REPORT

Subject: discussion on a dissertation thesis for the acquisition of an educational and scientific degree "Doctor of Philosophy" (PhD)

Author of the dissertation (PhD candidate): Assist. Prof. Apostol Georgiev Apostolov, Dipl. Eng.

Topic of the dissertation: "Innovative methods for separation and purification of bioactive substances"

Professional field: 4.2. Chemical Sciences

Scientific specialty: "Processes and Apparatus in Chemical and Biochemical Technology"

Scientific organization: Institute of Chemical Engineering at the Bulgarian Academy of Sciences (ICE – BAS), Laboratory of Biochemical Engineering

Scientific supervisor: Prof. Dragomir Simeonov Yankov, PhD

Prepared by: Assoc. Prof. Eng. Yordan Nikolaev Georgiev, PhD, Department of Chemistry, Biochemistry, Biology and Microbiology at the Faculty of Medicine of Prof. Dr. Assen Zlatarov State University of Burgas, Email address: yordan.georgiev@uniburgas.bg

By Order №09-69/21.11.2025 of the Director of the ICE-BAS, I was elected as a member of the scientific jury and appointed to prepare a report on this procedure, and in my capacity as such I present it below.

1. Brief biographical data of the candidate

Assist. Prof. Apostol Apostolov, Dipl. Eng., was born on 07.04.1995. In the period 2014-2020 he studied at the D. Mendeleev University of Chemical Technology of Russia, Faculty of Digital Technologies and Chemical Engineering, in the city of Moscow (Russian Federation), where he successively defended his Bachelor's and Master's degrees. From February 2019 to July 2020, the candidate was in an internship at the Institute of General and Inorganic Chemistry at the Russian Academy of Sciences. Dipl. Eng. Apostolov was appointed to the academic position of Assistant Professor at the ICE-BAS in 2020, and in the period 2021-2024 he was a full-time doctoral student at the same institute.

From the presented documents on the educational courses of the candidate during his studies at the university and at the BAS, and from his participation in scientific projects, his purposeful training as a young specialist in the field of industrial chemical-biological processes or food biotechnology is clearly visible.

2. Relevance of the problem developed in the dissertation

The dissertation thesis of Assist. Prof. Apostolov is dedicated to the development of modern extraction approaches for the isolation of valuable metabolites (organic acids) from model systems to complex fermentation liquids and their separation and purification through effective membrane technologies, as well as the application of combined membrane techniques for controlling the alcohol content of Bulgarian wines. The extractants obtained by

the dissertation are deep eutectic solvents (DES), which are biodegradable, recyclable, affordable, easy to prepare, non-volatile and capable of separating racemic mixtures. The industrial application of such extractants will lead to a reduction in the use of classical organic solvents, when conducting liquid-liquid extraction in biotechnological production. Although DES are not included in the EU Circular Economy Action Plan (2020-2050), they will help develop an environmentally friendly bioeconomy. It is more important to note that the testing, scaling up and implementation in production of the separation of racemic mixtures of the device developed at the institute by liquid membrane pertraction will help increase the competitiveness of the Bulgarian economy.

3. Review of the dissertation and analysis of the results

The detailed analysis in the literature review of a large number of extraction methods known to science for extracting biologically active compounds from natural sources and the illustration of the techniques with some technological schemes drawn by Dipl. Eng. Apostolov to explain the principle of the given extraction approach, as well as the tables summarizing the advantages and disadvantages of the methods in industrial conditions, make a very good impression. Perhaps modern chromatographic techniques (including ion exchange and affinity chromatography) should also have been presented in greater detail, because they are important for the purification of the extracted biomolecules and the characterization of the final bioproducts. The application of organic acids, which are the main subject of the dissertation, in nutrition, cosmetics and pharmacy should be also discussed.

The literature review is logically connected to the experimental work of the dissertation thesis and the subsequent goals and objectives. The goal of the study is relevant and justified in scientific and applied aspects, and the tasks are feasible within the terms of a full-time doctoral program in Bulgaria.

The section "Materials and Methods" is carefully prepared from an analytical point of view for reproducing the applied methods. There is a lack of explanation of some of the components in some of the presented mathematical equations (e.g., pages 49 and 51), as well as the source of the red wine used for the research in the dissertation and its abstract, but this omission has been corrected in the publications. I do not find the initial concentrations of fumaric and maleic acids in the individual and mixed aqueous solutions for conducting liquid-liquid extraction with the developed DES, but similar information is available when conducting the pertraction.

The main text of the dissertation in the section "Results and Discussion" is structured logically, in-depth and contains an adequate discussion of the results with other studies from the available international literature. The first part of the section presents results from the development and physicochemical characteristics (density, viscosity, thermal stability) of DES based on menthol and amines. The chemical interaction between the components in the individual solvents has been proven by FTIR spectroscopy. This is where my first question to the doctoral student arose: **What is the stability of the obtained solvents over time**

during their storage?

The extraction efficiency of L-lactic acid from the model 1% (w/v) aqueous solution in the menthol–dioctyl amine (2:1) system was calculated to be 82-86%, and the efficiency of the two-step re-extraction with 0.4 M NaOH led to a recovery of 97% of the acid, with the extractant maintaining stability even after a higher number of extraction and regeneration cycles. The extraction efficiency of the geometric isomers of butenedioic acid (maleic – *cis*-butanedioic and fumaric – *trans*-butanedioic acids) from their model mixture with DES menthol–salicylic acid (6:1) was highest for fumaric acid (35%), due to the increased hydrophobicity of the system and the stabilization of the *trans*-isomer. Again, the re-extraction from DES was also highly efficient (87.5%). This leads to my second question: **Is it possible to use both types of DES to extract L-lactic acid and fumaric acid from fermentation culture fluids of lactic acid bacteria and molds, respectively?**

It is important to note that pertraction with a membrane liquid of 1-decanol and pH=1.8 achieves over 85% extraction of fumaric acid and about 38% of maleic acid within 345 min. **What is the perspective of application of this laboratory method in industrial conditions?**

When combining diananofiltration and reverse osmosis, with industrial membranes, the most effective retention of the monosaccharides (glucose, fructose) and organic acids (citric, tartaric, malic, succinic, acetic) studied by HPLC-RID is achieved, and the reduction of ethanol and acetic acid in red wine produced from the red grape variety "Mavrud" is achieved. This leads to my fourth and fifth questions: **What efficiency of retention of resveratrol and other valuable phenolic compounds is expected, when applying the proposed combined filtration method for dealcoholization of the studied wine? On what principle were the membranes selected for dealcoholization of the wine?**

All technical gaps detected in the dissertation work were presented to Assist. Prof. Apostolov for educational purposes for his improvement and are of minor importance to the scientific value of his work.

4. Main fundamental and applied scientific contributions

Eighteen contributions are presented in the dissertation, however, they are not divided into purely fundamental and applied ones. Although some of the contributions can be combined, in essence they correctly reflect the actual results of the doctoral student's experimental work. In my opinion, the most valuable contributions of the dissertation, in a purely scientific aspect, are the development, physicochemical characterization and testing in model systems of new "green" and recyclable DESs based on menthol with organic amines and with salicylic acid for the separation of biogenic organic acids. In a scientific and applied aspects, in the dissertation are developed a laboratory pertraction process for the separation of geometric isomers and a two-stage membrane technology with commercial industrial membranes for the dealcoholization of red wines.

In conclusion 15, monosaccharides and organic acids are probably considered as high-molecular compounds in wine, but although they have a higher molecular weight than

the studied ethanol and acetic acid, from a chemical point of view, high-molecular compounds in wine are polyphenols, polysaccharides, glycoproteins (mannoproteins, arabinogalactans), proteins, etc.

5. Description and evaluation of the submitted materials

Assist. Prof. Apostolov has submitted electronically a set of documents for the procedure, which contains: an application for admission to the defense procedure, protocols for passed exams, a short professional biography, a copy of a Master's degree diploma, a dissertation, a dissertation abstract in English, a list of scientific publications on the dissertation, copies of the scientific publications and a list of noted citations on the dissertation, a list of participation in research projects, a list of participation in scientific forums, a statement of fulfillment of the additional criteria of the ICE-BAS for awarding the PhD degree, certificates and awards, as well as a similarity report from StrikePlagiarism.com. The documents on the procedure have been carefully prepared and are in accordance with the requirements of the Act on the Development of the Academic Staff in the Republic of Bulgaria (ADASRB), the Regulations for the Implementation of the ADASRB, the Regulations on the Conditions and Procedure for Acquiring Scientific Degrees and for Holding Academic Positions at the BAS for the Implementation of the ADASRB, the Regulations for the Activities of the Training Center and the Academic Council at the BAS, as well as the Methodology for the Growth of Scientists at the ICE-BAS of 05.11.2024.

The abstract for the dissertation is prepared in English, which meets the modern high scientific standards of the BAS, however, some text in Bulgarian is also found in it.

As a result of the development of the dissertation thesis, 2 experimental scientific publications have been published in the journals Applied Sciences (20 points. – Q2, Engineering, Multidisciplinary, IF=2.5, WoS, 2024) and Chemistry (20 points - IF=2.4, WoS, Q2, Chemistry (miscellaneous), Scopus, 2024) of the international publishing house MDPI and one review article in the journal Theoretical Foundations of Chemical Engineering (12 points - IF=0.6, WoS, Q4, Chemical Engineering, WoS, 2024) of the publishing house Springer Nature. Assist Prof. Apostolov is the first and second co-author in two of the three publications, which confirms his main contribution to the conducting of the experimental work and the preparation of the scientific publications. The candidate has accumulated a total number of 52 points, which exceeds the minimum requirements in the Additional Criteria of the ICE-BAS for acquiring the PhD degree. He has presented the results of his dissertation at a number of scientific forums in the country and abroad (Russian Federation and Czech Republic). The candidate is a participant in the working groups of 5 research projects (including an international project on bilateral cooperation Bulgaria-Russia), funded by the Scientific Research Fund at the Ministry of Education and Science in RB and the Center for Competence HITMOBILE, funded under the Recovery and Sustainability Plan of the Republic of Bulgaria and under the Next Generation EU (2021-2024). It is important to note that Assist. Prof. Apostolov is also a winner of the award for the youngest scientists under 30 years old of the BAS "Ivan Evstratiev Geshov" for achievements in the scientific field "Energy Resources and Energy Efficiency".

6. Reflection of the candidate's scientific publications in Bulgarian and foreign literature

One of the candidate's scientific publications (Ivanova, D., Apostolov, A., Tuleshkov, P., Novakov, C., Yankov, D. New Menthol-Based Hydrophobic Deep Eutectic Solvents as a Tool for Lactic Acid Extraction. Appl. Sci., 15(7), 2025, 3564, https://doi.org/10.3390/app15073564) has 2 citations in the foreign available scientific literature in the following journals with an impact factor: Bioresource Technology (https://doi.org/10.1016/j.biortech.2025.133706, 2026) and Journal of Environmental Chemical Engineering (https://doi.org/10.1016/j.jece.2025.120147, 2025). The citation in the same year of publication of the scientific work testifies to the relevance of the topic and the significance of the results obtained in the dissertation.

7. Critical notes and recommendations on the candidate's scientific works

I have no critical comments on the published scientific works of the candidate. I recommend the publication of the remaining results of the dissertation on the separation of geometric isomers of butenedioic acid with DES menthol-salicylic acid and the pertraction process. It is necessary to evaluate the efficiency of DES and the pertraction system with real fermentation liquids for obtaining bioproducts and to study the efficiency of retention of the two-component membrane system of main phenolic compounds in the studied red wine.

8. Personal impressions about the candidate

I do not know and I have not met the candidate personally and I am evaluating him only based on the scientific documentation presented.

CONCLUSION

The presented dissertation thesis is dedicated to current scientific issues in the field of processes and apparatus in the chemical and biotechnology industries. The contributions to the preparation, characterization and application of DES, the use of the pertraction laboratory apparatus for the separation of optically active biomolecules and the two-component membrane system for dealcoholization of wines, have a clear fundamental and applied scientific contributions with a real future application in the Bulgarian industry.

Due to all of the aforementioned, **I convincingly recommend a positive assessment of the dissertation thesis and propose to the esteemed jury to vote on the awarding of the educational and scientific degree Doctor of Philosophy (PhD) to Assist. Prof. Apostol Georgiev Apostolov, Dipl. Eng.**, in the field of higher education 4. Natural Sciences, Mathematics and Informatics, in the professional field: 4.2. Chemical Sciences and scientific specialty: "Processes and Apparatus in Chemical and Biochemical Technology".

22.12.2025 Member of the scientific jury:……
Burgas /Assoc. Prof. Yordan Georgiev, PhD/