REVIEW

of dissertation on topic:

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

Author: Assistant Professor Lilyana Nacheva

Scientific adviser: Assoc. Prof. Dr. Lyudmila Kabaivanova

Reviewer: Assoc. Prof. Dr. Zlatka Alexieva

The current dissertation of a doctoral student in self-study - Assistant Professor Lilyana Nacheva is presented for the conferment of the educational and scientific degree "Doctor" in the professional field 4.3. Natural sciences, Scientific specialty “Microbiology”. The elaboration is structured in 9 chapters and follows the accepted standards for the dissertation. It is written on 114 pages and is very well illustrated with 7 schemes, 4 tables and 46 figures, most of which demonstrate the results obtained. The list of used and cited scientific literature includes 181 sources, 3 of which are in Cyrillic, the rest in Latin. It should be noted that 22% of the cited materials were published after 2015. This undoubtedly reflects the wide interest and relevance of the issues addressed in the dissertation of Lilyana Nacheva.

It has long been known that biotechnologies based on microbial strains capable of absorbing and destroying harmful pollutants are a successful and cost-effective way to solve some of the most important problems related to a clean environment. The microbial capacity to break down a wide variety of xenobiotics has provoked efforts by researchers around the world to explore more opportunities to develop bioremediation technologies. Much efforts are being made to achieve effective microbial disposal of widespread chemical compounds in the environment, such as aromatic and polyaromatic compounds, which are harmful to human health and other living organisms.

The present study is devoted to the ability of free and immobilized cells of selected bacteria of the genera Rhodococcus, Nocardia, Micrococcus, Pseudomonas and others to degrade aromatic and aliphatic environmental pollutants.

The Introduction reveals in a synthesized form the serious problems of environmental pollution on a global and national level and provides a clear rationale for the relevance and relevance of the research to which this paper is devoted.

The literature review covers the scientific achievements in the field of biodegradation of xenobiotics, with an emphasis on the importance of microorganisms on a wide range of aromatic and aliphatic compounds. Serious attention is paid to physical, chemical, and biological factors influencing biodegradation processes. The catabolic schemes for the decomposition of phenol are presented as the main representative of the aromatic compounds, as well as the aliphatic compounds - n-alkanes in prokaryotic and eukaryotic microorganisms. The role of biosurfactants in the utilization of hydrocarbons is emphasized. Significant influence is given to the methods for immobilization of microbial cells, as an approach to increase their efficiency in the schemes for biotechnological removal of toxic organic pollutants.

To the clearly formulated main aim to study the ability of microbial species in free and immobilized form for biodegradation of aliphatic and aromatic xenobiotics are formulated 6 main tasks that clearly outline the focus of the dissertation, namely to study phenol degrading activity and adaptability of and immobilized cells of selected strains of bacteria from the genera listed above, as well as their participation in bioremediation of water and soil. Optimization of the conditions of a semi-continuous process with repeated feeding of xenobiotics; selection of the most effective approach for immobilization and repeated use of the selected immobilized preparations. Analysis of the potential of the selected strains for simultaneous degradation of aliphatic and aromatic (hydrophobic and hydrophilic) compounds with the participation of free and immobilized cells, as well as monitoring their activity for production of biosurfactants and their role in the process of biodegradation.

The chapter Materials and Methods is presented in 8 parts, which present classical and modern approaches to analysis used in the course of development. The methods for preparation of carriers and immobilization of cells are described in detail. The use of all described methods for the development of this dissertation characterizes the PhD student as a highly qualified experimenter with a multidisciplinary arsenal for research activities.

The Results chapter presents significant data on the biodegradation potential of free and immobilized cells of strains Rhodococcus wratislawiensis BN38 and Nocardia farcinica BN26 with respect to phenol and n-hexadecane, as the only sources of carbon in the nutrient medium. The experiments were performed under conditions of semi-continuous cultivation. The influence of different adaptation times at different initial densities and phenol concentrations was studied. The significant activity of the studied strains in the absorption of phenol and n-hexadecane has been proven. Significantly increased efficiency of their assimilation in the immobilization of cells of the strain R. wratislawiensis BN38 was reported.

In the immobilized strain Nocardiafa rcinica BN26 cells, a decrease in the time for their active assimilation was reported.

Of considerable interest are the results obtained from similar experiments conducted for the analysis of the cooperative biodegradation of phenol and n-hexadecane. The significant potential of the strain R. wratislawiensis BN38 to degrade the two compounds with practically the same total degree of assimilation of xenobiotics has been revealed. Only phenol - 11 g/l within 22 cycles. Together phenol + n-hexadecane - 8 + 2.4 + 10.4 g/l, within 16 cycles. Strain N. farcinica BN26 also co-degrades the two compounds, but with significantly lower efficiency. Using immobilized cells, it was found that the active degradation of phenol (20 g/l) and n-hexadecane (20 g/l) was maintained for forty cycles - a total of 40 g/l.

Original results were obtained by studying the degradation potential of a newly isolated strain of BN 66, taxonomically defined as Bacillus cereus. The ability of the strain to degrade aliphatic compounds, constituents of crude oil, as the only source of carbon, has been established. A parallel decrease in the surface tension of the nutrient medium has been established, which implies the synthesis of surfactants. This is also confirmed by the value of the emulsification index (EI24). Chromatographic analysis of the aliphatic fraction of crude oil (3 g / l) showed that in 2 days, n-alkanes were almost completely degraded (93%) by free cells. The results with immobilized cells of B. cereus BN 66 strain show that immobilization is significantly more effective for a long period of time - 47 days at high rate and stability. Better performance was obtained using PAAm cryogel synthesized from a high molar mass polymer.

Characterization of the activity of the strain with respect to some highly toxic aromatic compounds (n-hexadecane, benzene, biphenyl, toluene, naphthalene, anthracene, and phenanthrene) has also been performed.

In the chapter Discussion the obtained experimental results are summarized and discussed, and a comparison with similar results obtained by other researchers is made. This chapter substantiates and formulates the main scientific and applied scientific achievements as a result of the conducted experimental activity, which logically leads to a clear formulation of 9 conclusions and 4 contributions, which correspond to the results obtained in the course of the presented research.

- The experiments were performed under conditions of semi-continuous biodegradation, allowing long-term observations of the catabolic potential of cells. The

adaptive potential to aromatic compounds is expressed in the multiplied number of cycles and the ability of the studied strains to carry out very active biodegradation during the stationary phase. High adaptability of the cells of the studied strains was achieved in conditions of semi-continuous biodegradation. The repeatedly increased biodegradation potential of the studied strains Rhodococcus wratislawiensis BN38 and Nocardia farcinica BN26 is clearly expressed in the significantly increased number of cycles, leading to complete depletion and mineralization of the imported xenobiotic.

- Simultaneous degradation of aromatic and aliphatic xenobiotics has been established. Intensification of the degradation process of phenol and hexadecane was achieved by immobilizing the microbial cells of strain Rhodococcus wratislawiensis BN38 in cryogel carriers based on methylcellulose and strain Micrococcus luteus BN56 on polyethylene oxide cryogel carriers.

- The high catabolic potential of immobilized cells of Rhodococcus wratislawiensis BN38 compared to hydrophilic and hydrophobic xenobiotics is extremely stable over time, largely due to the choice of cryogel as a carrier, which allows to preserve the vitality and metabolic abilities of cells. This could find application in the real processes of biodegradation of industrial wastewater.

- A new bacterial strain BN66, identified as Bacillus cereus, has been isolated. Its ability to synthesize surfactants and degrade 93% of the aliphatic fraction of crude oil in 48 hours has been proven. An immobilizing matrix derived from high molar mass polyacrylamide capable of retaining cells in the carrier for a long time, stable even after 47 days, was selected, and the rate of degradation by immobilized cells was maintained at a high level until the 22nd cycle of work in semi-continuous operation, compared to only three cycles performed by free cells.

CONCLUSION

I reckon that the presented dissertation on "Biodegradation of aromatic and aliphatic xenobiotics from free and immobilized bacterial cells" fully reflects the implementation of the tasks. The presented results are significant in volume and are interpreted in detail and convincingly. Original and significant results and achievements are presented. The development has a complete look and meets all scientific requirements.

A significant amount of experimental work has been done and original scientific and applied results have been achieved with regard to the biodegradation of hydrocarbon xenobiotics.

The curriculum is fully covered by the PhD student (263 credits out of 200 required), and the publishing activity (one report and four publications - three of which in Q3, IF of 2,421 and 25 open citations) definitely exceeds the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the Regulations for its implementation at the Institute of Microbiology of the Bulgarian Academy of Sciences for obtaining the scientific degree "Doctor".

For these reasons, I confidently give my positive assessment and invite the respected members of the Scientific Jury to vote positively for the adjudication of the Educational and Scientific Degree "Doctor" to Assistant Professor Lilyana Vasileva Nacheva.

April 17, 2022 Signature:

(Assoc. Prof. Zlatka Aleksieva)