OPINION

by **Prof. Tania Pencheva, PhD**
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Regarding a procedure of promotion to the **educational and scientific degree "Doctor of Philosophy"**
Area of Higher Education: **4. Natural Sciences, Mathematics and Informatics**
Professional Field: **4.2. Chemical Sciences**
Doctoral Programme: **Processes and Apparatus in Chemical and Biochemical Technology**

Author of the dissertation thesis: **Lidia Plamenova Tsigoriyna**
Title: **Producing 2,3-butanediol from Inulin through a Modified Non-pathogenic Producer**

According to Order No. 587/14.11.2023 of the Director of the Institute of Chemical Engineering (ICE) at the Bulgarian Academy of Sciences (BAS), I was appointed member of the Scientific Jury for defence of the dissertation thesis by the PhD student **Lidia Plamenova Tsigoriyna** for the acquisition of the educational and scientific degree "Doctor of Philosophy".

As a member of the Scientific Jury, I have been provided with:

1. A dissertation thesis for acquisition of the educational and scientific degree "Doctor of Philosophy"
2. A synopsis of the dissertation thesis
3. Copies of the publications, which the thesis is based on
4. Other procedure-related documents

The dissertation thesis of **Lidia Tsigoriyna** is 102 pages long and comprises an Introduction, five chapters, conclusions and formulated contributions, a Bibliography of 206 literature sources and a list of 3 publications on the thesis, as well as a list of citations. The thesis has been illustrated by 16 tables and 19 figures. In the provided form, the dissertation thesis corresponds to the specific requirements defined in Art. 27(2) from the Council of Ministers' Regulations on the Implementation of the Academic Staff Development Act in the Republic of Bulgaria (ASDARB), except **the lack of a Declaration for originality of the results**.

The aim of the dissertation thesis is to develop a biotechnological process for microbial production of 2,3-butanediol (2,3-BD) from inulin by the genetically modified strain *Bacillus licheniformis* 24.

In order to accomplish the **aim** of the dissertation thesis, the following particular **objectives** have been outlined:

1. Optimization of the nutrient medium for production of 2,3-BD.
2. Optimization of the process parameters for production of 2,3-BD.
3. Detection of the ability of the wild strain *B. licheniformis* 24 to convert inulin into 2,3-BD.

4. Heterologous expression of Inulinase Gene (EC 3.2.1.80) from *Lacticaseibacillus paracasei* B41 (DSM 23505) into *B. licheniformis* 24;
5. Determination of the maximum ability of the modified strain *B. licheniformis* 24 to produce 2,3-BD from inulin-containing chicory flour.

The timeliness of the dissertation thesis stems from the fact that that in recent decades there has been a continuous increase in the production volume of 2,3-butanediol, caused by its growing demand due to its versatile application. In order to develop an efficient process for the production of 2,3-BD, the research in the presented thesis is targeted at finding cheaper carbon sources, developing new extraction methods, as well as finding efficient producers with a non-pathogenic nature.

The presented list of publications on the topic of the dissertation thesis by **Lidia Tsigoriyna** includes 3 publications in co-authorship, all of them in journals with an impact factor, in Q1, Q2 and Q4 quartiles. The total impact factor of **Lidia Tsigoriyna's** papers is 9.75. The PhD student is the first author in all of these publications, which unequivocally demonstrates her contribution to the achieved and presented research results. The presented Reference for 6 citations is yet another undeniable and independent testimonial for the timeliness of the thesis under discussion and the results achieved therein.

The presented synopsis of the dissertation thesis is 56 pages long in its Bulgarian version and 52 pages long in English. It fully covers the essence and contents of the dissertation thesis, including the aim and the tasks set, as well as their execution and the achieved results.

On the basis of the research elaborated in the dissertation thesis, **Lidia Tsigoriyna** has articulated three contributions, which I endorse in the form the PhD student has formulated them.

In general, **Lidia Tsigoriyna's** dissertation thesis impresses very strongly, and in different aspects. Despite the relatively small volume, the dissertation does not suffer from a lack of important information. The PhD student's relatively concise but comprehensive style of expression is also impressive. Special attention should also be paid to the huge amount of experimental as well as analytical work, which shows the arsenal of experimental methodologies and analytical methods mastered by the PhD student in the course of her studies to obtain the educational and scientific degree "Doctor of Philosophy".

Despite the indisputable qualities of the PhD student **Lidiya Tsigoriyna** and her dissertation thesis, I would like to pay **special attention to the so-called dissertation *credentials*. I consider it highly recommended that the relevant *area of higher education* (in my opinion 4. Natural sciences, mathematics and informatics), *professional field* (4.2. Chemical sciences) and *doctoral programme/scientific specialty* ("Processes and Apparatus in Chemical and Biochemical Technology"), as well as the educational and scientific degree "Doctor of Philosophy" should be unified in the dissertation thesis and the accompanying synopsis**. I use this opportunity to recommend that the ***Declaration for originality of the results***, which is required in Art. 27(2) of the Regulations of the Council of Ministers on the Implementation of the ASDARB, should be added. Also, in the pursuit of a perfect exposition, I would like to recommend another reading of the thesis to clear up some technical inaccuracies, such as: 1) spelling errors in the dissertation thesis; 2) Table 3 is

not cited in the text; 3) the citation of Figure 13 in the text should be in parentheses, while many tables are cited in parentheses without this being necessary; 4) need for standardising the citation of the *inu* gene (*inu* is not in italics everywhere), etc. I should immediately point out that this opinion of mine has the force of **recommendation**, and it **solely refers to the manner of thesis presentation**, which in **no way diminishes the achieved and appropriately presented results** pursuant to the accomplishment of the aims and tasks of the thesis.

I haven't had the opportunity to know **Lydia Tsigoriyna** personally, but the acquaintance mediated by her dissertation thesis leaves me convinced that she is a very promising young scientist. This opinion of mine is also confirmed by the fact that **Lydia Tsigoriyna** is the winner of the BAS Award "Ivan Evstratiev Geshov" for the youngest scientists under 30 years of age in the 2023 competition, as well as the Diploma for the best work of a young Bulgarian microbiologist in 2021 (II place) of the "Acad. Prof. Dr. Stefan Angelov" Foundation. I sincerely hope that her potential, as demonstrated in the high quality dissertation thesis presented herewith to allow her a fast track in the academic career.

In forming the overall assessment of the dissertation thesis, one should render account of the requirements formulated in the ASDARB and the Regulations of ICE-BAS on its implementation. The PhD student **Lidia Tsigoriyna** not only **covers** but certainly **exceeds** the requirements for acquisition of the educational and scientific degree "Doctor of Philosophy", as indicated in the Regulations of ICE-BAS on the implementation of the ASDARB.

With respect to all of the above, I certify that **the requirements for awarding the educational and scientific degree "Doctor of Philosophy" (PhD) set in the ASDARB and in the Regulations of ICE-BAS on its Implementation have been fulfilled**. After getting familiar with the presented dissertation thesis and the publications it is based on, which give visibility to the results achieved, and after analysis of the significance of these publications and the scientific and applied science results contained therein, I justifiably give my **positive evaluation** and recommend the respected members of the Scientific Jury to vote for awarding **Lidia Plamenova Tsigoriyna** of the **educational and scientific degree "Doctor of Philosophy"** in professional field **4.2. Chemical Sciences**, doctoral programme **Processes and Apparatus in Chemical and Biochemical Technology**.

29 January 2024
Sofia

Signature: [redacted]
(Prof. Tania Pencheva, PhD)