REVIEW

Concerning: Dissertation work submitted for acquisition of the **ESD "Doctor"** in higher education field 4. "Natural Sciences, Mathematics and Informatics", Professional direction 4.2. "Processes and devices in chemical and biochemical technology" at the Institute of Engineering Chemistry - BAS

Prepared by: Prof. Dr. Lyudmila Kabaivanova, Institute of Microbiology "Stephan Angeloff" - BAS, Sofia, selected as a reviewer in a scientific jury, determined by order No. 15-587 of 14.11.2023

Doctoral student: Lidiya Plamenova Tsigoriina, with scientific consultant Prof. Kaloyan Petrov, DSc

Dissertation topic: **"OBTAINING OF 2,3-BUTANEDIOL FROM INULIN THROUGH A MODIFIED NON-PATHOGENIC PRODUCER"**

Evaluation of the dissertation work and compliance of the candidate with the mandatory requirements

As a member of the scientific jury, I received all the necessary documents for the disclosure of the procedure for the defense of the dissertation work according to the LDASRB and the regulations for its application. I was given a dissertation with author Lidia Plamenova Tsigoriina, with content I got acquainted myself with in detail and found that it presents in a finished form the results of an in-depth study with objective discussion and assessment.

Lidiya Tsigoriina received her Master's degree in "Industrial Biotechnologies" at the Faculty of Biology of Sofia University "St. Kliment Ohridski" with the Diploma topic: "Optimization of a fermentation process for obtaining 2,3-butanediol from glucose". Currently, she is an assistant in the "Biochemical Engineering" laboratory at the Institute of Engineering Chemistry - BAS.

An important moment in her biography are the two awards received - for the youngest scientist "Ivan Evstratiev Geshov" of the BAS and for the best work of a young Bulgarian microbiologist of the Institute of Engineering Chemistry - BAS.

The results of the doctoral student's research activities are summarized in a compact work of 102 standard pages, 19 figures and 16 tables. It contains all the necessary sections of a dissertation - introduction, complete literature review of the topic (33 pages), aim and objectives, materials and methods, results and discussion and conclusions arising from the results achieved (13).

The topic of the dissertation work is current and important, considering the fully justified interest and importance of obtaining alternative fuels, due to the depletion of fossil natural resources while increasing the level of industrialization worldwide. No less important is the increased concern about rising carbon emissions, as efforts to replace petroleum-based chemical production with environmentally friendly ones are increasing.

The abstract contains all the necessary sections in a volume of 55 pages and clearly and concretely shows the results obtained, showing the contributions achieved.

The literary awareness of the author regarding the ways of obtaining 2,3-butanediol, its properties and applications is complete. This is evident in the presented overview, and this awareness is a prerequisite for the most accurate formulation of tasks and experimental approaches in conducting the research.

The aim of the presented dissertation is the development of biotechnology for the microbial production of 2,3-butanediol from inulin, a widely available and cheap plant substrate, by means of a genetically modified strain of *Bacillus licheniformis* 24, and the microbiological production has its advantages - relatively high efficiency with maximum preservation of the environment.

Formulated tasks are placed in a logical sequence and are directly related to the set goal. They include:

- Optimizing the composition of the nutrient medium for obtaining 2,3-BD;

-Optimization of process parameters for obtaining 2,3-BD;

-Establishment of the ability of the wild strain *Bacillus licheniformis* 24 to convert inulin into 2,3-BD;

-Cloning of a cell-bound inulinase gene (EC 3.2.1.80) from *Lacticaseibacillus paracasei* B41 (DSM 23505) into *Bacillus licheniformis* 24;

-Determination of the maximum ability of the modified strain *B. licheniformis* 24 to produce 2,3-BD from inulin-containing chicory flour.

The exact research work reflected in the dissertation is a real and complex optimization of the process for obtaining 2,3-butanediol from inulin-containing chicory flour, by means of the non-pathogenic producer *Bacillus licheniformis* 24, with application of optimization procedures in order to increase the efficiency of the process and by introduction into the producer of a gene for cell-bound inulinase, which would lead to a further increase in its inulinase activity.

The methodology used includes identification, sequencing, cloning, determination of the concentration of metabolites by high-performance liquid chromatography, reading of refractometric index, enzyme analyses. The methods for evaluating the obtained results include and require approaches characteristic of biotechnological, microbiological, genetic, biochemical and biophysical research.

At the end of the dissertation, thirteen important conclusions from the research are presented.

I estimate the author's contributions establishing the creation of a modified strain of *Bacillus licheniformis* 24 T26 with the added quality to produce 2,3-butanediol from inulin in a process of simultaneous saccharification and fermentation.

On the subject of the dissertation, Lidia Tsigoriina has presented three scientific papers that fall into quartiles and have received 6 citations, which is proof of the significance of the obtained results and their reflection in the international scientific space.

The doctoral student participated and presented her thesis work at two scientific forums.

According to the regulations for the activities of the Training Center and the Academic Council at the Bulgarian Academy of Sciences, the doctoral student has collected credits under item 1 - **130 points** with a mandatory minimum of 130 points, under item 2 - **57 points** with a

mandatory minimum of 40 points and under item 3 – **56 points** out of mandatory 30 points. From the total amount of **243 points**, with 200 points required, it can be seen that the received credits are not only sufficient, but also exceed the required ones.

CONCLUSION

The dissertation work submitted to me for review contains scientific and scientific-practical results representing significant scientific contributions. Possibilities and prospects for new developments are revealed on the investigated topical problem concerning the use of renewable sources for the production of alternative energy carriers. The work fully complies with the requirements of LDASRB and the regulations for its implementation, the Regulations for acquiring scientific degrees and holding academic positions in the Bulgarian Academy of Sciences and the Institute of Engineering Chemistry - BAS.

My assessment of the dissertation work, the abstract, the scientific results and publications, as well as the credits achieved by Lidia Tsigoriina, is convincedly positive and I propose the acquisition by Lidia Plamenova Tsigoriina of the educational and scientific degree "Doctor" in the field of higher education 4. "Natural sciences, mathematics and informatics", Professional direction 4.2. "Processes and devices in chemical and biochemical technology".

10.01.2024. Prepared the review: [redacted]

Prof. Dr. Lyudmila Kabaivanova