Application of a voltammetric enzymatic biosensor based on crude extract of Marasmiellus colocasiae for the detection of phenolic compounds in drinking water

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ABSTRACT

The chemical and pharmaceutical industries are the main generators of residues, such as phenolic microcontaminants, including catechol, resorcinol, p-nitrophenol and 4-chlorophenol. Therefore, this work aims to identify these microcontaminants through an emerging contaminant biosensor by means of an enzymatic biosensor constructed with an enzymatic extract from the fungus Marasmiellus colocasiae. Based on the differential pulse voltammetry electrochemical technique, the biosensor was used to analyze the patterns of catechol, resorcinol, p-nitrophenol and 4-chlorophenol. The analysis of a sample prepared with these standards in water from the public supply network was also carried out. As a result, it was possible to verify that the biosensor developed in this study is more sensitive than conventional methods and has a greater affinity for catechol. In the sample prepared with the standards, it was possible to qualitatively identify the presence of 4-chlorophenol, resorcinol and catechol. The proposed biosensor was sensitive and has potential for application in the analysis of microcontaminants in the environment with the detection limit = 0.17 µmol L⁻¹, and the quantification limit = 0.52 µmol L⁻¹.

Keywords: electrochemical biosensor, micropollutants, polyphenol oxidases, potable water.
Aplicação de um biosensor enzimático voltamétrico à base de extrato bruto de *Marasmiellus colocasiae* para a detecção de compostos fenólicos em água potável

**RESUMO**

As indústrias químicas e farmacêuticas são as principais geradoras de resíduos, como microcontaminantes fenólicos, dentre eles catechol, resorcinol, p-nitrofenol e 4-chlorophenol. Diante disso, este trabalho objetiva identificar estes microcontaminantes através de um biossensor de contaminantes emergentes por meio de um biossensor enzimático construído com extrato enzimático do fungo *Marasmiellus colocasiae*. A partir da técnica eletroquímica de voltametria de pulso diferencial, o biossensor foi utilizado para analisar os padrões de catechol, resorcinol, p-nitrofenol e 4-chlorophenol. Também foi realizada a análise de uma amostra preparada com estes padrões em água da rede de abastecimento coletivo. Como resultado, foi possível verificar que o biossensor desenvolvido nesse estudo é mais sensível que os métodos convencionais e apresenta maior afinidade para o catechol. Na amostra preparada com os padrões foi possível identificar qualitativamente a presença do o 4-chlorophenol, resorcinol e catechol. O biossensor proposto foi sensível e apresenta potencial para aplicação na análise de microcontaminantes no meio ambiente com o limite de detecção = 0.17 µmol L⁻¹ e limite de quantificação = 0.52 µmol L⁻¹.

**Palavras-chave:** biossensor eletroquímico, micropoluentes, polifenol oxidases.

1. **INTRODUCTION**

Chemical and pharmaceutical industrial activities produce micropollutants resistant to biodegradation, most of them hazardous compounds, that are often discharged into water resources through untreated or improperly treated wastewaters. Many of these compounds are not removed by traditional wastewater treatment technologies, and their presence in natural waters, even in small concentrations, limits their use, which can cause public health risks and negative environmental impacts (Gosset *et al.*, 2018; Ooi *et al.*, 2020).

Many compounds have a high potential for contamination, even at low concentrations, for water, soil and air, and are called "emerging contaminants" (ECs) or "emerging pollutants", with increasing concern for the water quality of water resources and drinking water supplied to the population (Conama, 2013).

Currently, it is estimated that there are more than 700 emerging pollutants in the water resources, compounds of chemical and synthetic origin, with dangerous potential for the environment and public health, and whose monitoring is deficient. Within these, there should be highlighted the phenolic compounds such as catechol, 2-chlorophenol, 4-nitrophenol, resorcinol, which are documented as being highly toxic to biota (Carvalho and Orlanda, 2017).

There is an urgent need for the development and application of tools for detecting and quantifying these micropollutants in the environment, with biosensor technology emerging as a potential resource for this purpose. These biosensors detect molecules by means of biological components such as enzymes, DNA, antibodies and aptamers with which they interact, forming measurable electrochemical and optical signals (Lobsiger *et al.*, 2019).

Enzymatic biosensors are defined as biologically modified sensors where enzymes selectively interact with the substrate. The laccase enzyme (EC 1.10.3.2) is an oxidoreductase, which acts mainly on diphenols and has oxygen as its final electron acceptor, being then widely used as an analytical tool for food, environmental and biomedical analysis (Kavetskky *et al.*, 2019). This enzyme has a multi-colored active site interacting with phenolic compounds and
can be immobilized by physical adsorption in biosensors. Laccase is found in plants and in some microorganisms (Simón-Herrero et al., 2019).

Marasmiellus colocasiae is a basidiomycete that has ligninolytic enzymes such as laccases, total peroxidases and metal chelating micronutrients, characterized as a pathogen of the araceae Colocasia esculenta, popularly known as taro and coconut yam, and is an important source of amino acids, minerals, vitamins and proteins (Capelari et al., 2010; Vidigal et al., 2016).

The European Union and the American Environmental Protection Agency have cataloged several emerging phenolic micropollutants originating from wastewaters from the pharmaceutical and cosmetic industries, such as the catechol, 4-nitrophenol and resorcinol, which can be harmful to health and can cause skin irritations, respiratory and ophthalmic problems and can be precursors of cancerous diseases or act as endocrine disruptors. The 2-chlorophenol, generated in the paper and cellulose industries, is toxic and bioaccumulative in aquatic species (Song et al., 2019).

This work aimed to develop an enzymatic biosensor with M. colocasiae extract, which presents a different designer of sensor optimized to detect and quantify the presence of catechol, resorcinol, p-nitrophenol and 4-chlorophenol in water preparations prepared with these compounds.

2. MATERIALS AND METHODS

2.1. Origin and maintenance of the fungus

The fungus Marasmiellus colocasiae CCIBT 3388, isolated in Domingos Martins-ES/2005, was obtained from the Basidiomycete Culture Collection (CCB) of the São Paulo Institute of Botany. The identification of the obtaining source ex situ of genetic heritage, with the information contained in the records, is in accordance with § 1 of the Article 22 of the Decree No. 8.772 of 2016.

The microorganism was placed in Petri dishes containing 1.5% of agar culture medium (Biolog®), 2% of malt extract (Biolog®), 1% of dextrose (Greentec®) and 0.1% of bacteriological peptone (Biolog®), in an oven at 28°C for 7 days, greenhouse SX350 Sterilifer® obtained in the United States of America.

2.2. Enzyme production

For the production of the crude enzymatic extract, 15 discs (5 mm in diameter) of M. colocasiae mycelo were used, in a 250 ml conical flask, containing 100 ml of the culture medium (2% of malt extract, 1% of dextrose and 0.1% of bacteriological peptone). Sterilization conditions were carefully maintained. Then, the flasks were incubated and kept in an oven at 28°C for 15 days.

After the incubation period, the content obtained was filtered with the aid of a vacuum pump (Prismatec-131®, Brazil), in a filtration system with 0.45 µm cellulose nitrate membrane and 47 mm in diameter (Unifil®). The enzymatic extract was used for the determination of enzymatic activity and for the composition of the biosensor.

2.3. Enzymatic activity determination

2.3.1. Total Polyphenoloxidase Determination (TPO)

To determine the enzymatic activity of total TPO, the reaction solution contained 3000 µL, with 750 µL of 100 mM sodium acetate buffer pH 5.0; 1800 µL and enzymatic extract of M. colocasiae; 150 µL of 2 mM H2O2 (Synth®); 300 µL of 0.03% ABTS (Sigma Aldrich®). For some extracts, it was necessary to dilute the sample in a buffer for proper determination.

The enzymatic activity was carried out in a spectrophotometer at 420 nm and a reaction time of 10 min. An enzyme unit was defined as the amount of enzyme needed to oxidize 1 µmol of substrate per liter per minute (Machado and Matheus, 2006; Zeraik et al., 2008).
2.3.2. Laccase activity determination

For the determination of the enzymatic activity of the laccase, the reaction solution contained 3000 µL, with 850 µL of 100 mM sodium acetate buffer pH 5.0, 1800 µL and enzymatic extract of M. colocasiae and 50 µL of commercial bovine catalase (Sigma Aldrich®). After 5 min of reaction with the catalase, 300 µL of 0.03% ABTS (Sigma Aldrich®) was added. For some extracts, it was necessary to dilute the sample in a buffer for proper determination. The enzymatic activity was monitored for 10 min in a spectrophotometer at 420 nm (Machado an Matheus, 2006; Zeraik et al., 2008).

2.3.3. Total peroxidase activity determination

To determine total peroxidases, the following formula was used: Total peroxidases = total TPO – Laccases, in which there is a subtraction of enzymatic activities (Eggert et al., 1996).

2.4. Biosensor preparation

A proportion of 50 µL of crude enzyme extract and 70 mg of graphite powder (Sigma Aldrich®) was used. The mixture was homogenized and dried at room temperature (28 ± 2°C). Then, 30 mg of mineral oil (Sigma Aldrich®) was added, forming an agglutinated paste, which was vigorously homogenized and added to a support electrode with a cavity of 2 mm in diameter and 0.5 mm in depth. As a control, a sensor containing only graphite powder and mineral oil was prepared. The carbon paste was manually renewed for each analysis, in order to guarantee the effectiveness and reproducibility of the results.

2.5. Electrochemical determination

Voltammetric analyses were performed on a PGSTAT® Model 204 potentiostat/galvanostat (Metrohm Autolab, Utrecht Netherlands) integrated with the NOVA 2.1® software. The experiments were carried out in an electrochemical cell with a capacity of 10 mL, containing 2 mL of 100 mmol L⁻¹ of sodium acetate buffer solution, at pH 5 and analyte, in a three-electrode system, with the biosensor being the electrode of work (Ø = 2 mm), the Pt wire the auxiliary electrode and finally the Ag/AgCl/KCl sat 3 mol L⁻¹ the reference electrode.

For conditioning the working electrode, a cyclic voltammetry technique was used. The operating conditions were 10 successive cycles in a 100 mmol L⁻¹ sodium acetate buffer solution, pH 5.0, with a sweep speed of 50 mV s⁻¹ and a potential range from 0 to 1 V. The differential pulse voltammetry technique was chosen for the analyses. Operating conditions included a pulse amplitude of 50 mV, pulse width of 0.5 s and scan speed of 10 mV s⁻¹. The experiments were carried out at room temperature (28 ± 2°C), in triplicate (n = 3), and the electrolyte solution used was a buffer solution of sodium acetate (100 mol L⁻¹, at pH 5.0). The results obtained were analyzed and treated with the aid of the Origin9® software.

2.6. Determination of phenolic micropollutants, calibration curve, detection limit and analysis in synthetic water sample

The phenolic micropollutants selected in this investigation were the 4-chlorophenol (PM = 127.5 g mol⁻¹), p-nitrophenol (PM = 139.11 g mol⁻¹), catechol (PM = 110.11 g mol⁻¹) and resorcinol (PM = 110.11 g mol⁻¹), purchased from Sigma Aldrich®. An initial solution of 10 mmol L⁻¹ of each compound was prepared for the tests. Through differential pulse voltammetry, the biosensor was used for checking the improvement of the analytical signal. The calibration curve and the detection limit were obtained with the catechol compound in the range of 50 to 300 µmol L⁻¹, in a sodium acetate buffer solution (100 mol L⁻¹, at pH 5.0).

The synthetic water was collected at the Faculty of Pharmacy, at the Federal University of Goiás - UFG, composed of elements with compounds mg L⁻¹ Al 0.5, As 0.8; B 0.07; Ba 0.02; Ca 40; Cu 1.0; K 2.0; Li 0.02; Mg 17.0; Mn 0.03, Mo 0.09; Na 29; Pb 0.2; S 4.4; Sb 0.04; Sr 0.4; U 0.03, Zn 0.5. The compounds Ag, Be, Co, Cr, Fe, Hg, Ni, P, Sn, Ti, V did not present...
significant amounts.

Tests were carried out on simulated samples, with the four compounds being added to 2 mL of water from the public supply network in the city of Goiânia-GO, collected in the building of the Faculty of Pharmacy (UFG). The compounds were added in concentrations of 1300 µmol L\(^{-1}\), for the 4-chlorophenol and the p-nitrophenol, 160 µmol L\(^{-1}\) for the catechol and 330 µmol L\(^{-1}\) for the resorcinol.

Different concentrations were chosen because the biosensor has a different affinity for each micropollutant, since it is an enzymatic matrix. From the prepared samples, aliquots of 100 µL, 150 µL, 250 µL and 350 µL were added to the electrochemical cell containing 2 mL of sodium acetate buffer solution (100 mol L\(^{-1}\), at pH 5.0) to perform analysis in triplicate by the biosensor, the concentrations of the micropollutants were obtained by means of serial dilution, using a 1mM mother solution.

3. RESULTS AND DISCUSSION

The crude enzymatic extract obtained from the *M. colocasiae* used showed enzymatic activity of 1286.20 ± 183.91 U L\(^{-1}\) for phenoloxidase enzymes, 389.79 ± 66.31 U L\(^{-1}\) for laccase enzymes and 896.41 ± 190.27 U L\(^{-1}\) for peroxidase enzymes. Therefore, the optimal conditions for the development of the biosensor were reached (Figure 1).

![Figure 1](image1.png)

**Figure 1.** Optimization of biosensors with crude enzymatic extracts. A: enzymatic extracts obtained from minimal liquid medium (ME) in different proportions (25, 50, 100 or 200 µL in 70 mg of graphite) in the construction of biosensors, tested with the 10 µmol L\(^{-1}\) catechol substrate. Insertion: CP-50ME and CP without modification in the analysis of the 10 µmol L\(^{-1}\) catechol. B: Different enzyme reaction times of the biosensors in the 10 µmol L\(^{-1}\) catechol solution, before electrochemical analysis. All testicles were performed in 100 mmol L\(^{-1}\) sodium acetate buffer pH 5.0.

The sodium acetate buffer pH 5.0 was used in accordance with the literature that uses the variation of 4.5 to 5.0 for the use of fungal enzymes in the use of electrochemical remedies and in biosensors, optimizing the utility of the enzyme, resulting in better oxidation (Kolomytseva *et al.*, 2017; 2019; Mishra *et al.*, 2017).

Through the Differential Pulse Voltammetry technique, the enzymatic sensor and the biosensor were tested for the detention of the four micropollutants (catechol, resorcinol, p-nitrophenol and 4-chlorophenol) under the same conditions, as shown in Figure 2.

The minimal liquid culture medium of *M. colocasiae* was incubated in order to obtain crude enzymatic extracts containing high TPO activity. Biosensors with different options were tested with the catechol substrate (Figure 2-A). From these results, which show the best
analytical responses for electrochemical reduction of the substrate with the biosensors with the culture medium, CP-50ME was selected for analysis of the enzymatic reaction times of the biosensor with the substrate, to promote biochemical oxidation, before electrochemical reduction (Figure 2-B).

![Figure 2](image)

**Figure 2.** (A) Affinity of the biosensor for different phenolic compounds. (B) Determination of phenolic compounds at 100 μmol L⁻¹ in a sodium acetate buffer solution (100 mmol L⁻¹, pH 5.0) by differential pulse voltammetry for sensor (--), enzymatic biosensor (—) and control (—).

Thus, it was possible to notice a higher biosensor specificity for the catechol (100%), followed by the 4-chlorophenol (21.45%), the p-nitrophenol (19.20%) and the resorcinol (15.96%) (Figure 2 - A). It was also possible to observe an improvement of about six times for each compound analyzed with the biosensor, when compared to the conventional sensor (Figure 2 - B). Catechol, as a diphenol, may explain the greater specificity of enzymes present in the biosensor, according to other polyphenol oxidases present in the literature (Benaceur *et al.*, 2019; Han *et al.*, 2019). The same is not applied to the resorcinol, another diphenol, which may be related to decreased affinity due to the position of the hydroxyls in the aromatic ring when in contact with the active site of the enzymes present in this crude extract.

In Figure 3, it is possible to observe that each compound has a reduction potential, being 0.15 V for the resorcinol, 0.30 V for the catechol, 0.88 V for the 4-chlorophenol and 1.08 V for the p-nitrophenol. Gan *et al.* (2019), using an electrochemical sensor for detecting different phenolic compounds, have observed oxidation potentials of 0.36 V for 4-aminophenol, 0.89 V for the 4-chlorophenol and 1.17 V for the 4-nitrophenol. These compounds are reversible, since phenols are oxidized by enzymes and reduced by electrochemical technique in similar potentials.

Through serial dilution using a 1mM mother solution, concentrations of 1300 μmol L⁻¹ were obtained for 4-chlorophenol and p-nitrophenol, 160 μmol L⁻¹ for catechol and 330 μmol L⁻¹ for resorcinol. The catechol showed better responses and through it a calibration curve was made, since this compound is considered the gold standard in micropollutant analyses (Han *et al.*, 2019).

A calibration curve (Figure 4) was developed based on the best response presented to the biosensor, the catechol compound, in order to assess whether the proposed method presented a linear response to the increase in analyte concentration. The Table 1 presents the results obtained in this work in comparison with those observed in other similar works.
Application of a voltammetric enzymatic biosensor based on a polyphenol oxidase immobilized electrode as a detector for catechol.

Figure 3. Different reduction ranges for the phenolic compounds: resorcinol, catechol, 4-chlorophenol and p-nitrophenol obtained by the enzymatic biosensor in a 100 µmol L\(^{-1}\) of sodium acetate buffer solution (100 mmol L\(^{-1}\), pH 5.0).

Figure 4. Calibration curve with the enzymatic biosensor for the catechol (50 to 300 µmol L\(^{-1}\)). Detection limit = 0.17 µmol L\(^{-1}\); Quantification limit = 0.52 µmol L\(^{-1}\).

Table 1. Comparison of the results obtained with the biosensor developed in this research with other polyphenol oxidases immobilized electrodes towards catechol detection.

| Enzyme source | Enzymatic activity | Method | Linear range (µmol L\(^{-1}\)) | Limit of detection (µmol L\(^{-1}\)) | Reference |
|---------------|--------------------|--------|-------------------------------|--------------------------------------|-----------|
| *M. colocasiae* | 1286.20 U L\(^{-1}\) | DPV | 50 – 300 | 0.17 | This work |
| Jenipapo Fruit (Genipa americana L.) | 593 U mg\(^{-1}\) | DPV | 10 – 310 | 7 | (Antunes et al., 2018) |
| Mushrooms | 1530 UI mg\(^{-1}\) | Amperometric | 0.5 - 24.0 | 0.3 | (Hervás Pérez et al., 2006) |
| *Manilkara Z.* (sapota) Fruit | 620 U | Chronoamperometric | 1.0 – 15.0 | 0.47 | (Sandeep et al., 2019) |
| *Agaricus bisporus* | - | Amperometric | 0.5 – 101 | 0.15 | (Sethuraman et al., 2013) |
| Purchased from Sigma | 1050 U mg\(^{-1}\) | Cyclic voltammetry | 1.0–100 | 0.01 | (Chen et al., 2013) |
Thus, using drinking water, four phenolic micropollutants and a biosensor based on *M. colocasiae*, a very good detection and quantification of catechol in concentrations from 15.71 µmol L\(^{-1}\) (100 µL of solution) to 49.15 µmol L\(^{-1}\) (350 µL of solution) was observed. Resorcinol and 4-chlorophenol were also detected, whereas their quantification was hampered by the low sensitivity. In fact, the expected concentrations would be below the limit of quantification of the proposed biosensor. Regarding p-nitrophenol, the low sensitivity of the biosensor for this compound, and its poor aqueous solubility, hampered its detection (Ramesh *et al.*, 2018).

The results showed that the proposed methodology and *M. colocasiae*-based biosensor are sensitive for the detection of catechol, resorcinol and the 4-chlorophenol micropollutants, for low detection limits and a wide range of linearity. The biosensor may exhibit different behaviors in contact with the analyzed compounds. The enzyme may therefore have greater affinity for a substrate with greater solubility (Figure 5).

![Figure 5. Detection curve for the four compounds in water samples from the public supply network, prepared in different concentrations in a sodium acetate buffer solution (100 mmol L\(^{-1}\), pH 5.0).](image)

4. CONCLUSIONS

The *M. colocasiae*-based biosensor developed for this research work was efficient in the detection and quantification of catechol and in the detection of resorcinol and 4-chlorophenol, in samples prepared with water from the public supply network. The biosensor covers the linear range of 50 to 300 µmol L\(^{-1}\), with the detection limit = 0.17 µmol L\(^{-1}\), and the quantification limit = 0.52 µmol L\(^{-1}\). Thus, this biosensor can be considered innovative and can be an interesting technology for the detection and quantification of micropollutants in water resources and water supply systems.

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6. REFERENCES

ANTUNES, R. S. et al. Development of a polyphenol oxidase biosensor from Jenipapo fruit extract (Genipa americana L.) and determination of phenolic compounds in textile industrial effluents. *Biosensors*, v. 8, n. 2, 2018. https://doi.org/10.3390/bios8020047

BENACEUR, F. et al. Purification and characterization of catechol oxidase from Tadela (Phoenix dactylifera L.) date fruit. *International Journal of Biological Macromolecules*, v. 125, p. 1248–1256, 2019. https://doi.org/10.1016/j.ijbiomac.2018.09.101

CAPELARI, M. et al. A new pathogenic species of Marasmiellus from Brazil. *Cryptogamie, Mycologie*, v. 31, n. 2, p. 137–142, 2010.

CARVALHO, J. A.; ORLANDA, J. Heat stability and effect of pH on enzyme activity of polyphenol oxidase in buriti (Mauritia flexuosa Linnaeus f.) fruit extract. *Food Chemistry*, v. 233, p. 159–163, 2017. https://doi.org/10.1016/j.foodchem.2017.04.101

CHEN, H. et al. A new catechol biosensor immobilized polyphenol oxidase by combining electropolymerization and cross-linking process. *International Journal of Polymeric Materials and Polymeric Biomaterials*, v. 62, n. 12, p. 620–626, 2013. https://doi.org/10.1080/00914037.2012.761629

CONAMA (Brasil). Resolução nº 460 de 30 de dezembro de 2013. Altera a Resolução CONAMA nº 420, de 28 de dezembro de 2009, que dispõe sobre critérios e valores orientadores de qualidade do solo quanto à presença de substâncias químicas e dá outras providências. *Diário Oficial [da] União*: seção 1, Brasília, DF, n. 253, p. 153, 31 dez. 2013.

EGGERT, C.; TEMP, U.; ERIKSSON, K. E. L. The ligninolytic system of the white rot fungus Pycnoporus cinnabarinus: Purification and characterization of the laccase. *Applied and Environmental Microbiology*, v. 62, n. 4, p. 1151–1158, 1996.

GAN, T. et al. Morphology–dependent electrochemical activity of Cu2O polyhedrons and construction of sensor for simultaneous determination of phenolic compounds with graphene oxide as reinforcement. *Sensors and Actuators B: Chemical*, v. 282, p. 549-558, 2019. https://doi.org/10.1016/j.snb.2018.11.102

GOSSET, A. et al. Xurography-based microfluidic algal biosensor and dedicated portable measurement station for online monitoring of urban polluted samples. *Biosensors and Bioelectronics*, v. 117, p. 669–677, 2018. https://doi.org/10.1016/j.bios.2018.07.005

HAN, Q. Y. et al. Comparison of biochemical properties of membrane-bound and soluble polyphenol oxidase from Granny Smith apple (Malus × domestica Borkh.). *Food Chemistry*, v. 289, n. 17, p. 657–663, 2019. https://doi.org/10.1016/j.foodchem.2019.02.064

HERVÁS PÉREZ, J. P. et al. Amperometric tyrosinase biosensor based on polyacrylamide microgels. *Biosensors and Bioelectronics*, v. 22, n. 3, p. 429–439, 2006. https://doi.org/10.1016/j.bios.2006.05.015

KAVETSKYY, T. et al. Dependence of operational parameters of laccase-based biosensors on structure of photo cross-linked polymers as holding matrixes. *European Polymer Journal*, v. 115, p. 391–398, 2019. https://doi.org/10.1016/j.eurpolymj.2019.03.056
KOLOMYTSEVA, M. et al. Rapid identification of fungal laccases/oxidases with different pH-optimum. Process Biochemistry, v. 62, p. 174–183, 2017. https://doi.org/10.1016/j.procbio.2017.07.027

KOLOMYTSEVA, M. P. et al. Laccase isoform diversity in basidiomycete Lentinus strigosus 1566: Potential for phenylpropanoid polymerization. International Journal of Biological Macromolecules, v. 137, p. 1199–1210, 2019. https://doi.org/10.1016/j.ijbiomac.2019.07.056

LOBSIGER, N. et al. YestroSens, a field-portable S. cerevisiae biosensor device for the detection of endocrine-disrupting chemicals: Reliability and stability. Biosensors and Bioelectronics, v. 146, n. July, p. 111710, 2019. https://doi.org/10.1016/j.bios.2019.111710

MACHADO, K. M. G.; MATHEUS, D. R. Biodegradation of Remazol brilliant blue R by ligninolytic enzymatic complex produced by Pleurotus ostreatus. Brazilian Journal of Microbiology, v. 37, n. 4, p. 468–473, 2006. https://doi.org/10.1590/S1517-83822006000400013

MISHRA, V. et al. Improvement of selective lignin degradation in fungal pretreatment of sweet sorghum bagasse using synergistic CuSO4-syringic acid supplements. Journal of Environmental Management, v. 193, p. 558–566, 2017. https://doi.org/10.1016/j.jenvman.2017.02.057

OOI, L. et al. Application of the cellular oxidation biosensor to Toxicity Identification Evaluations for high-throughput toxicity assessment of river water. Chemosphere, v. 247, p. 125933, 2020. https://doi.org/10.1016/j.chemosphere.2020.125933

RAMESH, A. V. et al. A Facile plant mediated synthesis of silver nanoparticles using an aqueous leaf extract of Ficus hispida Linn. f. for catalytic, antioxidant and antibacterial applications. South African Journal of Chemical Engineering, v. 26, n. November 2017, p. 25–34, 2018. https://doi.org/10.1016/j.sajce.2018.07.001

SANDEEP, S. et al. Detection of catechol using a biosensor based on biosynthesized silver nanoparticles and polyphenol oxidase enzymes. Portuguese Electrochimica Acta, v. 37, n. 4, p. 257–270, 2019. http://dx.doi.org/10.4152/pea.201904257

SETHURAMAN, V.; MUTHURAJA, P.; MANISANKAR, P. Fabrication of an efficient polyaniline-polyphenol oxidase based biosensor for catechol. Analytical Methods, v. 5, n. 22, p. 6523–6530, 2013. https://doi.org/10.1039/C3AY41341A

SIMÓN-HERRERO, C. et al. Immobilized laccase on polyimide aerogels for removal of carbamazepine. Journal of Hazardous Materials, v. 376, p. 83–90, 2019. https://doi.org/10.1016/j.jhazmat.2019.05.032

SONG, Y. et al. Simultaneous electrochemical determination of catechol and hydroquinone in seawater using Co3O4/MWCNTs/GCE. Materials Chemistry and Physics, v. 234, n. May, p. 217–223, 2019. https://doi.org/10.1016/j.matchemphys.2019.05.071

VIDIGAL, S. M. et al. Yield performance of taro (Colocasia esculenta L.) cultivated with topdressing nitrogen rates at the Zona da Mata region of Minas Gerais. Revista Ceres, v. 63, n. 6, p. 887–892, 2016. http://dx.doi.org/10.1590/0034-737x201663060019

ZERAIK, A. E. et al. Desenvolvimento de um spot test para o monitoramento da atividade da peroxidase em um procedimento de purificação. Química Nova, v. 31, n. 4, p. 731–734, 2008. http://dx.doi.org/10.1590/S0100-40422008000400003