Reception: **June 5, 2024** | Acceptance: **November 15, 2024** | Publication: **February 1, 2025**

Application of an electrochemical process to treat liquid waste from GRAM staining tests

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To quote this article:
Velasco Ortiz, C. F. de J., López Aguilar, C. E., Nájera Aguilar, H. A., Gutiérrez Hernández, R. F., & Araiza Aguilar, J. A. Aplicación de un proceso electroquímico para tratar residuos líquidos de pruebas de tinción de GRAM. *Espacio I+D, Innovación más Desarrollo, 14*(39). https://doi.org/10.31644/IMASD.39.2025.a02

— Abstract—

The experimental activities of teaching or research laboratories in higher education institutions can lead to the generation of wastewater that is complex to treat due to its high organic load and low biodegradability, such as those from Gram stain tests (wastewater composed of by the mixture of different dyes such as methyl orange, methylene blue, and gentian violet, among others). For the treatment of these effluents, advanced oxidation processes (PAO) can be a good option as they can achieve complete mineralization of the contaminants, as is the case of Anodic Oxidation (AO). Thus, the effectiveness of OA was tested using two types of electrodes; graphite as cathode and boron-doped diamond (BDD) as anode. The efficiency of the process was tested following the behavior of the chemical oxygen demand (COD) and color as response variables. The tests were carried out under a 3^2-experimental design, that is, different current intensities (0.10, 0.20, and 0.30 A) and pH values (3, 5, and 7). The influent started with initial COD and Color concentrations of 623 mg/L and 234 Pt-Co, respectively. Of the treatments tested, the best was at pH 7 and 0.30 A, with 100% removals in both parameters and up to 96.7% in suspended solids, for a reaction time of 90 minutes. In this way, OA proved to be efficient in oxidizing contaminants present in liquid waste from Gram Stain tests, so it can be a real treatment option for this complex mixture of dye waste generated in school environments.

Keywords:

Anodic oxidation; BDD electrode; Gram stain tests; laboratory wastewater.

In recent years, the publication of numerous studies carried out on bodies of water have warned of the presence of a variety of chemical products such as phenols, sulfides, and chromium, among others, as well as refractory organic compounds present in industrial effluents, such as dyes from wastewater from the textile industry. The latter, especially azo dyes, have a direct and negative effect on aquatic systems, even at low concentrations (Bermeo & Tinoco 2016; Hanane et al., 2020). Azo dyes are composed of two nitrogen molecules joined by a double bond, with high chemical stability and low biodegradability so when faced with this type of molecules, authors such as Barrera-Andrade et al. (2023) and Brdaric et al. (2024), mention that conventional treatment methods such as adsorption, flocculation, activated sludge, among others, are not efficient for decolorizing wastewater effluents. One option for the treatment of this type of compound is the so-called advanced oxidation process (AOP) (Nidheesh et al., 2018).

Among the different treatments that make up the AOP is anodic oxidation (AO), which consists of the generation of sufficient amounts of hydroxyl radicals (•OH) from water oxidation (Velázquez 2015; Xie et al., 2022). Essentially, authors such as Moreira et al. (2017) and Sánchez et al. (2020) point out the characteristics of these radicals; firstly, they represent the second most reactive species in nature, i.e., they have a redox potential of 2.8 V; secondly, they react non-selectively with most organic compounds, therefore, mineralization of the contaminants can be achieved, which will also depend on the type of electrode used and the contaminants treated. One of the most effective electrodes to achieve the combustion of pollutants are the so-called boron-doped diamond anodes (DDB), which have a high overpotential value for oxygen evolution, thereby favoring complete oxidation mechanisms up to CO_2 (Klidi et al., 2018).

AOPs can provide complete mineralization of contaminants in the problem water, as is the case with AO. This is a completely ecological process where there is no transfer of pollutants or sludge production (Sanchez et al., 2020). AO is a process where organic species and electrodes interact when an electron transfer occurs, this can occur at the anode by the generation of active oxygen physisorbed (•OH), or chemisorbed to obtain oxygen in metal oxides (Barrera-Díaz et al., 2014; Bermeo & Tinoco, 2016).

The AO process has been tested in problem waters contaminated with dyes, such is the case of the study reported by Yingying et al. (2021), who tested the removal of azo dyes such as crystal violet, with Ti/BDD electrodes with current densities between 2.5 y 15 mA cm^{-2}, under constant agitation and neutral pH, and with 95% TOC removals. Another study is the one performed by La Rosa and Ponce (2007), where the color removal of methyl orange was evaluated using Ti/Co_3O_4, Ti/PbO_2, and graphite electrodes, with a current density of 2.5 mA cm^{-2} and different pH values (2, 5, and 8). NaCl at 2% was used as the supporting electrolyte. In general, these tests showed color removal above 80%. Results obtained with other types of dyes are also reported, as in the case of the study conducted by Petrucci et al. (2015), using anodic oxidation applied to green dye 19, achieved in 15 minutes of reaction 100% color and 53% TOC removals using DDB electrodes, under the following conditions: current intensity of 300 mA, pH 7, and with 100 mg/L Na_2SO_4 as electrolyte support. In the case of the application of AOP in wastewater from Gram staining tests, as far as the literature review was possible, only the work of Granda-Ramirez et al. (2018) is reported, who applied heterogeneous photocatalysis with TiO_2, working with 10% diluted samples. The reaction time was 2 h and the COD was reduced by 40% and the color by 75%.

Thus, in the present study, unlike most of the reported works, where the AOPs have been applied to a specific dye, the objective of the present document was to evaluate the efficiency of COD and color removal through the OA method, for a mixture of liquid wastes from the Gram stain tests performed in the Environmental Engineering teaching laboratory of the Universidad de Ciencias y Artes de Chiapas (UNICACH). This mixture is mainly composed of dyes such as methylene blue, crystal violet, iodine, safranin, lactophenol blue, and methyl orange, as well as other compounds such as acetone and alcohol.

METHODOLOGY

Characterization of problem water

For this study, the problem water generated semi-annually in the laboratory as a by-product of the Gram staining microbiology practices was characterized with the following parameters: COD, color, total suspended solids (TSS), and pH. The COD test was quantified using the closed reflux micro method, digesting the sample at 150°C for 2 hours, and then reading at 620 nm in a HACH DR-5000 spectrophotometer. A HACH DR/890 colorimeter was used to determine color, and the gravimetric method was used to determine SST. Finally, the pH was only adjusted towards the beginning of the process and was re-determined at the end of the process, using the HI 3220 HANNA equipment. All parameters analyzed were carried out following standardized methods (APHA, 2012).

Experimental tests

Electrodegradation tests were carried out in an undivided electrolytic cell (150 mL beaker) operated under a batch regime. The system consisted of a plate at the top to hold the electrodes, where the anode (working electrode) and cathode (wear electrode) were made of boron-doped diamond and graphite, respectively. The dimensions of both were equivalent, i.e., 2.5 cm wide, 5 cm high, and 1.15 mm thick. The electrodes were placed parallel to each other 2 cm apart and with a submerged area of 6 cm^2. We made a hole at the top of the support plate that allowed the sample volume needed for color and COD measurement to be removed.

The current was provided by a power source brand EXTECH model 382270, in which cables and alligators were used to conduct the current to the electrodes. The electrolyte medium was maintained on a CORNING PC420D plate with constant agitation for 2 hours (Figure 1). For pH adjustments, a H_2SO_4 al 10% solution was used.

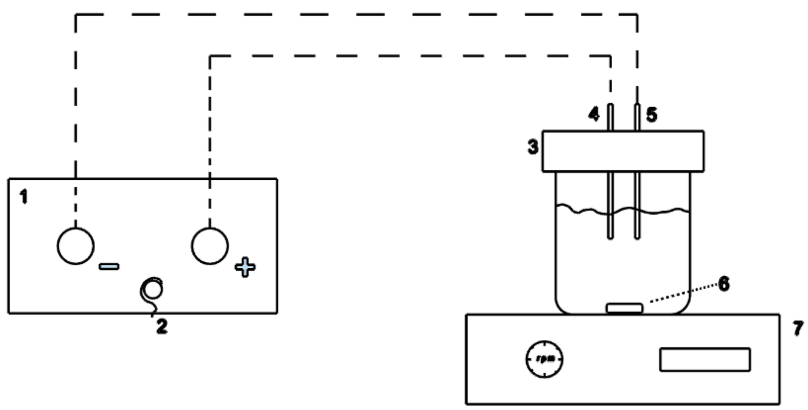

Figure 1. Experimental assembly of the electrochemical process. Where: 1. Power supply, 2. Ground wire, 3. Electrode adapter, 4. Anode (DDB), 5. Cathode (Graphite), 6. Magneto, 7. Stirring grill

Experimental design and data analysis

An experimental design of 3x3 was carried out, taking as study variables the current intensity (*i*) and the pH, each with three levels (Table 1). Levels were established as reported in other studies (Yingying et al., 2021, and Barrera-Díaz et al., 2014). The agitation speed was managed as a fixed factor at 300 rpm, a speed selected based on the report by Sánchez et al. (2020) and Chiliquinga et al. (2020).

Table 1
Experimental design used for the anodic oxidation process. Where: I (Current intensity), A (Ampere)

		pH		
		3	5	7
	0.10	T_1	T_2	T_3
I (A)	0.20	T_4	T_5	T_6
	0.30	T_7	T_8	T_9

The sample volume used was 100 mL for each test, under a 1:50 dilution, which was selected after preliminary tests, additionally, to make the application of the process more feasible, it was worked at room temperature. Finally, to favor the flow of electric current in the electrolytic medium, 0.05 M sodium sulfate was added. This electrolytic agent under this concentration, according to Rubí et al. (2023), favors the removal of color and organic matter (COD and TOC) in the reaction medium, as also shown by what was reported by Yungying et al. (2021), who evaluated the effect of different types of electrolytes on the removal of contaminants, and sodium sulfate was the best of them. It is important to mention that preliminary tests carried out with and without the induction of electric current and electrolyte support, allowed to see the influence of both factors for the electrochemical process to be carried out.

Regarding the response variables (color and COD), equation 1 was used to determine the removal efficiencies achieved, starting from the results of initial concentration (C_i) and final concentration (C_f). The results obtained were analyzed using the statistical program SigmaPlot 12.0, using an analysis of variance where the confidence interval was 95 % and each treatment was carried out in triplicate. In each case, the analysis was performed after checking the assumptions of normality (Shapiro-Wilk), independence, and homoscedasticity. When the analysis showed the existence of a significant difference between treatments, the multiple comparison procedure (Tukey's test) was performed.

$$\% \text{ Remoción} = [(C_i - C_f) / C_i] \times 100 \qquad \text{Ec. (1)}$$

Where:
C_i = *Initial concentration*
C_f = *Final concentration*

RESULTS AND ANALYSIS

Characterization of problem water

The results of the physicochemical characterization of the problem water used in the tests under 1:50 dilution are shown in Table 2, as well as their comparison with values reported by other authors.

Table 2
Physicochemical characterization of the problem water (mixture of residues from the Gram staining technique)

Parameters	Units	UNICACH's problem water	Nidheesh et al., (2018)	Yusuf and Reza (2012)
		Gram stain	Textile wastewater	Black Dye 5
pH	--	9.3	9.5- 12.5	10.17
Conductivity	µs/cm	1320	NR	NR
Color	Pt-Co	234	NR	100
DQO	mg/L	613	1835-3828	NR
SST	mg/L	450	60-416	NR

Note: NR: Not reported.

From Table 2 it can be seen that the pH value obtained (9.3) is similar to that found by Nidheesh et al. (2018) and close to what was reported by Yusuf and Reza (2012), who also carried out tests on the elimination of dyes by an oxidation process. In all cases, the pH values are in the basic range.

In appearance, the hue of the laboratory residue was purple and slightly viscous (somewhat characteristic of basic substances), with a COD reading of 613 mg/L and 234 Pt-Co in color. In the case of COD, the value shown in Table 2, was lower than that reported by Nidheesh et al. (2018), otherwise in the color, where the reported value is higher than that mentioned by Yusuf and Reza (2012) although the value of the problem water becomes much higher if we start from its concentrated value.

In general, it is observed that Gram staining residues represent a complex problem water with important values in both COD and color, finding a good part of its contaminants in suspended form.

COD Behavior and Removal

For COD removal results, Table 3 shows that the differences in mean values between most treatment groups are greater than would be expected by chance; that is, there is a statistically significant difference according to the analysis of variance performed (GL = 8, $\alpha = 0.009$, F = 3.950).

Table 3

Mean and standard deviation (SD) of COD removal percentage

	Treatments								
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**
	pH 3, 0.10 A	pH 5, 0.10 A	pH 7, 0.10 A	pH 3, 0.20 A	pH 5, 0.20 A	pH 7, 0.20 A	pH 3, 0.30 A	pH 5, 0.30 A	pH 7, 0.30 A
Mean	63.000	33.333	77.000	76.333	71.667	70.333	81.000	85.000	88.333
SD	26.889	3.055	4.359	34.298	24.786	25.146	3.000	25.981	20.207

The test procedure shown in Table 4, revealed that of 36 comparisons made, significant differences were obtained in 5 of them, where the results obtained under this test are statistically significant when presenting P values ≤0.05.

Table 4

COD Multiple Pairwise Comparison Procedures (Tukey's Test)

Comparison	Average differences	*P*	P<0.050
T9 pH 7, 0.30 A vs. T2 pH5, 0.10 A	55.000	0.005	Si
T8 pH 5, 0.30 A vs. T2 pH5, 0.10 A	51.667	0.009	Si
T7 pH 3, 0.30 A vs. T2 pH5, 0.10 A	47.667	0.018	Si
T3 pH 7, 0.10 A vs. T2 pH5, 0.10 A	43.667	0.035	Si
T4 pH 3, 0.20 A vs. T2 pH5, 0.10 A	43.000	0.039	Si

Regarding the behavior in the removal of the COD parameter, Figure 2 shows a tendency to improve the removals in this parameter as the current intensity was increased, mainly for the most favorable reaction time (90 min) where maximum removals were achieved (100%), that is, Figure 2c (pH 7 at 0.30 A). This shows current intensity as one of the operating parameters that has the greatest impact on the degradation of complex organic molecules (González et al. 2011; Cruz 2013), being able to favor the interaction of organic species and electrodes, through the transfer of electrons. Mechanisms that, according to Bermeo and Tinoco (2016), come to occur at the anode, mainly with physisorbed hydroxyl radicals (•OH).

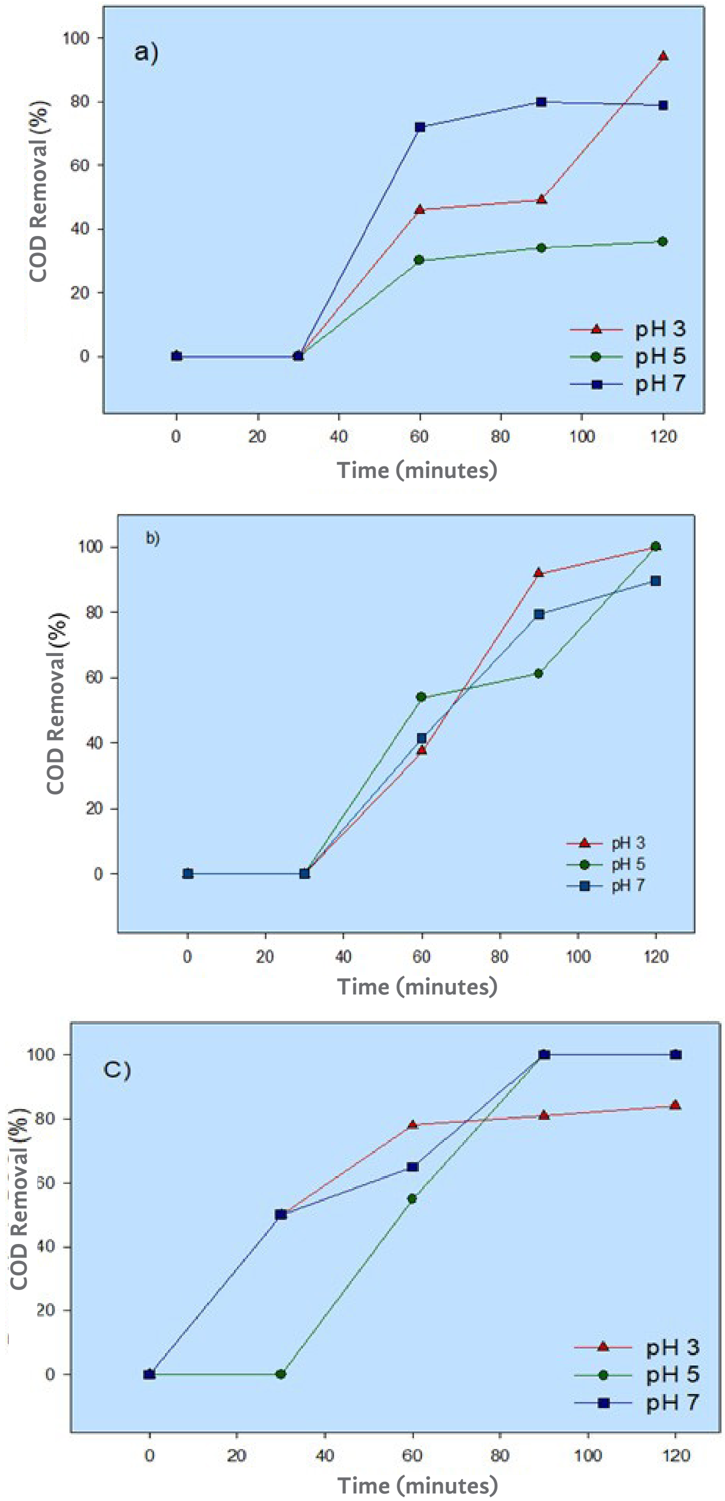

Figure 2. Removal of COD at different pH values and current intensities: a) 0.10 A, b) 0.20 A y c) 0.30 A

Figure 2a (0.10A) shows that the highest COD removal (94%) was obtained at pH 3 around 120 minutes of reaction. The remaining two treatments at pH 5 and 7 were below 80%.

For condition 0.20A (Figure 2b), in the treatments at pH 3 and 5, a 100% COD removal was achieved, with a final pH of 4, and for the treatment at pH 7, although the pH value in the final effluent remained neutral, the removal achieved was lower (89% COD).

Figure 2c shows that the highest removal (100%) was achieved at pH 5 and 7 and with only 90 minutes of reaction. In general, the results obtained for the best treatments (100% COD removal), reflect higher removals than those obtained in other studies, such as those reported by Bermeo and Tinoco (2016), who achieved slightly lower removals (96% COD), when working with synthetic water from the textile industry, or the study reported by Yingying et al. (2021) who report removals of the azo dye AV7 below 75% COD removal.

Behavior and color removal.

For the color removal results, an analysis of variance was also applied as shown in Table 5. Evidence of significant difference between treatments was observed in this analysis (GL = 8, α = 0.013, F = 3.120).

Table 5
Mean and standard deviation (SD) of color removal percentage

	Treatments								
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**
	pH 3, 0.10 A	pH 5, 0.10 A	pH 7, 0.10 A	pH 3, 0.20 A	pH 5, 0.20 A	pH 7, 0.20 A	pH 3, 0.30 A	pH 5, 0.30 A	pH 7, 0.30 A
Mean	48.500	16.250	31.500	52.000	58.250	62.000	63.750	62.000	80.750
SD	23.331	4.272	19.192	20.050	31.298	21.894	20.966	19.579	24.102

Thus, the source of variation of the significance level "P" indicates that in some of the means there is a significant difference since P=0.013, in short, the significance level is less than 0.05. To isolate the group or groups that differed from the others, the Tukey test was used (Table 6).

Table 6

Multiple pairwise comparison procedures for color removal (Tukey test)

Comparison	Average differences	P	P<0.050
T9: pH 7, 0.30 A vs. T2: pH 5, 0.10 A	64.500	0.006	Si

In this sense, the analysis of variance performed on the 36 comparisons allows us to see that the difference between some of the removal percentages achieved by the treatments evaluated is significant in only one comparison. According to the analysis using Tukey's test, it was observed that there is a significant difference between the means of the comparisons in the treatment T9 vs T2; that is, pH 7, 0.30 A vs. pH 5, 0.10 A.

In the case of the removal of the color parameter, Figure 3 shows that at low current intensity, the results were below 70%. The opposite was the case with the rest of the current intensities, where the results were above 80% removal.

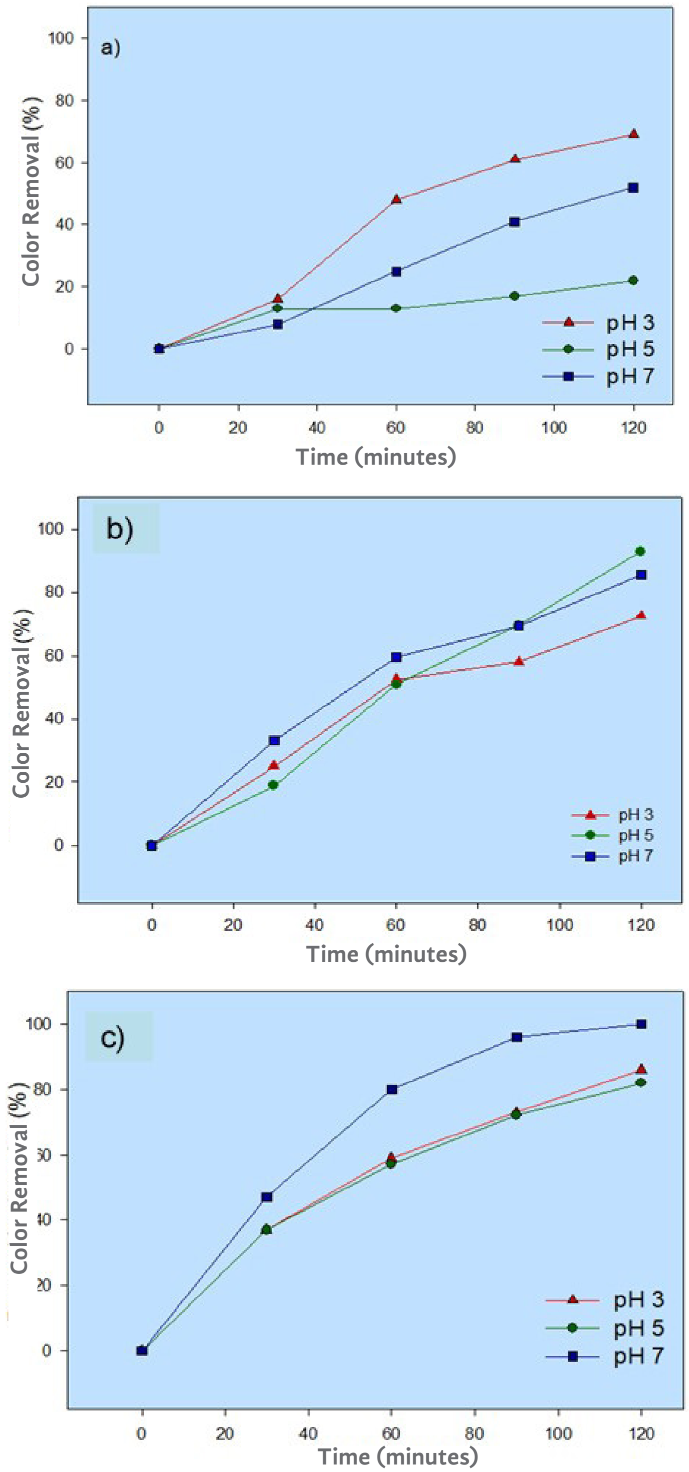

Figure 3. Color removal at different current intensities a) 0.10 A, b) 0.20 A, and c) 0.30 A

From Figure 3, it can be seen that the 3 treatments tested at a current intensity of 0.10 A (Figure 3a) show the lowest color removal efficiencies, and in general in the range of 22 to 69.26%.

For Figure 3b (current intensity of 0.20 A), it can be seen that for a reaction time of 120 minutes and at pH 5 and 7, the highest removals were recorded, i.e., 93 and 85%, respectively.

Finally, for Figure 3c (current intensity of 0.30 A), the maximum removals (100%) were obtained at pH 7, for the 90 and 120-minute reaction times. As with COD, the influence that current intensity can have on the degradation of complex organic molecules can also be seen with this parameter (González et al. 2011; Cruz 2013).

In general, in the decomposition of complex organic molecules, the •OH produced in the reaction medium, according to authors such as Barrera-Andrade et al. (2023) and Milam and Planalp (2024), are effective in the decomposition of dyes such as methylene blue, present in the dye mixture that characterizes Gram staining residues. The process begins by adding hydroxyl radical to the ring or the oxidation of the sulfur atom. After several additions of hydroxyl radicals to the phenyl rings, the ring structure may break down into phenolic moieties, giving way to further oxidations up to possible mineralization of the molecule (CO_2 and H_2O).

On the other hand, the analysis of the final characterization of the influent and effluent was carried out for the best combinations of pH and the different current intensities. Results are shown in Table 7.

Table 7
Characterization of wastewater (influent and effluent)

			a pH 3 (0.10 A)		b pH 5 (0.20 A)		c pH 7 (0.30 A)	
Parameters	Unit	Influent	Effluent	Removal %	Effluent	Removal %	Effluent	Removal %
pH	---	---	3.4	---	7	---	5	---
Color	(Pt-Co)	234	69.26	70.4	85	63.7	0	100
DQO	(mg/L)	612	36.72	94	61	90	0	100
SST	(mg/L)	450	390	13.3	75	83.3	15	96.7

From the table, it is observed that both the color and COD recorded 100% removals under the combination of pH 7 and 0.30 A, when recording values of 0 towards the end of the treatment. It also highlights the low presence of SST in the final effluent (15 mg/L). The removals achieved in the present study were much higher than those reported in the only work found (Granda-Ramírez et al. 2018), where a PAO such as heterogeneous photocatalysis with TiO_2 has been applied to the residues of the Gram staining test, and only 40% of the COD and 75% of the color were removed.

Behavior of the evaluated parameters and the statistical error obtained

The results of the color removal and COD concerning the error obtained are shown in Figures 4 and 5.

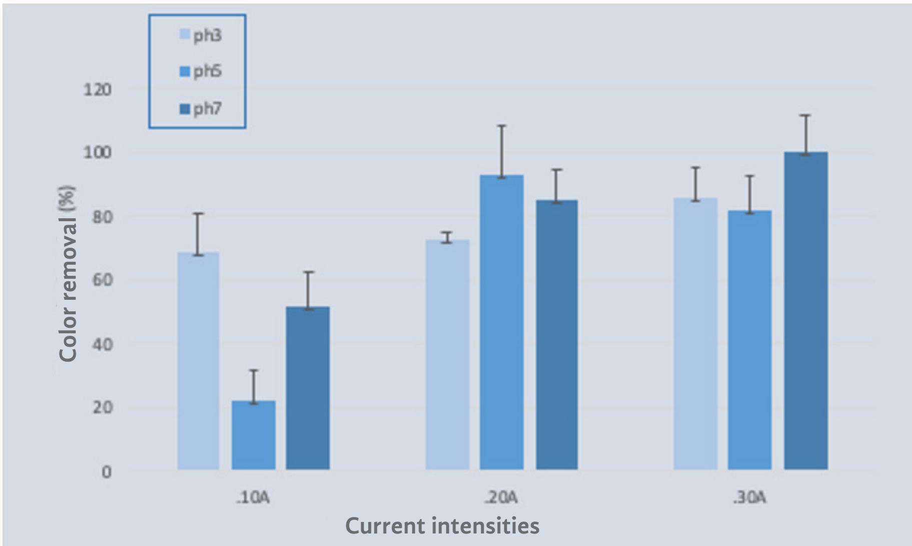

Figure 4. Color removal with the error obtained

As can be seen and under the conditions evaluated, the color removal values are not dispersed, they are even more concentrated at the pH 3 value under 0.20 A. In any case, it is convenient to highlight the direct relationship between the color removal and the applied current intensity; in fact, the higher the current intensity, the color removal result was more significant.

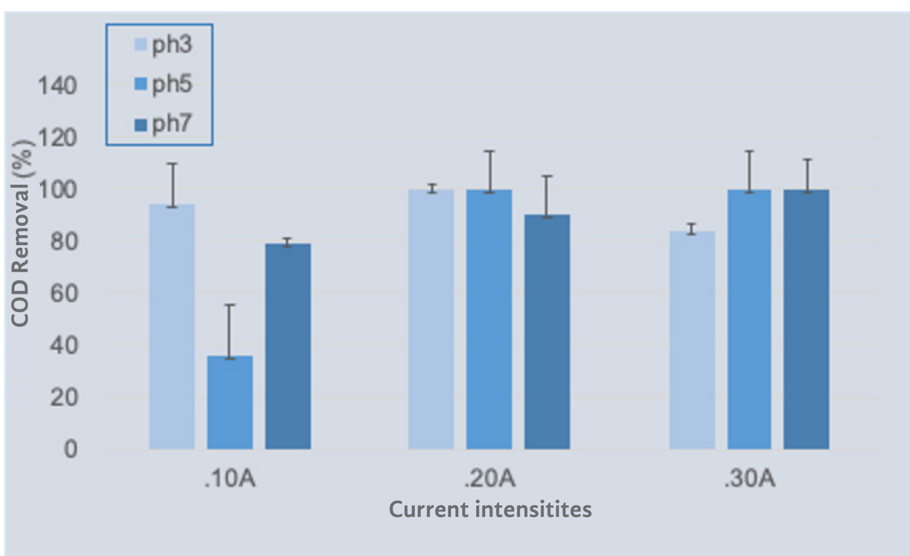

Figure 5. Removal in COD with the error obtained

For the COD removal treatability tests, it was observed that for the conditions evaluated (0.10 A and pH 7, 0.20 A, and pH 3, and 0.30 A and pH 3), the error values obtained were concentrated and the removals were above 80%.

CONCLUSIONS

The OA using the DDB electrode as an anode and the graphite electrode as a cathode, proved to be efficient in oxidizing the contaminants present in liquid waste from the Gram staining tests, so it can be a real treatment option for this complex mixture of dyes generated in school environments.

The ANOVA demonstrated the existence of significant differences between treatments. Tukey's test showed that the best removal conditions were 100% in COD and color, and 96.7% in SST when the system was operated with 300 mA and neutral pH.

Finally, considering that the UNICACH Environmental Engineering teaching laboratory generates an average of 2 L of Gram staining liquid waste every six months, it would be necessary to treat around 100 L of the already diluted test water, which could be discharged with values of zero mg/L in COD and zero Pt-Co units in color under the best conditions found in this study.

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