Degradation of Chloramphenicol in Synthetic and Aquaculture Wastewater Using Electrooxidation

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Abstract

Chloramphenicol (CAP) is a broad-spectrum antibiotic widely used in animal farming and aquaculture industries. Despite its ban in many countries around the world, it is still used in several developing countries, with harmful effects on the surrounding aquatic environment. In this study, an electrooxidation process using a Ti/PbO_2 anode was used to investigate the degradation of CAP in both synthetic solution and real aquaculture wastewater. A central composite design was used to determine the optimum conditions for CAP removal. Current intensity and treatment time had the most impact on the CAP removal. These two factors accounted for ~90% of CAP removal. The optimum conditions found in this study were current intensity of 0.65 A, treatment time of 34 min, and CAP initial concentration of 0.5 mg L^{-1}. Under these conditions, 98.7% of CAP removal was achieved with an energy consumption of 4.65 kW h^{-1} m^{-3}. The antibiotic was not present in the aquaculture wastewater, which received 0.5 mg L^{-1} of CAP and was treated (by electrooxidation) under the optimum conditions. A complete removal of CAP was obtained after 34 min of treatment. According to these results, electrooxidation presents an option for the removal of antibiotics, secondary compounds, and other organic and inorganic compounds from solution.

Core Ideas

- Antibiotics cause microbial resistance.
- Electrooxidation removes antibiotics.

S INCE THE 1950S, aquacultural production has grown steadily around the world. World aquaculture production reached 66.6 Tg in 2012, an increase of 33.5% since 2007 (FAO, 2002). The same study predicted a 40.5% rise in production by 2030. A significant amount of antibiotics is used to maintain this high production. The main purpose of these antibiotics is to prevent and treat infections, as well as to promote animal growth. However, overuse of antibiotics could lead to severe human health and environmental issues. Since these compounds are not totally metabolized, their residues can be found in animal tissues intended for human consumption, and a significant amount of antibiotics enter the surrounding aquatic environment in aquacultural effluent (Lu et al., 2009). Several studies have sounded the alarm regarding antibiotic resistance in pathogenic bacteria caused by the widespread use of antibiotics in aquaculture (McPhearson et al., 1991; Miranda and Castillo, 1998; Huys et al., 2000; Schmidt et al., 2000; Cabello, 2006; WHO, 2014).

The antibiotic chloramphenicol (CAP), which is obtained naturally from *Streptomyces venezuelae* Ehrlich or produced synthetically, has a solubility of 2.5 g L^{-1} and a pKa of 9.5 (Huang et al., 2006; Tan et al., 2017). It has been widely used in aquaculture and animal-farming industries because of its broad-spectrum antimicrobial activity (Lu et al., 2009). Exposure to CAP can trigger severe allergic reactions, especially aplastic anemia (Zhang et al., 2013; Tan et al., 2017; US National Library of Medicine, 2017). Its use is now prohibited in many countries because of its adverse effects on human health (Nicolich et al., 2006). Despite this restriction, CAP is still used in aquaculture in some developing countries because of its low cost (Lu et al., 2009). Hence, harmful concentrations of this antibiotic have been reported in aquacultural effluent, the nearby aquatic environment, and aquatic animal tissues (Collette, 2006; Hassan et al., 2013; Lu et al., 2009). Any presence of residual CAP in aquatic-animal tissue points to the use of this product in a farming pond. Chen et al. (2015) found concentrations of CAP up to 28.4 ng L^{-1} in urban water supplies in Shanghai, China, and Molina-Avila (2015) found 0.103 mg L^{-1} in aquaculture wastewater effluent in the south of the Sonora, Mexico. Mitchell et

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Abbreviations: CAP, chloramphenicol; RNO, *p*-nitrosodimethylaniline.

(2015) found that hydrolysis of CAP under ambient conditions did not deteriorate its structure.

Some studies report high removal rates of CAP and other drugs using electrochemical methods. For example, Chen et al. (2015) eliminated 87.3% of CAP after 2.5 h using a current intensity of 30 mA cm^{-2} and an Al anode doped with PbO_2. The electrooxidation consists of generating, by electrochemical means, very powerful oxidizing agents such as the OH$^-$ radical), which can interact and degrade organic pollutants. The main advantages of this process are the absence of sludge production, easy automation, and the possibility of degrading the organic pollutants until the final step of mineralization (Moreira et al., 2017; Dominguez et al., 2018; Garcia-Segura et al., 2018). This approach can help to eliminate residual antibiotics in the aquacultural effluent to decrease bacterial resistance and effects on the environment and to restore water quality sufficiently high for nonpotable purposes.

The main objective of this study was to investigate the efficiency of electrooxidation in the removal of CAP using a Ti electrode coated with PbO_2 (Ti/PbO_2). A response surface methodology had been used to determine the optimum conditions of CAP degradation in synthetic wastewater. Thereafter, these conditions were applied to treat actual aquacultural wastewater. In addition, determination of total oxidants, the kinetics of degradation, and the contribution of direct and indirect oxidation to CAP removal were investigated.

Materials and Methods

Electrooxidation Reactor

We conducted electrooxidation experiments using a 600-mL Plexiglass parallel-piped batch reactor agitated by a magnetic stirrer (Fig. 1) The working volume was 550 mL, and the inter-electrode distance was 1 cm. Current was applied using a Xantrex XFR40-70 power supply (Xantrex Technology). The rectangular anode and cathode materials were made of PbO_2 and Ti (Baoji Qixin Titanium Company), and they both measured 11 cm long by 10 cm wide. The electrodes in the form of expanded metals had a solid active area of 366 cm^2 for the anode and 320 cm^2 for the cathode. We used Na_2SO_4 or NaCl as electrolyte salt supports during the synthetic solution experiments. Experiments were conducted at room temperature (22 ± 2°C).

Synthetic Solution and Aquacultural Effluent

The synthetic solution was made by adding 155 mg of CAP (97% purity from Fisher Bioreagents) to 1000 mL of distilled water (150 mg L^{-1}) and agitating with a stirring bar for at least 1 h to ensure complete dissolving. This stock solution was stored at 4°C and used to make dilutions for the experiments. Depending on the experiment, Na_2SO_4 (100% purity, Fisher Scientific), NaCl (100% purity, Fisher Scientific), or $NaNO_3$ (99.8% purity, Fisher Scientific) were added to the working solution as an electrolyte salt support.

Actual aquacultural wastewater was collected in the general effluent from an aquaculture farm north of Obregon city in Sonora State, Mexico. The CAP concentration in the effluent was measured prior to treatment, and it was not detected. Hence, the effluent was later spiked with 0.5 mg CAP L^{-1}, and optimal conditions were applied.

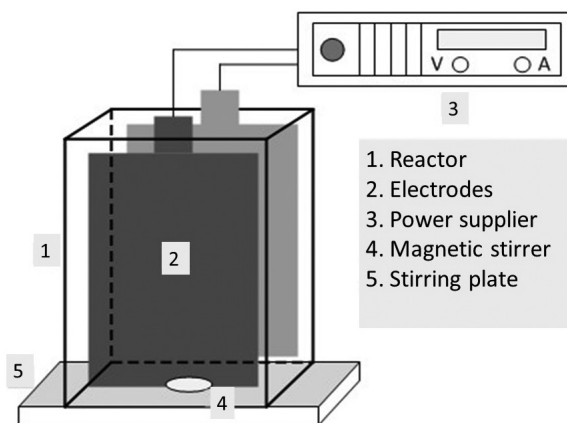Fig. 1. Diagram of the electrooxidation experimental system.

1. Reactor
2. Electrodes
3. Power supplier
4. Magnetic stirrer
5. Stirring plate

Experimental Design

Degradation of CAP in the synthetic solution was performed using a response surface methodology. Treatment time (X_1), initial CAP concentration (X_2), and current intensity (X_3) were the three independent variables in the study. The domains were: X_1, 20 to 40 min (the central value [$U_{i,0}$] = 30 min); X_2, 0.5 to 1 mg L^{-1} ($U_{i,0}$ = 0.75 mg L^{-1}); and X_3, 0.5 to 1.0 A ($U_{i,0}$ = 0.75 A). Two levels were assigned to each factor (a 2^3 plan), leading to 20 experiments each comprising eight runs for the factorial design and 12 runs for the central composite design (including six replicates at the center point and six runs for the extreme high and extreme low). Chloramphenicol removal (%) and energy consumption were the two investigated responses. Design Expert 7.0.0 (StatEase, 2007) was used to generate the quadratic polynomial model.

Analytical Details

The formation of hydroxyl radicals was assessed using the *p*-nitrosodimethylaniline (RNO) bleaching method employed by Daghrir et al. (2013). *p*-Nitrosodimethylaniline (97% purity, Sigma Aldrich) is an organic dyestuff that can be bleached out by some oxidants, such as OH$^-$ radicals, O_3, HClO, ClO$^-$, and others, by chemical oxidation (Daghrir et al., 2013). However, given the electrodes' nature (Ti/PbO_2 and Ti) and the electrolyte (Na_2SO_4) used in this study, production of these oxidants was expected, except for OH$^-$ radicals. Hence, RNO bleaching can be mainly associated with OH$^-$ radical production. The degradation of RNO (initial concentration: 4.8×10^{-5} M) was followed at regular intervals using an ultraviolet-visible spectrophotometer (Cary 50, Varian Canada) at 440 nm, corresponding to the optimum wavelength for RNO absorbance (Kraljić and Mohsni, 1978). The experiments were performed in buffer solution (pH 7) using Na_2HPO_4 and KH_2PO_4 (99% purity, Fisher Scientifics) at 9.45 and 9.06 g L^{-1}, respectively.

The concentration of indirect oxidants was determined using the Wessler method adapted from Zaviska et al. (2011). An excess of 0.5 g of KI was added to a 25-mL of sample, and the I_2 formed from the oxidation of I$^-$ ions was titrated back with a 0.1 M $Na_2S_2O_3$ solution. A starch solution was used as a color indicator to detect the endpoint of the titration. During this experiment, no organic material was added in the electrooxidation reactor. The intermediary oxidants accumulated in the reactor for later analysis, whereas the OH$^-$ radicals disappeared given their short life span.

The CAP measurement was performed by liquid chromatography tandem mass spectrometry (Thermo TSQ Quantum Access). The liquid chromatography separation was achieved in a HyperSil Gold C18 100-mm × 2.1-mm column (Thermo Scientific) heated to 35°C. Two mobile phases were used for the gradient elution process: Phase A (water–0.1% acetic acid–5 mM ammonium acetate) and Phase B (acetonitrile–0.1% acetic acid–5 mM ammonium acetate) at a flow rate of 0.2 mL min⁻¹ for 15 min, then 0.4 mL min⁻¹ for 5 min to equilibrate the column. The liquid chromatography was associated with a TSQ Quantum Access mass spectrometer equipped with an electrospray ionization source and operated in negative ionization mode. Nitrogen was used as a sheath and auxiliary gas. The spray voltage was 4000 V and capillary temperature was 350°C. The CAP detection limit was 1 μg L⁻¹.

Chemical oxygen demand was analyzed using the colorimetric method (APHA, 1998). The NO_x (NO_3^- and NO_2^-), NH_4^+, and PO_4^{3-} were determined by using the standard methods (APHA, 1999). The pH was measured using a pH meter (HI 2550, HANNA Instruments).

Results and Discussion

Formation of Oxidant Agents during Electrolysis

Direct and indirect oxidation are the two main ways of pollutant degradation in the electrooxidation cell. Direct oxidation, which occurs at the electrode surface, is generally attributed to the hydroxyl radicals generated by water oxidation and adsorbed onto the surface (Comninellis, 1994). In contrast, indirect oxidation occurs in the bulk solution. Certain ion species such as halides, SO_4^{2-}, CO_3^{2-}, dissolved O_2, etc., can be electrolyzed and generate some oxidants in solution (Drogui et al., 2007). The corresponding electrogenerated species are also called intermediate oxidants. Before investigating CAP degradation, we considered it important to characterize the electrooxidation reactor in terms of oxidant production.

Formation of Hydroxyl Radicals

These oxidants are mainly constituted by the OH^- radicals generated and accumulated on the anodic surface. The RNO bleaching method was used to quantify their production rate.

The RNO concentration decreased sharply in the first 20 min and kept decreasing slightly until the end of the experiment. The removal of RNO in the electrooxidation cell followed a first-order kinetic reaction with a rate constant (k) of 0.06 min⁻¹. The disappearance rate of RNO, corresponding to the production rate of OH^- radicals, was calculated at 4.8×10^{-3} mM min⁻¹, with an initial concentration of 45 mg RNO L⁻¹ and 60 min of reaction time. By increasing the initial concentration from 45 to 300 mg L⁻¹, the reaction rate became 3.2×10^{-2} mM min⁻¹ (data not shown). However, the reaction rate constant stayed the same (0.06 min⁻¹). This result is consistent with the finding of García-Gómez et al. (2014), who used a PbO_2 anode to determine OH^- radicals with the RNO bleaching method. In their study, the reaction kinetic rate was 0.07 min⁻¹.

Production of Other Oxidants

The thiosulfate/iodine titration method was used to assess the production of other oxidants (such as HClO, $H_2S_2O_8$, H_2O_2, etc.) that can be generated during electrolysis. We used NaCl and Na_2SO_4 as supporting electolytes. The two electrogenerated oxidants produced from these salts are HClO and $H_2S_2O_8$, respectively:

$$Cl^- + 2H_2O \rightarrow HClO + H_3O^+ + 2e^- \qquad [1]$$

$$2SO_4^{2-} + 2H^+ \rightarrow H_2S_2O_8 + 2e^- \qquad [2]$$
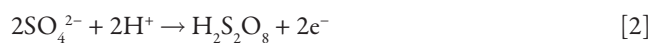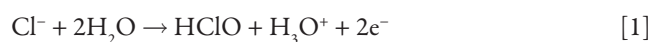
The production of oxidants using NaCl was much greater than that with Na_2SO_4. At the same initial concentration (1 g L⁻¹) and current intensity (1 A), the maximum production of HClO was 2.1 mM, whereas only 0.16 mM of $H_2S_2O_8$ was recorded. This is probably due to the generation of a Cl^- ion, which is a highly oxidizing agent, and the HClO produced, as illustrated by Eq. [1] (Tran and Drogui, 2013: Moreira et al., 2017). The concentration of the oxidants increased with higher concentration of the corresponding salt. For NaCl, after maximum production of oxidants at 90 min of electrolysis, a decline followed, possibly explained by the reaction between HClO and some reactive O_2 species such as H_2O_2 (Eq. [3]) (Tran and Drogui, 2013). In fact, OH^- radicals formed from the water oxidation can react among themselves to form H_2O_2, following Eq. [4] (Michaud et al., 2003):

$$HClO + H_2O_2 \rightarrow HCl + O_2 + H_2O \qquad [3]$$

$$2OH^- \rightarrow H_2O_2 \qquad [4]$$
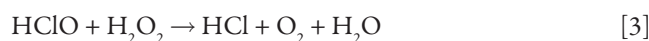
This result was consistent with a study by Awad and Galwa (2005), who found that ClO^- ions are preferentially produced over persulfate ions on the lead dioxide anode. However, the use of Cl^- is generally associated with a potential formation of organochlorine compounds, which are suspected to be carcinogenic. For this reason, Na_2SO_4 was selected as the electrolyte support to study the degradation of CAP in the synthetic solution.

Degradation of Chloramphenicol in Synthetic Solution

The degradation of CAP in the synthetic solution was evaluated using a central composite design. The central composite matrix allows exploration of the whole experimental domain and makes it possible to determine the coefficients of a mathematical second-order polynomial equation. There are three quantitative variables: treatment time (U_1, min), CAP concentration (U_2, mg L⁻¹), and current intensity (U_3, A). Chloramphenicol removal (Y_1, %) and energy consumption (Y_2, kW h⁻¹ m⁻³) were the dependent variables (responses). For the three variables, 20 experiments (Table 1) were performed. These tests comprised six experiments performed at the center of the experimental domain (central point), eight experiments corresponding to the factorial design (2^3), and six other experiments performed around the experimental domain (low and high extremities). Chloramphenicol removal (R) and energy consumption (E) were calculated using Eq. [5 and 6], respectively:

$$R\ (\%) = [(C_0 - C_f)/C_0]100 \qquad [5]$$

$$E = (IUt/V)10^{-3} \qquad [6]$$

where C_0 is the initial concentration of CAP (mg L⁻¹), C_f is the final concentration CAP (mg L⁻¹), I is the current intensity (A), U is the electrical potential (V), t is the treatment time (h), and V is the treated water volume (m³).

Table 1. Factorial and central composite experimental matrix in the 2³ design

Run	Experiment design			Experiment plan			Actual removal efficiency (Y₁ᵃ)	Predicted removal efficiency(Y₁ᵇ)	Relative deviation (Y₁ᵃ – Y₁ᵇ)	Energy consumption (Y₂)	Energy efficiency
	X_1	X_2	X_3	U_1	U_2	U_3					
				min	mg L⁻¹	A	———— % ————			kW h⁻¹ m⁻³	kW h⁻¹ g⁻¹
							Factorial design				
1	−1.0	−1.0	−1.0	20.0	0.50	0.50	59.32	59.57	−0.25	1.87	6.30
2	−1.0	−1.0	+1.0	20.0	0.50	1.00	83.05	81.87	1.18	5.11	12.30
3	−1.0	+1.0	−1.0	20.0	1.00	0.50	54.61	53.60	1.01	1.80	3.29
4	−1.0	+1.0	+1.0	20.0	1.00	1.00	74.61	80.86	−6.25	5.55	7.43
5	+1.0	−1.0	−1.0	40.0	0.50	0.50	91.69	89.12	2.57	3.71	8.09
6	+1.0	−1.0	+1.0	40.0	0.50	1.00	99.28	103.97	−4.69	9.98	20.25
7	+1.0	+1.0	−1.0	40.0	1.00	0.50	75.38	80.23	−4.85	3.60	4.77
8	+1.0	+1.0	+1.0	40.0	1.00	1.00	96.61	100.04	−3.43	10.68	11.05
							Central composite matrix				
9	0.0	0.0	0.0	30.0	0.75	0.75	98.25	96.71	1.54	5.13	6.96
10	0.0	0.0	0.0	30.0	0.75	0.75	88.78	96.71	−7.93	4.92	7.38
11	0.0	0.0	0.0	30.0	0.75	0.75	98.25	96.71	1.54	5.25	7.12
12	0.0	0.0	0.0	30.0	0.75	0.75	96.41	96.71	−0.30	5.03	6.95
13	0.0	0.0	0.0	30.0	0.75	0.75	99.81	96.71	3.10	5.31	7.09
14	0.0	0.0	0.0	30.0	0.75	0.75	97.87	96.71	1.16	5.06	6.89
15	0.0	−1.3	0.0	30.0	0.33	0.75	99.99	101.07	−1.07	5.13	15.70
16	0.0	+1.3	0.0	30.0	1.17	0.75	99.00	92.74	6.26	5.26	4.54
17	0.0	0.0	−1.3	30.0	0.75	0.33	55.55	56.42	−0.87	1.37	3.28
18	0.0	0.0	+1.3	30.0	0.75	1.17	97.90	91.83	6.07	10.81	14.72
19	−1.68	0.0	0.0	13.1	0.75	0.75	55.40	54.61	0.79	2.25	5.41
20	+1.68	0.0	0.0	46.8	0.75	0.75	99.99	95.59	4.41	7.81	10.51

The following second-order equation gives the predicted response in the all experimental field (Eq. [7]) (García-Gómez et al., 2014):

$$Y = b_o + \sum_{i=1}^{k} b_i X_i + \sum_{i=1}^{k} b_{ii} X_i^2 + \sum_{j}\sum_{i=2}^{k} b_{ij} X_i X_j \quad [7]$$

where Y is the experimental response; b_o is the average of experimental response; coefficients b_i, b_{ii}, and b_{ij} are, respectively, the linear, quadratic, and interaction effects between the factors X_i and X_j for the response Y, where $i = 1$ to 3, $j = 1$ to 3, and $i \neq j$.

After performing the experiments, the results in terms of actual CAP removal (Y_1^a) and energy consumption (Y_2) are presented in the Table 1. The corresponding second-order polynomial equations models are given by Eq. [8 and 9] for CAP removal and energy consumption respectively:

$$Y_1 = 96.71 + 12.18 X_1 - 2.48 X_2 + 10.53 X_3$$
$$- 0.73 X_1 X_2 - 1.86 X_1 X_3 + 1.24 X_2 X_3 \quad [8]$$
$$- 7.64 X_1^2 + 0.068 X_2^2 - 7.98 X_3^2$$

$$Y_2 = 5.12 + 1.68 X_1 + 0.086 X_2 + 2.65 X_3$$
$$+ 0.028 X_1 X_2 + 0.8 X_1 X_3 + 0.17 X_2 X_3 \quad [9]$$
$$- 0.067 X_1^2 - 8.252 \times 10^{-3} X_2^2 + 0.31 X_3^2$$

The coefficients of the equation models were calculated using the half difference between the arithmetic average of the values of the response when the variable is coded at the levels −1 and +1 (García-Gómez et al., 2014; Carabin et al., 2016). These coefficients could be positive or negative depending on the way they affect the investigated response. Hence, a positive coefficient reflects a positive effect on the response, whereas a negative coefficient reflects a negative effect on the response.

The coefficient $b_o = 96.71$ represents the average CAP removal obtained from all the experiments. The coefficient $b_1 = +12.18$, corresponding to the operation time (X_1), indicates that CAP removal increased on average by 24.36% (2×12.18) when the electrolysis time was 20 to 40 min; likewise, it is the most influential variable for CAP removal. The second most influential variable is current intensity (X_3). According to its coefficient ($b_3 = +10.53$), CAP removal increased on average by 21.06% (2×10.53) when the current intensity increased from 0.5 to 1 A. With respect to CAP concentration (X_2), the negative value of the coefficient ($b_2 = -2.49$) corresponds to a decrease in its contribution by 4.96% (2×2.48) when the concentration was increased from 0.5 to 1 mg L⁻¹. Among the interactions between the variables, the interactions $X_1 X_3$ and $X_2 X_3$ are more significant, with associated coefficients of −1.86 and +1.24, respectively.

Treatment time and current intensity had the greatest effect on CAP removal, at 54.6 and 34.4%, respectively. The large effect of current intensity is a reflection of this parameter's control of the amount of OH⁻ radicals and other oxidants produced in the reactor (Zaviska et al., 2013; García-Gómez et al., 2014; Chen et al., 2015). However, the impact of the pollutant's initial concentration (X_2) on the investigated response was relatively low (6.75%). In addition, the effects of the different interactions ($X_1 X_2$, $X_1 X_3$, and $X_2 X_3$) on the response were insignificant (0.22, 1.45, and 0.64%, respectively).

The effects of treatment time (X_1) and current intensity (X_3) on CAP removal are shown by the three-dimensional plot depicted in Fig. 2. These two variables have a significant impact on CAP removal response, especially in the ranges 0.5 to 0.8 A and 20 to 35 min, respectively. Steeply slopes were observed in these ranges

before reaching a cap where maximum CAP removal was achieved. Similar trends were noted regarding energy consumption.

To determine the optimum conditions for the electrooxidative process, a certain compromise in terms of CAP removal and energy consumption was established and was assigned an importance of five out of five to CAP removal and three out of five to the minimization of energy consumption. The independent factors such as CAP concentration, treatment time, and current intensity were left in their respective ranges. Given these preferences, the Design Expert software was able to generate the following optimum conditions: treatment time = 34.06 min, CAP concentration = 0.5 mg L^{-1}, and current intensity = 0.65 A. The predicted CAP removal and energy consumption were 98.7% and 4.65 kW h^{-1} m^{-3}, respectively. The desirability value gives information about the compromises made by the software to satisfy the imposed preferences. A desirability value close to one indicates that few compromises were made to satisfy the request, whereas a desirability value close to zero means that a lot of compromises were made to meet the desirable result. In this study, a desirability value of 0.89 indicates that a few compromises were made by the software to satisfy the imposed preferences.

To verify the truthfulness of the model, a triplicate experiment was performed using the predicted optimum conditions. The results showed complete CAP removal (100%) for each experiment. Even though a difference of 1.3% was recorded between the prediction and the actual result, the model can be considered as precise and valid. Although here we studied CAP removal with electrooxidation, in the study by Chen et al., (2015), 87.3% of CAP removal and 52.1% of mineralization (500 mg L^{-1} initial CAP concentration) was reached after 2.5 h electrolysis with 0.2 mol dm^{-3} of Na$_2$SO$_4$ at a current density of 30 mA cm^{-2} and an Al/PbO$_2$ electrode. The corresponding half-life was 49.8 min under these conditions. The use of electrooxidation for the removal of other drugs has been studied, with high elimination efficiencies. Rahmani et al. (2018) used this process to eliminate 0.2 Mm of ciprofloxacin with a Ti/PbO$_2$ anode; after 120 min and 32 mA cm^{-2}, 70% removal was achieved. García-Gómez et al. (2014) achieved 88 ± 1.2% carbamazepine removal with 1.37 A, a Ti/PbO$_2$ anode, and 101 min of operating time.
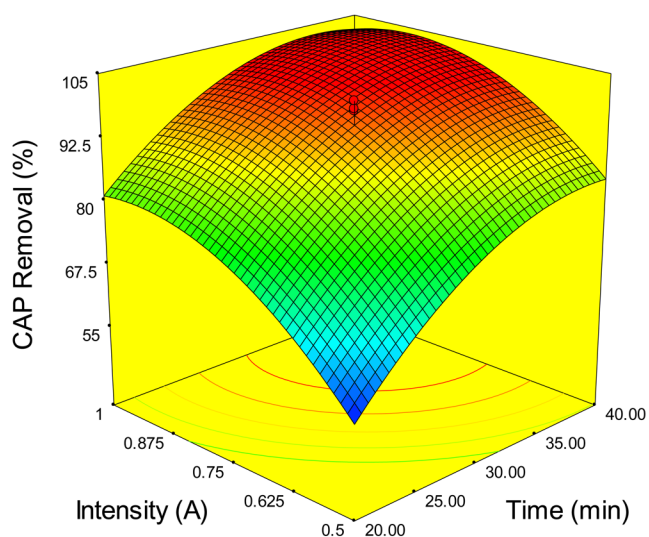

Fig. 2. Three-dimensional plot representing the effect of operation time (X_1) and current intensity (X_3) on chloramphenicol (CAP) removal (Y_1).

Contribution of Direct and Indirect Oxidation on Chloramphenicol Removal

This investigation aimed to estimate, under the optimum conditions, the contribution of direct (OH$^-$) and indirect (H$_2$S$_2$O$_8$) oxidation on CAP removal. The purpose was to determine the leading factor in this process. Two experiments with different electrolyte salts were conducted. The first experiment was performed using 1 g L^{-1} of NaNO$_3$ in the presence of CAP to estimate the effect of direct oxidation, since no electrogenerated oxidant was expected to be formed from the oxidation of NO$_3^-$ ions (only OH$^-$ is produced according Eq. [10–12]; Kalaruban et al., 2017). The second experiment was conducted using Na$_2$SO$_4$ (producing OH$^-$ and H$_2$S$_2$O$_8$). Therefore, the difference between CAP removals (calculated with Eq. [5]) in these two experiments can be attributed to indirect oxidation. Results are shown in Fig. 3, which shows that direct oxidation had much more influence on CAP removal than indirect oxidation did. For example, after 12 min of electrolysis, 76% of CAP removal was due to direct oxidation, in contrast with 24% for indirect oxidation. At 24 min, the removal percentages were 97% for direct and 3% for indirect oxidation; after 34 min (optimal time), the contribution was 100% for direct and 0% for indirect oxidation. This phenomenon of increasing the role of direct oxidation and decreasing that of indirect oxidation could be explained by a difference in reactivity between the OH$^-$ radicals and the persulfate toward CAP. In fact, the OH$^-$ radicals are very reactive, with half-lives of 10^{-9} s, and will immediately react with CAP. Furthermore, these radicals were constantly generated in the electrooxidation cell whenever electric current was applied to the electrodes; the greater the current, the higher the amount radical OH$^-$. On the other hand, the electrogenerated persulfate ions are less reactive than the OH$^-$ radicals, and their production is limited by mass transport phenomena and the SO$_4^{2-}$ availability in the reactor. These results were consistent with those obtained above dealing with the assessment of the production of direct and indirect oxidants. While investigating the use of Al-doped PbO$_2$ for CAP removal, Chen et al. (2015) concluded that this antibiotic was mainly removed by OH$^-$ radicals generated from anodic water oxidation. Based on cyclic voltammogram tests, bond dissociation energy theory, and analysis of intermediate compounds by ion chromatography or gas chromatography mass spectrometry, the authors proposed a possible CAP degradation pathway. The degradation follows three steps: radical reaction, ring-opening reaction, and mineralization. At the earlier degradation stage, the free electrogenerated OH$^-$ radicals attack CAP's molecular structure to disrupt chemical bonds with lower bond dissociation energy such as phenyl-nitryl, O-H, and C-Cl bonds. This attack leads to the formation of multiple intermediate chemicals, such as 4-(2-amino-1,3-dihydroxy-propanyl)-nitrobenzene, 4-hydroxybenzoic acid, hydroquinone, 1,4-benzoquinone, etc. Thereafter, the ring-opening reaction is followed by benzene ring cleavage, forming products such as maleic, fumaric, and succinic acids, which are further oxidized to acetic, oxalic, and formic acids. Finally, these latter compounds are mineralized to form water and CO$_2$ as end products:

$$NO_3^- + H_2O + 2e^- \rightarrow NO_2^- + 2OH^- \qquad [10]$$

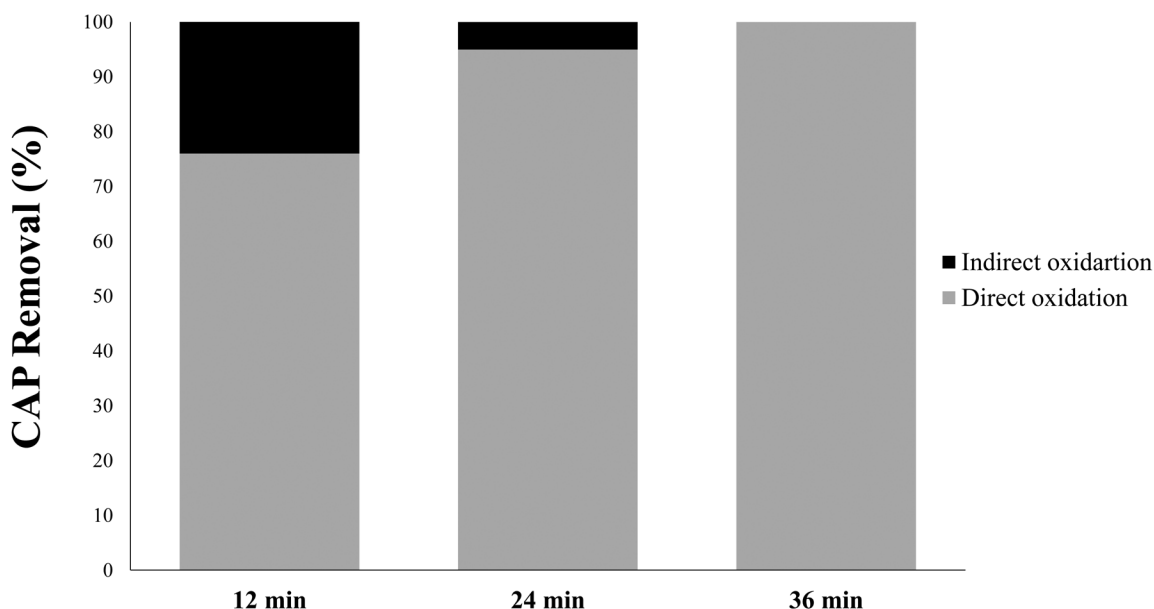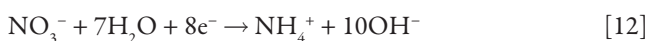$$NO_3^- + 3H_2O + 5e^- \rightarrow 0.5N_2 + 6OH^- \qquad [11]$$
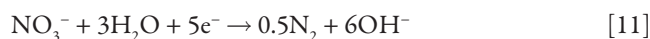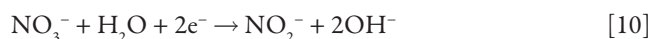
Fig. 3. Contribution of direct and indirect oxidation on chloramphenicol (CAP) removal. Anode = PbO2, cathode = Ti, intensity = 0.65 A, and CAP initial concentration = 0.5 mg L⁻¹.

$$NO_3^- + 7H_2O + 8e^- \rightarrow NH_4^+ + 10OH^- \qquad [12]$$

Degradation of Chloramphenicol in the Aquaculture Wastewater

After treatment (34 min), analysis revealed a complete degradation of CAP (Table 2). This result was somewhat unexpected because of an eventual competition between CAP degradation and the degradation of organic material present in the aquacultural wastewater. The faster CAP removal in the aquacultural wastewater might be explained by the high salinity of the influent. The conductivity of the wastewater was measured at 44.2 mS cm⁻¹, which was similar to that obtained with seawater (34—52 mS cm⁻¹) (Krainara et al., 2014). This salinity could promote the formation of indirect oxidants, which accelerates the degradation of CAP and other organic material. Active Cl species present in the aquacultural wastewater are the main indirect oxidation agents used in wastewater treatment. The Cl_2 ions at the anode can yield Cl_2, following Eq. [13], which indirectly reacts to form HClO via Eq. [1]. Up to pH 3, the predominant active Cl species is Cl_2; at pH 3 to 8, the dominant species is HClO, and for pH >8, ClO^- prevails (Ksiazek et al., 2017; Moreira et al., 2017; Garcia-Segura et al., 2018). In this study, the wastewater pH was not modified; it was ∼7.4 to 7.9 (Table 2) after the electrooxidative

process. In addition, the formation of organochlorinated species (chloramines, halomethanes, haloacetonitriles, chloromethanes, trichloroethanes, chloroamides, and others) during electrooxidation has been reported by reaction of active Cl species with different functional groups (amines, ethers, ketone, halogens, nitro compounds, aldehydes, and others) of organic matter at low pH, which are very toxic and usually recalcitrant (Gendel and Lahav, 2012); Moreira et al., 2017). The structure of CAP has two Cl molecules, which can promote the formation of this type of compound. Due to the high concentration of salt in the influent, the assessment of organic matter removal could not be achieved because of the possible interference of Cl⁻. In respect of other pollutants, a slight increase in PO_4^{3-} and SO_4^{2-} concentration was recorded, which may be due to oxidation of the initial organic compound (different residual nutrients added during feeding in the aquacultural activity), producing an inorganic form that was not detected in the initial analysis. However, the NH_3 concentration decreased after treatment. This decrease could be explained by the reaction between HClO acid (produced from Cl⁻ oxidation) and NH_4^+ ions, according to the following reaction (Eq. [14]) (Szpyrkowicz et al., 2005; Ksiazek et al., 2017):

$$2Cl^- \rightarrow Cl_2 + 2e^- \qquad [13]$$

$$NH_4^+ + 1.5HOCl \rightarrow 0.5N_2 + 1.5H_2O + 2.5H^+ + 1.5Cl^- \qquad [14]$$
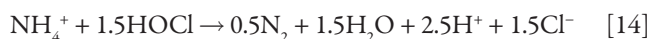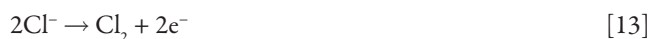
Overall, we conclude that the high salinity of aquaculture wastewater could benefit electrooxidative treatment. This high salinity induces greater production of indirect oxidants, which participate in the degradation of pollutants and lower the treatment time. The current efficiency will also increase, helping to reduce treatment cost.

Conclusion

In this study, we examined the effects of CAP concentration, treatment time, and current intensity in the electrooxidative breakdown of CAP. The last two variables had a greater effect on response variables (54.6 and 34.4%, respectively).

Table 2. Performance of the electrooxidation (EO) process during the treatment of aquaculture wastewater (intensity = 0.65 A, time = 34 min, anode = Ti/PbO₂, working volume = 550 mL).

Parameters	Before EO	After EO	Removal
			%
Chloramphenicol (µg L⁻¹)	0.5	<0.01	<99.9
pH	7.4	7.9	+6.75
NO₂⁻ (mg L⁻¹)	0.076	<0.05	<34.21
NO₃⁻ (mg L⁻¹)	0.056	<0.05	<10.71
SO₄²⁻ (mg L⁻¹)	2.84	3.05	+7.39
PO₄³⁻ (mg L⁻¹)	0.128	0.309	+141.40
NH₄⁺ (mg L⁻¹)	7.268	4.815	33.75
Conductivity (mS cm⁻¹)	44.5	44.2	30

Chloramphenicol removal was attributed mainly to direct oxidation by OH radicals (76–100% during 12 and 34 min).

Optimal oxidative conditions for the synthetic solution were applied to aquacultural wastewater (current intensity = 0.65 A, treatment time = 34 min, and CAP initial concentration = 0.5 mg L^{-1}, to obtain 98.7% of CAP removal with 4.65 kW h^{-1} m^{-3} of energy consumption). Removal reached 100% at 1.42 kW h^{-1} m^{-3}. For this type of wastewater, the treatment time can be less than other effluents due to the high salt concentration, which contributes to the formation of major oxidants and lower energy consumption. Future applications for aquacultural wastewater can use sodium thiosulfate as a dechlorinating agent after CAP removal to reduce Cl and NH_2Cl species. This study underlines the effectiveness of electrooxidation for the removal of CAP and other organic compounds in different wastewater types.

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