CHEMICAL ENGINEERING TRANSACTIONS
VOL. 38, 2014
A publication of AIDIC
The Italian Association of Chemical Engineering
www.aidic.it/cet
Guest Editors: Enrico Bardone, Marco Bravi, Taj Keshavarz

ISBN 978-88-95608-29-7; **ISSN** 2283-9216
DOI: 10.3303/CET1438074

Application and Construction of a Biosensor Using Graphite Rod and Bean, Phaseolus Vulgaris L., for Phenol Detection

Francisca das C. S. S. Mihos, Lívia M.C. Silva, Andrea M. Salgado, Mariana R. Maggesissi

Laboratory of Biological Sensors E-122, Biochemical Engineering Department, Chemistry School, Technology Center, Federal University of Rio de Janeiro – Avenida Horácio Macedo, 2030 – Cidade Universitária - Ilha do Fundão – 21949-909 – Rio de Janeiro, RJ, Brasil
franciscasobral@ufrj.br

This paper presents the possibility of fabrication and assembly of an amperometric biosensor for the phenol detection. The sensor was designed by immobilizing the bean powder containing peroxidase enzyme, with graphite rod as a conductor. Using the peroxidase activity, phenol can be oxidation in presence of hydrogen peroxide. The performance of the biosensor to phenol detection was based on the electron transfer detected by cyclic voltammetry. The proposed biosensor has a very sensitive response to phenol compounds, an applied potential of -0.2 to +0.8 mV *vs* Ag/AgCl. The manufacturing process parameters were optimized for the electrode. Experimental conditions that influenced the biosensor performance, such as pH, were investigated and evaluated. The response time of the biosensor was about 10 seconds.

1. Introduction

The presence of phenolic compounds in the environment has been an issue in different countries (Geiger et al. 2010). These compounds are present in pesticides that are used in pest control in diverse crops (Odukkathil & Vasudevan 2013). The indiscriminate use of these compounds can cause serious damage to health and to the environment, therefore the need for a strict control over the use of phenol and its derivatives (Silva et al. 2011). Many scientific studies have been developed to obtain methods capable of detecting these pollutant compounds such as phenolic derivatives present in pesticides. Biosensors are useful for this purpose because they have important features as: automation, miniaturization, selectivity, sensitivity and speed of analysis (Zhang et al. 2014). These devices are capable of performing “in situ” detection besides being a low-cost technique compared with chromatographic methods (Zhou et al. 2006). This paper presents the development of a biosensor for easy construction from a cheap and abundant raw materials such as beans (*Phaseolus vulgaris L.*). This study is in early stage of development, considering that the beans used has not suffered any pre-purification treatment, only the bean fabric and its extract were used as a biological component, being able to identify the phenol in the presence of a solution of known concentration, which demonstrates its applicability for development of a biosensor.

2. Materials and methods

2.1. Obtaining the enzymatic bean extract (*Phaseolus vulgaris L.*)

Bean powder was obtained by milling the grains at a blender and sieving in the first crushing, the powders obtained from the bean hulls were discarded. The disposal was made due to the presence of phenolic compounds in the seed coat, which may reduce the activity of the enzyme (Fatibello-Filho 2002). 50 g of the powder obtained was homogenized in a blender with 150 ml of phosphate buffer 0.2 mol L^{-1} (pH 6.0). Then, the homogenate solution was filtered through four layers of cheesecloth and centrifuged at 4000 rpm for 30 minutes at 4 ° C.

Please cite this article as: Mihos F., Silva L., Salgado A., Maggesissi M.R., 2014, Application and construction of a biosensor using graphite rod and bean, phaseolus vulgaris l., for phenol detection, Chemical Engineering Transactions, 38, 439-444
DOI: 10.3303/CET1438074

2.2. Determination of enzyme activity

The activity of the enzyme extract was determined using guaiacol as substrate. The substrate solution was prepared by solubilizing 2.5 g of guaiacol in phosphate buffer 0.2 mol.L^{-1} (pH 6.0). 1.0 ml of the enzyme extract was diluted ten times in phosphate buffer solution (0.2 mol.L^{-1}).

At a test tube were added 2.0 mL of guaiacol, 0.5 ml of diluted enzyme extract and 0.5 ml of hydrogen peroxide (3% v/v) at 25 ° C. The solution was homogenized and the absorbance was read at 470 nm for visible spectrometer (UV / VIS) (Jiang et al. 2011). Before the reading, control tests (blanks) of substrate and enzyme were performed to verify that they did not absorb the same wavelength of the product formed (tetraguaiacol). The enzymatic activity was calculated by equation 1.

$$U/mL = \frac{Abs1 - Abs2 \times 1000 \times DF}{T1\text{-}T2 \times V} \quad (1)$$

Being U/mL: Each enzyme activity and Abs1, Abs2 - initial and final absorbance; DF - Dilution Factor T1 and T2 - initial and final time (sec) and V - Volume of the sample (mL).

One unit of the peroxidase enzyme activity was regarded as the amount of enzyme that catalyzes the oxidation of guaiacol (1.0 mol) causing an increase of 0.001 absorbance unit per minute of reaction, under the test conditions (Esteves et al. 1999). The specific activity (U/mg protein) was calculated as the ratio of enzyme (U/mL^{-1}) activity and total protein content (mg/mL^{-1}).

2.3. Determination of total enzyme protein by Bradford method

A solution was prepared with BSA (bovine serum albumin) at 1.25 mg/mL and dilutions were made from this solution in different concentrations. 0.1 mL was removed and the standard solutions and were added to test tubes with 3 mL of Coomassie Brilliant Blue G-250 reagent. After 10 minutes, the readings were done using UV-visible spectrophotometer at 595 nm. The analysis was performed in duplicate.

2.4. Construction of the electrode containing peroxidase enzyme

The working electrode was designed to be easy to handle and transport, besides being economically viable. The device was built using a PVC support (polyvinyl chloride) by making a hole of diameter graphite rod, as is schematized in Figure 1.

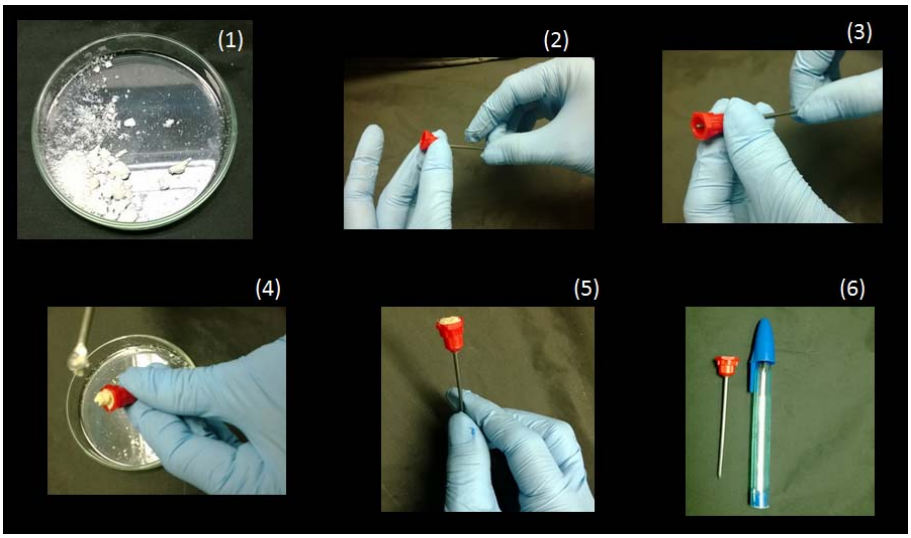

Figure 1: Construction of an amperometric biosensor using powdered bean (Phaseolus vulgaris L.) for phenol identification. (1) 0.5 g of the powdered pulp obtained from the beans support (2) PVC support, (3) graphite rod fitting and addition of bean paste powder, (4) finishing assembly and (6) size of 9.0 cm and 2.0 mm diameter device.

For electrode the construction, was weighed 0.5 g of bean powder and added to 200 µl of a glutaraldehyde solution (1%). This solution was prepared by solubilizing 40 µl of glutaraldehyde (25%) in 960 mL of phosphate buffer solution 0.2 mol L^{-1}, pH 6.0. After homogenization, the slurry was added in PVC support

containing graphite rod previously sanded with sandpaper. The working electrode was stored at refrigerator until the use.

2.5. Construction of the electrochemical system

Electrochemical measurements were obtained using a potentiostat/galvanostat (Autolab PGSTAT 302). The system was assembled on an electrochemical vat using three electrodes: the working electrode (with the immobilized enzyme), silver silver chloride (Ag|AgCl (KClsat)) as reference electrode and a platinum auxiliary electrode.

3. Results and Discussion

3.1. Enzyme activity and protein content

Many scientific papers have specific ways to evaluate enzyme activity without some important factors, in which readers may mistakenly play in their experiments, such as the enzyme/substrate proportion. Preliminary study of the variation of substrate relative concentration to the enzyme is of great importance as it can avoid the saturation of the active site of the enzyme, resulting in loss or impairment of the enzymatic activity. To evaluate the behavior of the enzyme peroxidase activity, the guaiacol (substrate) concentration was ranged as: 2.5, 2.0, 1.5 and 1.0 g/mL by diluting the extract ten times in phosphate buffer solution (0.2 mol.L^{-1}). The results are shown in Figure 2.

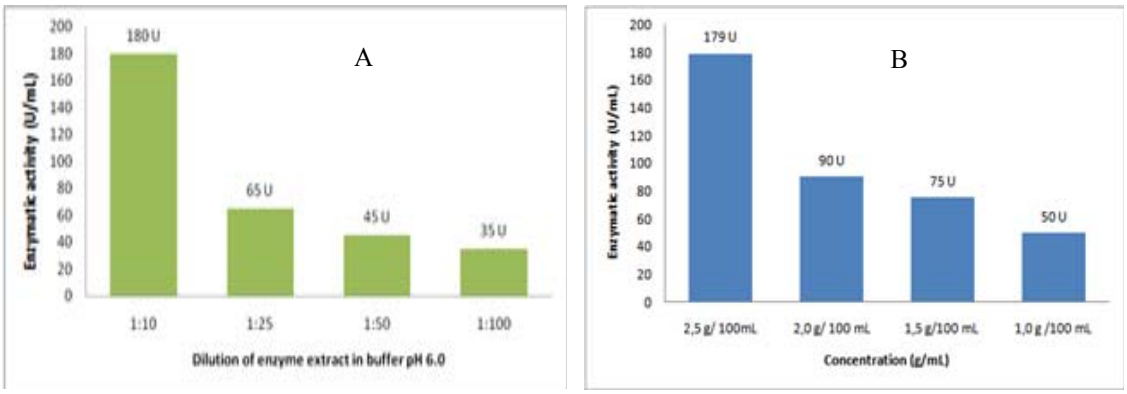

Figure 2: A - Enzymatic activity obtained ranging the enzyme concentration in the reaction medium. B - Enzyme activity at different guaiacol concentrations.

The results presented in Figure 2 show that increasing the guaiacol concentration is possible to obtain better monitoring of the product formed in the tetraguaiacol reaction, when using crude enzyme extract diluted ten times the latter being more available to the enzyme active site and not causing posterior saturation.

Thereby, the conditions, for monitoring the enzymatic activity of the crude extract used in the construction of the biosensor, were standardized at the following conditions: 2.5 g/100 mL of the substrate to extract diluted ten times. From the calibration curve constructed with a solution of bovine serum albumin, was possible to determine the concentration of 11.22 mg/mL this protein in the enzyme extract, by the equation of the standard curve: y = 0.693x – 0.020 (R^2 = 0.996). The concentrations of the solutions for calibration curve construction were: 0.25, 0.50, 1.0, and 1.25 mg/mL

3.2. Response of the biosensor using the enzyme extract containing peroxidase

Initially, tests were performed on white only with the addition of sodium phosphate buffer solution (0.2 mol.L^{-1}, pH 6.0), and 30 ml of standard solution of phenol (5ppm), without the presence of H_2O_2 as shown in Figure 3A. The range of potential was +0.8 to -0.2 V and scan rate of 50 mV/s. It is observed in Figure 3B, that in presence of hydrogen peroxide, natural peroxidase substrate, unchain a reaction, where an oxygen molecule in the H_2O_2 oxidizes the enzyme active site, which is followed by reduced phenol. The chain-reduction potential of the processes on the graphite rod surface (working electrode) enzyme voltammetric profile shows a cathodic current I_{pc} = 4,0x10^{-5} potential close to 0.79 V. The experiment was

repeated under the same conditions using 0.5 g powder immobilized in PVC support (bean) containing the graphite rod in Figure 4A.

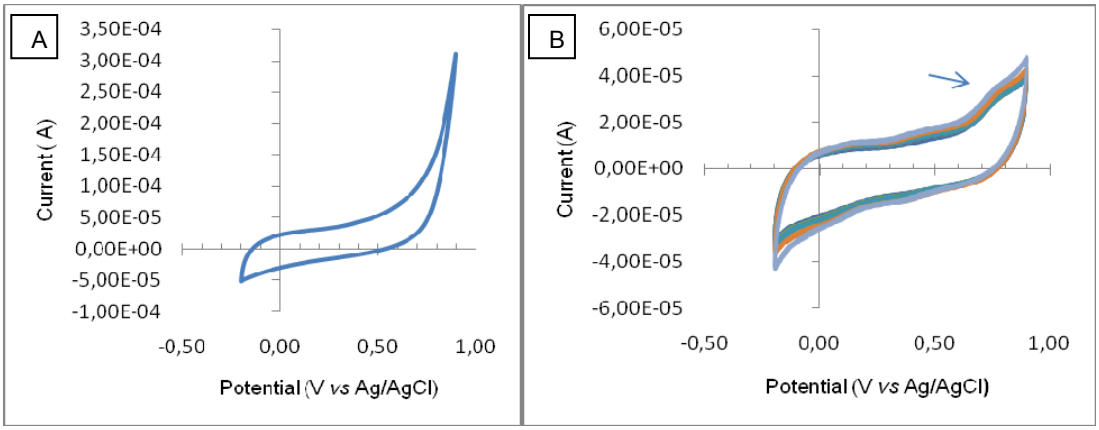

Figure 3: A - Blank test without the addition of hydrogen peroxide. B - Repeated voltammograms for the biosensor in the presence of 5 ppm phenol solution with the addition of 5 ml of 20 mM H_2O_2.

It is observed in Figure 3B, that in presence of hydrogen peroxide, natural peroxidase substrate, unchain a reaction, where an oxygen molecule in the H_2O_2 oxidizes the enzyme active site, which is followed by reduced phenol. The chain-reduction potential of the processes on the graphite rod surface (working electrode) enzyme voltammetric profile shows a cathodic current I_{pc} = 4,0x10^{-5} potential close to 0.79 V. The experiment was repeated under the same conditions using 0.5 g powder immobilized in PVC support (bean) containing the graphite rod in Figure 4A.

The biosensor containing immobilized bean tissue with 1% glutaraldehyde at pH 6.0, Figure 4A, showed similar response to the device containing the enzyme extract under the same conditions, Figure 3B. In these experiments, tests were conducted initially with blank phosphate buffer solution (0. 2molL^{-1}). One can observe that no current peak is related to the enzyme reduction. The experiments containing bean powder were also performed in phosphate buffer solution (0.2 mol L^{-1}) at pH 7.0, in the presence of 30 ml of phenol solution (5 ppm) and 5 mL of H_2O_2 (20 mM). At this pH has been observed that a reduction potential in the range of 0-0.4V, 7V, according to literature (Rosatto et al. 2001). For comparison, the commercial enzyme horseradish peroxidase (HRP) was used, with reduction potential close to 0.8 V, being close to that obtained with the enzyme extracted from beans, *Phaseolus vulgaris L*.

4. Conclusion

The proposed biosensor showed a positive response to phenol identification and can be further used for this purpose. It is important to highlight that the bean fabric can be used only after characterization and quantification of the peroxidase enzyme present, through processes of pre-purification and lyophilization. The biosensor kept its characteristics, phenol oxidation in the same pH 7.0. Such tests have been proposed for the development of a biosensor capable of quantifying real samples containing phenol through a calibration curve.

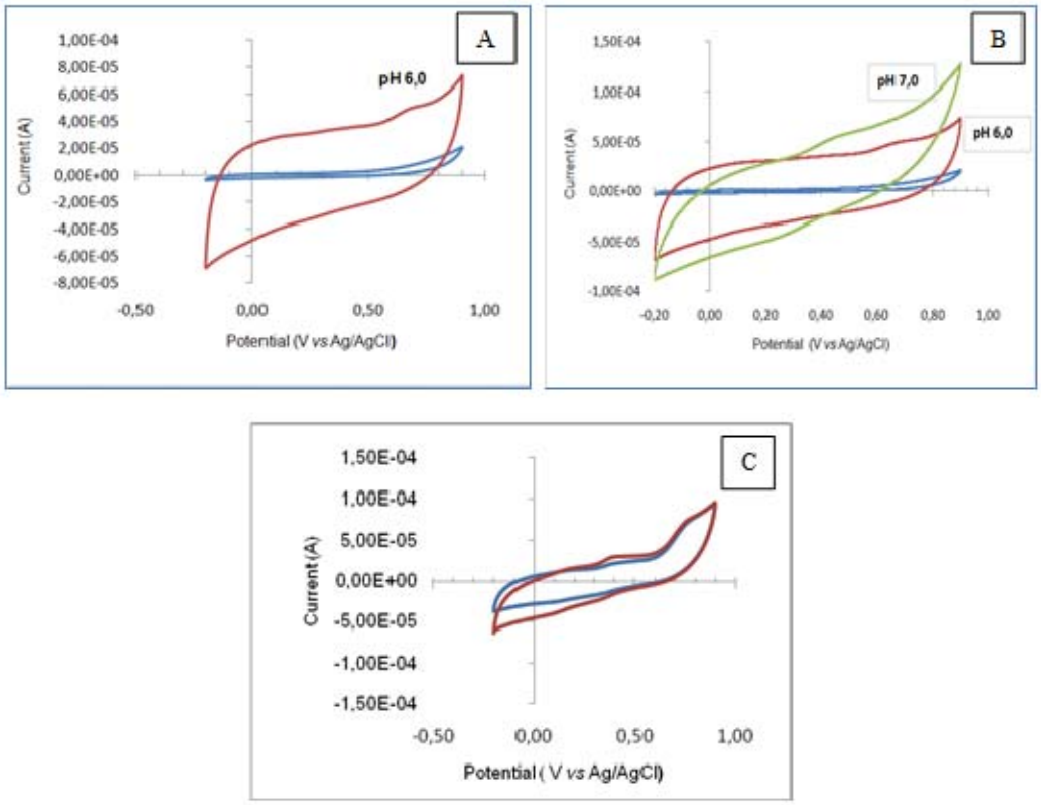

Figura 4: 4A - Voltammogram obtained with immobilized bean tissue in the graphite rod; 4B - Voltammogram obtained with immobilized bean tissue graphite rod with pH 6.0 and pH 7.0; 4C - Voltammogram obtained with 180 U/mL enzyme solution from commercial enzyme horseradish peroxidase (HRP) immobilized on the graphite rod.

Acknowledgements:

The authors thank the financial support of the National Council for Scientific and Technological Development (CNPq), and National Institute of Metrology, Quality and Technology (INMETRO).

References

Esteves, A.N.A.M. et al., 1999. chemical and enzimatic comparision of six bean lineagens (Phaseolus vulgaris L .). , 26(5), pp.999–1005.

Fatibello-Filho, 2002. “Analytical Use of tissues and plant crude extracts as enzyme source.” New Chemistry. , 25(3), pp.455–464.

Geiger, F. et al., 2010. Persistent negative effects of pesticides on biodiversity and biological control potential on European farmland. *Basic and Applied Ecology*, 11(2), pp.97–105.

Jiang, J. et al., 2011. UV – visible spectroscopic properties of dissolved fulvic acids extracted from salined fl uvo-aquic soils in the Hetao Irrigation. , pp.670–679.

Odukkathil, G. & Vasudevan, N., 2013. Toxicity and bioremediation of pesticides in agricultural soil. *Reviews in Environmental Science and Bio/Technology*, 12(4), pp.421–444. Available at: http://link.springer.com/10.1007/s11157-013-9320-4 [Accessed February 10, 2014].

Rosatto, S.S. et al., 2001. Amperometric biosensors for phenolic compounds determination in the environmental interess samples. , 24(1), pp.77–86.

Silva, L.M. da C., Salgado, andréa M. & Coelho, M. alice Z., 2011. Amperometric Biosensor for Phenol Determination. *Chemical Engineering Transactions*, 24, pp.1249–1254.

Zhang, W. et al., 2014. Nanomaterial-based biosensors for environmental and biological monitoring of organophosphorus pesticides and nerve agents. *TrAC Trends in Analytical Chemistry*, 54, pp.1–10.

Zhou, Q. et al., 2006. Determination of atrazine and simazine in environmental water samples using multiwalled carbon nanotubes as the adsorbents for preconcentration prior to high performance liquid chromatography with diode array detector. *Talanta*, 68(4), pp.1309–15. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18970465 [Accessed November 22, 2013].