OPINION
By: Prof. Iskra Vitanova Ivanova, PhD

By order No. PD-22-1277 of 27.05.2025 of the Rector of Plovdiv University "Paisiy Hilendarski" (PU), I am appointed as a member of the scientific jury.

Subject: evaluation of a dissertation for the acquisition of the educational and scientific degree "Doctor"
Field of higher education: 4. Natural sciences, mathematics and informatics
Professional field: 4.3. Biological sciences;
Doctoral program: Biochemistry
Author of the dissertation:
ANGEL ILIEV PESHKOV

Title of the dissertation:
"STUDY OF THE PROPERTIES OF OXIDOREDUCTASES IMMOBILIZED IN BIOCOMPATIBLE MATTRISES

Scientific supervisors:
Prof. Dr. Iliya Nikolov Iliev
Assoc. Prof. Dr. Nina Dimitrova Dimcheva

1. RELEVANCE AND SIGNIFICANCE OF THE DEVELOPED PROBLEM
The presence of these pollutants in the environment and water sources affects all living organisms and disrupts the ecological balance, and their removal is a serious challenge. The use of enzymes in the processes of detection of toxic substances is an important part of modern biotechnological processes for environmental purification. The development of practical biocatalytic applications of oxidoreductases is an important goal in biotechnology. In detoxification processes, the oxidoreductases laccase and catalase are widely used. Laccase and catalase are enzymes that play a significant role in biosensor technology due to their catalytic properties and potential for specific interactions with substrates. The prospect of using catalase and laccase in the construction of biosensors with multiple applications presupposes scientific research to develop new methods for immobilizing the enzymes and incorporating them into new biosensors.
All this gives me reason to assess the presented scientific work as up-to-date, with the potential for scientific achievements that can have a rapid practical implementation. The perspective of using catalase and laccase in the construction of biosensors with multiple applications presupposes scientific research to develop new methods for immobilizing the enzymes and incorporating them into new biosensors.
The two enzymes contribute to the development of sensitive, specific and environmentally friendly biosensors for applications in environmental monitoring, health diagnostics, food safety and bioelectronics.

2. VOLUME AND STRUCTURE OF THE DISSERTATION
The dissertation is presented on 137 standard pages of text. The generally accepted scheme and the recommended ratios between the individual parts of the work have been observed, as follows: Introduction – 2 pages; Literature review – 31 pages; Aim and objectives – 1 page; Materials and

methods – 16 pages; Results and discussion – 45 pages; Conclusions 2 pages. The results obtained are illustrated with 28 figures, 14 tables, 1 scheme and 238 literary sources.

3. LITERARY AWARENESS AND STATEMENT OF THE GOAL AND OBJECTIVES

This dissertation is complex and assumes a good knowledge of the literature sources and methods for solving it. The doctoral student has made a thorough review of the achievements of other researchers, which he managed to convey and analyze on 31 pages in the literature review. The review presents the state of the problem in detail and proves the need to develop the dissertation thesis. The literature review consists of three sections. The author consistently examines the oxidoreductases catalase and laccase, their properties, structure, classification, as well as their application in the food industry, in the pharmaceutical-food industry and in bioremediation. Data on the immobilization of catalase and laccase are presented, such as covalent immobilization, immobilization by crosslinking, adsorption and encapsulation of the studied enzymes. In the third section of the literature review, the author examines the information on catalase and laccase-based biosensors and their importance in bioremediation, the food industry and in the construction of biosensors.

The literature review is specific, structured correctly, and follows the logical connection of the information. The data from the review served to clearly and correctly define not only the goal, but also the formulation of the tasks. 5 well-founded experimental tasks were set for solving. The literature (both in the review and in the entire work) is closely related to the topic of the dissertation. The literature list includes an impressive number of titles in Latin. They are mainly from recent years. This speaks of the excellent theoretical awareness of the doctoral student and the aim of finding a new scientific challenge.

4. PURPOSE AND OBJECTIVES

The purpose of this dissertation is to investigate the capacity of the enzymes catalase and laccase, obtained from different sources, for inclusion in biosensors for conducting catalytic reactions under conditions of heterogeneous biocatalysis and in the presence of organic solvents.

5. EVALUATION OF THE METHODS AND MATERIALS USED

The "Materials and Methods" section demonstrates an impressive set of methods, tailored to the specific requirements of the experiments. They are modern and adequate for the implementation of the dissertation. They are described accurately and in detail, fully covering the multifaceted areas of work: from classical to modern research. Biochemical methods include extraction, concentration, purification and drying of the enzyme catalase Pen. Chrysogenum and determination of enzyme activity from various sources in homogeneous and heterogeneous media. The determination of laccase from various sources was carried out by spectrophotometric method in the presence of organic solvents. Tris-glycine SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and Tris-glycine native-polyacrylamide gel electrophoresis (Native-PAGE) were used. Modified electrode materials were used from electrochemical methods. Immobilization of catalase enzyme on glass substrates, electrografting of catalase enzyme on a gold electrode and covalent immobilization of catalase on the electrochemically modified gold electrode and electrochemical characterization of the modified glassy carbon or gold electrodes were carried out. Scanning electron microscopy was used in the development. All this allows me to give a high assessment of the scientific level and the excellent preparation, which manages to correctly combine a variety of classical and modern methods for the purposes of the dissertation, successfully solving the experimental tasks set.

6. EVALUATION OF THE OBTAINED RESULTS

The section "Results and Discussion" is well structured, supported by tabular and graphical material, with an appropriate interpretation of the results obtained by foreign scientific teams. The author consistently presents evidence for his scientific thesis, thus logically finalizing experimental work. A large-scale and diverse experimental work has been carried out within the framework of a complex study. The results obtained show that catalase from Penicilium immobilized on glucan type URE13-300 retains up to 76% of its initial activity in the presence of 5% ethanol and 63.5% of the initial activity. In the presence of low molecular weight aliphatic alcohols, it was found that the most resistant to the effects of the organic solvent is catalase from Penicilium chrysogenum. It retains 83% and 98% of its activity in the presence of 1% ethanol and 1% methanol, respectively, and when their content is increased to 5%, 63% and 57% are retained, respectively. An electrochemical method has been developed for determining the enzymatic activity of immobilized catalase from Penicilium chrysogenum in the absence and presence of methanol and ethanol, and a formula has been derived for determining the enzymatic activity. Two types of electrochemical biosensors were obtained by immobilizing the laccase enzyme from Trametes versicolor and Trametes pubescens in a polymer film and were characterized using electrochemical techniques such as cyclic and pulse voltammetry, as well as chronoamperometry at constant potential. The electrochemical behavior of the biosensors in the presence of di- and trihydroxy phenols was studied. The operating conditions of the biosensor guarantee low energy consumption by the biosensor and minimal interfering influences when analyzing complex matrices; The two laccase enzymes studied show a significant difference in their heterogeneous biocatalytic activity, with the enzyme from Trametes pubescens being characterized by a much higher specific activity; The biosensor method was applied to determine the total phenolic content in herbal extracts. When compared with chromatographic analysis of the phenolic content of the samples, a good correlation of the results of the chromatographic and biosensor analyses was established. The results presented in "Results and Discussion" logically follow the course of solving the tasks set. They are summarized and discussed in the light of published data from recent years. Both the idea and the volume of research conducted on the implementation of this task and throughout the work deserve high praise. The discussion of each experiment, the comparison of the results and the comparison with the literature data, once again emphasizes the qualities of the doctoral student in mastering experimental theory. With this, he proves that he has fully mastered the third stage of his training and is a complete experimenter.

7. CONTRIBUTIONS AND SIGNIFICANCE OF THE DEVELOPMENT FOR SCIENCE AND PRACTICE, NOTES AND QUESTIONS

I accept the contributions made!

Angel Peshkov is an author in 3 scientific publications, in two of which she is a leading researcher and nine participations in scientific forums, which shows her creative and research activity in their development and shaping.

I have a few questions for the dissertation:

1. What kind of intermediate agents, acting as mediators between the reagent or catalyst and the solid support (indirect immobilization) play a key role in facilitating the attachment of the reagent or catalyst to the support material, increasing the stability and efficiency of the immobilization process?
2. Which fermentation strategies are used for the production of laccases?

CONCLUSION

The topic is relevant, the doctoral student has mastered modern methods, the experiments are methodically set up correctly, the results obtained are reliable and are a solid basis for further scientific and applied developments. Based on the above, I can confidently state that the reviewed dissertation work represents an original scientific development, with theoretical and applied significance. The proposed dissertation is evidence that Peshkov has developed competencies necessary for the award of the doctoral degree, including theoretical preparation, methodological knowledge, independence and experience in planning experiments and the ability to analyze the results.

The dissertation work contains scientific, scientifically applied and applied results that represent an original contribution to science and meet all the requirements of the Act on the Development of the Academic Staff in the Republic of Bulgaria (ADSRB), the Regulations for the Implementation of the ADSRB and the relevant Regulations of the University "Paisiy Hilendarski" and I give my high assessment and recommend to the members of the scientific jury to award the dissertation candidate Peshkov the educational and scientific degree "Doctor" in professional field 4.3. Biological Sciences, Specialty Biochemistry.

31.07.2025 Signature:

Prof. Iskra Ivanova