OPINION

regarding a PhD thesis for the acquisition of the educational and scientific degree "Doctor" in the Professional field: 4. Natural sciences, mathematics and informatics, 4.2. Chemical Sciences "Processes and Apparatus in Chemical and Biochemical Technology"

Scientific Jury formed by Order No. 15-587 of 14.11.2023 of the Director of the Institute of Chemical Engineering at BAS

Author: Lidia Plamenova Tsigoriyna
Title of the PhD thesis: Production of 2,3-butanediol from inulin by a modified non-pathogenic producer.
Scientific consultant: prof. D.Sc. Kaloyan Petrov
Reviewer: Assoc. Prof. Maria Gerginova Gerginova, Institute of Microbiology, "Stefan Angelov", BAS

The materials presented to me for review are a complete set of the necessary documents, according to the Regulations of the Bulgarian Academy of Sciences and the Law on the Development of the Academic Staff in the Republic of Bulgaria.

1. Relevance and significance of the PhD Thesis

The production of metabolic compounds by biotechnological methods is becoming a key trend in industry related to the development of green, sustainable technologies. One such compound is 2,3-butanediol, a chemical with wide applications which can be produced by bacterial fermentation. The production of 2,3-butanediol by a fermentation production process has attracted a lot of interest due to the higher concentration of the final product that can be obtained. Many research efforts have been focused on improving strains to produce 2,3-BD in high yields, using low-cost substrates to reduce the cost of the raw material, and optimizing the operating mode to make the process more efficient.
It is in this context that the research presented in this thesis is carried out and is therefore relevant and timely.
The dissertation is organized according to the classical model adopted in Bulgaria, with 19 figures, 16 tables and 206 references cited in 102 pages. The individual sections of the dissertation are balanced, the achievements in the world to date are thoroughly presented, and this allows a precise interpretation of the results obtained in fulfillment of the formulated goal.

2. Knowledge of the state of the problem

The dissertation is written in a good scientific style, with accurate use of terminology which shows that the PhD student has a professional knowledge of the subject matter. The literature review offers an in-depth analysis of the problem , correctly reporting what has been achieved to date and presenting the challenges facing scientists associated with the biotechnological production of 2,3 butanediol. The literature review is clearly structured, reads with ease and is directly relevant to the thesis aim and objectives. The aim is clear, well formulated and unifies all areas of experimental work. To realize this goal, 5 specific, interrelated and logically following tasks are formulated.

3. Compatibility of the chosen research methodology with the stated aim and tasks of the dissertation work

Materials and Methods section are an important part of the dissertation that shows the level of research. A variety of classical and modern microbiological, analytical, molecular and bioinformatics are presented. The methods are described lucidly and fully enough to be reproducible, which is indicative of the level of knowledge of Assistant Professor Tsigoriyna. The methods applied are adequate to the research and set the stage for correct results. The multidisciplinary nature of the research has allowed the PhD student to acquire new methodological skills in the implementation of the experimental work and in this sense the purpose of the PhD as an educational degree has been fulfilled.

4. Results, discussion and contributions of the PhD Thesis

In the "Results" and "Discussion" sections, Assistant Professor Tsigoriyna describes and discusses the results of the experiments in a logical sequence according to the set tasks. The capacity of the non-pathogenic strain *Bacillus licheniformis* 24 to generate 2,3-butanediol is described. The biosynthesis and secretion of 2,3-butanediol are affected by several fermentation parameters, such as the nature and initial concentration of the carbon source used, the incubation temperature, the pH of the medium and the aeration applied. Assistant Professor Tsigoriyna used a specific approach to optimize the biotechnological process for the biosynthesis of 2,3 butanediol, by planning the experiments with the Plackett-Burman method. The PhD student's last experiment, which was designed to validate the applied model, produced a high percentage of overlap between the theoretically anticipated parameters and the experimentally observed findings.

In response to the search for accessible and inexpensive substrates, the PhD student turned to the readily available chicory flour with 90% inulin content to monitor the synthesis of 2,3-butanediol by *B. licheniformis* 24. Inulin is the third most abundant polysaccharide in nature, making it extremely attractive for bioconversion. Applying methods and techniques from molecular biology, Assistant Professor Tsigoriyna took a different and original approach to enhance the weak natural inulinase activity of the strain under study. After cloning and expression of the *inu*

gene from *Lacticaseibacillus paracasei* DSM 23505 in host *B. licheniformis* 24 and successful transformation, two recombinant clones of *B. licheniformis* 24 with altered characteristics for the synthesis of 2,3-butanediol from insoluble inulin.

The conclusions and contributions are a logical consequence of the experimental data and provide the necessary information on the value of the studies conducted.

All this is evidence of excellent work that presents a complete, efficient laboratory technology for the production of the valuable microbial product, 2,3-butanediol.

5. Assessment of the publications on the PhD Thesis

Assistant Professor Tsigorina presents 3 published materials in good scientific journals - 1 from the first quartile , 1 from the second quartile and 1 from the fourth quartile. Citations of one of the publications (6 items) are also provided.

In all articles and in 2 contributions to scientific forums, Assistant Professor Tsigoriyna is ranked first, which proves her significant contribution to the development of the dissertation.

6. Fulfillment of formal requirements

Minimum national requirements:

- Group of indicators "A" 50 points against the required 50 points.
- Group of indicators "D" 57 points against the required 30 points.

7. Conclusion

The reviewed dissertation meets the quality and scope volume requirements for a PhD thesis for the educational and scientific degree "Doctor" and the minimal national requirements are covered. The main results have been published in well-known international journals.

Based on the analysis made above, I give a positive evaluation of the developed dissertation work and consider it justified for the Scientific Jury to award the education and science degree "Doctor"to Assistant Professor Tsigorina in the scientific field 4. Natural Sciences, Mathematics and Informatics, professional field 4.2. Chemical Sciences

Date: 17.01.2023

Reviewer: [redacted]

/Assoc. Prof. Maria Gerginova/