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CONSTRUCTION OF BIOREACTOR FOR REMOVING OF THE PHENOL COMPOUNDS AND BIOSENSOR FOR THEIR ASSAY

Abstract. In this study, we describe the fabrication of sensitive biosensor for the detection of phenolic substrates using laccase immobilized onto HexaCyanoFerrate (HCF) modified graphite electrode. The results of amperometric analysis revealed that the Pt-HCF-modified laccase bionanoelectrode possesses better electrochemical behavior for laccase than non-modified carbon electrodes (control). The bioelectrode have demonstrated 4 folds enhanced maximal current at substrate saturation (Imax) values, 5 folds increased sensitivity and twice wide linearity compared with control bioelectrode. A laboratory prototype of a bioreactor based on chitosan beads with encapsulated bioelement: laccase-Fe_3O_4 – for degradation of diclofenac, which can be successfully used for biodegradation of xenobiotics (DF) in model solutions, was constructed.

Key words: HCF, laccase, ABTS, amperometric biosensor, xenobiotics.

DISTRIBUTION OF XENOBIOTICS IN THE ENVIRONMENT

One of the most important environmental problems facing today is the pollution of soil, water and air with toxic chemicals. Due to the rapid pace of industry and the widespread use of plastic, detergents, pharmaceuticals, pesticides in agriculture, environmental pollution is causing a serious problem. The United States produces 80 billion pounds of hazardous organic matter annually, and only 10% of it is disposed of safely [1].

Monitoring of phenolic compounds in the food industry and for environmental and biomedical analyzes using portable, cost-effective devices has become an area of growing interest over the past decade. Phenolic compounds are widespread in nature. They can be found in fruits and vegetables, and they are responsible for the organoleptic properties of some foods, such as wine and olive oil [2; 3]. Their antioxidant properties help prevent cancer and cardiovascular disease [4]. Phenols are also the breakdown products of natural organic compounds such as humic substances, lignins and tannins. However, some phenols are ubiquitous pollutants that enter natural waters with chemical effluents. Industrial activities, such as refineries, pharmaceuticals, production of resins, paints, textiles, petrochemicals and cellulose, including phenol production [5; 6].

Thus, aquatic organisms, including fish, are exposed to these contaminants [7], and due to their toxicity, some phenolic compounds are subject to regulation as water contaminants. In this context, the European Commission (EC) and the US Environmental Protection Agency (US

EPA) have established lists and classifications to prioritize hazardous substances for monitoring in drinking or natural waters, including special attention to phenolic compounds [7-9].

Phenol compound is the basic structural unit that is part of the structure of various organic molecules and without which the existence of modern industry and modern human economy in general would be impossible [9; 10]. But at the same time phenol and its derivatives occupy the first places in the list of pollutants of the US Environmental Protection Agency and the US Toxic Substances and Diseases Registry. Toxic phenolic compounds are formed from several sources such as partial degradation of phenoxy herbicides, use of wood preservatives and waste generation in petroleum industries (refineries, gas and coke industries), pharmaceuticals, phenol-formaldehyde production of resins, plastics, etc. [9-11].

Of particular concern are the emerging pollutants, mainly those contained in phenolic compounds that have an endocrine effect, and are represented by chlorophenols and their derivatives [9-11]. Chemicals that disrupt the endocrine system are substances that mimic the effects of hormones [11, 12], which has an adverse effect on the reproduction of wildlife and man; some phenolic compounds, such as bisphenol A, nonylphenol and their alkylphenol derivatives, triclosan, genistein and others, are widely used in industrial and domestic applications, produce estrogenic activity [11-13].

Ingestion of phenol and its derivatives leads to toxic effects on blood, liver, kidneys and heart [14]. For example, the heart rate decreases, blood pressure drops and heart failure occurs. Various physicochemical methods are used to remove phenol from polluted waters, such as ionization, adsorption, reverse osmosis, electrolytic oxidation, use of hydrogen peroxide, photocatalysis, etc. [14-17].

Removal of phenol by biological methods has an advantage over physico-chemical methods due to their environmental friendliness and economic efficiency and the possibility of complete mineralization of the substrate. To date, the biodegradation of phenol has been studied in detail using pure and mixed cultures of suspended bacteria. However, due to the inhibitory effect of phenol on microorganisms, the biological treatment of phenol-containing wastewater is a problem. Therefore, for the biodegradation of high concentrations of phenol from effluents, it is especially important to identify a suitable microorganism that can withstand such conditions.

BASIC PRINCIPLES OF BIODEGRADATION OF XENOBIOTICS

The main strategy of biodegradation of xenobiotics by microorganisms is their transformation into products that can be used for catabolic and anabolic processes (for energy production and synthesis of cellular substances).

Biodegradation and complete mineralization can be carried out using aerobic and anaerobic microorganisms. Aerobes oxidize organic compounds with redox enzymes. In turn, anaerobes decompose xenobiotics by fundamentally different mechanisms. The terminal acceptor of electrons in anaerobes is not oxygen, but nitrate, sulfate or carbon dioxide.

A group of different microorganisms is more effective in destroying foreign substances than monocultures. The ability to biodestruct organic xenobiotics is inherent in both gram-positive (Nocardia spp., Mycobacterium spp., Corynebacterium spp., Arthrobacter spp., Bacillus spp.) And gram-negative (Pseudomonas spp., Acinetobacter group, Cynetobacter spge. Xanthomonas spp.) Organisms and fungi [18; 19]. Most xenobiotics are insoluble in the aqueous phase, so it is difficult for prokaryotes to transport them into the cell because they are insoluble in the soil. Bacteria have developed special mechanisms for this.

The cell synthesizes rhamnolipids, which are exometabolites. According to their physicochemical properties, they are amphiphilic compounds, ie one part of the rhamnose molecule is hydrophilic, and the lipid part of the molecule is hydrophobic. These molecules form micelles, which contain hydrophobic organic compounds and in this form penetrate into prokaryotic cells.

Industrial production of biocatalysts based on technical enzymes is economically viable. Various enzymes are widely used in industrial biocatalytic processes, while laccase is of particular interest [20]. The broad substrate specificity of these enzymes and their ability to use atmospheric oxygen as an electron acceptor makes laccase a promising component of various industrial processes – bleaching and decolorization of tissues, delignification of pulp, production of antibiotics and anticancer drugs, wastewater treatment from organic pollutants [21]. The study of biocatalytic alternative methods of wastewater treatment from pollutants today helps to reduce the impact of toxic substances on the environment [22] and is relevant because in Ukraine, unfortunately, we have a very difficult environmental situation

CHARACTERISTICS OF LACCASE AND THEIR FUNCTIONS

Laccase (CF 1.10.3.2, para-diphenol: oxygen oxidoreductase) contains four copper atoms at its catalytic center [21]. Depending on the source of the enzyme, the substrate specificity and catalytic activity of laccase may vary [23-26]. They are able to catalyze the oxidation of a wide range of substrates of organic and inorganic nature, which results in four-electron reduction of molecular oxygen to water [21, 21]. Yakhida was first discovered in the sap of the Japanese lacquer tree Rhus vernicifera [27]. Subsequently, laccase and laccase-like oxidase have been found in many plants [28; 29] and some bacteria [30, 31], where they are involved in pigmentation and pathogenesis [32]. However, most of the laccase described so far have been isolated from different species of fungi [24; 33]. The most studied of these are the enzymes of basidiomycetes, which cause white rot of wood. These fungi include Trametes versicolor, Trametes hirsuta, Trametes ochracea, Trametes villosa, Trametes gallica, Trametes maxima, Coriolopsis polyzona, Lentinus tigrinus, Pleurotus eryngii and others [34]. Laccase catalyzes the oxidation of various organic substrates with the formation of radical products. Following this process, the oxidative coupling reaction plays a key role in the processing of many biologically important compounds: lignins, melanins, alkaloids, as well as humic and tannins [35]. In this regard, the main functions of laccase in living organisms are the formation of fruiting bodies of fungi, participation in pathogenesis, as well as degradation and biosynthesis of lignin [34].

The efficiency of the use of microorganisms or enzymes for the biodegradation of xenobiotics remains a major challenge for environmentalists and bioengineers. Some strategies have been proposed to overcome this issue, such as the use of immobilized cells of microorganisms or enzymes (laccase).

The development of fast, simple and relatively inexpensive methods of screening and accurate determination of the content of phenolic derivatives in the environment is an urgent problem of modern analytical chemistry and biotechnology. In particular, biosensor methods of analysis that can provide inexpensive, sensitive and selective analysis in real time. Biosensors are currently considered to be the most promising of all the methods of toxin detection proposed by analytical biotechnology. Thus, the development of simple sensitive selective methods for the analysis of phenol and its derivatives, including enzyme biosensors, is very important for human safety.

IMMOBILIZATION OF LACCASE

Enzymes have a number of features that make their use better than conventional chemical catalysts. However, there are a number of practical problems that reduce their service life, such as their high cost of separation and purification, their reuse, the instability of their structures and their sensitivity to specific process conditions. Many of these undesirable limitations can be solved with the help of immobilized enzymes. Immobilization is achieved by fixing enzymes or in solid substrates, resulting in inhomogeneous immobilized enzyme systems. In immobilized

form, enzymes are more reliable and more resistant to environmental changes, which allows easy recovery and reuse [36].

Laccase immobilization has been studied with a wide variety of different methods and substrates. For example, laccase immobilized on TiO_2 nanoparticles mixed with a polyester sulfonated membrane is used. TiO_2 functionalized PES membrane showed better efficiency of the immobilization enzyme than non-functionalized membrane. Optimal performance was observed PES membrane containing 4% by weight of TiO_2. These biocatalytic membranes also exhibit good enzyme stability, tolerance to a wider pH range under the harsh filtration conditions required when used for water treatment. Kinetic studies have also shown that the affinity of the enzyme for the substrate is preserved after immobilization compared to the chemical and physical stabilization of the enzyme [26].

The enzyme isolated from Trametes versicolor is immobilized on silicon dioxide, which is chemically modified with imidazole groups and Amberlite IRA-400. This allows the enzyme to remain active for several weeks [19].

Laccase was immobilized on magnetic chitosan microspheres with glutaraldehyde as a cross-linking reagent. The immobilized enzyme shows maximum activity at pH 3.0. It is shown that the indicators of thermal stability and storage stability for the immobilized enzyme exceeded those of the free enzyme [20].

MATERIALS AND METHODS

Characteristics of used reagents and drugs.

All aqueous solutions used in the work were prepared on double distillate. Working solutions of hydrochloric acid were prepared by diluting the corresponding concentrated acids of qualification "h.ch.", acetic – from acetic acid (glacial). The work used solutions of glutaraldehyde, which was prepared by dissolving the reagent Sigma-Aldrich were used in the study: 2,2`-azinobis (3-ethylbenzothiazoline-6-sulfonate) diammonium salt (ABTS), phenol, and other inorganic salts.

Sigma-Aldrich polymer chitosan was used in the work. All buffers and standard solutions were prepared using Milli-Q purified water (Millipore).

The composition of the environment and cultivation conditions.

Strains of microorganisms were grown in a mineral medium of the following composition (g/l): KNO_3 – 2.5 g, KH2PO4 – 2 g, NaCl – 0.5 g, $MgSO_4 \cdot 7H_2O$ – 0.5 g, mineral elements – 2 ml, yeast extract – 0.2 g, which was dissolved in distilled water. The source of carbon, depending on the purpose of the experiment were: glycerol – g, glucose – 10 g, maltose – 10 g.

Strains were grown at 20-2°C or 28°C in 500 ml flasks on a shaker with constant aeration (150 and 200 rpm).

Laccase *Trametes zonate* was obtained from the culture fluid. To do this, up to 80% of ammonium sulfate was added to 100 ml of extracellular laccase culture fluid, kept cold and unscrewed. The obtained laccase preparation was used to construct a bioreactor.

Determination of laccase activity in solution using ABTS.

Determination of laccase activity was performed in 50 mm acetate buffer, pH 4.5, containing 1 mm ABTS, in a final volume of 1 ml. The test sample was added to the reaction mixture and the reaction product formed at 420 nm was analyzed by colorimetric method. The unit of activity (Unit) was taken to be the amount of enzyme that provides the formation of 1 μmol of colored product in 1 min under the conditions described above.

Design of amperometric biosensor.

Amperometric biosensors were designed on the basis of standard amperometric potentiostats using a three-electrode sensor configuration: silver-silver-silver Ag/AgCl/KCl (3 M) was used as a reference electrode, and as an auxiliary - a platinum rod electrode. The working electrodes were

designed on the basis of graphite rods (RW001, diameter 3.05 mm, Ringsdorff Werke, Bonn, Germany), placed in a glass tube and sealed with epoxy glue. Before direct use, the working surface of the carbon electrodes was polished with sandpaper and washed with water in an ultrasonic bath for 10-15 minutes.

Amperometric measurements.

Amperometric measurements were performed at room temperature in a 50 ml electrochemical cell filled with 15 ml of 50 mM acetate buffer solution, pH 4.5 (background). In the case of chronoamperometric analysis, the chemoelectrodes were placed in a vigorously stirred solution and, after establishing the baseline signal, increasing concentrations of catechol added to the cell. The results of measurements in units of current were recorded and processed with the help of amperometric potentiostat.

Methods of forming chitosan balls.

A solution of chitosan (0.5 ml) with a concentration of 20 g/l was taken mixed with 0.05 ml of laccase solution (1 mg/ml) and 0.1 ml of suspension pre-treated with ultrasonic Fe_3O_4-NP (30 mg/ml), stirred vigorously for 15 minutes at room temperature. The crosslinking agent was glutaraldehyde (GA). Therefore, the resulting mixture was collected in a volume of 0.01 ml and added dropwise to 0.05 M solution of GA, which was kept for 0.5 h to form beads, then washed and used for analysis.

Statistical analysis.

The experiments were performed in three to four replicates. The arithmetic mean (M) was determined for each sample. The calculation of statistical indicators and plotting was performed using the program Origin 7.5. The linearization of the graphs was performed according to the regression equation Y = A + BX (A and B are the parameters of the equation), calculated the linear correlation coefficient R and the level of reliability of the relationship p (for the event R = 0).

RESULTS AND DISCUSSION

Construction of a laccase biosensor using Pt-HCF *nanoparticles for catechol analysis.*

Catechol (1, 2 dihydroxybenzene) is an industrial feedstock and acts as one of the environmental pollutants causing various health issues like skin irritation, eye damage etc. It is a challenge to the researchers to design efficient sensing material for catechol. The following aspects must be taken into account when determining phenolic compounds: 1) the toxicity of phenolic compounds significantly depends on the structure, so their determination must be selective; 2) phenolic compounds are characterized by rapid chemical and biochemical oxidation, so the methods of their determination must be express; 3) The MPC of phenol compound in water is 0.001 mg / l, so the methods of its determination must be highly sensitive. Monitoring of phenolic compounds in the food industry, as well as for environmental and medical applications has become more relevant recently. Conventional methods for detecting and quantifying these compounds, such as spectrophotometry and chromatography, are time consuming and expensive. However, laccase biosensors are a rapid method for monitoring these compounds on-line and in situ.

We were the design a biosensor using Pt-HCF and laccase for catechol analysis. 10 µl of laccase solution from *Trametes zonata* (with a concentration of 1 mg · ml-1 and a volumetric activity of 12 Units · ml^{-1}, in 50 mm acetate buffer, pH 4.5) was applied dropwise to the surface of the working electrodes. 5 µl of a colloidal solution of Pt-HCF (1 mg · ml^{-1}) and dried the resulting mixture (enzyme + nanoparticles) cover 1% aqueous neutralized Nafion solution for 10 min in air at room temperature. Before use, the bioelectrodes were stored in 50 mm acetate buffer pH 4.5 at +4°C. The cyclic voltamperometric analysis using catechol as a model laccase substrate was performed to estimate the optimal working potential of the reduction of electroactive products of the laccase's reaction with a bioelectrode based on Pt-HCF-Nafion bio-nanocomposite. It has been shown

that the addition of catechol to measuring cell resulted in an increase in the redox currents of the bio-nanoelectrode. The significant increase in the redox currents clearly indicates the high efficiency of electron transfer between the electroactive product of the laccase reaction and Pt-HCF-Nafion-modified surface of the working electrode. In addition, it allows estimating the optimal operating potential for the biosensor (laccase/Pt-HCF/GE electrodes), which corresponds to 230 mV vs Ag/AgCl reference electrode. The potential of 230 mV vs Ag/AgCl was chosen as optimal for further work to study the characteristics of the designed bio-nanoelectrodes laccase/Pt-HCF/GE.

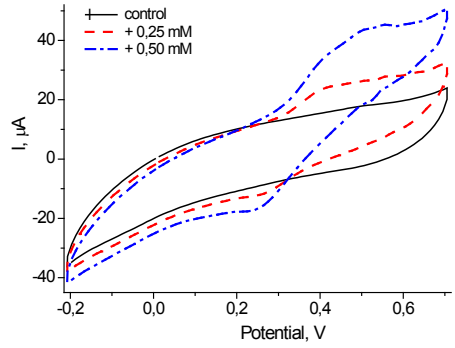

Fig. 1. Cyclic voltamperograms the laccase/Pt-HCF/GE electrodes

The bioanalytical characteristics of the bioelectrode were investigated by chronoamperometric approach, using as the operating potential 230 mV against the Ag/AgCl reference electrode. The choice of such working potential is due to on the study of optimal working potential (Fig. 2). In the designed sensor, laccase was a selective element. A copper-containing enzyme does not require hydrogen peroxide as a co-substrate or additional cofactors for the enzymatic reaction. In a typical laccase reaction, the phenolic substrate undergoes one-electron oxidation to form an aryl radical, which is converted to quinone in the next step of the enzymatic reaction. The principle of detection of amperometric biosensor based on laccase is based on the reduction of oxidized electroactive products formed as a result of laccase enzymatic reaction.

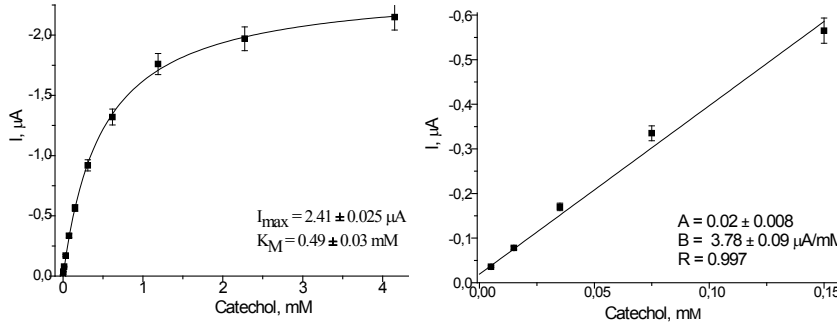

Fig. 2. The chronoamperometric calibration curve of bionanoelectrode (laccase/Pt-HCF/GE electrode) response for substrate saturation (a) and within the linear range (b) toward subsequent addition of increasing catechol concentration. Abbreviations: B – slope of the curve; R – the correlation coefficient for linear regression

The dependence of the operating parameters of Pt-HCF-Nafion-modified laccase bioelectrodes of was performed using chronoamperometric analysis (Fig. 2). Evaluation of the dependence of the operating parameters of Pt-HCF -Nafion-modified laccase bioelectrodes relative to

the control (without using Pt-HCF) was performed on four main parameters: I_{max} – the maximal response of the biosensor at substrate saturation; K_M^{app} – the apparent Michaelis-Menten constant; linearity and sensitivity. The bioelectrodes based on Nafion membrane without of any Pt-HCF nanoparticles and bio-nanoelectrodes based on nanoparticles (Pt-HCF) (Fig. 2). The modified bioelectrode were characterized by I_{max} value of 2.41±0.06 μA, K_M^{app} – 0.49±0.02 mM with an upper linearity limit of 0.17 mM toward catechol, and sensitivity – 516 $A \cdot M^{-1} \cdot m^{-2}$. The bionanoelectrodes' sensitivity was calculated taking into account the area of the working electrode surface which was equal to 7.3 mm^2. Modification of nanoparticles leads to a 5-fold increase in sensitivity, which makes them promising for use in biosensors as nanomediators.

Construction of a laboratory prototype of a bioreactor based on laccase for the degradation of xenobiotics in model solutions.

For the construction of the bioreactor, laccase was used as a degrading element due to its wide range of substrates, the ability to oxidize organic and some inorganic substrates, as well as the ability to remove xenobiotics from industrial effluents and change the color of dyes. In addition, compared to the peroxidase enzyme, the laccase enzyme does not require hydrogen peroxide (H_2O_2), but can oxidize organic compounds with oxygen (O_2) as a substrate [20].

To build a laboratory prototype of the bioreactor, strong chitosan beads with encapsulated laccase and magnetic nanoparticles (Fe_3O_4 – NPs) were used. Used magnetic nanoparticles (MNPs), had the advantage due to their properties of magnetism, it is possible to control their movement and accumulation at a distance. Chitosan was chosen for the formation of balls due to its high sorption properties to organic compounds and mechanical stability.

The schematic diagram of the formation of chitosan beads with encapsulated enzyme and MNC is presented in Fig. 3, the balls were formed according to the procedure described in Zheng et al. 2016.

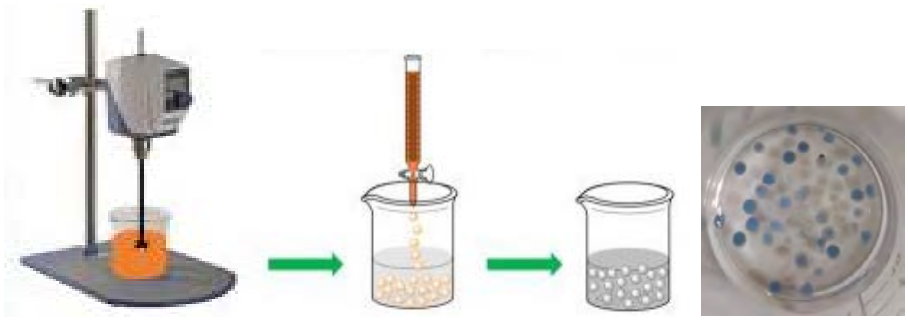

Fig. 3. Schematic diagram of the formation of chitosan beads with encapsulated bioelement: laccase-Fe_3O4-NPs.

A solution of chitosan (0.5 ml) with a concentration of 20 g/l was taken mixed with 0.05 ml of laccase solution (1 mg / ml) and 0.1 ml of suspension pre-treated with ultrasonic Fe3O4-NP (30 mg/ml), stirred vigorously for 15 minutes at room temperature. The crosslinking agent was glutaraldehyde. Therefore, the resulting mixture was collected in a volume of 0.01 ml and added dropwise to 0.05 M solution of alkaline aldehyde (HA), which was kept for 0.5 h to form beads, then washed and used for analysis.

Photo of the appearance of the formed chitosan balls with a bioelement: laccase-Fe_3O_4-NPs is presented in Fig. 4.

The obtained laccase / chitosan beads were packed in column-type tubes and used as a bioreactor in experiments for bioremediation of diclofenac (DF) in model solutions (Fig. 5).

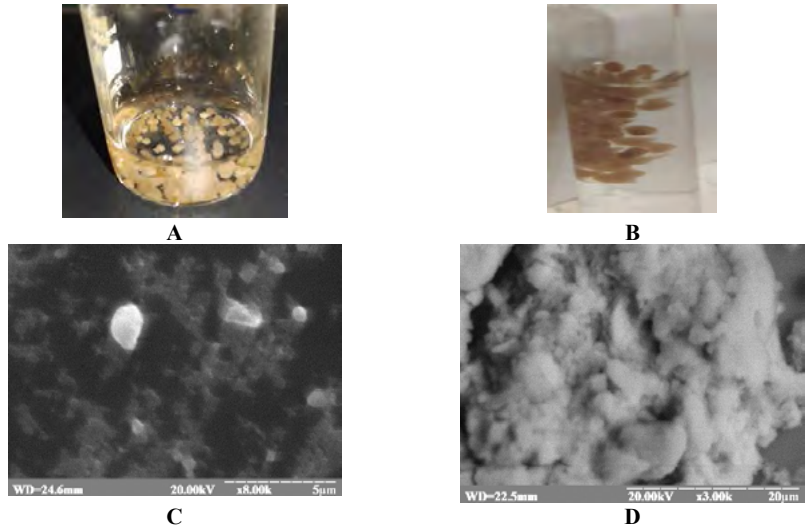

Fig. 4. Photo of chitosan beads with encapsulated bioelement: laccase-Fe_3O_4-NPs. A – encapsulated with laccase-Fe_3O_4-NPs in buffer; B – test tube near the magnet with encapsulated laccase-Fe_3O_4-NPs; SEM images: laccase-Fe_3O_4-NPs (C) and encapsulated laccase-Fe_3O_4 (D)

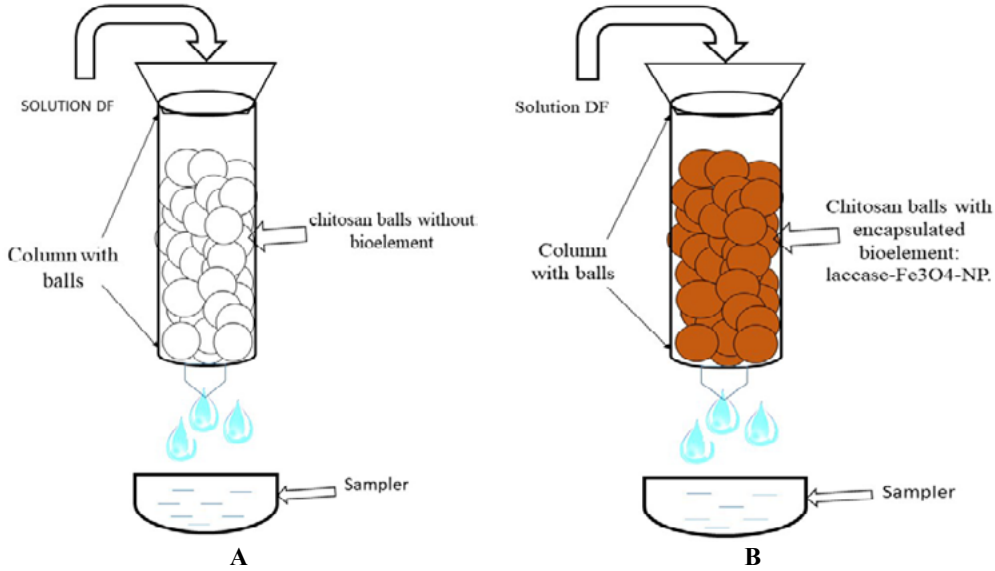

Fig. 5. Photo of column type laboratory bioreactor with chitosan beads with and without bioelement. A – control column without laccase-Fe_3O_4-NPs; B – experimental column with an encapsulated element of laccase-Fe_3O_4-NPs

The bioreactor with chitosan beads without laccase and MNPs served as a control. Model solutions of diclofenac at a concentration of 0.05 mm were slowly passed (with a flow rate of 0.005 ml / (min · cm2)) through column-type bioreactors filled with beads with and without a bioelement. Then in the resulting solutions that passed through the column analyzed the content of diclofenac. The scheme of the experiment is shown in Fig. 5.

DF concentrations during degradation in the bioreactor were measured by HPLC (Dionex UltiMate 3000) with an ultraviolet detector (Dionex UltiMate 3000 RS Variable wavelength detector) using a moving phase of 2 mM acetic acid: methanol (30:70 rpm), detected UV at 274 nm.

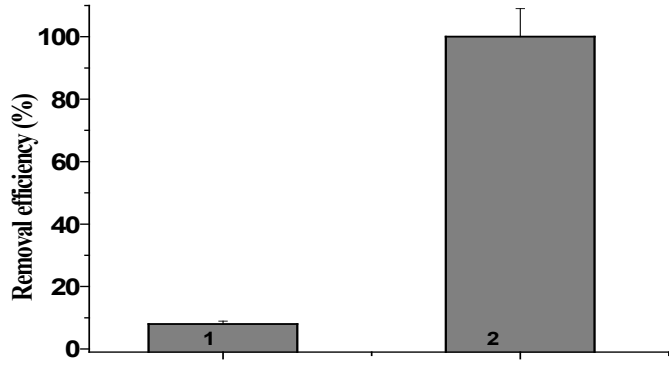

Fig. 6. Biodegradation of DF in laccase bioreactor. 1 – experimental column with encapsulated element laccase-Fe_3O_4-NPs; 2 – control column without laccase-Fe_3O_4-NPs

The dynamics of DF bioremediation in the bioreactor was studied in both variants: from the control column and from the column with laccase and MNPs (Fig. 5). In the solutions obtained from the control column there was no complete oxidation of DF, while in the solutions from the experimental column the concentration of DF decreased 3 times and was 0.015 mm (Fig. 4). It was shown that the designed column had the ability to neutralize phenol for 2 weeks, with daily (1 time per day) use. The obtained results emphasize the importance of laccase in its ability to oxidize phenolic compounds.

Designed bioreactors can be used to treat pharmaceutical industrial effluents.

CONCLUSION

The construction of laccase-based bionanoelectrode on Pt-HCF-modified graphite electrode has been developed. The laccase/Pt-HCF/GE electrode was characterized by I_{max} value of 2.41±0.06 μA, K_M^{app} – 0.49±0.02 mM with an upper linearity limit of 0.17 mM toward catechol, and sensitivity – 516 $A \cdot M^{-1} \cdot m^{-2}$. The laccase bionanoelectrode possesses better electrochemical behavior for laccase than non-modified carbon electrodes (control). The bioelectrode have demonstrated 4 folds enhanced maximal current at substrate saturation (I_{max}) values, five folds increased sensitivity and twice wide linearity compared with control bioelectrode.

A laboratory prototype of a bioreactor based on chitosan beads with encapsulated bioelement: laccase-Fe_3O_4 – for degradation of diclofenac, which can be successfully used for biodegradation of xenobiotics (DF) in model solutions, was constructed.

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АНОТАЦІЯ

КОНСТРУКЦІЯ БІОРЕАКТОРА ДЛЯ ВИДАЛЕННЯ ФЕНОЛЬНИХ СПОЛУК ТА БІОСЕНСОРА ДЛЯ ЇХ АНАЛІЗУ

Збереження і відновлення водних ресурсів являє собою величезну проблему для сучасного суспільства. Феноли та деякі з ксенобіотиків фенольної природи, окрім стічних вод очисних споруд, виявляються також у поверхневих і підземних водах, оскільки вони лишень частково видаляються в процесі існуючих технологічних схем очистки стічних вод. Надходження похідних фенолу до природніх водойм призводить до зменшення здатності водного об'єкта до саморегенерації за допомогою наявного геобіоценозу і унеможливлює в подальшому дезактивацію інших забруднень. Мінімальна токсична доза похідних фенолу, що зменшує кількість мікроорганізмів, які забезпечують знешкодження небезпечних сполук у воді на 50%, становить 22,1 мг/дм^3. Фенольні похідні є поширеними забруднювачами, вони надходять у поверхневі води зі стоками підприємств нафтопереробної, сланцепереробної, лісохімічної, коксохімічної, а також зі стоками гідролізної промисловості. Для прикладу, ксенобіотик бісфенол А – це мономер, що використовується для виробництва полікарбонатного пластику і епоксидних смол – сировини для продукції пакувальних матеріалів для харчових продуктів та напоїв. Внаслідок гідролізу ефірних зв'язків у цих полімерах, в навколишнє середовище вивільняється бісфенол А, що спричинює широкомасштабний негативний вплив на людину та тварин. Головними джерелами ксенобіотиків фенольної природи є стічні води міст, підприємств та тваринних комплексів. В Україні централізованим водовідведенням у сільській місцевості забезпечено близько 14% населення, а міські водоочисні споруди знаходяться здебільшого у незадовільному стані. Навіть після очистки ґрунтових вод, у них залишається досить високий вміст похідних фенолу та фармацевтичних препаратів. Феноли та ксенобіотики фенольної природи класифікуються як канцерогени, є токсичними для здоров'я сполуками, що зумовлюють порушення імунної та ендокринної систем людини та тварин, а тому розробка нових підходів моніторингу цих небезпечних речовин у стічних водах є дуже актуальною проблемою сьогодення. Запропоновані дослідження складають особливу маркетингово-соціальну цінність, зокрема, для майбутнього їх впровадження при контролі рівня забруднення стічних та ґрунтових вод, а також для оцінки якості питної води.

У цьому дослідженні ми описуємо виготовлення чутливого біосенсора для виявлення фенольних субстратів з використанням лаккази, іммобілізованої на модифікованому гексаціаноферратом (HCF) графітовому електроді. Результати амперометричного аналізу показали, що Pt-HCF-модифікований лакказний біонаноелектрод має кращу електрохімічну поведінку для лаккази, ніж немодифіковані графітові електроди (контроль). Біоелектрод продемонстрував у чотири рази збільшений максимальний струм при значеннях насичення субстрату (I_{max}), у п'ять разів підвищену чутливість та удвічі ширшу лінійність порівняно з контрольним біоелектродом. Створено лабораторний прототип біореактора на основі хітозанових кульок з інкапсульованим біоелементом: лакказою-Fe_3O_4 – для деградації диклофенаку (ДФ), який можна успішно використовувати для біодеградації ксенобіотиків у модельних розчинах.

Ключові слова: HCF, лакказа, ABTS, амперометричний біосенсор, ксенобіотики.