Pharmaceutica Analytica Acta
ISSN: 2153-2435

Maallah and Chtaini, Pharm Anal Acta 2018, 9:3
DOI: 10.4172/2153-2435.1000580

Commentary Open Access

Bacterial Electrode for the Oxidation and Detection of Phenol

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Abstract

Voltametric degradation of phenol was carried out at microbial electrode. This electrode is based on graphite carbon and natural phosphate modified by bacteria inserted in the phosphate matrix, the whole is covered by a polymer developed *in situ* on the surface. This electrode, designated subsequently by bacteria-NP-CPE, Showed stable response and was characterized with voltametric methods, as cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). The experimental results revealed that the prepared electrode could be a feasible for degradation of hazardous phenol pollutants.

Keywords: Modified electrodes; Cyclic voltammetry; Caprolactone ; Naturel phosphate; Polymer; Impedance spectroscopy; Bacteria; Phenol

Introduction

Phenol is known to be the simplest toxic product; it is the basis of the majority of the most common toxic environmental pollutants and comes mainly from industrial processes such as pharmaceuticals, formaldehyde resins, pesticides, textiles, petroleum refineries, chemical industry and agricultural activities. Phenol is one of 129 chemical compounds considered important pollutants listed by the Agency for Environmental Protection (EPA) [1,2].

The combination of electrochemical and biological methods can add value to the degradation of toxic products such as phenol. Microbial electrodes have the advantage of combining a highly efficient biological method for the degradation of phenol, biodegradation, and electrochemical methods that have proven effective at oxidizing phenol [3]. Microbial electrodes have generated a lot of interest in recent decades. They have been introduced in several biotechnological processes, starting with the production of electrical energy from organic waste (bacterial fuel cells) [4], also used effectively in organic and pharmaceutical electro synthesis [5]. The microbial electrodes can act as electron acceptors or electron donors depending on the reaction envisaged.

In the present work, a new electrochemical biosensor using bacterial electrode was developed for the oxidation and the detection of phenol. The bacteria were immobilized on the phosphate matrix.

Experimental

Reagents and apparatus

Electrochemical measurements were performed using a Volta lab potentiostat (model PGSTAT 100, Eco Chemie B.V., Utrecht, The Netherlands) driven by the general purpose electrochemical systems data processing software (Volta lab master 4 software) run under windows 2007. The three electrode system consisted of a chemically modified carbon paste electrode as the working electrode a saturated calomel electrode (SCE) serving as reference electrode, and platinum as an auxiliary electrode. Phenol solution: was purchased from Merck. Potassium Chloride (KCl), Hydrochloric acid and sodium hydroxide were purchased from Aldrich (Milwaukee, USA)

The natural phosphate samples were obtained from CERPHOS, Morocco.

Bacterial cultivation

The bacterial strain used in this study was *Staphylococcus aureus* ATCC 25923. The strain was cultured in Luria Burtani broth at 37°C for 24 h after culture; the cells were harvested by centrifugation for 15 min at 8400 xg and were washed twice with and resuspended in a KNO_3 solution of 0.1 M ionic strength [6]. Oxygen was removed by sparging the nitrogen for 10 minutes. The suspension of resuspended bacteria was diluted with distilled water to obtain the necessary suspension of different concentrations before use.

Electrodes preparation

The working carbon paste electrode was prepared by mixing appropriate weight of natural phosphate (NP) with a graphite powder (CP) to give an appropriate ratio NP-CP. The whole cell modified carbon paste was subsequently packed firmly into the PTFE cylindrical tube electrode cavity (0.1256 cm^2) and polished to a smooth shiny finish by gently rubbing over an ordinary weighing paper. Electrical contact was established with a bar of carbon. The modified electrodes were immersed in a cell containing 2 ml of caprolactone monomer and a quantity of the bacterium for 15 minutes. The resulting electrode is hereby denoted as bacteria-NP-CPE.

Procedure

In a first step, the electrode developed is characterized in an electrolytic medium 0.1 M NaCl, and then tested for the electro oxidation of phenol, added in the measurement cell. The mixture solution was kept for 20 seconds at open circuit and deoxygenated by bubbling pure nitrogen gas prior to each electrochemical measurement. The cyclic voltammetry was recorded in the range from -2 V to 2 V. Optimum conditions were established by measuring the peak currents in dependence on all parameters. All experiments were carried out under ambient temperature. The electro polymerization of monomer is *in situ*.

Provisions were taken for deoxygenation by splashing the solution with nitrogen gas during approximately 5 min. In order to obtain

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Received March 02, 2018; **Accepted** March 20, 2018; **Published** March 26, 2018

Citation: Maallah R, Chtaini A (2018) Bacterial Electrode for the Oxidation and Detection of Phenol. Pharm Anal Acta 9: 580. doi: 10.4172/2153-2435.1000580

reliable and reproducible results, a new electrolyte was prepared for each handling.

Results and Discussion

Natural phosphate characteristics

The composition of the natural phosphate used as a modifier was analyzed, respectively, by scanning electron microscopy, XRD and IR [7] (Figures 1-3). These results show that the NP used has the composition of fluoro apatite. Indeed, in a natural environment, all the calcium phosphates will evolve towards the apatite structure [8].

Preparation of bacteria-polymer-NP-CPE

The developed electrodes are based on phosphate and graphite carbon powders, which are susceptible to dissolution, to remedy this problem we propose to cover them with a polymer developed *in situ*, from ε-caprolactone. Figure 4 represents a series of voltammograms obtained during the electropolymerization of ε-caprolactone on a graphite carbon electrode modified with natural phosphate (CPE-NP), recorded with a scanning rate of 80 mV / S, in the NaCl solution (0.1 mol / l) containing 2 ml -caprolactone, in a potential range between -2 V and 2 V.

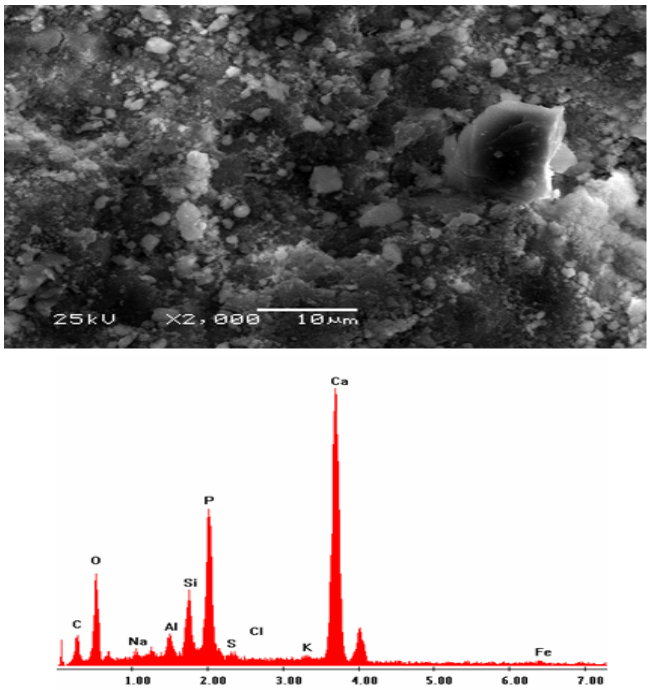

Figure 1: Scanning electron micrograph of natural phosphate.

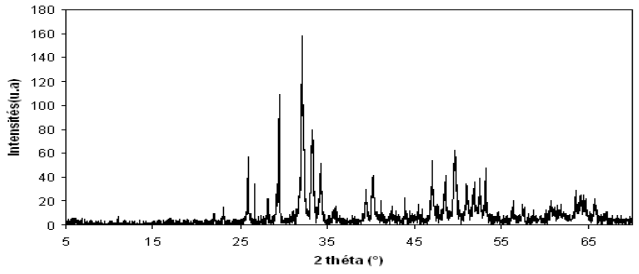

Figure 2: XRD pattern of the natural phosphate.

It can be seen that the presence of an anodic peak at about 0.34 V (2.34 mA/cm^2). This peak is attributed to the oxidation of the monomer (ε-caprolactone). The decrease in the intensity of the currents during the sweeps accounts for the growth of the film deposited on the surface of the electrode with the number of cycles, the densities Voltammogram currents tend towards 0 mA/cm^2 indicating that the developed polymer has a non-conducting character [9].

The voltammograms recorded for the two electrodes, in an electrolytic medium, are different, which suggests that the CPE-NP electrode is well modified by the polycaprolactone film.

Immobilization of bacteria on polymer

The graphite carbon electrode modified with natural phosphate (NP-CPE) is polymerized according to the previous procedures. The electrode obtained (polymer-NP-CPE) is immersed in a solution containing a suspension of bacteria; the contact time is 15 min.

Figure 5 show the cyclic voltammograms recorded respectively for the electrodes, polymer-NP-CPE and bacteria-polymer-NP-CPE, in electrolytic medium NaCl (0.1M) at a scanning rate of 100 mV/s.

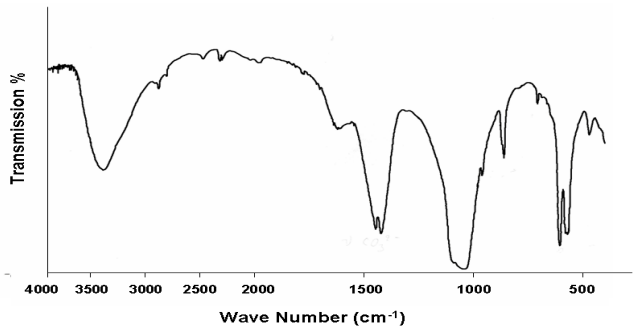

Figure 3: IR spectra of natural phosphate.

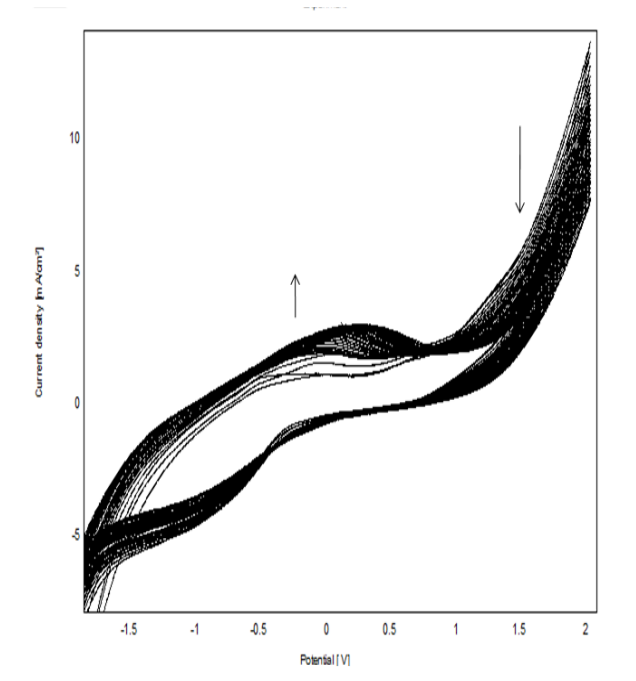

Figure 4: Voltammogram of electropolymerization of ε-caprolactone in electrolyte of 0.1 mol NaCl at pH=7, on CPE-NP with a scanning speed of 80 mV /.

We can notice that the presence of microorganisms on the surface of the polymer-NP-CPE electrode leads to the increase of the current densities, in particular those of growth of the peak corresponds to the oxidation of the polymer [10]. Probably, because of the improved conductivity of the electrode surface.

Electro oxidation of phenol at bacteria-polymer-NP-CPE

As can be seen in Figure 6, in absence of phenol, no peak is observed on the Voltammogram (a). On the other hand, the presence of phenol in the electrolytic medium leads to the appearance of two anodic peaks attributed to the oxidation of phenol, the first at about 0.15 V and the second towards 0.96 V.

Scan rate effect

The influence of scan rate on the oxidation peak potentials (Ep) and peak current (Ip) of phenol, were studied by CV (Figure 7). The current density, of phenol oxidation peak, increase considerably with increasing scan rate [11]. The Figure 8 shows the linear relationship between the scan rates and the current density of anodic peak (P2), suggesting that the oxidation of phenol at bacteria-polymer –NP -CPE is adsorption controlled reaction (Figures 7 and 8).

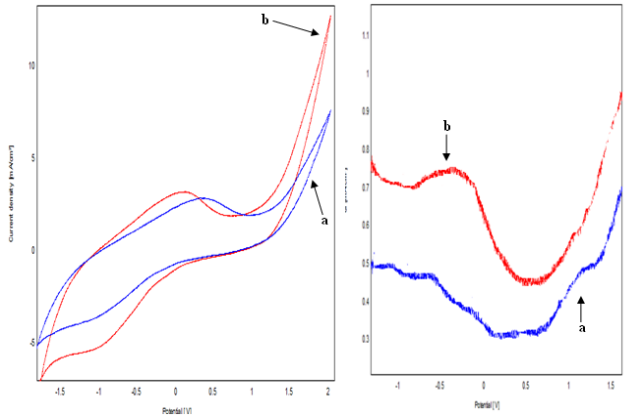

Figure 5: Cyclic voltammograms obtained by CPE-NP-polymer (a) and CPE-NP-polymer-bacteria (b) in 0.1M NaCl (pH = 7) with a scanning rate of 100 mV / S.

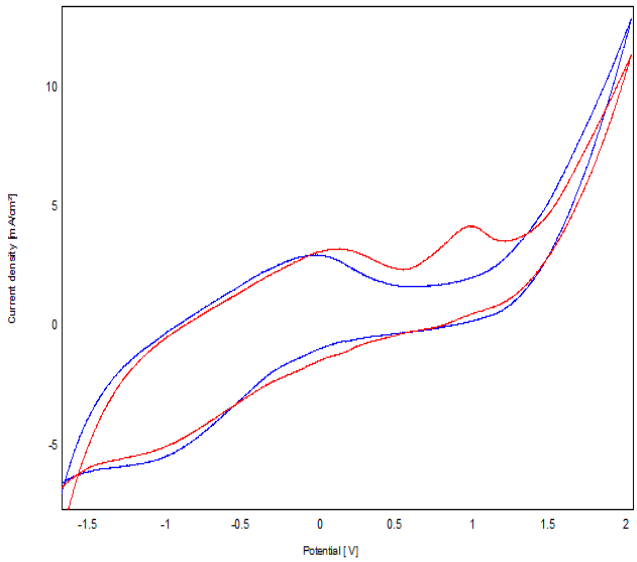

Figure 6: Cyclic voltammograms obtained by CPE-NP-polymer-bacteria in the absence (a) and in the presence (b) of 4 mM phenol in 0.1 M NaCl (pH = 7) with a scanning rate.

Calibration graph

The variation of the anodic peak intensity as a function of phenol concentration was followed by cyclic voltammetry (Figure 9), and impedance spectroscopy (EIS) (Figure 10). Peak densities increase with the concentration of phenol, which shows that the new surface of the electrode has a large number of active sites [12]. The Figures 11 and 12 shows the linear relationship between the concentration and the current density of anodic peaks.

This result is confirmed by the impedance diagrams, which show high-frequency semicircle curves attributed to the transfer of electrons (Rt), the diameter of these semicircles corresponds to the electron transfer resistance. The increase in phenol concentration lowers the value of Rt.

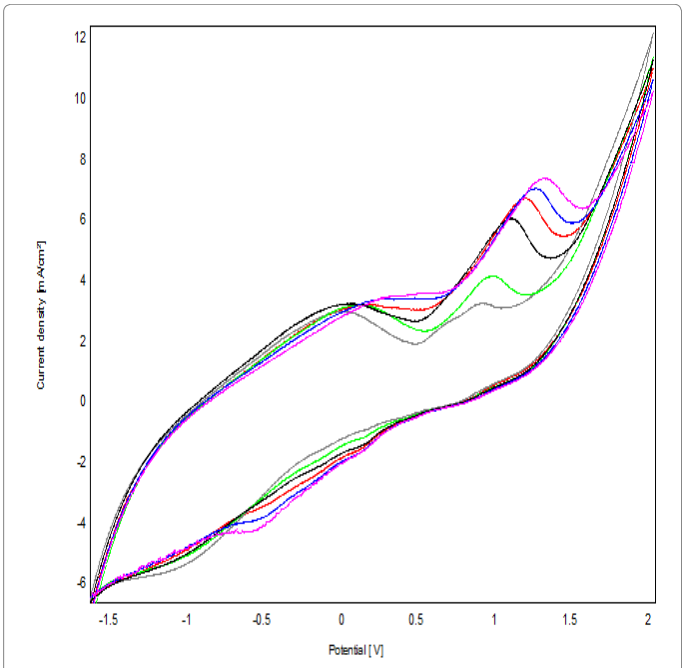

Figure 7: Influence of the scanning rate on the intensity of phenol oxidation reduction peaks for CPE-PN-polymer-bacteria.

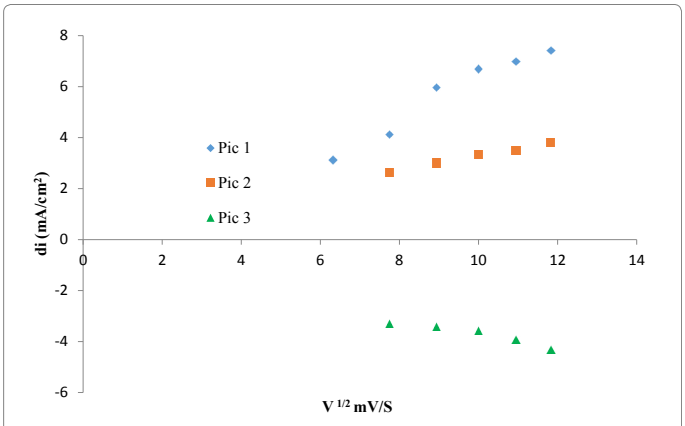

Figure 8: Influence of the scanning rate on the intensity of phenol oxidation peaks for CPE-PN-polymer-bacteria.

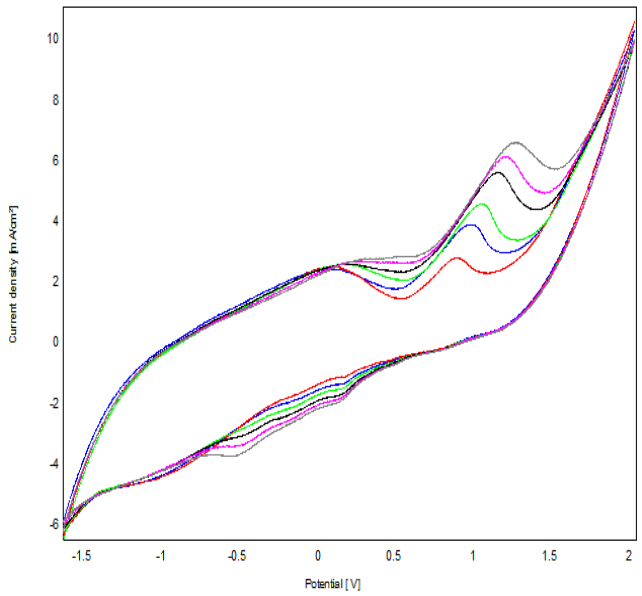

Figure 9: Cyclic voltammograms at different concentrations of phenol (from 2 mM to 12 mM) in 0.1 M NaCl (pH = 7) on CPE-NP-polymer-bacteria, V = 100 mV.S^{-1}.

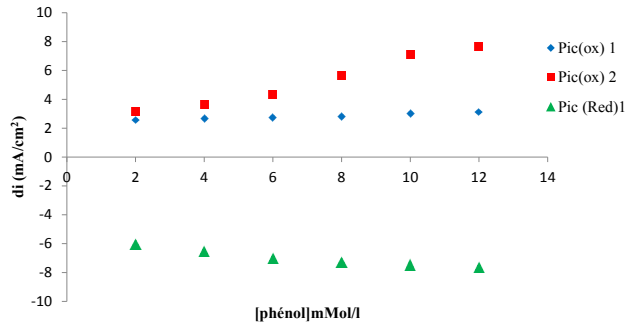

Figure 10: Effect of concentration on the intensity of the redox peaks of phenol for CPE-PN-polymer-bacteria.

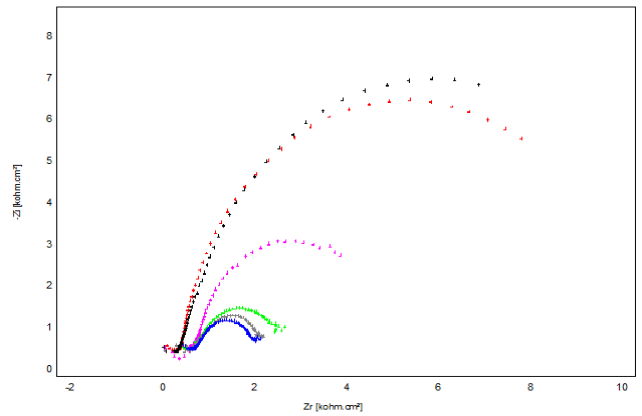

Figure 11: Impedance diagram at different concentrations of phenol (from 0 to 10 mM) in 0.1 M NaCl (pH ~ 7) on bacteria-polymer-NP-CPE, V = 100 mV.S^{-1}.

The activity of the immobilized bacteria

The calibration curves were plotted respectively for the polymer-NP-CPE and bacteria-polymer-NP-CPE electrodes, from the square-wave voltammograms (Figure 13). These experiments are carried out in the concentration range, which varies between 4 and 12 mmol/L (Figure 14). Table 1 group together the correlation equations and the detection and quantification limits calculated, for the two CPE-NP-polymer and CPE-NP-polymer-bacteria electrodes. We find that the presence of bacteria improves the sensitivity of the electrodes (Figure 15) [13].

The bacterial activity immobilized on the polymer-NP-CPE electrode surface calculated for the oxidation of phenol is :

$$\alpha = (1 - \frac{Ibact}{I}) \times 100 = 59.18\%$$

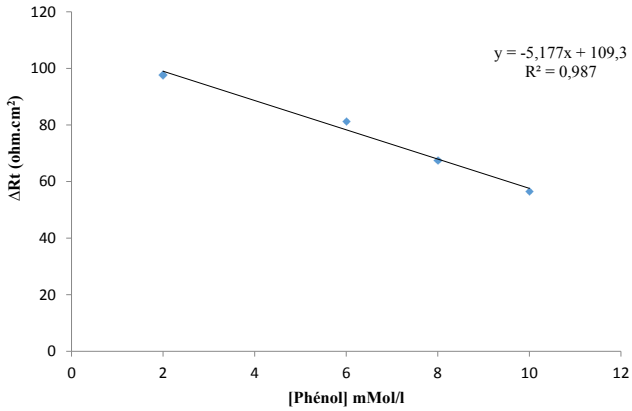

Figure 12: Evolution of the charge transfer resistance as a function of phenol concentration.

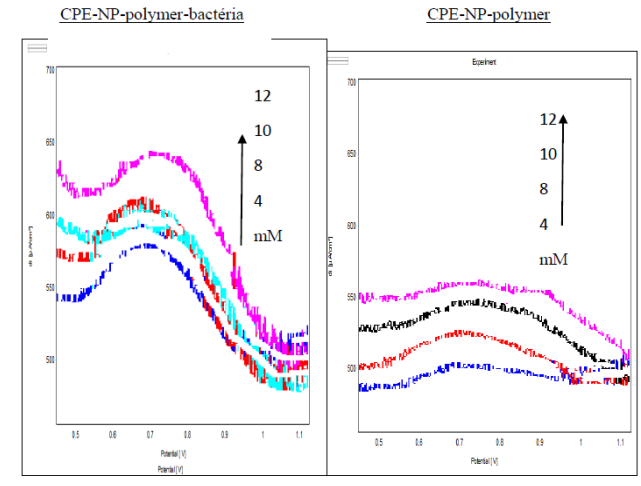

Figure 13: Influence of the concentration of phenol on the intensity of an oxidation peak obtained by recorded by the electrodes CPE-NP-polymer and CPE-NP-polymer -bacterium.

Characterization by DRX:

The compositional analysis of the elaborated electrode surface was performed by DRX, The results obtained, respectively, for the natural phosphate modified carbon paste electrode (NP-CPE) (Figure 16) and for polymer coating natural phosphate modified carbon paste electrode (polymer-NP-CPE) Figure 17, show that the surface composition is mainly composed of the composition of the surfaces consists mainly of graphite carbon analysis, fluoroapatite in addistion of the poly carbolactone in case of polymer-NP-CPE.

Morphological characterization

The surface of the polymer-NP-CPE electrode, after detection of phenol was observed by optical microscopy. The presence of the polymer on the surface of the electrode increases its porosity and therefore facilitates the adsorption of phenol in the phosphate-polymer matrix (Figure 18).

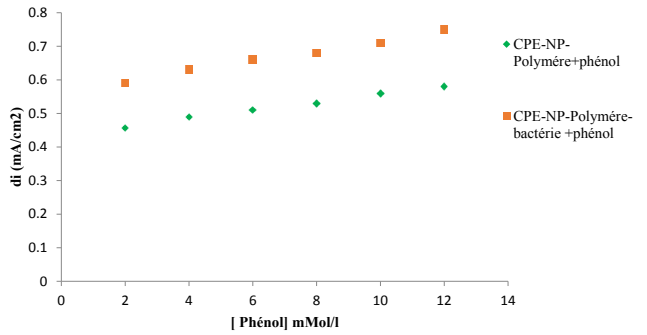

Figure 14: Activity of the two elaborate bioelectrodes CPE-NP-Polymer-Bacteria and CPE-NP-Polymer-Bacteria for Biodegradation of Phenol.

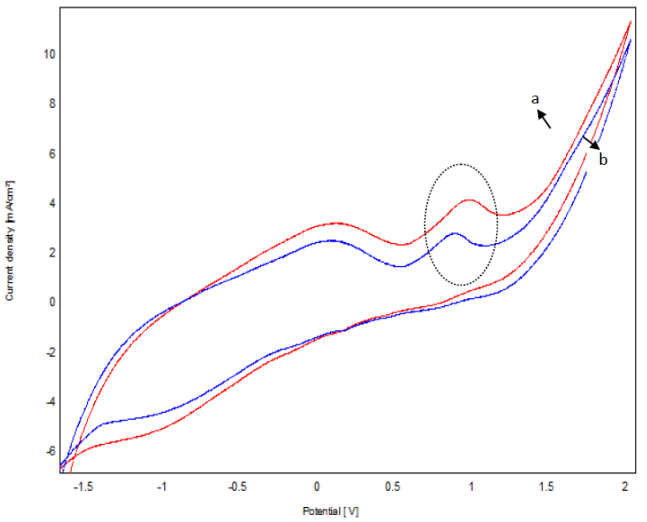

Figure 15: Cyclic voltammograms obtained by CPE-NP-polymer-bacteria (a) and CPE-NP-polymer (b) in the presence of 6 mM phenol in 0.1 M NaCl (pH = 7) with a scanning speed 100 mV / S.

Electrode	Equation	R^2	D.L ($mol.L^{-1}$)	Q.L ($mol.L^{-1}$)
CPE-NP-Polymer+Phenol	di = 0,015x+0,564	R^2 = 0,990	$2.762.10^{-6}$	$4.561.10^{-5}$
CPE-NP-Polymer-bacteria+phenol	di = 0,012x+0,436	R^2 = 0,993	$5.009.10^{-8}$	$3.962.10^{-7}$

Table 1: The correlation equations off the limits of detection and quantification, obtained by the CPE-NP-polymer and CPE-NP-polymer-bacteria electrode.

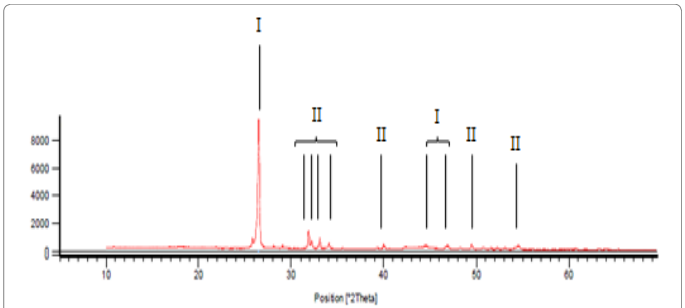

Figure 16: The DRX spectra recorded by the surface of the CPE-NP electrode.

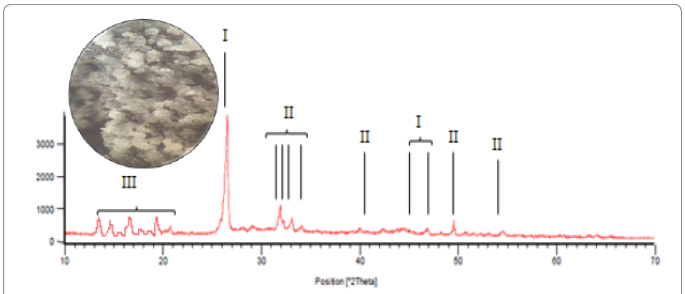

Figure 17: The XRD spectra recorded by the surface of the CPE-NP-Polymer-phenol electrode.

Peaks I: correspond the graphite carbon
Peak II: correspond to the structure of fluoroapatite of natural phosphate
Peak III: correspond the overlap of phenol and polycaprolactone peaks

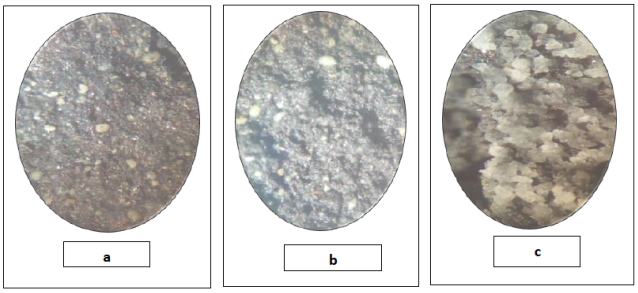

Figure 18: Natural phosphate- carbon paste electrode (a), after modification by polymer (b) and after detection of phenol (c).

Conclusion

In this work, we have proposed a microbial electrode for the oxidation of phenol. This electrode is based on natural phosphate and graphite carbon powders coated with a polymer that has made it possible to protect the surface of the electrode against dissolution. The presence of the polymer at the surface has resulted in the loss of electrode conductivity. Subsequently we modified this electrode by microorganisms deposited on the surface by self assembly; the pre-concentration time is 15 minutes. The presence of bacteria on the surface of the electrode to improve the activity of the electrode with respect to the oxidation of phenol.

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