REVIEW

on a dissertation work for obtaining the scientific degree "doctor" at the Institute of Engineering Chemistry at the BAS

Author of the dissertation: Master Lidiya Plamenova Tsigoriyna

Dissertation topic: Production of 2,3-butanediol from inulin by a modified non-pathogenic producer

Direction: 4.2. Chemical Sciences

Scientific specialty: Processes and apparatus in chemical and biochemical technology

Reviewer: Prof. Dr. Venko Nikolaev Beshkov

1. Biographical data about the candidate

Mrs. Lydia Pl. Tsigoriyna graduated from the Faculty of Biology of the University of St. Cl. Ohridski" in 2020 with the scientific and educational degree "Bachelor" in the specialty "Biotechnologies". Immediately after that, he started working as a technologist and later as an assistant at the Institute of Engineering Chemistry at the BAS, where he currently works. He received his master's degree in the same specialty in the same faculty in 2021 on a topic similar to the topic of the current dissertation. She participated in 5 research projects under the supervision of Prof. Kaloyan Petrov (scientific supervisor of her dissertation work) and one under the supervision of Prof. Dragomir Yankov. The topic of the projects is close to that of the dissertation.

Mag. L. Tsigoriyna is the author (co-author) of 10 scientific publications in journals with a high impact factor (IF>4) with quartiles Q1 and Q2. 78 citations were noted on the publications.

Her interests are in the field of biotechnology related to the application of bioprocess and metabolic engineering approaches and the microbial production of valuable metabolites of industrial importance.

Mag. L. Tsiguriina is the winner of two awards: of the foundation "Acad. Stefan Angelov" (2021) for a young Bulgarian microbiologist and the "Iv. Evst. Geshov" of the BAS for the youngest scientist (2023).

2. Timeliness of the problem being developed

Interest in this kind of research is determined by the threat of climate change associated with energy using fossil fuels. As a result, there are huge emissions of carbon dioxide with a greenhouse effect. The prospect of replacing carbon-containing fuels with renewable energy sources is tempting, including bioproducts such as bioethanol, biogas, biodiesel, etc. The compound 2,3-butanediol (2,3-BD) is in this category. In addition to being a fuel, it can be used

as a starting material for various syntheses and productions, as a solvent, as an additive to other fuels, etc. From this point of view, 2,3-butanediol fits perfectly into the concept of a biorefinery product, due to the perspective that to be produced by fermentation from natural raw materials (carbohydrates) instead of oil derivatives. This approach using glucose, fructose and inulin is the subject of the peer-reviewed dissertation.

In the dissertation, 206 literary sources are cited, of which one hundred and forty-six of them are after 2000, and fifty are after 2015. This shows that the interest in these studies is high and that the topic is current and the author is familiar with the latest advances in the field..

3. Does the dissertation student know the state of the problem and creatively evaluate the literary material?

In the literature review, the author dwells on current questions about the fermentation production of 2,3-BD. These are the metabolic pathways for different producer strains, the conditions for obtaining high-yield optical isomers, racemic mixtures, strategies to increase the efficiency of fermentation processes for this product, producer strains and their genetic modifications.

Much attention has been given to the importance of microbial shams known in the literature and their ability to produce 2,3-BD in different ratios of the optically active isomers and the racemic mixture. The achievements in genetic modification of strains of the genus Bacillus and the strains of Escherichia coli and Saccharomyces cerevisiae are also reviewed.

The nutrient media and process parameters that have an impact on the fermentation processes for the production of the target product are examined.

The aim and tasks of the dissertation work are derived from the literature review and are very well formulated. The overall impression is that the dissertation student has a good command of the literary material and handles it freely.

4. Brief analytical description of the nature and assessment of the credibility of the material on which the contributions of the dissertation are based

The author logically presents the methodology for conducting experimental research, starting with the methods of the planned experiment. After the selection of the Bacillus licheniformis 24 strain, the significant components of the nutrient medium are first screened, followed by optimization of the specified nutrient medium and the various process parameters for the production of 2,3-BD (pH, temperature and degree of aeration). During the experimental proof of the model in a batch mode during the optimization of the conditions of the fermentation process, very high concentrations of the final product (91.2 g/l) were achieved with a yield close to the theoretical one. A high productivity of 1.94 g/l/h was achieved. The achieved values

mentioned above are close to those predicted according to the mathematical model derived from the planned experiment, which proves its adequacy.

Glucose, fructose and inulin were used as substrates-carbon sources. The studies were conducted in batch mode and fed-batch mode. The comparison of the obtained results for intermittent regimes shows that with glucose and fructose substrates, high product concentrations (for glucose) and high yields (for fructose) are achieved. In both cases, the yield of 2,3-BD is close to the theoretical one. In a feeding regime, fructose appears to be the more suitable substrate.

Experiments with inulin as a substrate show the weak activity of the strain, which even after genetic modifications is inferior to its activity on monosaccharide substrates.

In this regard, I believe that the title of the dissertation is incomplete, since the most significant results obtained were using monosaccharides, not inulin.

In all cases, the main by-product is glycerol. In this regard, I think it would be good if the text included a diagram showing the place of glycerol in the metabolic pathways to produce 2,3-butanediol.

Page 78 (below) states that "...the effect of the presented complex optimization of media composition and process parameters is most evident when comparing the results of batch fermentation from glucose before and after optimization". What were the process parameters "before" optimization?

5. What are the scientific and scientific-applied contributions of the dissertation work?

The dissertation work has a scientific and practical character, and the contributions in it are mainly scientific and fundamental. They are divided into "conclusions" and "contributions". For me, what is defined as "conclusions" has the value of contributions. More generally, they are as follows.

• Through the methodology of planning the experiment, the most significant components of the nutrient medium for the production of 2,3-BD by the strain B. licheniformis 24 were established. Through the planning of the experiment, the optimal concentrations of these components were established.

• By planning the second-order experiment, the optimal values of the process parameters for the production of 2,3-BD by B. licheniformis 24 (temperature; pH; aeration rate) were established.

• In the fermentation of glucose by B. licheniformis 24, with increasing temperature, the following increase: (a) the rate of glucose uptake; (b) rate of 2,3-BD formation (productivity); (c) the production of the by-product glycerol, as the yield of 2,3-BD decreases.

• In the case of glucose as a substrate, increasing the aeration increases the yield of 2,3-BD, its productivity does not change, but the rate of glucose absorption and the formation of glycerol - decrease.

• In a fed-batch process with a glucose substrate, the maximum amount of 2,3-BD that the strain can produce under optimal conditions is 138.8 g/L, with a yield close to the theoretical (0.478 g/g) and a productivity of 1.16 g/L/h .

• In a batch process with fructose substrate feeding, the maximum amount of 2,3-BD produced under optimal conditions is 156.1 g/L, with a yield of 0.46 g/g and a much higher productivity - 2.17 g/L/h.

• B. licheniformis strain 24 has natural inulinase activity that is not sufficient to hydrolyze inulin from Frutafit® HD chicory flour to fermentable sugars. Introduction of the inu gene from Lacticaseibacillus paracasei DSM 23505 (GenBank KP663715.1) encoding a cell-bound fructan-β-fructosidase (EC 3.2.1.80) into B. licheniformis 24 resulted in an 8.7-fold increase in enzyme activity (transformant T26). Introduction of the inu gene from Lacticaseibacillus paracasei DSM 23505 into B. licheniformis 24 without its enzyme cell wall binding domains to render the enzyme extracellular resulted in a 4.7-fold increase in enzyme activity (transformant T14).

• The modified strain B. licheniformis 24 T26 was able to hydrolyze 140 g/L inulin, but the maximum amount of 2,3-BD obtained was only 18.5 g/L, due to the accumulation of sucrose in the culture liquid.

The contributions contain useful recommendations on the conditions and possibilities of obtaining 2,3-butanediol for practical purposes. These recommendations contain the applied value of the dissertation work.

6. Is it possible to assess to what extent the dissertation work and contributions represent the personal work of the dissertation student?

The presentation of the texts of the dissertation and the author's abstract show that the development of the dissertation is the work of the doctoral student.

7. Evaluation of publications on the dissertation work

The dissertation is based on three scientific publications, two of which are in journals with a high impact factor (4.501 and 5.11) and quartiles Q1 and Q2. The third is in a journal with an impact factor of 0.14 and quartile Q4. There are also 2 participations in conferences and other scientific forums. 6 citations were noticed on one of the scientific publications.

The scientometric data fully satisfy the requirements for the awarded educational and scientific degree "Doctor" according to the rules of IIH-BAS.

8. Is the abstract made according to the requirements, does it correctly reflect the main points and main contributions of the dissertation work?

The abstract correctly reflects the content of the dissertation work.

9. Conclusion

What has been stated so far allows me to confidently recommend to the honorable jury to award the scientific degree "doctor" to a master. Lydia Plamenova Tsigoriyna.

Sofia, January 19, 2024. REVIEWER:

(Prof. PhD Venko N. Beshkov)