S C I E N T I F I C O P I N I O N

on Ph.D. Thesis of **Lilyana Vasileva Nacheva** entitled:

"**Biodegradation of aromatic and aliphatic xenobiotics from free and immobilized bacterial cells**" to obtain the educational and scientific degree "Doctor" in scientific area 4. Natural Sciences, Mathematics, and Informatics, professional field: 4.3. Biological sciences, scientific specialty: Microbiology.

From: Prof. Penka Mladenova Petrova, Department of General Microbiology, Stefan Angelov Institute of Microbiology - BAS.

1. Relevance of the topic, general data for the dissertation

Pollution of the environment by toxic chemical compounds is one of the great problems of our time. It is estimated that today, in just one month, the human body is exposed to more toxins than the bodies of people from the beginning of the last century for their entire lives. Some of the main sources of xenobiotics in the environment come from large industries: pharmaceuticals, fossil fuels, wastewater after bleaching pulp and paper, and pesticides used in agriculture. They can be synthetic (organochlorides, polyaromatic hydrocarbons), but also natural derivatives such as some fractions of crude oil and coal. Many xenobiotics are resistant to degradation because they are aromatic compounds containing a benzene nucleus or aromatic heterocyclic rings in their molecule.

Biotechnological approaches for the detoxification of waters and soils are especially valuable both ecologically and economically. The methods of using microorganisms in wastewater treatment are based on their ability to decompose various organic substances, incl. and toxic substances. In addition, microorganisms are able to adapt to xenobiotics and use such compounds as energy sources. Bioremediation through selected strains or isolation of natural microorganisms that degrade xenobiotics is particularly relevant and its potential is hoped for by a number of polluting industries.

The Thesis contains 109 pages and the necessary sections - literature review (26 pages), materials and methods (7 pages), results (42 pages), and discussion (9 pages). The evidence is presented in 5 tables and 46 figures. 181 literature sources are cited. At

the end of the dissertation is included a list of articles related to its topic and a list of citations to these articles.

2. Literary review

The review is comprehensive and detailed and shows a good knowledge of the topic. The biotechnological approaches to solving problems related to environmental pollution, the possibilities for impact on xenobiotics, and the mechanisms of biodegradation and biotransformation are considered. The factors that influence the biodegradation process depending on the nature of the pollutant are described in detail, as well as the microorganisms with proven qualities as bio-degradants of xenobiotics. Directly related to the topic, the methods for immobilization of microbial cells are also considered.

3. Methodology

The Materials and Methods section is short, precise and clear. The applied methods are both classical microbiological and biochemical, and possibly the latest in the field such as scanning electron microscopy (SEM). The advantage of the exhibition is the lack of unnecessary details regarding methods that are already established in laboratory practice. Statistical analyzes have been performed where necessary.

4. Obtained results and scientific contributions

In the dissertation comparative research of the biodegradation activity of xenobiotics of the bacteria of the species *Rhodococcus wratislawiensis, Nocardia farcinica*, and *Micrococcus luteus* is made. The studies were performed with both free cells and immobilized cultures. Specific cryogels (consisting of hydroxypropyl cellulose and N-isopropyl acrylamide) have been prepared for this purpose. Cell attachment and biofilm formation are illustrated by excellent quality micrographs. The conditions for conducting phenolic biodegradation have been optimized by comparative monitoring of biodegradation processes with a gradual increase in the initial concentration of phenol and in combination with hexadecane. The relationship between cellular hydrophobicity and surface tension in the process of biodegradation and biosurfactant production has been studied.

The dissertation has a number of novelties and contributions. The most significant would be the demonstration that *Rhodococcus wratislawiensis* BN38 is able to degrade both hydrophilic and hydrophobic xenobiotics, as well as the isolation of a

new strain *Bacillus cereus* BN66 and the demonstration of degradation of aromatic and aliphatic cultures in xenophobia. immobilized).

5. Remarks and questions

I have no remarks on the work. It is written in a fascinating way, in a good scientific style.

Questions: Do you envisage patenting the immobilization procedure related to the inclusion of *Bacillus cereus* in polyacrylamide gel? Could this approach to immobilization have an industrial application, having given the cost of starting reagents?

6. Publications related to the dissertation

There are four publications related to the topic of the dissertation, already published in authoritative journals with a total IF - 2,421, and the doctoral student is the first author in one of them. The articles are cited 28 times. The doctoral student is a participant in research projects and in a conference report.

7. Conclusion

The presented work outlines Lilyana Nacheva as a long-term researcher with good knowledge in the field of microbiology. Based on the novelties and achievements of the dissertation and the scientometric indicators, I strongly recommend that she be awarded the scientific and educational degree "Doctor".

April 17, 2022 Signature:

(Prof. Penka Petrova, DSci)