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Experimental Design Applied to the Inactivation of *Escherichia coli* in Water through Electrooxidation with Ti/IrO$_2$ Anodes

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Abstract

An electrooxidation process using Ti/IrO$_2$ anodes was studied for the inactivation of *Escherichia coli* (*E.coli*) through an experimental design. This statistical methodology was used to evaluate the influence of electrooxidation parameters electrolyte support (NaCl and NaHCO$_3$) and current density, on *E.coli* inactivation. The electrolyte support significantly influenced the response variable, in contrast to the current density that showed no significant effect. Microorganism inactivation was more efficient with an increase in NaCl concentration and a decrease in NaHCO$_3$ concentration. The experimental design allowed to determine the optimum values for the electrooxidation under the conditions studied, obtaining a high NaCl concentration (0.45 mol/L) and low NaHCO$_3$ concentration (0.35 mol/L), in combination with low current intensity (6 mA/cm^2), for an *E.coli* inactivation. The predicted values of the empirical model were consistent with the experimental values.

Keywords: Factorial design, electrodisinfection, disinfection

1. Introduction

Water-borne diseases are among the leading causes of deaths in developing countries, affecting mainly children [8]. The common strategy to eliminate these pathogenic microorganisms in water is through chlorination; however they are currently evaluating other alternatives due to the potential formation of disinfection byproducts such as trihalomethanes (THMs) [7, 21]

Electrooxidation is an electrochemical technique which allows the degradation of different pollutants through direct or indirect oxidation, using electrodes as graphite, Pt, Ti, DSA® and BDD, several investigations have shown that the electrooxidation is a promising alternative for the removal coliforms microorganism in aqueous medium [3, 5, 6, 19].

An adequate form to evaluate this type of physicochemical and operative parameters in an electrochemical process is with an experimental factorial design. This statistical methodology facilitates the evaluation of two or more relevant factors and its impact on an important response variable for a given process, generating a minimum number of experiments without sacrificing the reliability of the results. The final outcome of the experimental design is to obtain an empirical regression model of the evaluated process [11, 12]. The application of this statistical tool brings advantages like rapid experimentation, a simpler evaluation of the interaction between the factors, determination of optimal conditions and therefore process improvements. Several authors have studied the application of experimental design for the degradation of different contaminants in water including microorganisms [4, 17]. However, to date there have not been reported studies in experimental design for the elimination of microorganisms by electrooxidation. Therefore, the main objective of this study was to evaluate the influence of electrooxidation parameters, such as electrolyte support concentration and current density, on *E.coli* inactivation using a factorial experimental design.

2. Methodology

2.1 Reagents

Sodium bicarbonate (Merck) and sodium chloride (Honeywell), were used as supporting electrolytes. The microbiological reagents, peptone water, and endo agar were purchased from Oxoid. Distilled water was used to prepare aqueous solutions.

2.2 Electrochemical experiments

Electrochemical tests were performed in a 200 mL batch reactor equipped with Ti/IrO_2 anode, with an effective area of 6.25 cm^2, and 1 cm interelectrodic space. The electrodes were connected to a direct current power supply (MCP Lab electronics), operated at different current densities (Table 1). The system was continuously stirred at 200 rpm.

2.3 *E.coli* preparation and quantitation

A suspension with native *E.coli* in peptone water was prepared and incubated at 37 °C until the sample was at 10^5-10^6 CFU/mL, according to McFarland standard. The samples were analyzed using the filtration membrane technique in accordance with standard methods [2]. Sterile cellulose membranes (Advantec MFS) with a pore size of 0.45 µm 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 (2k) with two replicate center points was used; the factors considered for this study were initial electrolyte support concentration (NaCl and $NaHCO_3$) and current density. The levels for the factors studied are shown in Table 1. The response variable was *E.coli* inactivation, 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 assays were performed in a random order in triplicate, and the data were analyzed using the software Statgraphics®plus, using a 95% confidence level.

Table 1. Factors and levels used in the experiments.

Variables	Low Level, -1	Center points, 0	High Level, +1
Electrolyte support (mol/L)	0.35	0.4	0.45
Current density - j (mA/cm^2)	16	32	48

3. Results and discussion

Table 2 shows the design matrix generated using the software Statgraphics®plus, it includes factors, levels and the results of the response variable for each experiment, which is defined as *E.coli* inactivation after electrochemical treatment for 5 min. The *E.coli* inactivation was superior to 3.5-log when $NaHCO_3$ was used and 4.2-log when NaCl was used, this behavior can be explained by the formation of oxidant species with disinfectant potential [10], like active chlorinated species formed due to the catalytic activity of IrO_2 anode in presence of chloride [14, 15], or the formation of oxidants such as percarbonate as a result of the indirect reduction of carbonate ions [1, 13].

Table 2. Experimental design applied to *E.coli* removal through electrooxidation.

Assays	Electrolyte support (mol/L)	Current density (mA/cm^2)	$NaHCO_3$ Log (N_t/N_o)	NaCl Log (N_t/N_o)
1	0.35	48	-3.853	-4.243
2	0.4	32	-3.756	-4.720
3	0.4	32	-3.677	-4.720
4	0.45	48	-3.677	-4.720
5	0.35	16	-3.853	-4.243
6	0.45	16	-3.677	-4.720
7	0.35	48	-3.978	-4.419
8	0.4	32	-3.756	-4.720
9	0.4	32	-3.501	-4.419
10	0.45	48	-3.610	-4.720
11	0.35	16	-3.853	-4.118
12	0.45	16	-3.756	-4.720
13	0.35	48	-3.853	-4.419
14	0.4	32	-3.677	-4.720
15	0.4	32	-3.610	-4.720
16	0.45	48	-3.756	-4.720
17	0.35	16	-3.978	-4.243
18	0.45	16	-3.756	-4.720

3.1 Effect of current density and type and concentration of electrolyte support

The primary-effects plot shows the influence of electrolyte support and current density on the log-inactivation of *E.coli*. As can be evidenced in Figure 1, the corresponding lines to the current intensity for the NaCl and $NaHCO_3$ solution, did not show a steep slope, therefore the current density was not a significant factor in the inactivation of *E.coli*. The electrolyte support concentration has an important effect on the microorganism inactivation depending of the electrolyte employed. It can be observed that the increase of NaCl concentration improves the inactivation of *E.coli*; on the contrary an increase in $NaHCO_3$ concentration decreases the inactivation of the microorganism. The effect of NaCl can be explained by a rise in the generation of active chlorinated species with bactericide effect [18, 20], according to the equations [14]:

$2Cl^- \rightarrow Cl_2 + 2e^-$ (1)

$Cl_2 + H_2O \rightarrow HOCl + H^+ + Cl^-$ (2)

$HOCl \rightarrow H^+ + OCl^-$ (3)

While the opposite effect of $NaHCO_3$ could be related to the adsorption of bicarbonate on the electrode surface [9, 16].

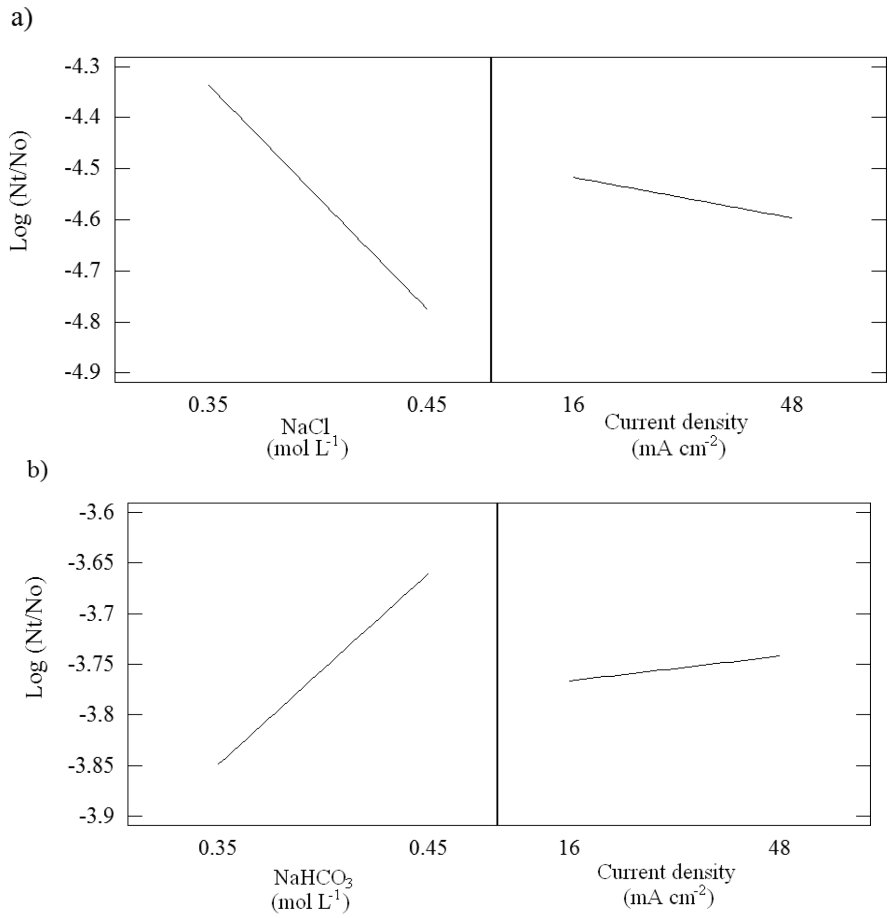

Figure 1. Primary-effects plot for *E.coli* inactivation through electrooxidation. a) Effect of NaCl, b) Effect of $NaHCO_3$

Figure 2 represents the Pareto chart, this is used to evaluate the most significant factors and their interactions, showing the magnitude and importance of the effects on the response variable. The chart comprises a reference line, and any factor that extends past this line is potentially important. As can be evidenced only the factor corresponding to electrolyte support exceed the dotted line, therefore the NaCl and $NaHCO_3$ concentration presents a significant effect on the response variable, as had been observed previously in Figure 1. The current density (B) and the interaction between the electrolyte concentration and current density (AB) were not significant on the *E.coli* inactivation with IrO_2 anodes.

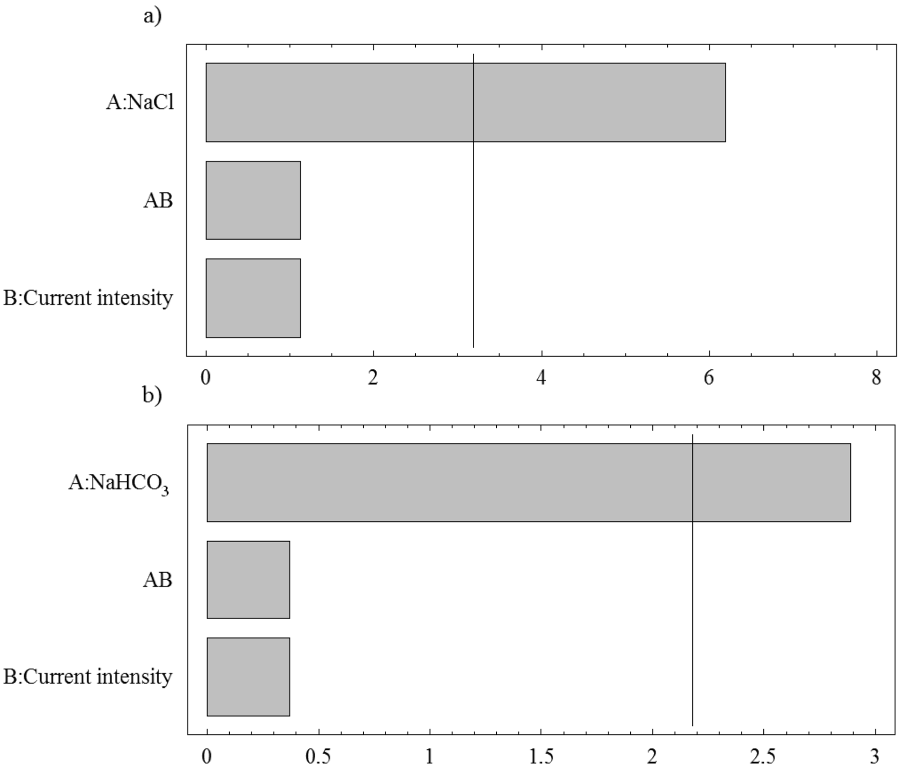

Figure 2. Pareto chart for *E.coli* inactivation through electrooxidation. a) Effect of NaCl, b) Effect of $NaHCO_3$.

3.2 Reduced empirical model, comparison with the experimental results and determination of the most suitable conditions to *E.coli* inactivation

The experimental design used herein lead to establish a reduced model that directly relates the response variable with the factors to facilitate subsequent evaluation of the data. The reduced empirical model for *E.coli* log-inactivation through electrooxidation with IrO_2 anodes is shown in Equation (4) for NaCl and (5) for $NaHCO_3$. To determine the significance of the model was used the lack of fit test, the result indicate that p-value was greater than 0.05, therefore the models appear to be suitable for observed data (95.0% confidence level).

$$\text{Log}\,(N_t/N_o) = -\,2.800 - 4.392\,[NaCl] \qquad (4)$$
$$\text{Log}\,(N_t/N_o) = -\,4.512 - 1.893\,[NaHCO_3] \qquad (5)$$

A comparison between the *E.coli* log-inactivation, predicted by Equation (4) and (5), and the values generated from each experiment (Table 2) is presented in Figure 3. The observed results indicated a very good agreement with the experimental data.

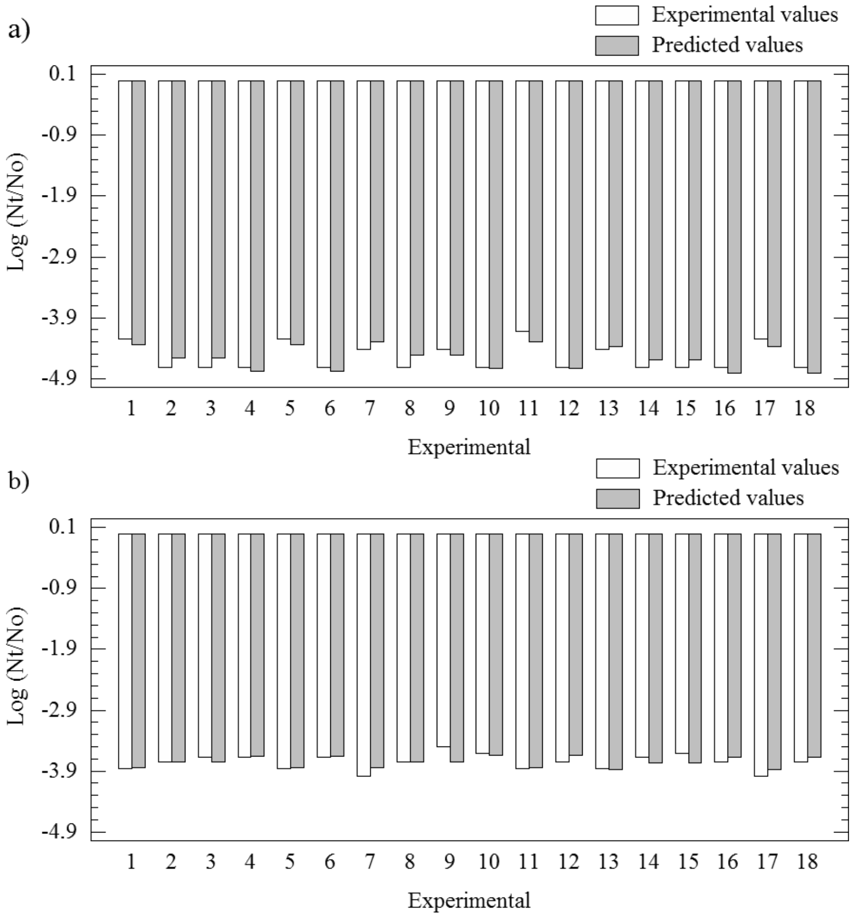

Figure 3. Comparison of the experimental and predicted values for *E.coli* inactivation through electrooxidation using the empirical reduced model. a) Effect of NaCl, b) Effect of $NaHCO_3$

Figure 4 shows the response surface plot used to determine the optimal conditions to reach an adequate *E.coli* inactivation. The best results for inactivation were obtained at high NaCl concentration (0.45 mol/L) and low $NaHCO_3$ (0.35 mol/L). As can be also observed in Figure 4 and as stated in the precedent sections, under the range of conditions evaluated, the effect of current density is negligible. Therefore, under the work conditions 16 mA/cm^2 is the most suitable current density to *E.coli* inactivation in order to keep the lowest energy consumption.

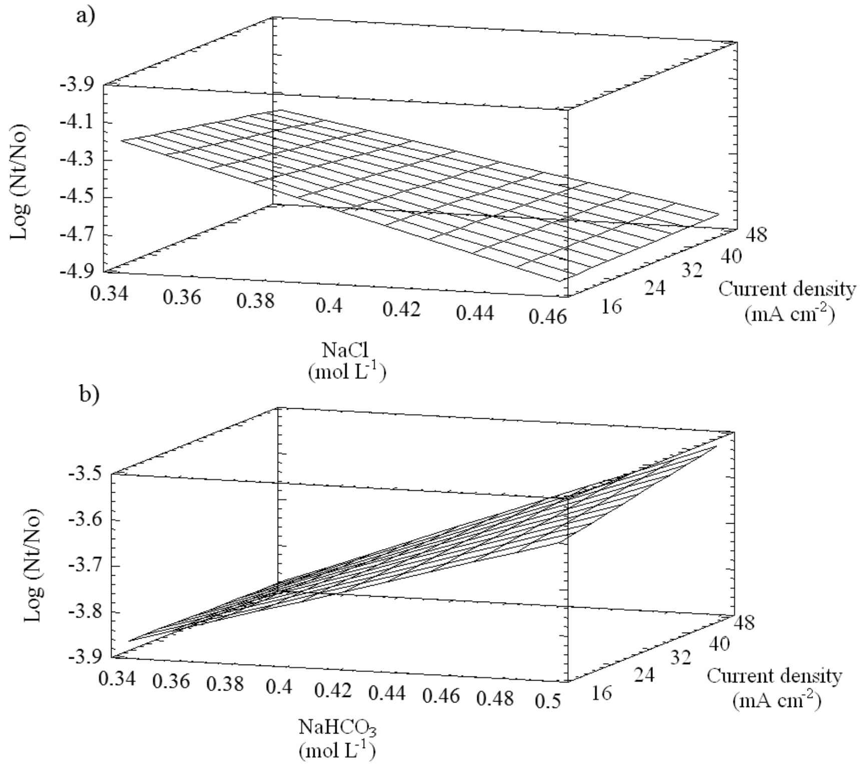

Figure 4. Response surface plots for the *E.coli* inactivation through electrooxidation as a function of electrolyte support concentration and current density. a) Effect of NaCl, b) Effect of $NaHCO_3$.

4. Conclusion

The application of a factorial experimental design allowed to observe the influence of electrolyte support concentration (NaCl and $NaHCO_3$) and current density on *E.coli* inactivation through electrooxidation with Ti/IrO_2 anodes. It was possible to reach adequate values of inactivation employing NaCl and $NaHCO_3$, however NaCl presented better inactivation efficiency, probably due to increase in the electrogeneration of chlorinated species with bactericide effect. Results indicated that the electrolyte support concentration (NaCl and $NaHCO_3$) affected the microorganism inactivation, which improved with a decrease in $NaHCO_3$ concentration an increase in NaCl concentration. An empirical model was established that describes *E.coli* inactivation using electrooxidation with Ti/IrO_2 anodes.

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