REVIEW

by Prof. Dr. Velizar Kostadinov Gochev, "Paisii Hilendarski" University of Plovdiv

of the dissertation thesis for awarding the educational and scientific degree " Doctor of Philosophy"

in: Higher education area 4. Natural Sciences, Mathematics and Informatics

Professional field 4.3 Biological Sciences, Doctoral program in Biochemistry.

Author: Angel Iliev Peshkov

Title: Investigation of the properties of oxidoreductases immobilized in biocompatible matrices

Supervisors: Prof. Dr. Iliya Nikolov Iliev and Assoc. Prof. Dr. Nina Dimitrova Dimcheva

1. General description of the materials presented

By Order No. PD-22-1277 of 25th May 2025 of the Rector of "Paisii Hilendarski" University of Plovdiv (PU), I was appointed as a member of the scientific jury to ensure the procedure for the defense of a dissertation on the topic „Investigation of the properties of oxidoreductases immobilized in biocompatible matrices“ for the acquisition of the educational and scientific degree " Doctor of Philosophy" in the field of higher education 4. Natural Sciences, Mathematics, and Informatics, professional field 4.3 Biological Sciences, doctoral program in Biochemistry. The author of the dissertation is Angel Iliev Peshkov, a full-time doctoral student at the Department of Biochemistry and Microbiology, supervised by Prof. Dr. Iliya Nikolov Iliev and Assoc. Prof. Dr. Nina Dimitrova Dimcheva from Plovdiv University.

The set of materials on paper presented by Angel Iliev Peshkov is in accordance with Art. 36 (1) of the Regulations for the Development of Academic Staff at PU and includes the following documents:

– application to the Rector of PU for the initiation of the procedure for the defense of a dissertation;
– curriculum vitae in European format;
– dissertation;
– abstract;
– list of scientific publications on the topic of the dissertation;
– copies of scientific publications;
– a declaration of originality and authenticity of the attached documents;

All documents submitted comply with national and institutional regulatory requirements, which allows me to determine the procedure as lawful.

2. Brief biographical information about the doctoral student

Angel Iliev Peshkov graduated from University of Plovdiv and obtained a master's degree and a bachelor's degree from the Faculty of Chemistry, specializing in Food Chemistry and Medical Chemistry, respectively. In 2021, he was enrolled as a full-time doctoral student in the Faculty of Biology, doctoral program "Biochemistry," and since the same year, he has been a researcher at the Technology Center at Plovdiv University. During his doctoral studies (2022), he completed a one-month specialization in "Biosensors and Bioelectronics" at the University of Malmö, Sweden.

3. Relevance of the topic and appropriateness of the goals and objectives

One of the most serious problems of modern society is the ecological one, manifested in the growing anthropogenic pressure on the environment and the accumulation of a wide range of chemical pollutants in various terrestrial and aquatic ecosystems. In recent years, there has been increased interest in the possibilities of applying biotechnological approaches and "green chemistry" methods to solve the problem of xenobiotic degradation. The design and application of enzyme biosensors for ecological monitoring, clinical research, etc. is also the subject of increased scientific interest. All this gives me reason to categorically define the topic of this dissertation research as relevant.

4. Knowledge of the subject

The literature review on the topic is presented on 31 pages and is based on the use of over 200 literary sources. The review begins with a general description of the class of oxidoreductases, but with an emphasis on the structure, properties, and application of the enzymes catalase and laccase, which are the subject of the dissertation. The various approaches to enzyme immobilization are very well presented, with the respective advantages and disadvantages of each method. Various types of catalase- and laccase-based biosensors described in the literature are discussed in sufficient detail, as are their possible applications in the food industry, medicine, and wastewater treatment processes. As a major strength of the review, I would highlight the fact that the cited literature and analysis of the literature data are strictly focused on the enzymes catalase and laccase. I believe that the doctoral student has an in-depth understanding of the problem, which allows him to formulate the goal and tasks and carry out the present study. The review would benefit if, at the end, in some synthesized form, it highlighted the still insufficiently researched problems and challenges in the subject area, which would help to motivate the need for the development of this dissertation and more clearly define the objective of the study.

5. Methodology

The Materials and Methods section is presented on 17 pages and describes in detail a wide range of spectrophotometric, enzymatic, electrochemical, microscopic, and statistical methods. The chosen research methodology allows the set goal and research tasks to be achieved. The way the section is structured allows for the reproducibility of the experiments and shows that the doctoral student has successfully completed the educational component of the educational and scientific degree of "Doctor of Philosophy".

6. Characteristics and evaluation of the dissertation

The dissertation is structured in the accepted manner, including the following main sections: *Introduction* (2 pages), *Literature Review* (31 pages), *Aims and Objectives* (1 page), *Materials and Methods* (17 pages), *Results and Discussion* (46 pages), *Conclusions* (2 pages), *Contributions* (1 page), and *List of References* (20 pages). The proportions between the individual sections of the dissertation are optimal. The introduction fulfills its function by highlighting the significance of the problem of environmental pollution and the need to seek effective biotechnological approaches to solving it, in particular enzymatic biodegradation. The characteristics of the *Literature Review* section were discussed in section 4. The aim of the dissertation and the tasks for its achievement are clearly formulated, the experimental scheme applied is well structured and suggests that the aim of the research will be achieved. *The Materials and Methods* section shows that the doctoral student has successfully mastered a wide range of experimental methods. Regarding this section, I have a comment on section 2.1, because it is clear from the *Results and Discussion* section that the doctoral student works with a ready-made enzyme preparation of mold catalase from *Penicillium chryzogenum,* rather than obtaining it in the course of the dissertation, and in this line of thought, point 2.1 is unnecessary. There are some inaccuracies in the use of terms such as “homogeneous enzyme activity” instead of “enzyme activity in a homogeneous environment” and “heterogeneous enzyme activity” instead of “enzyme activity in a heterogeneous environment,” etc. The method described in section 2.3 is the same spectrophotometric method described in section 2.2 and did not need to be separated into a separate section. The most significant omission in the *Materials and Methods* section is the lack of a described procedure for immobilizing enzymes on glucan, which is in fact the only biocompatible matrix studied, which should be the main focus of the work, based on the title. The immobilization procedure is explained, but in the *Results and Discussion* section. The results are presented in 14 tables and 28 figures and are correctly commented on. The experimental work begins with determining the activity of catalase of different origins (bacterial, fungal, and bovine) in native and immobilized form. The effect of different concentrations of ethanol and metha-

nol on the activity of free and immobilized catalase is investigated. Similar experiments are conducted with the enzyme laccase of different origins, with a technical commercial preparation of laccase from *Aspergillus niger* being purified and concentrated prior to immobilization. The commentary on the results contains some factual errors and terminological inaccuracies, for example, it is written, and I quote, "mold laccase is more stable than enzymes obtained from eukaryotic organisms," while molds belong to *Regnum Fungi, Imperia Eucariota*. Enormous efforts have been made in the development of electrochemical methods for determining the activity of catalase and laccase. The work on the dissertation continues with the construction of a biosensor for the electrochemical determination of gallic and caffeic acid based on immobilized laccase from basidiomycete fungi. The biosensor is applied to determine the content of total phenols in herbal extracts. The results are compared with those from HPLC analysis. Nowhere, neither in the *Materials and Methods* section nor in the *Results and Discussion* section, are the types of herbal extracts, the conditions for their preparation, and the conditions for chromatographic determination specified. Based on the results obtained, five conclusions and two contributions are formulated. The conclusions are categorically based on the results obtained, but are formulated too generally, especially 3, 4, and 5.

Overall, the dissertation is written in good scientific language with correctly commented experimental results.

7. Contributions and significance of the study for science and practice

The contributions are correctly formulated with a clearly defined scientific and applied character.

8. Assessment of publications related to the dissertation

Three scientific publications are presented in connection with the dissertation, two of which have the doctoral student as the lead author. Two of the publications are in Q3 quartile journals and one is in a Q4 quartile journal. The publications reflect significant results from the dissertation and generate 42 points for the doctoral student, which fully meets and exceeds the minimum national requirements. Angel Peshkov has participated with 9 posters and oral presentations reflecting the results of the dissertation, both in Bulgaria and abroad, which I consider positive.

9. Personal participation of the doctoral student

I consider the doctoral student's personal participation in the realization of the dissertation and scientific publications to be relevant to his level of competence.

10. Abstract

The abstract has been prepared in accordance with the requirements and reflects the main results achieved in the dissertation.

11. Critical remarks, recommendations, and questions

The main critical remark regarding the dissertation is related to the lack of a sense of conceptual coherence between the individual parts of the dissertation. The topic of the dissertation suggests that the main focus of the experimental work, and respectively the conclusions and contributions, should be in the direction of immobilizing enzymes in biocompatible matrices, and these are definitely in the part concerning the development of an electrochemical method for determining the activity of catalase and laccase and the construction of biosensors. The only biocompatible matrix studied in the dissertation is glucan. The introduction and overview are very well written, but they create an expectation for the work to focus on the ecological aspect, yet such research is not present in the work, neither in the tasks set nor in the results obtained. The focus of the research and the results obtained is on electrochemical methods for determining enzyme activity, but this claim is missing from both the title and the aim of the thesis. In the comments on the results of the individual research tasks, no appropriate links were found between the individual steps of the experimental work, and in a sense, they remain relatively independent of each other. The main contribution of the dissertation is the construction and application of an enzyme biosensor based on immobilized laccase for the determination of phenols (gallic and caffeic acid), but the main analytical characteristics of the biosensor, such as LOD and LOQ, have not been determined, and there is no quantitative evidence of the advantages of the constructed biosensor.

Despite these comments, the overall style of the dissertation, the way the results are presented and interpreted, make it a complete scientific study with a contribution to the field.

I have the following question for the doctoral student:

What do you understand by electrochemical determination of catalase activity in the abiotic and biotic stages?

12. Personal impressions

My personal impressions of Angel Dimitrov Peshkov are positive.

CONCLUSION

The dissertation meets all the requirements of the Law on the Development of Academic Staff in the Republic of Bulgaria (ZRASRB), the Regulations for the Implementation of ZRASRB, and the relevant Regulations of "Paisii Hilendarski" University of Plovdiv.

The dissertation shows that Angel Peshkov has theoretical knowledge and professional skills in the scientific field of "Biochemistry" and demonstrates qualities and skills for conducting independent scientific research.

In view of the above, I give my positive assessment of the research presented in the dissertation reviewed above, abstract, achieved results, and contributions, and I propose that the distinguished scientific jury award Angel Dimitrov Peshkov the educational and scientific degree of "Doctor" in the field of higher education: 4. Natural Sciences, Mathematics, and Informatics, professional field 4.3 Biological Sciences, doctoral program in Biochemistry.

14.07.2025 г. Reviewer: .....

Prof. Dr. Velizar Gochev