CHEMICAL ENGINEERING TRANSACTIONS Volume 24, 2011
Editor Sauro Pierucci
Copyright © 2011, AIDIC Servizi S.r.l., ISBN 978-88-95608-15-0 ISSN 1974-9791
DOI: 10.3303/CET1124209

Amperometric Biosensor for Phenol Determination

Lívia Maria da Costa Silva[1], Andrea Medeiros Salgado[1*], Maria Alice Zarur Coelho[2]

[1]Laboratory of Biological Sensors, EQ/UFRJ
[2]BIOSE, EQ;UFRJ
Avenida Horácio Macedo, 2030 – Technology Center, Chemistry School (Block E),
Departament of Biochemical Engineering (DEB), Cidade Universitária, cep: 21949-909.
andrea@eq.ufrj.br .

The measurement of the quantities of phenols present in many industrial effluents is quite important due to the high toxic pollutant impact in the environment. Usually the analyses of the phenolic species have been carried out by the chromatographic and spectrophotometric methodology. The present work is, indeed, an effort to develop an analitical method, which intends to demonstrate that a continuous monitoring of phenols is achievable by using the mushroom tissue of *Agaricus bisporus* through the activity feature of the tyrosinase. Meanwhile the oxygen electrode would operate as a transducer. The experimental methodology adopted in the development in this work aimed to construct the biosensor. The linear relationship between the variation of dissolved oxygen and concentration of phenol in aqueous solution was investigated. For the calibration of the instrument, studying the time of reaction and amount of tissue needed to promote reliable answers and with less error from the analysis. The possibility of the reuse of biocomponent and the influence of saturation of the solution with air before reading were also studied. During the preparation of a biosensor system, results showed that 5g of mushroom tissue during 1 minute reaction time were best biosensor response in range of phenol concentration 5-10ppm. The biocomponent did not be reuse in the analysis, however initial air saturation caused less variation among all of them.

1. Introduction

A considerable number of organic pollutants, which are found widely distributed in the environment, have phenolic structures. Phenols and their derivatives, are well known because of their high toxicity and are common compounds in industrial effluents, coming from the activities related to production of plastics, dyes, drugs, antioxidants, polymers, synthetic resins, pesticides, detergents, disinfectants, oil refinery and mainly pulp and paper. Phenols have been defined as hazardous pollutants due to their high toxicity and persistence in the environment, and are found in the list of hazardous substances and priority pollutants of the European Commission and the U.S. Environmental Protection Agency (EPA) (Rodriguez-Mozaz et al., 2006).
Until present days those compounds determination have been achieved mainly by spectrometric, chromatographic and fluorometric methods. These methods do not allow

Please cite this article as: Silva L.M.D.C., Salgado A.M. and Coelho M.A.Z., 2011, Amperometric biosensor for phenol determination, Chemical Engineering Transactions, 24, 1249-1254
DOI: 10.3303/CET1124209

easily continuous monitoring in situ due to the fact that they are expensive, quantification takes a long time and high skill operators are needed to achieve it.
In a view of global concern in environmental issues, considerable attention has been given to versatile detection systems to monitoring environmental pollutants. In other words, biosensors use new techniques to building sensors that mix high biological specificity and many possibilities of electronic circuits. These seem to be potential tools to supplement the techniques that are in use, due to some particular characteristics as selectivity, low build and storage cost, miniaturizing potential, easily to become automatic and apparatus are simple to be build and portable.
Biosensor works, usually involves specificity and high sensibility between target substrate and biological compound. Then, the product results from reactions between biological molecule and substrate promotes one or more variations in physicochemical parameters. These parameters became electrical signals that can be measured and processed by a suitable transducer.
In a search for economic and efficient biological components to be used in phenolic compounds biosensors, polyphenol oxidases, in particular, tyrosinase (EC 1.14.18.1) has been investigated in last years. Literature reported that many biosensors been developed using tyrosinase from *Agaricus bisporus* as biocomponent (Kochana et al., 2008; Zejli et al., 2008). Otherwise, this enzyme was not used naturally immobilized in mushroom tissue. The purpose of this work was to continue the work of Silva et al. (2010) in the development of an amperometric biosensor for phenol detection using in natura *Agaricus bisporus* mushroom tissue as tyrosinase source and oxygen electrode.

2. Material and Methods

2.1 Biosensor development: study of time reaction and biocomponent amount

Initial tests had the objective to choose the best mushroom tissue amount and enzymatic reaction time that are able to generate a variation on oxygen-dissolved concentration and it should have lower standard deviation within replicates. Assays were carried out at room temperature, in a 50mL of 10ppm phenol standard solution into a batch reactor. Sodium phosphate buffer (pH 8.0) was used and fungi tissue was shaped as 1cm cubes. First the electrode was stabilized and then the measurements was achieved and repeated 5 times. Three different amounts of mushroom tissue were used: 1, 3 and 5g and time reaction range was 1 to 30 minutes.

2.2 Calibration tests

Assays for construction of standard curve of amperometric biosensor used a range of 0 to 10ppm of phenol solution. Measurements were carried out by the variation of dissolved oxygen. The assay was repeated 5 times and was carried out at room temperature. The electrode was immersed in a 50mL of phenol standard solution into a batch reactor with 5g of mushroom tissue. After the stabilization of electrode, measurements were made along the range of phenol concentration, using the reaction time determined.

2.3 Stability of biocomponent used in biosensor development

These tests were to evaluate the fungi tissue re-uses into the biosensor system, so the biocomponent was used for 5 times. A transducer was put in a batch reactor with 50mL phenol solution in sodium phosphate buffer (pH 8.0) in a range of 0 to 5ppm and 5g of fungi tissue at room temperature. Measurements were taken after 1 minute reaction time.

2.4 Influence of initial air saturation on samples solutions

Assays were achieved to evaluate the initial air saturation influence on sample solutions in biosensor measurements. Before each biosensor measures, the sample solutions were air saturated by bubbling air into them. The objective of this procedure was that all samples would have the same initial oxygen concentration and avoid interferences on final measure of dissolved oxygen variation. Assays were carried out as reported before without the fungi tissue and in addition the air bubbling initial for 5 minutes.

3. Results and Discussion

3.1 Studies of best conditions of fungi tissue amount and reaction time for biosensor development

Table 1 shows some test results for best biocomponent amount and time reaction. Results are the average module value of 5 replies tests and their standard deviation in each condition tested (1, 3 and 5g of fungi tissue and reaction time range of 0 to 30 minutes).

Table 1: Some results for best biocomponent amount and reaction time.

Fungi tissue (g)	Time (minutes) Average module of O_2 variation					
	1	5	10	15	20	30
1	0.72±0.34	0.74±0.98	0.74±1.18	0.62±1.19	0.46±1.07	0.36±1.13
3	0.22±0.34	3.27±0.52	3.70±0.79	4.23±0.91	4.23±1.01	4.27±1.18
5	0.18±0.10	4.20±0.38	5.20±0.46	5.65±0.6	5.95±0.68	6.45±1.24

Results showed that minor standard deviations occurred in smaller reaction times in any amount of fungi tissue used. In addition, the increasing of reaction times leads to enhance of numerical value of dissolved oxygen variation. This can be explained by the fact that dissolved oxygen electrode has a membrane that separates the internal electrolyte and the electrodes (anode and cathode) from the external medium. Oxygen can pass through this membrane by diffusion until platinum cathode. A redox reaction occurs with four electrons, and it generates a current, proportional to oxygen concentration, in reaction media (Figure 1). Therefore, the higher substrate concentration in the sample and/or the higher reaction time, the higher oxygen dissolved variation will be detected.

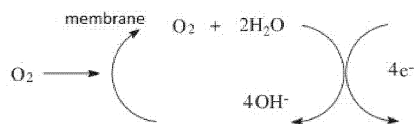

Figure 1: Scheme of operation mode of the oxygen electrode.

The results were analyzed and the system with 5g of fungi tissue and 1 to 3 minutes of reaction time showed the lesser deviation among 5 replications. Thus, to build an instrument with higher reliability, the combination 5g of fungi tissue and 1 minute of reaction was chosen for the following tests.

In literature, there are no studies using intact tissue of *Agaricus bisporus*, which is normally used as it homogenized and mixed with another compound for immobilization. Moreover, the tissue has also been used as a source of the enzyme tyrosinase extracted. Similar to the response time chosen in this work, Tomita et al. (2005) developed an amperometric biosensor for ascorbic acid, using oxygen electrode as transducer, where the best response time was 60 seconds and the settling time was also 60 seconds. And Campanella et al. (1993) using 3mg of tyrosinase placed under the oxygen electrode type Clark, obtained a response time of 2 minutes.

3.2 Study tests of influence of initial air saturation on samples solutions in oxygen electrode measurements

The initial condition (without air bubbling) don't promote a linear response in the range studied, then air bubbling into the samples solutions was achieved before measurement of initial time with biosensor and phenol concentration range was increased to 0-10ppm. Figure 2 shows the results obtained to observe the influence of this procedure in final measure of dissolved oxygen variation. The procedure used in the assay promoted the lesser variations and errors during the measurements in the phenol concentration range. Due to these observations it is possible that the following tests applying the bio-component after air bubbling should have more repeatability.

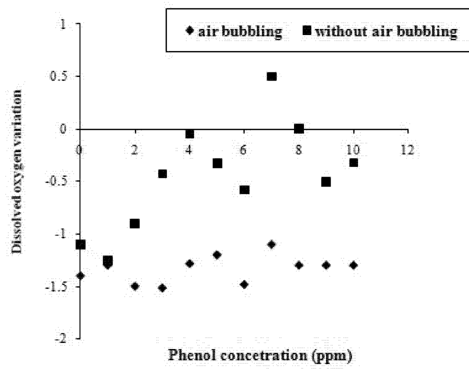

Figure 2: Tests comparing the influence of air bubbling before oxygen electrode measurements, when there isn't fungi tissue presence.

3.3 Studies of biosensor calibration

Figure 3 shows the calibration curve and it was drawn using a group of experimental data obtained from a value of transducer (oxygen electrode) exit signal that is a response of a value of entrance signal (phenol concentration range of 0 to 10ppm). The objective of this study was comparing if the analytical method had the same linear range of colorimetric method (Apha, 1992). A linear response would indicate a direct relation between input (phenol concentration) and output (oxygen electrode signal).

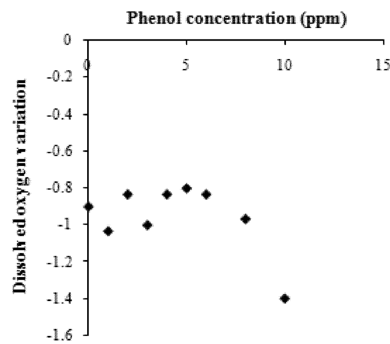

Figure 3: Results of preliminary analysis for biosensor's calibration using 5g of fungi tissue (1cm); room temperature; pH 8.0 and air bubbling before oxygen electrode measurements for 1 minute of reaction.

However, data repeatability using the same fungi tissue (1cm) under conditions selected during this work wasn´t observed, but the tests showed that there was response in the range of phenol concentration used (5-10ppm).
The no repeatability of biocomponent re-use could be due to melanin formation into interstices of fungi tissue used, that could be seen because it became dark. Quinone complex polymers formation leads to increasing in response times of biosensor and diminish of its sensibility. The polymer formed blocks the interstices of biological part of biosensor, obstructs the diffusion of the analyte and causes a decrease in tyrosinase catalytic activity present in the fungi tissue as well (Fatibelo-Filho and Vieira, 2002).

4. Conclusions

5g of fungi tissue and 1 minute time reaction showed best results in range of phenol concentration 5-10ppm for the biosensor system development. However, due to lack of data repeatability, the biocomponent cannot be re-used and in other tests will be used lyophilized *Agaricus bisporus*. In addition, the air bubbling procedure promoted lesser variation in measurements with oxygen electrode.

5. Acknowledgement

The authors gratefully acknowledge FAPERJ for financial support.

References

Campanella L., Beone T., Sammartino M.P. and Tomassetti M., 1993, Determination of phenol in wastes and water using an enzyme sensor, Analyst, 118, 979-986.

Fatibelo-Filho O. and Vieira I.C., 2002, Uso analítico de tecidos e de extratos brutos vegetais como fonte enzimática, Química Nova, 25, 455-464.

Greenberg A.E., Clesceri L.S. and Eaton A.O., Eds., 1992, Standard Methods for Examination of Water and Wastewater. American Public Health Association, Washington, United States.

Kochana J., Nowak P., Jarosz-Wilkołazka A. and Bieroń B., 2008, Tyrosinase/laccase bienzyme biosensor for amperometric determination of phenolic compounds, Microchemical Journal, 89, 171-174.

Rodriguez-Mozaz S., Alda M.J.L. and Barceló D., 2006, Biosensors as useful tools for environmental analysis and monitoring, Analytical and Bioanalytical Chemistry, 386, 1025-1041.

Silva L.M.C., Salgado A.M. and Coelho M.A.Z., 2010, *Agaricus bisporus* as a source of tyrosinase for phenol detection for future biosensor development, Environmental Technology, 31, 6, 611-616.

Tomita I.N., Manzoli A., Yamanaka F. and Yamanaka H., 2005, Amperometric biosensor for ascorbic acid, Eclética Química, 30, 2, 37-43.

Zejli H., Hidalgo-Hidalgo de Cisneros J.L., Naranjo-Rodriguez I., Liu B., Temsamani K.R. and Marty J.L., 2008, Phenol biosensor based on sonogel-carbon transducer with tyrosinase alumina sol–gel immobilization, Analytica Chimica Acta, 612, 198-203.