PHENOL DETERMINATION IN ENVIRONMENTAL INTEREST SAMPLES BY AN AMPEROMETRIC BIOSENSOR BASED ON LYOPHILIZED FUNGI TISSUE (*Agaricus bisporus*)

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ABSTRACT

A biosensor based on lyophilized fungi tissue (Agaricus bisporus) was used for the amperometric determination of phenol in samples of environmental interest. This fungi tissue contained the tyrosinase enzyme that is able to catalyze two sequential oxidation reactions with phenolic substrates. Both reactions involve molecular oxygen, therefore the Clark-type oxygen electrode commercial was selected as transducer. Two samples of environmental interest were analyzed by the biosensor: a sample of domestic wastewater and a sample of feed water of a membrane bioreactor (MBR) tank, provided by General Electric Company. The results of the biosensor were compared with the results of the Standard Method for phenol determination, colorimetric method. The application of the biosensor in the samples showed percentage difference of 2.29 for the domestic wastewater and of 6.95 for the feed water when compared to the standard method, which shows a promising future to the biosensor.

Keywords: amperometric biosensor; tyrosinase enzyme; samples of environmental interest; Clark-type oxygen electrode.

RESUMO

DETERMINAÇÃO DE FENOL EM AMOSTRAS DE INTERESSE AMBIENTAL POR BIOSENSOR AMPEROMÉTRICO BASEADO EM TECIDOS DE FUNGOS LIOFILIZADOS (*Agaricus bisporus*)

Para a determinação amperométrica de fenol em amostras de interesse ambiental, utilizou-se um biossensor a base de fungos liofilizados (Agaricus bisporus). Este tecido fúngico continha a enzima tirosinase capaz de catalisar duas reações de oxidação sequenciais com substratos fenólicos. Ambas as reações envolvem oxigênio molecular, assim um eletrodo commercial de oxigênio do tipo Clark foi selecionado como transdutor. Duas amostras de interesse ambiental foram analisadas pelo biossensor: uma amostra de águas residuais domésticas e uma amostra de água de alimentação de um tanque de biorreator de membrana (MBR), fornecido pela empresa General Electric. Os resultados do biossensor foram comparados com os resultados do Método Padrão para determinação de fenol, que é o método colorimétrico. A aplicação do biossensor nas amostras mostrou diferença percentual de 2,29 para as águas residuais domésticas e de 6,95 para a água de alimentação quando comparado ao método padrão, o que mostra um futuro promissor para o biossensor.

Palavras chave: biossensor amperométrico; enzima tirosinase; amostras de interesse ambiental; eletrodo de oxigênio tipo Clark.

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1. INTRODUCTION

The main problems related to pollution refer to increasing of production and use of chemicals intensified in recent decades. Phenolic compounds lie between the main organic contaminants in soil and water bodies, they are present in many effluents from chemical production of synthetic resins, plastics, rubbers, dyes, explosives, solvents, paint thinners, pulp and paper industries and a wide variety of aromatic derivatives (BEKER et al., 2010; GUPTA et al., 2006).

Therefore, the development of techniques for simultaneous detection and determination of these chemical species in different matrices is of great interest. Currently the analysis of phenolic compounds has been carried out mainly by spectroscopic and chromatographic methods that do not allow easily continuous monitoring "*in situ*". Besides that they are expensive, slow, need well-trained operators, and in some cases require pre treatment steps, which increase the risk of losing samples (Rodriguez-Mozaz et al., 2004; Rogers, 2006).

The Biosensors represent a promising tool to supplement the existing techniques, because of their unique characteristics such as high selectivity, relatively low construction cost and storage, potential for miniaturization, easy automation and construction of simple and portable equipment for a quick monitoring "in situ" (Rogers and Gerlach, 1996; Rodriguez-Mozaz et al., 2004; Rogers, 2006).

According to the International Union of Pure and Applied Chemistry (IUPAC, 1998), biosensors are integrated instruments able to provide specific analytical information, quantitative or semi-quantitative, by the use of a biological component and a transducer element (Thévenot et al., 1999).

The tyrosinase enzyme (EC 1.14.18.1) has been investigated in recent years as the biological component of biosensor for phenol detection, for presenting desired characteristics such as low cost and high efficiency in the detection and quantification of phenolics compounds (Kochana et al., 2008; Zejli et al., 2008).

In this context, the aim of this study is to continue the work of Silva and collaborators (2013) analyzing if the amperometric biosensor based on fungi tissue (Agaricus bisporus) can be applied to samples of environmental interest.

2. MATERIAL AND METHODS

Biocomponent: Agaricus bisporus tissue

As biological component of the amperometric biosensor for the detection of phenolic compounds, Agaricus bisporus tissue lyophilized in powder was used as a source of tyrosinase (EC 1.14.18.1). The mushrooms used in the preparation of the biosensor were purchased from Quality Horty Frutti. The mushrooms were stored at 4 ° C until use.

Biosensor system

The schematic set-up for biosensor system for phenol analysis is presented in Figure 1 (Silva, 2011). The set up consists of a sample recipient (1), peristaltic pump (2), reaction chamber (red rectangle) made from PVC pipe with biological component (3), transducer (oxygen electrode Clark-type) and data recorder (4) and discard sample (5). Silicone tubing was used for connections.

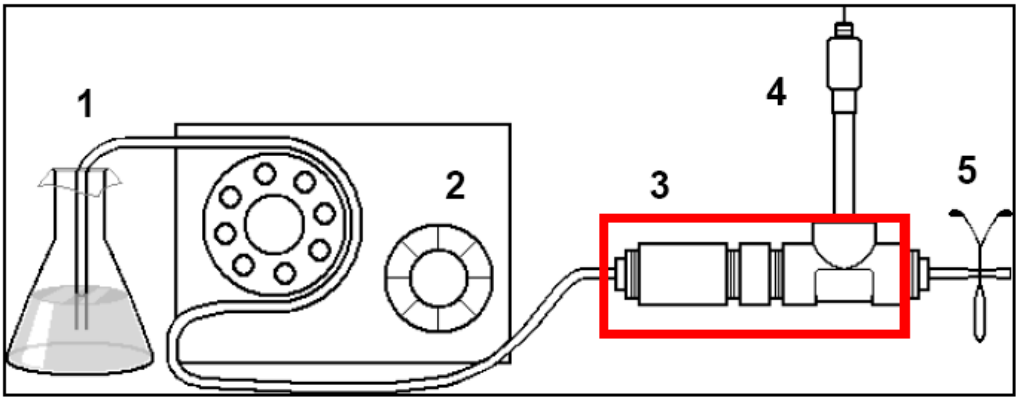

Figure 1 - Schematic set-up for biosensor system for phenol analysis (Silva, 2011).

The biosensor system configuration used 1.0 g of mushroom tissue lyophilized powder, flow rate of 40.00 mL*min^{-1} and reaction time of 10 minutes. This was the optimum configuration obtained in previous works (Silva, 2013).

Measurement procedure

For phenol analysis, calibration standards were prepared by dilution of phenol stock solution in phosphate buffer, pH 8.0. All measurements were carried out by injection of 50.00 mL standard sample (0.10 a 10.00 mg*L^{-1}) at a flow rate of 40.00 mL.min^{-1}. After the sample has completed the reaction chamber, the pump was turned off and calibrated oxygen

electrode was immersed. Then, data were collected after the reaction time of 10 min. After each sample analysis, the system was thoroughly rinsed with distilled water for 2 minutes. The amperometric measurements were made at room temperature (24 °C ± 1 °C). The procedure was the same for the environmental interest samples.

The biosensor response was defined as the dissolved oxygen variation in the sample over the 10 minute period, since the decrease of dissolved oxygen has a linear relation with the concentration of phenol present in the standard sample.

The calibration curve of the biosensor is made by plotting the phenol concentration in the samples versus the dissolved oxygen variation (initial dissolved oxygen in the sample - dissolved final oxygen in the sample).

Analysis of real effluents

The samples of crude domestic wastewater were donated by the Laboratório de Engenharia do Meio Ambiente (LEMA) and were collected at the Centro Experimental de Saneamento Ambiental (CESA), and the sample of feed water of a MBR tank of G.E. Company was donated by the Laboratório de Tratamento de Água e Reuso de Efluentes (LABTARE), both laboratory are located at UFRJ.

Standard Method: Colorimetric method

The phenol concentration of the samples was determined using the colorimetric method described in Standard Methods, based on obtaining the standard curve.

The standard curve was obtained as follows: 250 mL of ammonium hydroxide (NH_4OH) 0,5M was added to 10 mL of standard solutions, from 1 to 5 mg*L-1 of phenol and blank solution, which was composed of sodium phosphate buffer pH 8.0. Subsequently, 100 ml of 4-aminoantipyrine, 2% (w / v) solution and 100mL of potassium ferricyanide, 8% (w / v) were added to the solutions. The pH was adjusted to 7.9 (+0.1) by adding potassium phosphate buffer pH 6.8. After 15 minutes, the absorbance of the solutions was read on a spectrophotometer at wavelength (λ) of 500nm.

With the standard curve obtained, crude domestic wastewater and feed water of a MBR tank, were analyzed following the same procedure of obtaining the standard curve.

Application of amperometric biosensor in samples of environmental interest

For the analysis of the samples of crude domestic wastewater and feed water of the MBR by the biosensor, the same operating conditions and the same experimental procedure used in the construction of the standard curve were used. For the quantitative determination of phenol, calibration curves of the biosensor system prepared on the day of analysis were used. Analyses of both samples were made in triplicate.

3. RESULTS AND DISCUSSION

Determination of phenol concentration in Crude domestic wastewater

The phenol concentration found in crude domestic wastewater using the standard method was 1.31 mg*L^{-1}. Three samples of crude wastewater were passed through the biosensor system, biosensor response already converted to phenol concentration is show in the Table 1.

Table 1: Phenol concentration found in crude domestic wastewater by the biosensor

Sample of Crude Wastewater	Phenol concentration (mg*L^{-1})
1	1.29
2	1.23
3	1.31
Average	1.28

The average phenol concentration found by the passage of the samples by the biosensor system presented 2.29% of difference when compared to the concentration found by the standard method, that is, the use of the biosensor to determine the phenol concentration in the crude domestic wastewater samples presented a response similar to the response of the standard method without adding more chemicals during analysis.

Determination of phenol concentration in Feed water of the MBR Tank

The phenol concentration in the feed water of the tank MBR found by the standard method was above the range of the standard curve, being necessary to dilute the sample before the analysis. A 1:10 dilution ratio of the sample of feed water of the MBR Tank was used for the standard method analysis and the phenol concentration found was of 1.54 mg*L-1, indicating that the phenol concentration of the feed water was of 15.4 mg*L-1.

Three samples of feed water of the MBR Tank were passed through the biosensor system and the biosensor response already converted to phenol concentration is show in the Table 2:

Table 2: Phenol concentration found in diluted feed water of the MBR Tank by the biosensor

Sample of feed water of the MBR Tank	Phenol concentration ($mg*L^{-1}$)
1	2.14
2	2.13
3	2.07
Average	2.11

As one can observe, the values found by the biosensor were above the value found by the standard method. The average phenol concentration found by the passage of the samples by the biosensor system presented 37% of difference when compared to the concentration found by the standard method.

One possible reason for this adverse result is the fact that the sample contained much organic material, which can cause a fluctuation in the pollutants concentrations presents in the feed water. A new analysis of the sample using the standard method followed by passage of the same sample through the biosensor was taken. The result can be seen in the table 3.

Table 3: Phenol concentration found in diluted feed water of the MBR Tank by the biosensor and by the Standard method with both methods used in the same day.

Sample of Feed water with dilution ratio of 1:5	
Methods	Phenol concentration (mg*L-1)
Standard	2.44
Biosensor	2.61

As it can be observed when analyzing the sample on the same day, the phenol concentration of the sample obtained a difference percentage below 7% between the methods. Subsequent analyzes showed that the percentage difference between the phenol concentration determination methods increases over the time. This result, shown in Table 4, helps to demonstrate the natural variation of phenol concentration in the feed water sample.

These results demonstrate that analyses are necessary to determine the composition of this feed water in order to understand how this natural variation of phenol concentration occurs. This difference of values were found also when the standard method were used to determine the phenol concentration.

Table 4: Phenol concentration obtained by the biosensor and by the Standard method on different days

Sample of Feed water with dilution ratio of 1:5		
Methods	Phenol concentration ($mg*L^{-1}$)	Analysis realization
Standard	2.44	Same day
Biosensor	2.61	Same day
Biosensor	3.48	After 4 days
Biosensor	4.07	After 8 days

4. CONCLUSIONS

The biosensor showed satisfactory results when applied to both samples, obtaining results with the percentage difference under 7% relative to the standard method for determining the concentration of phenol, especially when applied to the sample of crude domestic wastewater where the percentage difference was under 3%.

The samples of the feed water of MBR tank presents some interferers that provoke a fluctuation of phenol concentration, which leads to a high variation of values between the phenol concentration determination methods when they aren't carried out in the same day.

The results show that the biosensor based on lyophilized fungal tissue provides an excellent alternative for the determination of phenolic compounds in crude domestic wastewater and in feed water of a MBR tank *in situ*.

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