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THE REVIEW OF THE DOCTORAL DISSERTATION

on *Lactate-based bioproduction of medium chain carboxylic acids via mixed culture fermentation* prepared by Filip Brodowski, MSc. Eng.

1. Formal and legal basis of the review

Formal basis: Resolution of the Scientific Council in the Discipline of Environmental Engineering, Mining and Energy from June 20th, 2023 (Letter of appointment as a reviewer No. WISIE.63.51.2023, June 23rd, 2023).

Legal basis: Regulation of the Minister of Science and Higher Education of July 20th, 2018 (Journal of Laws of 2023, item 742).

The subject of the review: PhD dissertation on *Lactate-based bioproduction of medium chain carboxylic acids via mixed culture fermentation* prepared by Filip Brodowski, MSc. Eng. and performed under the supervision of Prof. Piotr Oleśkiewicz-Popiel and auxiliary supervision of Mateusz Łężyk, PhD.

2. Description of the PhD candidate

Filip Brodowski as a PhD candidate at Poznań University of Technology has been working on bioconversion of organic waste through mixed culture formation to obtain added value products. Specifically, he has been working on bioproduction of medium chain carboxylates through the process of chain elongation to learn how the selected parameters of the process and the composition of a lactate-based substrate/substrates have the effect on the elongation of carboxylic chains. It has to be highlighted that Filip Brodowski was involved as a researcher in two research projects granted by the national funding agencies (i.e. the *National Centre for Research and Development* and the

National Science Centre). He has published four scientific papers in peer reviewed journals (four papers are indexed in the Web of Science and three papers are indexed in the Scopus databases). He shared the results of his research through oral and poster presentations at national and international conferences (e.g. in the USA and Germany). His scientific work is getting more attention which is reflected in the bibliometric parameters, i.e. the citation index and the H-Index according to the Web of Science are: 21 and 2, respectively, whereas according to the Scopus these parameters were: 17 and 3. This can be considered as an important accomplishment for an early stage researcher.

3. Evaluation of the PhD dissertation

The doctoral dissertation was based on three scientific publications published in the peer reviewed scientific journals (referred to as Paper 1, Paper 2 and Paper 3 in the doctoral dissertation), i.e.:

Paper 1

Brodowski, F., Łężyk, M., Gutowska, N., Oleśkiewicz-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>

Impact Factor: 10.754, 5 Year Impact Factor: 10.237, 200 points of MEiN.

Paper 2

Brodowski, F., Łężyk, M., Gutowska, N., Kabasakal, T., Oleśkiewicz-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>

Impact Factor: 10.754, 5 Year Impact Factor: 10.237, 200 points of MEiN.

Paper 3

Brodowski, F., Duber, A., Zagrodnik, R., Oleśkiewicz-Popiel, P., 2020. Co-production of hydrogen and caproate for an effective bioprocessing of waste. *Bioresource Technology* 318, 123895.

<https://doi.org/10.1016/j.biortech.2020.123895>

Impact Factor: 11.889, 5 Year Impact Factor: 11.139, 140 points of MEiN.

In all of these publications the PhD candidate was the lead author and his contribution primarily included conceptualization, conducting of the research, interpretation of the results and writing the manuscripts. The role of the PhD candidate in this work and his input into the publications was explained in detail. The degree of the scientific contribution of the PhD candidate in the development of listed publications is crucial. These scientific publications were published in the most influential academic peer reviewed journals (specifically for environmental sciences) with high impact factors (IF) and citations indexes (i.e. *Bioresource Technology* or *Science of the Total Environment*) resulting in the total of IF of 31.613 and the total of the MEiN points of 540. This is an outstanding accomplishment for the PhD candidate and the lead author.

The title of the work reflects the overall goal and the scope of the presented research work. The dissertation is organized and structured in a clear manner. The structure is typical for this type of scientific contribution and it consists of the following parts: *Introduction, Motivation and aim of the research, Materials and Methods, Results and Discussion, Summary and References*. These parts were preceded by: the abstracts (in Polish and in English), the list of the papers included in the dissertation, a brief description of scientific activity of the PhD candidate and the list of abbreviations. The copies of the scientific publications which are the basis for this dissertation (i.e. Paper 1, Paper 2, Paper 3) and the statements of co-authorship were also included. The dissertation complies with the standard requirements for PhD dissertations.

The *Introduction* part provides a concise – and rather brief – overview of the literature but sufficiently presents the current state of the art with clearly identified issues and problems, the research gaps which need to be addressed and explains the potentials of carboxylate platform using the lactate-based feedstock (as a second-generation biorefinery) and the potentials of caproic acid. The performed literature review shows that the PhD candidate has the ability of critical thinking and extracting and synthesizing the information and data. Thus, it proves that he has a very good understanding of the research area. The research topic selected by the PhD candidate is relevant, important and fully justified as there is a growing need for new solutions and technologies which aim towards circularity, sustainability and independence of non renewable resources.

In the part on *Motivation and aim of the research* the PhD candidate explains the reasons for undertaking the research work and how this work will go beyond the state of the art. The main objective of the research work, i.e. to identify the effect of the composition of a lactate-based substrate on chain elongation, was clearly stated. Two research hypotheses were formulated along with the specific objectives. The PhD candidate planned three long-term continuous and two batch experiments. The links between the main and specific objectives and the published papers were logically

presented in Figure 3. This shows that the PhD candidate carefully planned the research work, including the completion of the publication process of scientific papers in academic peer reviewed journals (i.e. the Paper 1, Paper 2 and Paper 3).

The *Materials and methods* part sufficiently describes how the research work was done. Table 1 gives the comprehensive overview of all the experiments with the selected process variables. This part includes the information on the substrates used for the experiments (characteristics of synthetic medium as well as acid whey as a model waste feedstock), bioreactor systems for continuous and batch experiments (which were described in greater detail in the Paper 1, 2 and 3), the microbiome analysis and the calculations. Detailed laboratory procedures, laboratory set-ups, operating conditions and analytical techniques and calculations were described in detail and supported with the references in the published papers. This will allow the replication of the continuous and batch experiments described in the doctoral dissertation.

The results published in the Paper 1, Paper 2 and Paper 3 were discussed in the part on *Results and Discussion*. The discussion of these results was constructed in view to the main objective and particular aims described in the section on *Motivation and aim of the research* and linked with the published papers (Figure 3). In reference to the particular aims and the obtained results presented in the papers it was confirmed that bioconversion of lactate being the sole carbon source to caproate demonstrated stability issues which resulted from lower availability of acetate. This, in turn was caused by higher loading rates of lactate. These findings and observations give better understanding of the process dynamics and overcoming its limitations. It was found that supplementation with acetate had a positive effect on lactate overloading limit and facilitated the production of caproate. It was also concluded that the production of caproate was primarily influenced by the concentration of lactate in the feedstock. The PhD candidate also studied the potentials of acid whey as a model feedstock with high contents of lactate and lactose for simultaneous production of hydrogen and caproate. He reported that the process itself was feasible despite the issues related to the accumulation of ethanol and lactate. In view to the results from the microbiome analysis it was confirmed that the use of lactate-based feedstock will contribute to the microbiome enrichment in the lactate-based chain elongating bacteria. The predominant microorganisms in mixed culture fermentation systems were identified and reported.

In summary, the main objectives and particular aims of the PhD dissertation were fully achieved. The results published in the Paper 1, Paper 2 and Paper 3 allow the confirmation of the research hypotheses and the conclusion that apart from operational process parameters (i.e. pH, temperature and hydraulic retention time) the composition of lactate-based substrates has the effect on the process dynamics and efficiency.

4. Specific comments and questions

The work performed within this doctoral dissertation undoubtedly will contribute in a significant way to the advancement of the state of the art by giving better understanding of the impact of substrate composition on process dynamics in the bioproduction of medium chain carboxylic acids through mixed culture fermentation. One of the potential waste feedstock that was selected and studied in the doctoral dissertation is acid whey (due to high concentration of lactose and lactate) from dairy industry which was used for simultaneous production of hydrogen and caproate. However, there is little information in the dissertation and in the published Paper 3 on this feedstock (regarding e.g. the consistency in composition and available quantities). Also, it would be interesting to know if the PhD candidate was considering other feedstocks which would not compete with food or feed.

It has to be pointed out that the obtained results demonstrate a great potential for practical use. Therefore, it would be interesting to learn what are/would be the challenges of scaling up the proposed solution, i.e. lactate-based bioproduction of medium chain carboxylic acids through mixed culture fermentation? Are there any plans for developing this technology to higher TRL and bringing this solution to the market?

5. Final conclusion

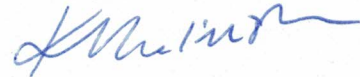
As the final conclusion of this review, I would like to state that the submitted PhD dissertation demonstrates novelty and high scientific value. The obtained results allow better understanding of the importance not only of the process parameters but also the composition of the substrate in lactate-based bioproduction of medium chain carboxylic acids by mixed culture fermentation, and thus make a significant contribution to the advancement of the state of the art. The proposed approach to use bioproduction of medium chain carboxylates through the chain elongation demonstrates high potential for scaling up and future commercialization. In addition, I would like to highlight that the submitted PhD dissertation confirms that the PhD candidate is able to conduct research independently and he has developed a set of skills and qualifications required for a PhD candidate, including critical thinking, data analyzing, scientific writing and presenting as well as the ability to work in an interdisciplinary research group and function in an international scientific community.

In view to the above, I am confirming that the presented doctoral dissertation on *Lactate-based bioproduction of medium chain carboxylic acids via mixed culture fermentation* prepared by Filip Brodowski, MSc. Eng., performed under the supervision of Prof. Piotr Oleśkiewicz-Popiel and auxiliary supervision of Mateusz Łężyk, PhD., is in

the scope of the discipline of environmental engineering, mining and energy and fulfills all the requirements set out in the Art. 187 (sec. 1-4) of the Act of July 20th, 2018 on the Law on Higher Education and Science (Journal of Laws of 2023, item 742).

Therefore, I am asking the members of the Council of the Science Discipline of Environmental Engineering, Mining and Energy at Poznań University of Technology to admit the PhD dissertation by Filip Brodowski, MSc. Eng. to the next stage of the doctoral procedure.

In my opinion the work performed by Filip Brodowski, MSc. Eng. within the doctoral dissertation demonstrates high scientific value and will significantly contribute to the advancement of the state of the art. Therefore, I would like to request for the distinction of the PhD dissertation by Filip Brodowski.



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