeability of the cell membrane of bacteria. These injured cells, which can, however, be regenerated in a favorable environment [49], are inactivated following the infiltration into the cytoplasm of chemicals. It causes dysfunction in the internal enzyme groups, and thus, it destroys the cells.

To these two effects, we can add the gradual death of bacteria that have been weakened or strongly stressed during the treatment. Cells with damaged cytoplasmic membranes have been shown to be inhibited by certain values of pH and/or salt concentration, which would normally allow growth [49].

The study of the elimination kinetics of *E. coli* after treatment by electrocoagulation shows the ability of this disinfection technique to maintain a residual concentration of disinfecting agents (its remanent power) after treatment, which is one of the fundamental characteristics sought for purification and production systems for drinking water. This study also makes it possible to consider the reduction of the application time of electrocoagulation for the total elimination of bacteria, which, in fact, allows reducing the treatment time and energy consumption, thus reducing the cost of treatment. Although it should be noted that at the end of the treatment, it still takes a certain time before obtaining the total removal. In other words, it saves processing costs while wasting a little more time.

4. Conclusion

The effects of initial pH on the removal of *E. coli* in artificial wastewater by electrocoagulation with ordinary steel and aluminum electrodes and elimination kinetics after treatment are systematically investigated, and the conclusions drawn are as follows:

- (i) The initial pH significantly affects the treatment efficiency, irrespective of the nature of the electrode used (ordinary steel or aluminum). For an optimal treatment, the choice of electrode material depends on the initial pH. The best removal rates are obtained for weakly acidic solutions and for alkaline pH. However, the elimination rate decreases for neutral and very acidic solutions.
- (ii) From the results obtained, the removal of *E. coli* could be attributed to the three following reasons: the free diffusion through the cytoplasmic membrane of the chemical species, the harmful effect of the alkaline pH on *E. coli*, and adsorption by the metallic hydroxides formed in the solution.
- (iii) The study of the kinetics of elimination of *E. coli* after treatment shows the remanent capacity of the electrocoagulation process. It could reduce treatment time and energy consumption, thus reducing the cost of treatment.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The authors wish to thank the International Foundation of Science (IFS) and the Organization for Prohibition of Chemical Weapons (OPCW) (project no. W/4395-1, Prof. Nanseu- Njiki C. P.) for their financial support. They also thank ISP for the grant offered to the African Network of Electroanalytical Chemists (ANEC).

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