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Experimental Design to Measure *Escherichia coli* Removal in Water Through Electrocoagulation

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The experimental design herein was used to evaluate the influence of electrocoagulation parameters, such as initial pH and electrolyte support (Na_2SO_4) concentration, on *Escherichia coli* (*E.coli*) removal. The initial pH and Na_2SO_4 concentration influenced the response variable. *E.coli* removal is more efficient with a decrease in pH and an increase in Na_2SO_4 concentration. The predicted values from the empirical model were consistent with the experimental values. In a final experiment under optimal conditions (2.5 mg L^{-1} of Na_2SO_4 and initial pH 4.0), the electrocoagulation with aluminum electrodes was able to remove 1-log after 40 min and 1.9-log in a contact time of 90 min of *E.coli*. The study shows electrocoagulation as a promising alternative to remove microorganisms in water.

Keywords: Factorial design, electrochemistry, microorganism, pH, electrolyte support

1. INTRODUCTION

Chlorination is the primary method for disinfecting water because it is effective and inexpensive, but this method is related to trihalomethanes (THMs) byproduct formations, which are recognized as a potentially carcinogenic substances [1, 2]. This requires evaluating alternative techniques that may serve as a pretreatment or chlorination replacement.

Electrocoagulation is an alternative for water treatment and reduces or removes contaminants in an aqueous medium by passing an electric current through iron or aluminum electrodes and generating coagulants at the anode that form flocs, which settle or float with the contaminants [3]. This technique has successfully removed inorganic and organic contaminants [4-9], including microorganisms such as algae, bacteria and viruses [10-12]. In previous studies using

electrocoagulation, Azarian, et al. [10] removed between 99.8% and 100% of the *Microcystis aeruginosa* in the final effluent from a water treatment plant, and Gao, Du, et al. [13] entirely removed the algae in a NaCl solution using aluminum electrodes. Ghernaout, et al. [14] removed between 80-100% of the bacteria *Escherichia coli* using aluminum and stainless steel electrodes; evaluated certain parameters, such as pH and current; and observed a positive impact on microorganism removal by using a neutral pH and increasing the current.

For this study, the influence of electrocoagulation parameters, such as initial pH and Na_2SO_4 concentration, on *E.coli* removal was studied using the experimental design described herein. This statistical methodology facilitates an assessment of two or more factors relevant to a response variable and generates an appropriate number of experiments; the results are expressed as a regression model [15].

2. EXPERIMENTAL

2.1 Reagents

Sodium sulfate and nitric acid were purchased from Sigma-Aldrich; the microbiological reagents, peptone water, and Endo agar were purchased from Oxoid. Sulfuric acid and sodium hydroxide were acquired from Merck and were used to adjust the pH. Distilled water was used to prepare the aqueous solutions and for the experiments.

2.2 Electrochemical experiments

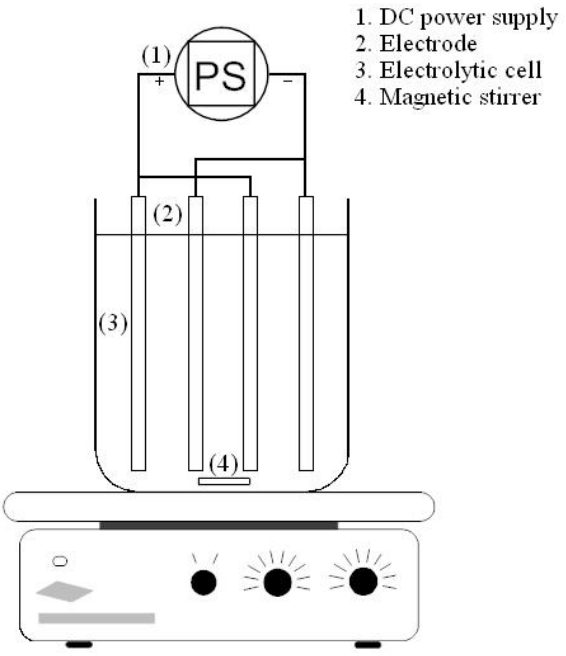

Figure 1. Schematic diagram of electrocoagulation experiment

Electrochemical tests were performed in a batch reactor, which comprised a 500 mL glass beaker equipped with four aluminum electrodes, a 44 cm^2 effective area, and a 1.0 cm interelectrodic space (Figure 1). The electrodes were connected to a direct current power supply (MCP Lab electronics) at 2.27 mA cm^{-2}. Before each experiment, the aluminum electrodes were washed, sanded, dipped in a nitric acid solution (0.1 mol L^{-1}), and rinsed with distilled water.

Na_2SO_4 was selected as the support electrolyte to avoid forming organic chlorine by-products [16]. Before the electrocoagulation process, the initial pH of the water was adjusted using NaOH or H_2SO_4 (0.1 mol L^{-1}). Samples were collected at different time intervals for microbiological analysis.

2.3 E.coli preparation and quantitation

A suspension comprising native *E.coli* in peptone water was prepared and incubated at 37 °C until the sample was at 10^5-10^6 cfu mL^{-1}. The samples were analyzed using the filtration membrane technique in accordance with standard methods [17]. Sterile cellulose membranes (Advantec MFS) comprising a 0.45 μm pore size were placed in a Petri dish with Endo agar (Oxoid). The *E.coli* colonies were counted after incubation at 35 °C for 24 h.

2.4 Experimental design

A two-level factorial design (2^k) with three replicate center points was used; the factors considered for this study were initial pH and support electrolyte (Na_2SO_4) concentration. The levels for the factors studied are shown in Table 1. The response variable was *E.coli* removal, which was defined as Log (N_t/N_o), where N_o is the initial *E.coli* concentration, and N_t is the remaining *E.coli* population at time t. The experiments were performed in a random order in duplicate, and the data were analyzed using the software Statgraphics®plus.

Table 1. Factors and levels used in the experiment

Variables	Low Level, -1	Center points, 0	High Level, +1
Initial pH	4.0	5.5	7.0
Na_2SO_4 (mg L^{-1})	0.25	1.38	2.5

3. RESULTS AND DISCUSSION

3.1 Experimental design applied to E.coli removal through electrocoagulation

Table 2 shows the design matrix generated using the software Statgraphics®plus. It includes the experimental conditions and results for each experiment and response variable, which is defined as

E.coli removal after electrocoagulation for 50 min. The study was conducted using a 95% confidence level.

Table 2. Design matrix and experimental results

Assays	Initial pH	Na_2SO_4 (mg L^{-1})	Log (N_t/N_o)
1	5.5	1.38	–0.83
2	5.5	1.38	–0.79
3	4.0	0.25	–0.18
4	7.0	0.25	–0.47
5	4.0	2.5	–1.36
6	5.5	1.38	–0.72
7	7.0	2.5	–0.22
8	5.5	1.38	–0.66
9	5.5	1.38	–0.79
10	4.0	0.25	–0.24
11	7.0	0.25	–0.46
12	4.0	2.5	–1.29
13	5.5	1.38	–0.51
14	7.0	2.5	–0.15

The primary-effects plot (Figure 2) shows the effect of each factor on the response variable. An increase in the Na_2SO_4 concentration had a positive effect on the response variable and may be explained by an increase in conductivity, which would improve microorganism removal [18, 19]. A decrease in initial pH also had an effect on *E.coli* removal, which is consistent with previous research that shows higher efficiency at acidic and neutral pH values using aluminum electrodes; in addition, an acidic pH can limit *E.coli* growth and survival [20-22].

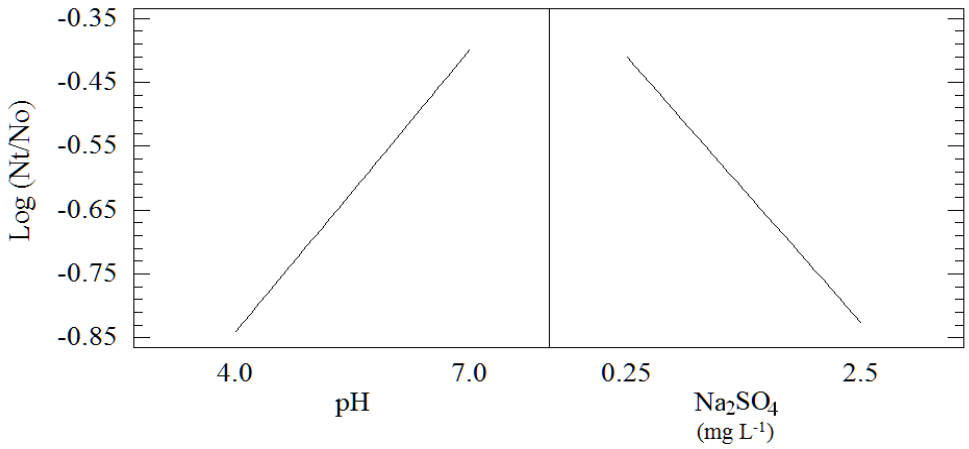

Figure 2. Primary-effects plot for *E.coli* removal through electrocoagulation

The Pareto chart (Figure 3) was used to draw conclusions on the most significant variables and variable interactions. This chart shows both the magnitude and importance of the effects (variables and interactions). The chart comprises a reference line (discontinuous vertical plot), and any effect that extends past this line is potentially important. An interaction between the initial pH and Na_2SO_4 (AB) as well as initial pH (A) and Na_2SO_4 (B) concentration is considered important in *E.coli* removal.

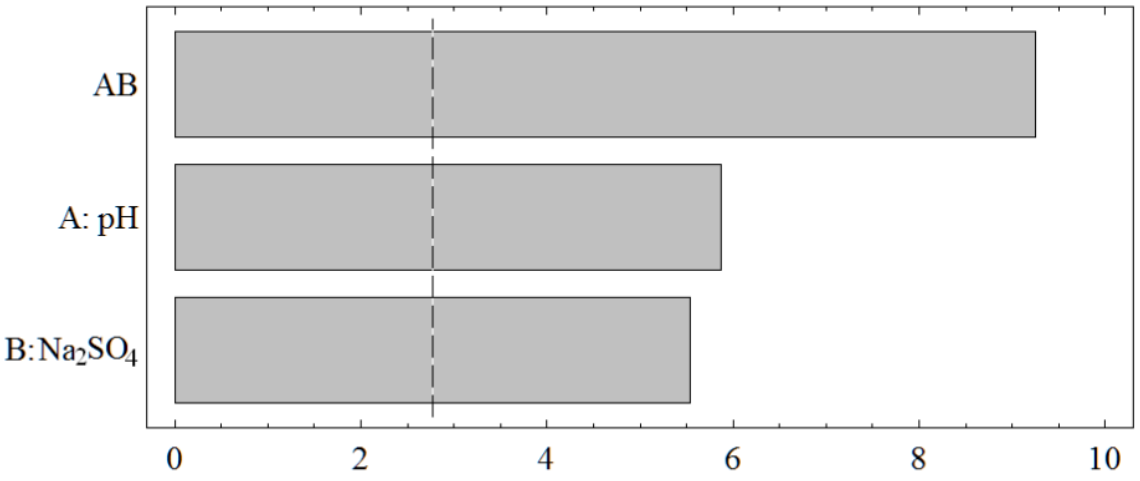

Figure 3. Pareto chart for *E.coli* removal through electrocoagulation

The experimental design used herein included a reduced model that directly relates the response variable with the factors to facilitate subsequent evaluation of the data.

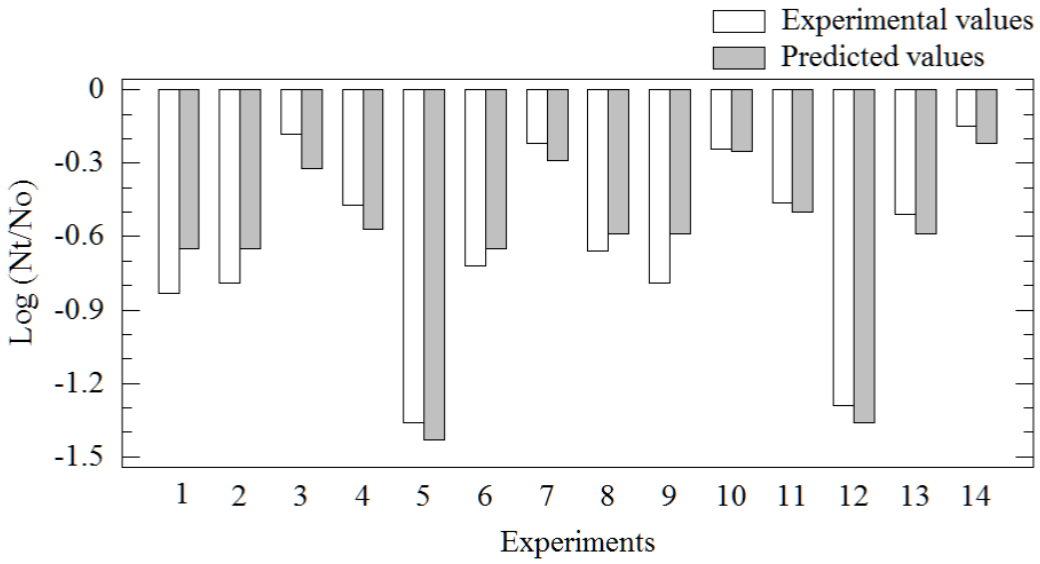

Figure 4. Comparison of the experimental and predicted values for *E.coli* removal through electrocoagulation using the empirical reduced model

The reduced empirical model for *E.coli* removal through electrocoagulation is shown in Equation 1. The R^2 value for Equation 1 is 0.9153, which indicates that 91.53% of the total variation in *E.coli* removal is attributed to the factors studied.

$$\text{Log } (N_t/N_o) = 0.388 - 0.137[\text{pH}] - 1.322\ [Na_2SO_4] + 0.207[\text{pH}][Na_2SO_4] \quad (1)$$

Figure 4 shows a comparison between the experimental values generated from each experiment (Table 2) and the predicted values for *E.coli* removal, which were calculated using Equation 1. The predicted values are consistent with the experimental data.

3.2 E.coli removal through electrocoagulation under optimal conditions

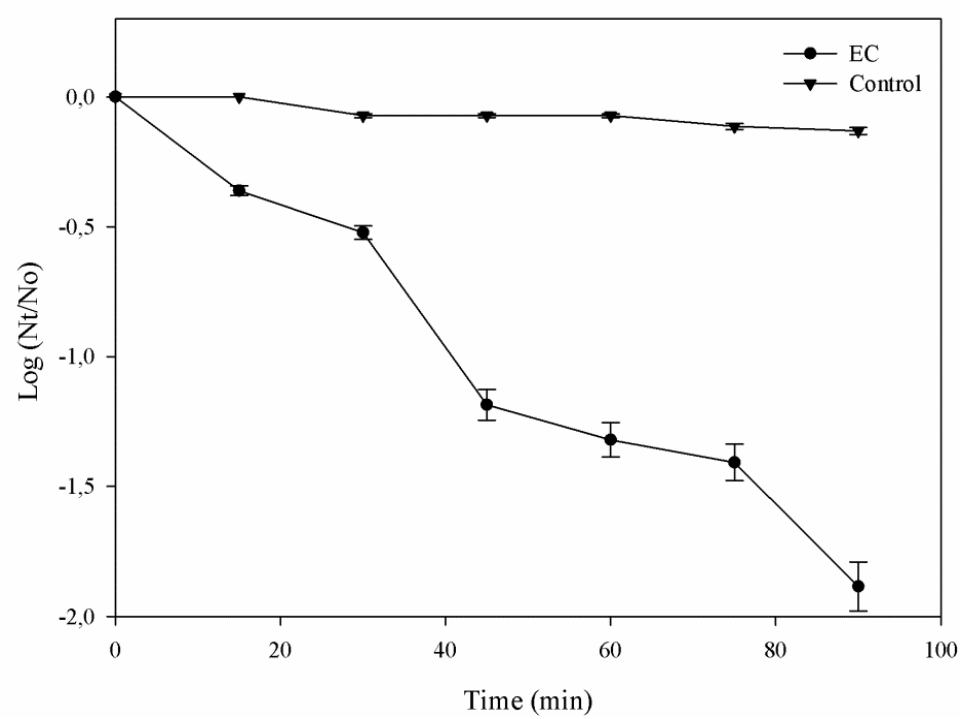

Figure 5. Variation of *E.coli* removal through electrocoagulation

In a final experiment, the removal of *E.coli* through electrocoagulation was conducted under optimal conditions (2.5 mg L^{-1} of Na_2SO_4 and initial pH 4.0) in distilled water. As seen in Figure 5, the electrocoagulation with aluminum electrodes is able to remove 1-log after 40 min and 1.9-log in a contact time of 90 min, similar results had been reported previously [14, 19]. The water without electrolysis (control) did not show significant effect on the growth of *E.coli*, indicating that removal of the microorganism is due electrocoagulation, this is attributed to the adsorption of *E.coli* in the flocs formed, which float to the surface or settle to the bottom of the reactor [12, 14, 22], as seen in the Figure 6. The microorganism can survive in the flocs, therefore is important to complement this technique for a complete elimination.

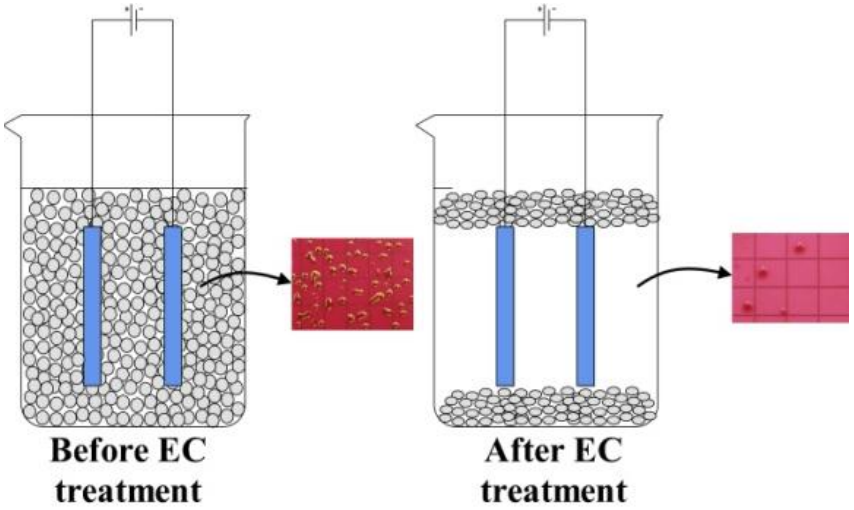

Figure 6. Schematic representation for *E.coli* removal through electrocoagulation

4. CONCLUSIONS

The experimental design, it allowed to observe the influence of the factors initial pH and electrolyte concentration support on the removal of the bacteria *E.coli* using electrocoagulation. The results indicated that the factors initial pH and electrolyte support (Na_2SO_4) concentration as well as the interaction between these factors affected microorganism removal, which improved with a decrease in initial pH and an increase in Na_2SO_4 concentration. An empirical model was established that describes *E.coli* removal using electrocoagulation. The removal of *E.coli* is attributed to the physical effect promoted by electrocoagulation.

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