

БЪЛГАРСКА АКАДЕМИЯ НА НАУКИТЕ
ИНСТИТУТ ЗА МИКРОБИОЛОГИЯ
№ 256/18 04.29.22
СОФИЯ

To the chairman of the scientific jury,
as defined by Order No 1-26/01.03.2022
of the Director of IM, BAS

STATEMENT REVIEW

of a thesis for the award of educational and scientific degree "Doctor" (PhD), Higher education area: 4. Natural sciences, mathematics and informatics, Professional field: 4.3. Biological sciences, Scientific specialty **01.06.12** „ Microbiology”

Candidate: **Lilyana Vasileva Nacheva**, PhD student in the Laboratory "Bioremediation and Biofuels", Department of Biotechnology at the Institute of Microbiology (IM) of the Bulgarian Academy of Sciences (BAS),

Thesis topic: „Biodegradation of aromatic and aliphatic xenobiotics from free and immobilized bacterial cells“

Supervisor: Assoc. Prof. Dr. Lyudmila Kabaivanova

Prepared by: Assoc. Prof. Nina Dimitrova Tsvetkova, PhD, National Center of Infectious and Parasitic Diseases, Sofia

I declare that I have no common scientific publications or conflict of interests of another nature within the meaning of para 1, items 3 and 5 of the Law on the Development of the Academic Staff in the Republic of Bulgaria (LDASRB) with the doctoral student.

Relevance of the PhD thesis topic

Xenobiotics are one of the most common and dangerous pollutants with detrimental effects on living organisms (microorganisms, plants, animals and humans). Biodegradation processes carried out with the participation of microorganisms are successfully used in the fight against chemical pollution by decomposition of compounds, subsequent absorption and removal from the environment. The ability of microorganisms in the process of their evolution to adapt to adverse environmental conditions is used in modern approaches to its purification from various pollutants (phenols, oil). Maintaining the catalytic activity of microbial cells for a longer period of time with the help of immobilization techniques on the one hand protects cells from the harmful effects of high concentrations of xenobiotics, and on the other hand allows easier separation and recovery of used cells, which allows their repeated application. All this shows that the topic chosen by the supervisor for the development of the PhD thesis is relevant and significant.

Characteristics and evaluation of the PhD thesis

The PhD thesis is developed in the required volume and corresponds to the LDAS in the Republic of Bulgaria and to the Rules for its application in the IM of BAS. It contains 105 standard

pages and is illustrated with 5 tables, 46 figures and 7 diagrams. The reference list includes 181 literary sources. More than 30% (60) of the publications cited in the literature review are from the last 10 years. Traditional thesis format has been followed. Based on the literature, the doctoral student has made an in-depth literature review (24 pages), which is proof that she is well acquainted with the problem related to the topic of the dissertation.

The formulated goals and the tasks arising from them are clear and feasible. To achieve the set tasks the doctoral student has mastered and applied 7 types of methods - immobilization method, scanning electron microscopy, colorimetric method, quantitative determination of biosurfactants, cellular hydrophobicity, biodegradation, genetic methods.

The **results** of the conducted research work correspond to the tasks set in the dissertation. Strains of the genera *Micrococcus*, *Nocardia*, *Pseudomonas* and *Rhodococcus* were selected to study the biodegradation activity to xenobiotics. Solid and liquid growth media with phenol at a concentration of 500 mg/L were used. Ability to grow on solid medium was found in strains *Rhodococcus wratislawiensis* BN38 and *Nocardia farcinica* BN26, and the most active biodegradation on liquid growth medium was observed in strain *R. wratislawiensis* BN38. In the process of optimizing the conditions under which the biodegradation of phenol takes place, it was found that in the strains *Rhodococcus wratislawiensis* BN38, *Nocardia farcinica* BN26 and *Micrococcus luteus* BN56 the highest rate was reached at 29°C. The influence of the initial microbial density on the rate of phenolic degradation was also studied. The dynamics of phenolic biodegradation after adaptation of strains *Rhodococcus wratislawiensis* BN38 and *Nocardia farcinica* BN26, biodegradation in the presence of aromatic and aliphatic toxic substances in the environment with ten times passaged cells of both strains, as well as the relationship between the processes of phenol biodegradation and immobilization of microbial cells of these strains. The ability of immobilized microbial cells to simultaneously biodegrade two xenobiotics was studied and the activity of free and immobilized cells was compared. The cells of the newly isolated bacterial strain *Bacillus cereus* BN66 have been shown to be able to degrade aromatic and aliphatic xenobiotics in the free and immobilized state, as well as to produce biosurfactant. The immobilization method has shown high efficiency in the biodegradation processes carried out by *Bacillus cereus* BN 66 for a long period of time (47 days) at high speed and stability.

The **conclusions** (10 pieces) are correctly formulated and reflect the implementation of the goal and the set tasks, while emphasizing the most important findings of the dissertation.

I accept the **contributions** formulated by the doctoral student. I believe that they are an objective reflection of the results of the thesis.

The **thesis summary** meets the requirements of the Law on the Development of Academic Staff in the Republic of Bulgaria and the Regulations for its application in the IM of BAS. It is written in a volume of 56 pages. Its structure reflects the essence of the dissertation.

Assessment of the doctoral student's publications and personal contribution

The results of the dissertation are published in 4 journal articles with a total IF 2,421 (1 of them Lilyana Nacheva is the first author) and in 1 international scientific forum. The citations of the publications on the topic of the dissertation are 28. The publication activity of the doctoral student in connection with the dissertation is sufficient and meets the requirements for this scientific

degree, according to the Rules of IM of BAS for application of educational and scientific degree "Doctor".

CONCLUSION

The PhD thesis of Lilyana Vasileva Nacheva is a relevant, excellently executed research work. The material is sufficient in volume, the tasks are completed. As a result of the conducted research with precisely selected research methods, original results of important scientific and practical significance have been obtained. I believe that Lilyana Nacheva fulfills the criteria for obtaining the educational and scientific degree "Doctor", in accordance with the Law on the Development of Academic Staff in the Republic of Bulgaria and the Rules for its implementation in the IM of BAS.

I confidently give my positive assessment and strongly suggest to the respected members of the scientific jury to vote for the award of the educational and scientific degree "Doctor" in the Professional field 4.3. Biological Sciences (Scientific specialty "Microbiology" – 01.06.10.) by Lilyana Vasileva Nacheva.

18 April 2022

Signature:..........

/ Assoc. prof. Nina Tsvetkova, PhD /