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REVIEW

The doctoral dissertation of Mr. Filip Brodowski, M.Sc. Eng., entitled “Lactate-based bioproduction of medium chain carboxylic acids via mixed culture fermentation”

1. FORMAL BASIS FOR THE PREPARATION OF THE REVIEW

The formal basis for the review was the letter of Prof. Dr. Hab. Eng. Zbigniew Nadolny, Head of the Discipline Council Environmental Engineering, Mining and Power Engineering of the Poznań University of Technology on 23.16.2023, in which I was asked to review the doctoral dissertation of M.Sc. Eng. Filip Brodowski entitled "Lactate-based bioproduction of medium chain carboxylic acids via mixed culture fermentation".

The review was prepared by the provisions of the Regulation of the Minister of Science and Higher Education of January 19, 2018, on the detailed procedure and conditions for conducting activities in the doctoral procedure, in the habilitation procedure, and in the procedure for conferring the title of professor (Journal of Laws of January 30, 2018, item 261), based on the received copy of the doctoral dissertation of M.Sc. Eng. Filip Brodowski in a paper form.

Under 187 Article of the Act of July 20, 2018, Law on Higher Education and Science, the doctoral dissertation presents the candidate's general theoretical knowledge in the discipline or disciplines and the ability to independently conduct scientific or artistic work. The subject of the doctoral dissertation is an original solution to a scientific problem, an original solution to apply the results of one's research in the economic or social sphere, or an original artistic achievement. A doctoral dissertation may be a written work, including a scientific monograph, a collection of published and thematically related scientific articles, a design, construction, technological, implementation, or artistic work, as well as an independent and separate part of a collective work.

The scope of this review includes the assessment of whether the doctoral dissertation meets the criteria set out in the Act, as well as the assessment of the relevance of the topic undertaken, the correctness of the formulated goal, verification of the research methodology used, research results presented in the work, conclusions formulated and the ability of the PhD student to independently conduct scientific work.

2. GENERAL CHARACTERISTICS AND EVALUATION OF DISERTATION

Doctoral dissertation of M.Sc. Eng. Filip Brodowski entitled "Lactate-based bioproduction of medium chain carboxylic acids via mixed culture fermentation" is a research work based on a series of three publications. The doctoral dissertation was prepared in English, with a summary in Polish and English. The work takes up a total of 79 pages, including 14 pages of introductory and organizational information. The introduction and purpose of the research are presented on 4 pages. The next 7 pages of the study are occupied by a collective presentation of research methods. On page 8, the results and discussion of the results are presented. Finally, a summary of research results and a list of cited literature are presented. The essential, substantive part of the work consists of three multi-author, original, research publications, in which the PhD student is the first author. These are:

- Brodowski, F., Łężyk, M., Gutowska, N., Oleskowicz-Popiel, P., 2022. Effect of external acetate on lactate-based carboxylate platform: Shifted lactate overloading limit and hydrogen co-production. *Science of The Total Environment* 802, 149885. <https://doi.org/10.1016/j.scitotenv.2021.149885>;
- Brodowski, F., Łężyk, M., Gutowska, N., Kabasakal, T., Oleskowicz-Popiel, P., 2022. Influence of lactate to acetate ratio on biological production of medium chain carboxylates

via open culture fermentation. *Science of The Total Environment* 851, 158171.
<https://doi.org/10.1016/j.scitotenv.2022.158171>;

- Brodowski, F., Duber, A., Zagrodnik, R., Oleskiewicz-Popiel, P., 2020. Coproduction of hydrogen and caproate for an effective bioprocessing of waste. *Bioresource Technology* 318, 123895. <https://doi.org/10.1016/j.biortech.2020.123895>;

The theoretical introduction to the work includes three subchapters: 1) The role of biorefineries in the future biobased economy, 2) Carboxylate platform concept, and 3) Lactate-based carboxylate chain elongation. Author explained the basics and importance of research in the context of the circular economy in a short but precise way.

Based on this description, the author formulated the basic aim of the work, i.e.: The study's main objective is to identify the influence of lactate-based substrate composition on CE (chain elongation). In order to achieve the research goal, the author conducted three long-term continuous processes (two based on synthetic medium and one based on lactate-based model waste stream, i.e. acid whey (AW), as well as two batch experiments (presented in the three publications).

Achievement of the main research objective planned through the implementation of the following particular aims and objectives of the research:

- characterize a process in which lactate is used as the sole carbon source in CE,
- describe the lactate overloading phenomenon in CE systems,
- identify the role of acetate in lactate-based CE,
- investigate the influence of lactate to acetate (L:A) ratio on CE,
- investigate the effect of complex lactate-based substrate composition on CE (using AW as a model lactate-based feedstock),
- identify key microorganisms responsible for lactate-based CE.

The author proposed two following hypotheses:

- 1) The composition of lactate-based substrates, especially the lactate (ED) and acetate (EA) concentration, will significantly influence the CE performance.
- 2) Utilizing a lactate-based substrate under CE-promoting conditions during mixed culture fermentation will lead to a microbiome enrichment in the lactate-based chain elongating bacteria.

The basic aim of the work together with the specific objectives related to it are presented in the form of a graphic diagram. At the same time, the diagram presents the links between individual specific objectives with articles included in the publication cycle. In my opinion, this diagram clearly shows the idea of the work and the organization of the research process.

In the Materials and Methods section, the PhD student presented the collective organization of research work described in detail in three scientific publications. At the same time, the description considers both the common elements of the methodology, which are repeated in all publications, and those characteristics for individual articles. The collective presentation of the research results along with the discussion is contained in six pages of the text, divided into five subchapters: 4.1. Lactate as a sole carbon source in CE; 4.2. The role of acetate in lactate-based CE; 4.3. Lactate to acetate ratio in substrate composition; 4.4. Complex lactate-based substrate composition; 4.5. Butyrate-caproate competition in lactate-based CE. Unfortunately, the layout of the sub-chapters does not accurately reflect the six specific objectives adopted. Such an editorial approach would facilitate the reception of the work and the assessment of the achievement of goals. The end of the descriptive part of the work is a long (two-page) summary. The author resigned from a short conclusion in the form of a short list of essential basic conclusions from the work carried out.

In my opinion, the research was properly planned and carried out. The adopted research methods and the analytical techniques used allowed to achieve the assumed research goal. It is worth emphasizing the wide range of modern, advanced analytical methods used.

The dissertation is a logical whole, which is confirmed by the skills of M.Sc. Eng. Filip Brodowski in formulating research goals and planning the research process. The thesis contains all the necessary elements required for doctoral dissertations. The overall assessment of the doctoral dissertation is very high.

3. EVALUATION OF THE SUBJECT SELECTION OF THE DISSERTATION

Research topics of M.Sc. Eng. Filip Brodowski is associated with the issues of bioeconomy in the circulation system, and the search for unconventional methods of using waste substances. An example of such processes may be the bioproduction of medium-chain carboxylic acids in the process of carbon chain elongation. The research focused on determining the effect of the lactic acid-based substrate composition on the production of medium-chain carboxylic acids (primarily caproic acid). The tested process of production of medium-chain carboxylic acids by chain elongation is an example of a new mixed culture fermentation technology, which is an alternative to the commonly used anaerobic digestion. Short-chain carboxylates which are intermediates accumulated in the acidogenesis and acetogenesis during anaerobic digestion can be also converted into medium-chain carboxylates through the chain elongation instead of forthgoing through methanogenesis to methane. Potentially, the carboxylate platform can play a significant role in the

bio-based products market. Especially caproic acid, containing six carbon atoms in the molecule, is shown as a big potential. It can be used directly as a food additive, antibacterial agent, and plant growth promoter, as well as an intermediate component in the production of lubricants, gums, dyes, paints additives, or pharmaceuticals. In his research, the PhD student focused on assessing the influence of the substrate composition, in particular the composition of electron donors and electron acceptors, on the process of carbon chain elongation. The main aim of the research is to identify the influence of lactate substrate composition on the process of chain elongation.

The subject of the doctoral thesis is current and important from the point of view of the development of the circular economy. Undoubtedly, the presented research work is the beginning of a long road. The commercial success of the technology for the production of carboxylic acids in the process of chain elongation will be determined by economic profitability. However, the potential and innovation of the undertaken research work should be absolutely noted.

I believe that the doctoral thesis submitted for review by M.Sc. Eng. Filip Brodowski fits well into the issues of waste valorization and closed-circuit management. The topic of the work was chosen correctly, and the formulated goal of the work has a significant practical aspect.

4. SOLVED SCIENTIFIC ISSUES

The most important and original issues constituting a scientific novelty of the work of M.Sc. Eng. Filip Brodowski include:

1. Demonstration of the possibility of using lactic acid as a substrate for the production of medium-chain fatty acids, especially caproic acid. At the same time, limitations for such a process (conversion of lactic acid as the only carbon source) were determined, resulting from the limited availability of acetic acid, which had to be first produced directly from lactic acid, and then it could be extended to butyric acid and caproic acid.

2. Demonstration of the positive effect of supplementation with acetic acid on the stable production of caproic acid and the possibility of achieving a higher permissible load of lactic acid in the bioprocess.

3. Research on the co-production of hydrogen and caproic acid from acid whey should be considered particularly valuable. The use of real waste in these works, and not chemically pure reagents, means that the application value of the information obtained is significantly greater. The observed accumulation of electron donors in the process of elongating the carbon chain, i.e. ethanol and lactic acid, is important knowledge for further research and practical application.

The research challenge undertaken by M.Sc. Eng. Filip Brodowski was a complex task. A number of experiments were carried out, and the way they were planned and implemented indicates a well-thought-out and consistently implemented research procedure. The presented experimental works confirm the PhD student's very good knowledge of fermentation technology and laboratory techniques. The content of the dissertation proves that he is well-placed in the subject matter. I do not find any significant shortcomings and I rate the knowledge of the subject matter very highly.

5. COMMENTS / QUESTIONS

While reading the dissertation, I have drawn attention to the following issues:

- How can the reproducibility of the results be assessed; to what extent a repeated experiment, even with the use of inoculum from the same source, will allow to obtain similar results?
- What determines the length of the individual phases used in research using CSTR reactors? Accumulation of metabolites and their impact on the further course of the process was repeatedly found in the presented experiments.
- How was the constant biomass level in the flow reactors provided? Was the hydraulic retention time related to the biomass generation time? To what extent the wash-out of slower-growing biomass could influence the formation of population variability?
- For batch experiments, three replicates were performed. If the presented values of carboxylate concentrations are the average of these repetitions, what was the variability of these results? Were statistical tools used to assess the significance of differences between variants?

Minor remarks and editorial suggestions that came to mind while reading the publication:

- There is no need to refer to the literature when providing obvious information of a general nature. For example, in publication 1, the introduction says: Population growth and consumerism affect the natural environment (Sanders and Langeveld, 2020). Certainly, the cited authors are not the first to make such a statement, and the PhD student is aware of the impact of human population growth on the environment.
- Why are there two different notations for millimole (one mmol C or mM C)?
- Why were no additional materials attached for publication 1? In the case of a doctorate in the form of a series of publications, all elements should be included in the paper version.

6. CONCLUSIONS

M.Sc. Eng. Filip Brodowski became interested in important issues related to the innovative method of neutralizing organic waste in the fermentation process. Research work has been undertaken, which leads to the development of a circular economy.

The research results presented by M.Sc. Eng. Filip Brodowski should be considered valuable, current and original from the point of view of further development of environmental engineering. The PhD student demonstrated his ability to independently formulate scientific problems and plan research leading to their solution, together with the presentation of results and their interpretation.

In my opinion, the doctoral thesis of M.Sc. Eng. Filip Brodowski meets the conditions set out in the Act of July 3, 2018, Law on Higher Education and Science (Journal of Laws of 2018, item 1669) and in the Regulation of the Minister of Science and Higher Education of January 19, 2018 on the detailed procedure and conditions for conducting activities in the doctoral thesis, in the habilitation procedure and in the procedure for conferring the title of professor (Journal of Laws of January 30, 2018, item 261), and thus I apply to the Discipline Council of Environmental Engineering of Mining and Power Engineering for admission to the next stages of the doctoral procedure.