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Electrochemical Characterization and Biosensors with the Coagulant *Moringa oleifera* Seed Lectin (cMoL)

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Abstract: Coagulant lectin from *Moringa oleifera* seeds (cMoL) was characterized by potentiometry and scanning electron microscopy (SEM) using MOF, Metal-Organic Frameworks of $[Cu_3(BTC)_2.H_2O)_3]_n$, to immobilize cMoL and construct biosensors. Developed aluminum-air batteries degraded indigo carmine dye formulated similar to a textile effluent; biosensors investigated cMoL interaction with specific galactose and monitored residual dye. SEM revealed components of electrode assembly steps. Oxide reduction reactions of batteries generated $Al(OH)_3$ promoting dye electrocoagulation. Cyclic voltammetry showed differentiated redox peaks related to dye residue quantification by cMoL. Electrochemical systems evaluated cMoL interaction with ligand and efficiently degraded dye; biosensors could be used for lectin characterization and monitoring dye residues in environmental textile effluents.

Keywords: lectins; Metal-Organic Frameworks (MOF); electrochemical systems

1. Introduction

Lectins are proteins that have binding sites, being able to recognize carbohydrates in a specific and reversible way, thus with biochemical, biomedical and biotechnological applications [1]. They are widely distributed in nature in different tissues of organisms and can adhere to carbohydrates present in the membrane or cell wall, agglutinating cells, recognizing glycoconjugates and complex glycans [2].

Currently, research demonstrates several applications of plant compounds including as coagulant in water treatment [3–5]. *M. oleifera* is a polyvalent tree [6–8] with therapeutic properties for alternative medicinal uses [9]. Extracts from its seeds showed efficiency in removing humic acid compared to the chemical component commonly used [6] and also has coagulant proteins applied in water treatment and industrial waste removal [10,11] such as textile effluents [12].

One of the coagulant molecules of *M. oleifera* seeds (cMoL) is a cationic lectin, specific for galactose, showed to be resistant to wide pH and high temperatures. The partially characterized lectin revealed a molecular profile of 26.5 KDa, approximate isoelectric point of 11.67, 101 amino acids [13] and the following biological activities: anticoagulant [14], insecticide [15], antiparasitic [16], cytotoxicity for tumor cells [17] and removal of water turbidity similar to aluminum sulfate treatment [13].

Electrochemical methods are sensitive, financially viable, and have a variety of instrumental styles and components [18]. These technics have been useful to detect a diversity of analytes [19–21]. Potentiometric and amperometric systems, can be used sequentially in the monitoring of industrial waste [22]; they detect, through measurable electrical signals, conformational changes of biomolecules when in contact with a ligand [23].

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Electrochemical biosensors are integrated and autonomous devices that use a biochemical receptor to obtain analytical information. These instruments have been designed with several applications [23–25]. Among them the monitoring of water pollutants, due to its high sensitivity and its application in real time; this feature has attracted the attention of researchers [26].

Considering that there are different physical, chemical and biological methods for wastewater treatment, it is difficult to choose a single procedure for efficient removal of dyes from the textile industry [27].

Coagulation corresponds to the first stage of water treatment, in the supply networks; it is used to reduce turbidity and coloration, as well as to eliminate pathogens [28]. One of the promising methods for this purpose is electrocoagulation [28,29], which consists of an alternative method to chemical treatment, occurs by destabilizing pollutant particles [30] through redox processes, promoted by an electric current applied in the electrochemical cell. The system is composed of metallic electrodes, which can be iron or aluminum electrolyte solutions [31]. The reaction generates coagulant substances, which can be metallic hydroxides or polyhydroxides, responsible for flocculation of these particles, which float on the surface [32].

With the intention of reducing the cost of electrocoagulation processes for treating contaminated water, the aluminum electrode used in these reactors can be replaced by recycled aluminum beverage cans by building an aluminum battery; this metal that constitutes these cans works as an anode [27,29,33].

MOF constitute Metal-Organic Frameworks with two- or three-dimensional structures that form coordination networks with metallic centers and organic ligands [34], which can be presented as nanochannels with adjustable shapes and sizes. Standing out for their qualities, such as the presence of these adjustable pores and providing a large surface area, in this way, they have several applications such as gas separation, emission purification and drug encapsulation [35]. Biosensors that use nanomaterials are paving the way for the development of devices with better performance for monitoring environmental parameters, such as water quality control [36].

In this work cMoL was characterized by a potentiometric biosensor and scanning electron microscopy (SEM). In addition, an amperometric biosensor was designed using cMoL immobilized in MOF of $[Cu_3(BTC)_2.(H_2O)_3$ with the objective of monitoring residual indigo carmine dye that simulates a textile effluent, degraded by electrocoagulation.

2. Materials and methods

2.1. Lectin isolation

The extract obtained from *M. oleifera* seeds in powder and saline solution was added with ammonium sulfate at 60% (w/v) saturation according to Green and Hughes [37]. The precipitated fraction (F 0-60%) was collected, resuspended and dialyzed against distilled water (4 h, with two liquid changes); then added to a column (10 x 1.0 cm) containing guar gel [38], equilibrated with 0.15 M NaCl. The chromatographic conditions used were the same as those reported by Santos et al. [13] to purify cMoL. A flow rate of 20 mL/h was maintained and, after a washing step with the equilibration solution, the adsorbed proteins were eluted with 1.0 M NaCl. Fractions of 2.0 mL were collected and evaluated for absorbance at 280 nm and hemagglutinating activity (HA). The protein concentration was estimated by the method of Lowry et al. [39] and the HA assay was used to evaluate the carbohydrate binding capacity of the lectin, according to Paiva and Coelho [40].

2.2. Electrochemical evaluations of cMoL immobilized on MOF/Platinum electrode surface using MOF, Metal-Organic Framework of $[Cu_3(BTC)_2.(H_2O)_3]_n$

The MOF $[Cu_3(BTC)_2.(H_2O)_3]_n$ is a porous and crystalline polymer composed of a metallic center, copper dimers (two copper II ions) and the paddlewheel unit formed by four carboxylate anions of the BTC-ligands. It was synthesized electrochemically according to the method developed by Silva et al. [41] using a solution of 1,3,5-benzenetricarboxylic acid, sodium nitrate and dimethylformamide in Millipore® water in a 1:1 ratio. The synthesis lasted about 17 min.

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2.3. Preparation of the reference electrode

The reference electrode was composed of a silver wire (Ag). Part of this wire was covered with silver chloride (AgCl) and this was inserted into a plastic tip containing a saturated conductive solution of KCl 3 mol/L.

2.4. Washing and preparation of the working electrode

The electrode consisted of a platinum plate with an area of 0.5 cm^2. Before mounting, it was submitted to chemical washing with immersion in a solution of nitric acid (HNO3) P.A. for 2 min, then washed with distilled water and dried at room temperature. To fix the MOF/cMoL on the platinum electrode, a paste composed of 0.0650 g of powdered carbon and 6 drops of mineral oil was used to build the modified working electrode, Pt/MOF/cMoL.

2.5. Immobilization and Potentiometry

In the immobilization process of lectin in MOF $[Cu_3(BTC)_2.(H_2O)_3]_n$, 0.0060 g of this polymer was weighed and 20 µl of cMoL 3 mg/L were added at a temperature of 4°C for 24 h.

After assembling the working electrode under the conditions mentioned above and in item 2.4, measurements of the electrochemical potentials of the following steps were taken:

1) Platinum electrode with MOF (Pt/MOF);
2) Platinum electrode with Pt/MOF/cMoL with 0.15 M NaCl in the electrolytic medium;
3) Pt/MOF/cMoL with 0.15 M NaCl and different concentrations of galactose: 10, 15 and 20 mM.

The platinum electrode together with the silver/silver chloride (Ag/AgCl) reference electrode and the saline solution in the electrolytic medium constituted the electrochemical cell; this system was connected to a digital voltmeter with a scale of 0 - 1000 V.

2.6. Scanning electron microscopy

The characterization was performed by obtaining images in the scanning electron microscope (SEM), equipment (TESCAN, Model: VEGA3) available at CENAPESQ/UFRPE.

2.7. Electrocoagulation

For the construction of the aluminum battery a solution was used that simulated an efluente containing indigo carmine at a concentration of 0.1 g/L^{-1}. In each can with copper wires, 1 m long and 2.5 mm thick, 19 g of sodium choride was dissolved in the dye solution.

The components of the electrochemical cell were iron electrodes (sacrifice) and electrolyte solution, containing the same solution as the aluminum battery.

The system was connected by an external energy source (aluminum battery) composed of batteries, whose electrodes were aluminum containers (anode) and copper wires (cathode); these batteries were connected in series, in which the copper was inserted inside each container by connecting it to the next container. The potential was measured by coupling the positive terminal of the multimeter to the first copper wire, and the negative terminal to the last container and was equivalent to the sum of the potential of each battery (Figure 1).

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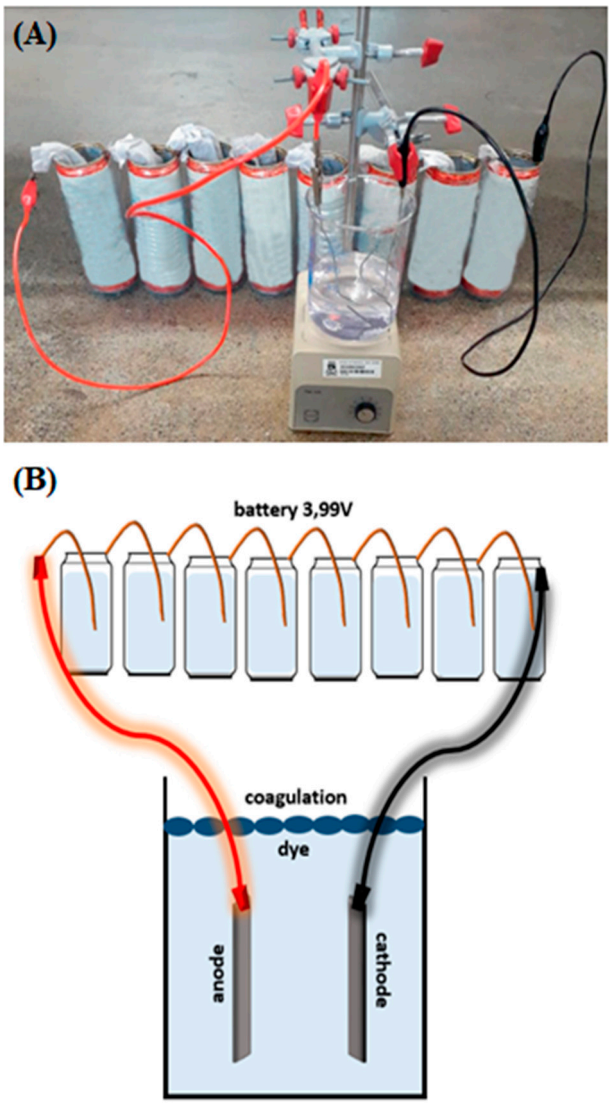

Figure 1. Electrochemical system showing the mechanism of electrocoagulation, resulting from reactions occurring at the anode and cathode (A); Representative scheme of the aluminum battery (B).

2.8. Cyclic Voltammetry

The electrochemical characterization was carried out by cyclic voltammetry in the potentiostat/galvanostat of Autolab Eletrochemical Instruments, located in the Laboratory of Chemical Analysis and Sensors–LAQIS/UFRPE. The measurement used the Ag/AgCl reference electrode, a platinum wire as counter electrode and the modified working electrode Pt/MOF/cMoL inserted into samples containing indigo carmine after treatment with aluminum containers. The treatment of the solution with dye, in aluminum containers, lasted for 1 h.

3. Results and discussion

Potentiometric measurements were performed using the working electrode and the reference electrode, both immersed in a 0.15 mol/L NaCl solution and coupled to a potentiometer.

The electrochemical potential was verified in all phases of this analytical system (Figure 2A,B). The higher electrochemical potentials result from the interaction between cMoL immobilized on the MOF-coated platinum electrode (Pt/MOF/cMoL) and the different concentrations of galactose, revealing that these alterations are the result of intrinsic conformational changes occurring on the surface of the immobilized lectin, without altering the native structure of cMoL.

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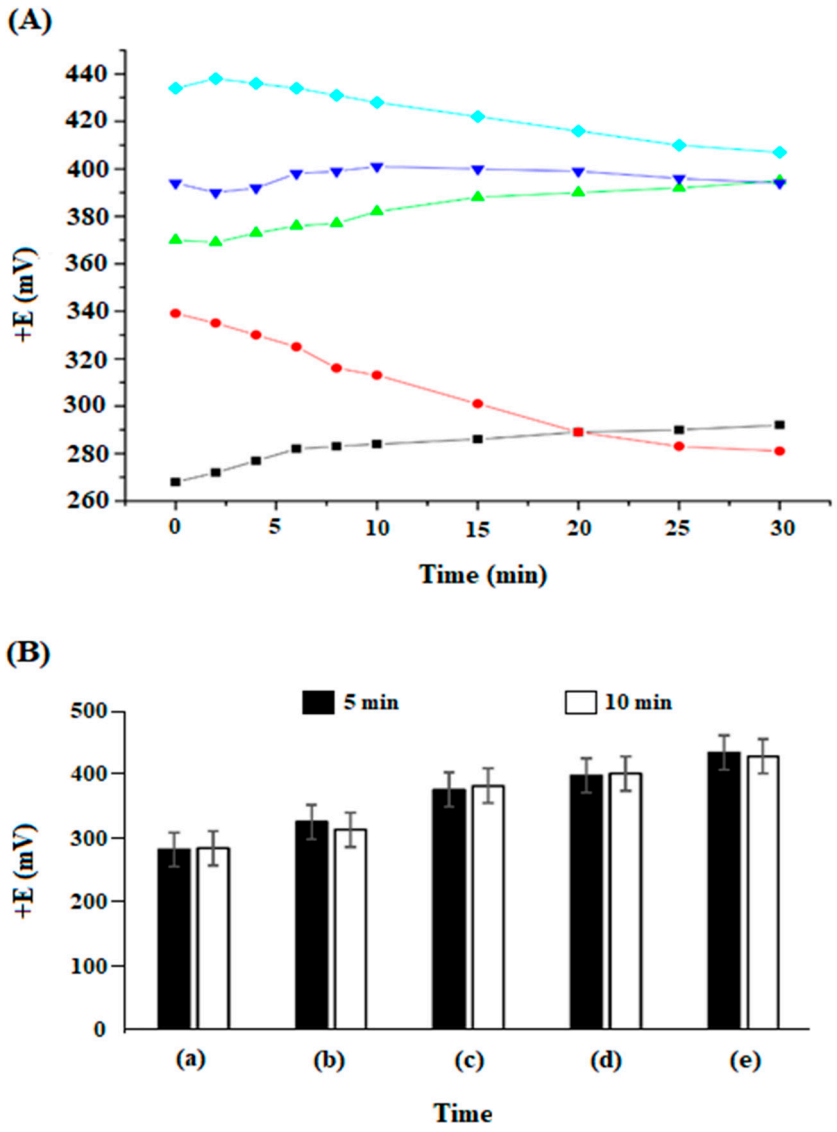

Figure 2. Electrochemical potential of platinum electrode coated with MOF, Pt/MOF (-■-), platinum electrode coated with MOF and immobilized cMoL, Pt/MOF/cMoL, (-•-); platinum electrode coated with MOF and immobilized cMoL, Pt/MOF/cMoL and interacting with different concentrations of galactose: 10mM (-▲-), 15 mM (-▼-) and 20 mM (-♦-) **(A)**. Application of bar errors (5%) in the results obtained at intervals of 5 and 10 min: electrochemical potentials of platinum electrode coated with MOF, Pt/MOF (a); platinum electrode coated with MOF and immobilized cMoL, Pt/MOF/cMoL (b); platinum electrode coated with MOF and immobilized cMoL, Pt/MOF/cMoL and interacting with different concentrations of galactose: 10 mM (c), 15 mM (d) and 20 mM (e) **(B)**. The number of repetitions made a total of five for the entire experiment.

In electrochemistry of biological molecules, a biosensor is considered efficient when the immobilized biological element does not undergo denaturation, therefore maintaining its activity [35,42,43]. This biocompatibility was also observed in electrochemical biosensors using polymers such as MOF and covalent organic frameworks (COFs) in assays with enzymes and antibodies [23] .

The electrochemical potential was verified in all phases of this analytical system for different concentrations of galactose observing the results in the time period of 5 and 10 min (Figure 2A); the mathematical model of bar errors (5%) was applied for these time parameters (Figure 2B). It was revealed a significant increase in the electrochemical potential in this time range in relation to the addition of carbohydrate in the electrolytic medium.

The characterization of *Craylia mollis* seed lectin (Cramoll) immobilized on a gold electrode coated with crystalline polymer MOF-$[Cu_3(BTC)_2.(H_2O)_2]_n$ with different concentrations of glucose in

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the electrolytic medium induced an increase in potential electrochemical, also detected between 5 - 10 min [45].

However, no variation was observed for each specific carbohydrate concentration between the respective analysis times (5 and 10 min), due to the stabilization of the potential generated by the platinum electrode coated with MOF (Pt/MOF/cMoL) (working electrode) in relation to the reference. A significant elevation in these electrochemical potentials is verified by the increase in the concentration of galactose in the electrolyte medium, revealing that this rise in the magnitude of the electrical signal produced in the system is the result of intrinsic conformational changes that occur on the surface of the immobilized lectin and also the biocompatibility exhibited by MOF for the manufacture of electrochemical biosensors.

The biological performance of a lectin correlating structural modifications and surface charge distribution was elucidated by potentiometry [45]. This redox electroactivity can reveal data characteristic of the equilibrium state, through electrochemical methods; the application interface with electrical charges, adsorbed on the surface of the electrode, can be evaluated in a simplified way [23].

The redox-active nature of MOF favors their application in electrochemical biosensors [34]. This property has an effect on the charge distribution on the surfaces of biomolecules in electrochemical systems that are relevant to understanding their interaction mechanisms and biological properties [44].

This elevation of the electrical signal generated through the electrodes of a biosensor by increasing the concentration of the analyte, in an electrochemical cell, was confirmed by Carvalho et al. [45], Mohankumar et al. [24] and Selzer et al. [46].

This electrochemical model with the lectin immobilized in MOF for the construction of a biosensor was also verified by Carvalho et al [45], when evaluating the results of the electrochemical potentials related to the interaction from Cramoll immobilized in MOF with different concentrations of glucose. The combination of lectins with semiconductors such as metallic polymers increases their recognition capacity in biosensors [47].

Immobilization of lectins for sugar recognition offers further advantages among biospecific protein/carbohydrate systems as it does not undergo structural changes after binding and also can provide independent carbohydrate binding domains [48,49].

In addition to the electrochemical biosensor the Scanning Electron Microscopy (SEM) was used to characterize MOF and investigate its interaction with cMoL and galactose (Figure 3).

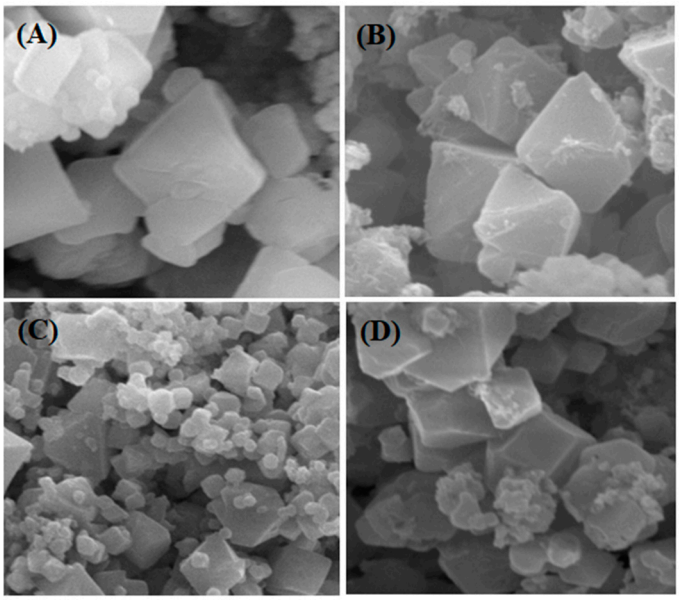

Figure 3. $[Cu_3(BTC)_2.(H_2O)_3]_n$ MOF crystals (A), $[Cu_3(BTC)_2.(H_2O)_3]_n$ MOF crystals with immobilized cMoL (B), $[Cu_3(BTC)_2.(H_2O)_3]_n$ with immobilized cMoL interacting with 10 mM galactose (C), and $[Cu_3(BTC)_2.(H_2O)_3]_n$ MOF crystals with 10 mM galactose (D).

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The geometric characteristic of the MOF Cu-BTC crystals is represented by a smooth octahedral structure, approximately 2 μm in size. Figure 3B shows galactose adhered to MOF surface, in Figure 3C a globular structure of cMoL adhered to MOF surface indicates the efficiency of immobilization on the surface of this Metal-Organic Frameworks and the complete MOF/cMoL/galactose system is represented in Figure 3D; this interaction model is confirmed by the evolution of electrochemical potentials, Figure 2.

SEM is a perfect technological tool that helps the characterization of biomolecules immobilized on MOF due to the production of high resolution images, capable of showing the target surface being analyzed [34,45,50,51].

After cMoL characterization by a potentiometric biosensor and the analysis of images generated by SEM, a system of aluminum-air batteries was developed for the degradation of indigo carmine dye in an electrolytic medium.

The success of electrocoagulation is already known for treating wastewater, such as recycling water contaminated by domestic sewage [29]. The advantages of this method over others that use electric current to coagulate suspended solids in contaminated water are the reduction in energy consumption and operating costs [27,29,33].

The treatment of dye residues in aluminum batteries was shown by the electrocoagulation mechanism (Figure 1). At the battery's cathode, oxygen dissolved in water undergoes reduction on the copper surface, forming hydroxyl. At the anode, aluminum undergoes oxidation releasing Al^{3+} which react with the hydroxyl to form $Al(OH)_{3(s)}$, a white precipitate responsible for the coagulation of the dye contained in the solution, according to the following equations:

$$\text{Anode: } Al_{(s)} \longrightarrow Al^{3+}_{(aq)} + 3e^-,\ E^0 = -1.66\ V \quad (1)$$

$$\text{Cathode: } O_2 + 2H_2O + 4\ e^- \rightarrow 4OH^-,\ E^0 = 0.82\ V \quad (2)$$

The potential generated by the battery was equivalent to 3.99 V and allowed electrolysis to treat new dye samples in the system formed by the beaker and iron electrodes (Figure 1). The container is the anode in the battery because it undergoes oxidation. The electrons formed in this oxidation travel through the external wire to the iron electrode which will promote the reduction of water forming hydroxide (OH^-) and hydrogen (H_2), while in the electrode of iron, connected to the copper wire, oxidation of the iron occurred forming Fe^{2+} which react with the hydroxide to produce $Fe(OH)_2$, a gelatinous precipitate capable of coagulating the dye contained in the solution.

$$Fe_{(s)} \rightarrow Fe^{2+}_{(aq)} + 2e^- \quad (4)$$

$$2H_2O + 2e^- \rightarrow H_2 + 2OH^- \quad (5)$$

The pH of samples with dye was measured to verify the formation of hydroxides in the solution (Table 1).

Table 1. Measurement of the pH values of the samples.

Samples with dye	pH
Sample without treatment	5.97
Sample after aluminum electrode treatment	6.24
Sample after iron electrode treatment	7.01

Measurement of the pH values of samples before treatment and after electrochemical treatment, with iron and aluminum electrodes.

There was an increase in the pH of the samples after treatment, suggesting that this increase in pH occurs due to the evolution of hydrogen in the cathode [52]. The techniques commonly applied in the treatment of effluents have a high cost of chemical components; the search for new methods

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for water treatment has been carried out [53]. Electrocoagulation methods are more advantageous than other treatments, since they use simple instrumentation, easy to handle, and the residual sludge formed can be retained more easily [54]; so this technique is widely used to remove color and decontaminate the effluent [55]. Despite of this, the method has some limitations, such as electricity consumption, so new improvement alternatives have been investigated [56]; the system composed of aluminum containers generating energy to activate the reaction degradation of the dye, represents, therefore, of economic and environmental importance.

Based on the results obtained, Figure 2, and carefully substantiated by the literature; it appears that the immobilization of cMoL in MOF of $[Cu_3(BTC)_2.(H_2O)_3]_n$ was efficient and facilitated the obtaining of electrochemical potentials, showing that the electrochemical system used with modified work electrode can be applied to study the interaction of this lectin with the indigo carmine.

In order to build a biosensor to detect dye residues in samples after electrocoagulation with aluminum batteries, cMoL was used to aggregate MOF crystals on the platinum electrode surface, providing stability for the use of this device.This aggregate effect was also verified with Cramoll lectin on the surface of the gold electrode containing MOF (45).

To identify the occurrence of interaction between cMoL and the indigo carmine dye, cyclic voltammetry was performed (Figure 4). According to Mohankumar et al. [24], the oxidation and reduction peaks shown in cyclic voltammograms result from the current variation generated on the surface of the electrodes in an electrochemical biosensor.

Electroanalytical methods such as cyclic voltammograms using electrodes modified with nanocomposites are increasingly required to identify the redox properties of biomolecules [57], such as the use of MOF due to their biocompatibility for glucose and H_2O_2 detection [34] .

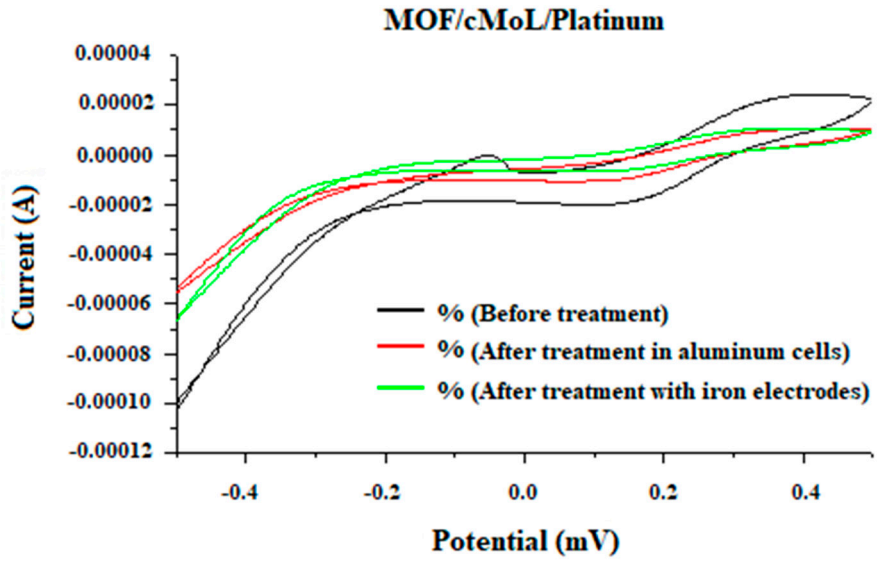

Figure 4. Cyclic voltammogram with samples before treatment, after treatment in the aluminum cell and also after treatment by electrolysis with iron electrodes.

In this work, through the voltammogram, the treatment efficiency was observed due to the difference in the redox peaks, in which there was a decrease in the current peaks in the treated samples, indicating that cMoL was able to monitor the residues before and after the treatment. No significant difference was verified between the samples treated in aluminum piles or after electrolysis. In a previous study, cMoL was purified and showed coagulant capacity to remove impurities in turbid water [13].

The peaks that stand out in the cyclic voltammograms are due to the redox process of the MOF/cMoL interaction with the dye in the untreated samples.

This is the first time that the MOF/cMoL/Platinum amperometric biosensor is utilized to detect dye residues after the treatment with aluminum batteries and electrolysis, a novelty to control water

residues. Electrochemical biosensors are currently considered a high-technology system for detecting target analytes, due to its ease of handling, sensitivity, selectivity, speed and reproducibility of its responses [23], due to this specificity and sensitivity; these biosensors have excellent applicability for determining contaminants that cause water pollution [36].

The electrocoagulation method was efficient in removing dyes, indicating the possibility of using systems containing reactors with aluminum and copper electrodes for the treatment of effluents from textile industries. The biosensor using cMoL can be applied in the recognition of dyes in textile industry effluents.

4. Conclusions

The coagulant lectin from *M. oleifera* seeds (cMoL) was characterized by electrochemical methods. The potentiometric data obtained demonstrate the interaction of cMoL with different concentrations of galactose, consequences of the conformational changes that occurred on the lectin surface. The electrocoagulation method was efficient in removing the dye and constitutes an conventional system. The biosensor using MOF/cMoL was able to monitor dye residues in samples treated with iron and aluminum electrodes.

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Conflict of Interest: The authors have no conflict of interest.

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