REVIEW

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On the dissertation for the award of Educational and Scientific Degree (ESD) "Doctor"

in the field of higher education: 4. Natural sciences, mathematics and informatics,

professional field: 4.3 Biological sciences,

scientific specialty 01.06.12 "Microbiology"

Author: Assistant Lilyana Vasileva Nacheva, PhD student in Bioremediation and Biofuels, Department of Biotechnology, Institute of Microbiology "Stefan Angelov" - BAS

Topic: "***Biodegradation of aromatic and aliphatic xenobiotics from free and immobilized bacterial cells***"

Scientific adviser: Assoc. Prof. Dr. Lyudmila Kabaivanova

1. **General presentation of the procedure and the doctoral student**.

By order I-26 / 01.03.2022 of the Director of the Institute of Microbiology - BAS I was appointed a member of the scientific jury in connection with the procedure for defense of dissertation on "Biodegradation of aromatic and aliphatic xenobiotics from free and immobilized bacterial cells "for obtaining the educational and scientific degree (ESD) " Doctor " in professional field 4.3 Biological sciences, from the field of higher education 4. Natural sciences, mathematics and informatics, scientific specialty 01.06.12 " Microbiology ". The author of the dissertation is assistant Lilyana Vasileva Nacheva - PhD student in independent form of education at the Institute of Microbiology "Stefan Angelov" - BAS. For the preparation of the review I received the materials required by the Regulations for the development of the academic staff of the Institute of Microbiology and in accordance with the rules for application of Law for the Development of the Academic Staff (LDAS) in the Republic of Bulgaria and BAS.

The doctoral student graduated in the period 1995-2001 the University of Chemical Technology and Metallurgy - Sofia. Her professional field is a biotechnology engineer with a master's degree in "Bioproduction Technology". The main competencies arising from her education are in the field of microbiology, biochemistry, organic and analytical chemistry, physical chemistry, nutrient chemistry and analysis of organic products.

From 2000 until today the doctoral student goes through all stages of successive career development through various positions, incl. laboratory assistant, biotechnologist, research associate, assistant.

Almost the entire professional biography of the doctoral student is related to the research and educational activities conducted at the Institute of Microbiology "Stefan Angelov" - BAS. Since January 2015 she has held the position of assistant, and to date she has been expelled from the doctoral program with the right to defend after successfully passing internal approbation - the dissertation was discussed and defended at a meeting of the National Scientific Seminar on Applied Microbiology and Microbial Biotechnology on February 17, 2022 at the Stefan Angelov Institute of Microbiology - BAS.

2. **Relevance of the topic.**

The dissertation topic is directly and indirectly related to the extremely important issues of environmental protection today. This is evident from the goal set for the development of this dissertation - to study the capacity for biodegradation of aromatic and aliphatic xenobiotics of certain microbial species in free and immobilized form. The proposed biotechnological approaches for detoxification of waters and soils are especially valuable in scientific, ecological and economic terms. The topic of the dissertation covers methodologies for wastewater treatment, using biological methods based on the ability of microorganisms to degrade various organic substances, including and toxic substances.

3. **Knowledge of the problem**.

The introduction and the literature review with the basic information about the role of biotechnology in solving problems related to environmental pollution demonstrate a very good knowledge of the state of the problem developed by the doctoral student in the dissertation. Issues related to the specifics of xenobiotic substances, the processes of biodegradation and biotransformation, the role of microorganisms degrading aliphatic hydrocarbons, the production and application of biosurfactants, as well as the promotion of methods for immobilization of microbial cells.

4. **Characteristics and evaluation of the dissertation and contributions**.

The dissertation is structured according to the generally accepted requirements - introduction, literature review, purpose and tasks, material and methods, results, discussion, conclusions, contributions and literature. It covers 105 pages, contains 7 diagrams, 46 figures and 5 tables. The bibliographic reference includes 181 literature sources. In the overall literary interpretation of the problem, the author considers the role of xenobiotics in progressive environmental pollution and the significant possible capacity for biodegradation and detoxification through microbial activity. The dissertation examines existing specific biotechnological solutions related to the problem of environmental pollution. This pollution is gaining negative significance on a global scale as a result of the demographic explosion, urbanization, scientific and technological progress and industrialization, which together unequivocally contribute to the progressive contamination of the biosphere.

A comprehensive overview of the processes of biodegradation and the factors influencing the biodegradation processes carried out by microbes is presented. The existence of this type of microbial biodegradation is seen as a unique approach successfully applied in the fight against

chemical pollution in general. The presented literature data confirm the important fact that microorganisms are the main factor in changing the structure of xenobiotics that we find in soils and waters. It is stated that the biodegradation capacity of microorganisms is important for their extremely high potential for adaptation and microbial transformations. The author provides data on the varieties of hitherto known microorganisms - biodegradants of xenobiotics. The analysis shows that xenobiotic aromatic compounds are degraded by a large group of microorganisms, primarily Gram (-) negative bacteria of the genus *Pseudomonas, Sphingomonas, Acinetobacter, Ralstonia and Burkholderia*; Gram (+) positive of the genus *Rhodococcus, Nocardia, Bacillus, Geobacillus*; yeasts of the genus *Candida* and *Trichosporon*, molds of the genus *Fusarium* and *Aspergillus*. It is emphasized that the most important role in the biodegradation of xenobiotics in soil and water is played by bacteria of the following genera *Pseudomonas, Achromobacter, Acinetobacter, Alcoligenes, Arthrobacter, Bacillus, Flavobacterium, Nocardia, Corynebacmeus, Cordionia*, etc. As a result of the accumulation of an increasing amount of microbiological information of this type, the author manages to analyze in her review data on the parallel biotechnological effects of acceleration, which leads to the introduction of biosurfactants in the system. The issue of application of cell immobilization and methods for immobilization of microbial cells using membrane technologies (immobilization in membrane reactors) is considered.

The performed multilayer microbiological and biomedical analysis of the problem is directly related to environmental protection. This allowed the doctoral student and her supervisor to determine the exact purpose of the study - to study the ability of microbial species in free and immobilized form to biodegrade aromatic and aliphatic xenobiotics.

The research material is sufficient in volume, properly selected and structured, well documented and accurately registered, which ensures the reliability of the results.

The evaluation of the experimental research approach shows that the doctoral student has used reliable and sufficient in volume methods to achieve the set goal, with which she receives an adequate answer to the tasks. From six of the tasks directly related to the goal arises a structured methodology for solving them: conditions for the cultivation of microorganisms, methods of immobilization, synthesis of cryogels from a mixture of hydroxypropylcellulose (HPC) and N-isopropylacrylamide (NIPAM), cryogel synthesis synthesized by precursors of polyacrylamide or high molar mass acrylamide, synthesis of cryogel polyethylene oxide (PEO) and polyamidine (PAA), scanning electron microscope (SEM), colorimetric methods, quantification of biosurfactants, assessment of cellular hydrophobicity, biodegradation of n-hexadecane, strain identification, etc. Statistical methods are appropriately selected with the information needed for the survey. The research related to the dissertation work was conducted in the Laboratory "Bioremediation and Biofuels" at the Institute of Microbiology "Stefan Angelov" - BAS.

The sections of the results and discussion occupy a total of 41 pages of the exhibition. The obtained experimental data are clearly shown and analyzed in the presented documentation. The selected strains of *Rhodococcus wratislawiensis BN38*, *Nocardia farcinica BN26* and *Micrococcus luteus BN56* have been shown to show pronounced biodegradation potential to the aromatic xenobiotic phenol. This potential of the studied strains is multiplied by adaptation,

which is expressed in the greater number of cycles during which the imported xenobiotic is completely degraded. The data obtained from the author of the dissertation show that the adapted cells of *Rhodococcus wratislawiensis BN38* have the best ability to degrade phenol in all studied concentrations, as the catabolic potential is not reduced but preserved during the stationary growth phase of the adapted strain. It was concluded that the strain *Rhodococcus wratislawiensis BN38* has a high biodegradation potential compared to hydrophilic (phenol) and hydrophobic (n-hexadecane) xenobiotics separately and simultaneously.

There is a clear stability and durability of the studied processes. Cellular immobilization in cryogels of a 1: 1 mixture of hydroxypropylcellulose and N-isopropylacrylamide preserves the active degradation of phenol (20 g / l) and n-hexadecane (20 g / l) within forty cycles - a total of 40 g / l . After in situ immobilization in polyethylene oxide matrices of Micrococcus luteus cells, 40 cycles of repeated successful simultaneous biodegradation of aliphatic hexadecane and aromatic xenobiotic phenol (20 g / l + 20 g / l) were achieved, with biodegradation not hindered neither of the two xenobiotics.

A new bacterial strain of *BN66* has been isolated, which has been identified as *Bacillus cereus* and has been shown to degrade the constituents of crude oil, an original contribution to the study. Immobilization of *Bacillus cereus BN66* strain in a high molecular weight polymer (PAAm) cryogel synthesized leads to stability and intensification of biodegradation processes after immobilization, confirmed by the high degree of degradation - up to 100% of the aliphatic fraction after 20 cycles without inhibition.

The author of the dissertation summarizes the data and the results of her experimental work in five clearly formed scientific and applied contributions. They are related to the screening of the processes of biodegradation of hydrocarbon xenobiotics. The original contribution of the dissertation is the proven possibility for application of the adapted and immobilized cells of strain *Rhodococcus wratislawiensis BN38* and *Micrococcus luteus BN56* in real processes of biological wastewater treatment or contaminated soils. For the first time, cells of the newly isolated strain *Bacillus cereus BN66* were immobilized in a cryogel carrier based on polyacrylic amide as a way to increase the intensity of the process, which is applicable in the bioremediation of oil-contaminated sites. The use of the immobilization technique has been shown to be a successful approach, as demonstrated by the high efficiency of the immobilized cells of *Rhodococcus wratislawiensis BN38*, *Micrococcus luteus BN56* and *Bacillus cereus BN66*. The immobilization of viable microbial cells, achieved by attachment, insertion into the volume or capture, locates the cells in a certain region and allows the repeated use of their catalytic activity. The high efficiency of the immobilized cells and the high affinity between the matrix and the substrates lead to efficient biodegradation. The immobilization of living cells in supermacroporous matrices offers great advantages for their reuse and stability during continuous operation.

Finally, I must point out that for the visualization of cryogel matrices and the cells immobilized in them, the doctoral student uses modern morphological methods, such as the method of scanning electron microscopy (SEM) of cells and matrix. Observations with scanning electron microscopy morphologically confirm that the cells retain their shape and uniform distribution

below the matrix surface in the formed channels and appear to be partially embedded in the matrix pores after many cycles of use.

5. **Assessment of the publications and personal contribution of the doctoral student.**

The main results of the research have been published not only in Bulgarian (Comptes Rendus de L'academie Bulgare des Sciences) but also in foreign scientific journals (Polish Joumal of Microbiology, Biotechnology &. Biotechnological Equipment). The publications are co-authored, but my personal impressions of the work of the team under the leadership of Assoc. Prof. Lyudmila Kabaivanova give me reason to have no doubt that Assistant Nacheva has a major contribution to most of the results.

The reference made for fulfillment of the minimum requirements for points by groups of indicators for acquisition of " ESD „Doctor" according to LDAS and its Regulations shows full coverage (50 points) by group A and high indicators in groups D, E, E, although they are not required for the acquisition of this scientific degree.

The total number of citations from the publications on the topic of the dissertation is 28.

The total number of credits obtained under the educational doctoral program is 263, with 200 mandatory.

There are four publications related to the dissertation, and the impact factor (IF) from this publication activity of the doctoral student is considerable: 2,421.

6. **The abstract**

is structured correctly, reflects the content, main results and contributions of the dissertation.

7. **Recommendations**

In the further research activity of the doctoral student, I want that she will work both for future expansion of the types of studied xenobiotic factors and for extensive study of previously unexplored bacterial strains in terms of their capacity for biodegradation.

8. **CONCLUSION**

The dissertation of assistant Lilyana Vasileva Nacheva considers a microbiological problem, the solution of which has not only scientific but also concrete practical effect in the field of ecology and protection of natural resources. Results have been obtained that enrich the technologies for restoring the purity of biosphere systems. For the first time in Bulgaria, the author creates a detailed comparative microbiological characteristic of the biodegradation capacity of certain strains, and in addition to its identification, she offers optimal working biotechnologies.

The layout of the dissertation and the abstract is very good. Large and difficult work has been done. This shows that the doctoral student has theoretical knowledge and professional skills in the scientific specialty, as well as experience in conducting independent research. The materials and documents on the procedure meet the requirements of the Law on the Development of

Academic Staff in the Republic of Bulgaria, the Regulations for implementation of this law and the Regulations for acquiring ESD "Doctor" at the Institute of Microbiology "Stefan Angelov" - BAS.

Based on the above, I give a positive assessment of the research reflected in the dissertation, proposing to the esteemed scientific jury to award the assistant Lilyana Vasileva Nacheva educational and scientific degree "Doctor" in higher education 4. Natural Sciences, Mathematics and Informatics, professional field: 4.3 Biological sciences, scientific specialty 01.06.12 "Microbiology".

На основание
чл. 2 от ЗЗЛД

Prof. Dr. Dimitar Kadiysky, MD, PhD, DSci

April 19, 2022