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THE REVIEW OF DOCTORAL DISSERTATION

written by **Aleksandra Gęsicka MSc. Eng.**

entitled „**Conversion of methane into selected
polyhydroxyalkanoates with the use of
methanotrophic microorganisms**”

prepared under the scientific supervision of

Prof. Piotr Oleśkiewicz-Popiel

and

Mateusz Łężyk, PhD

1. The basis for the preparation of the review

The formal basis for the preparation of the review is a letter from the Dean of the Faculty of Environmental Engineering and Energy, Poznan University of Technology, Zbigniew Nadolny, Eng., PhD, DSc, dated July 08, 2024.

Following Article 187 of the Law of July 20, 2018. - Law on Higher Education and Science (Journal of Laws 2018, item 1668, as amended), the doctoral dissertation presents the candidate's general theoretical knowledge in a discipline or disciplines and the ability to conduct scientific or artistic work independently. In addition, the subject of the doctoral dissertation is to be an original solution to a scientific problem, an original solution to the application of the results of one's scientific 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, and an independent and isolated part of a collective work. According to the guidelines of the Council for Scientific Excellence¹, "the opinion on a given dissertation should include the following elements:

¹ Rada Doskonałości Naukowej. 2022. Recenzje w postępowaniach o awans naukowy. Poradnik.

- 1) an assessment with a justification of whether the doctoral dissertation demonstrates the general theoretical knowledge of the applicant for the doctoral degree in a specific discipline or disciplines;
- 2) an evaluation with a justification of whether the doctoral dissertation demonstrates the ability of the applicant to conduct scientific or artistic work independently;
- 3) an assessment with the justification of whether the doctoral dissertation represents an original solution to a scientific problem, an original solution to the application of the results of one's scientific research in the economic or social sphere, or an original artistic achievement."

The review was prepared following the above recommendations to the extent specified in the contract for the preparation of the review in the doctoral proceeding.

2. Thesis characteristics

The dissertation submitted for review is a collection of published (two manuscripts prepared for sending to scientific journals at the time of dissertation submission of the dissertation) and thematically related research articles, comprising:

- Gęsicka, A., Oleskowicz-Popiel, P., Łężyk, M., 2021. Recent trends in methane to bioproduct conversion by methanotrophs. *Biotechnology Advances* 53, 107861. <https://doi.org/10.1016/j.biotechadv.2021.107861>. (3 authors, Aleksandra Gęsicka M.Sc. is the first author) - review paper;
- Gęsicka, A., Gutowska, N., Palaniappan, S., Oleskowicz-Popiel, P., Łężyk, M., 2024. Enrichment of mixed methanotrophic cultures producing polyhydroxyalkanoates (PHAs) from various environmental sources. *Science of the Total Environment* 912. <https://doi.org/10.1016/j.scitotenv.2023.168844>. (5 authors, Aleksandra Gęsicka M.Sc. is the first author) - research paper;
- Gęsicka, A., Gutowska, N., Palaniappan, S., Oleskowicz-Popiel, P., Łężyk, M., 2024. Sequential feast-famine process for polyhydroxyalkanoates production by mixed methanotrophic culture under different carbon supply and pH control strategies. The manuscript is ready to be submitted to a scientific journal (5 authors, Aleksandra Gęsicka M.Sc. is the first author) - research manuscript;
- Gęsicka, A., Gutowska, N., Palaniappan, S., Oleskowicz-Popiel, P., Łężyk, Influence of valerate addition during the fed-batch process for PHBV synthesis by pure and mixed methanotrophic cultures. The manuscript is ready to be submitted to a scientific journal (5 authors, Aleksandra Gęsicka M.Sc. is the first author) - research manuscript.

The total number of points for published papers according to the MEN scoring system is 400, however, papers are published in highly ranked Q1 journals, *Biotechnology Advances* with IF=12.1, and *Science of the Total Environment* with IF=8.2. The Ph.D. student has also prepared an abstract in Polish and English and an introduction, in which she briefly discusses the content of the individual components of the dissertation. Additionally, the summarizing chapter titled Outlook, contains conclusions, recommendations, and perspectives for future work.

3. The significance of the research topic undertaken for the development of the discipline of environmental engineering, mining, and energy

In my opinion, the research problem undertaken, in the reviewed work, is an important and topical issue, falling within the discipline of environmental engineering, mining, and energy. One of the identified problems is the emission of greenhouse gases, including methane. Capturing and utilization of methane, a component of biogas, are challenging, especially, when it is sourced from dispersed origins, like wastewater treatment plants, composting sites, and old landfills. Usually, methane is utilized for energy purposes, however, it requires a high (>45%) content of CH₄ in biogas to make it economically feasible. If the quality of biogas is not good enough, it is just burned in flares. That solution doesn't lead to the generation of value-added products except electricity and heat. However, the utilization of methanotrophic bacteria may simultaneously lead to the oxidation of methane and the production of organic compounds including biofuels (butanol), biopolymers (polyhydroxyalkanoates), or single-cell protein. One of the new scientific, developmental, and commercialization trends is the production of bioplastics from organic matter contained in wastewater and waste. It has been proved that specific microorganisms, under specific conditions, can accumulate organic matter in the form of polyhydroxyalkanoates (PHB, PHBV). This phenomenon has been used in wastewater treatment plants in the process of removal of phosphorous, where accumulated polyhydroxyalkanoates are used for the production of polyphosphates. Numerous research groups, work on the optimization of the conversion of organic matter contained in the wastewater to polyhydroxyalkanoates. One of the branches of this activity is the application of methanotrophic bacteria for the production of polyhydroxyalkanoates from simple molecules of methane. Biological oxidation of methane is widely recognized, as a phenomenon occurring both on natural and anthropogenic sites. Methane is biologically oxidized in wetlands, peat bogs, natural gas extraction, and storage sites, and landfill covers. It has been found that due to that methane can be converted into polyhydroxyalkanoates. This is a solution that meets the

goals of sustainable development and circular bioeconomy, by returning carbon to the form of biopolymers which have properties similar to fossil plastics. It may bring solutions for the utilizing of the low methane content emission points (landfill sites) both for the mitigation of GHG emission, and production of value-added biopolymers. The proposed solutions are synergistic and represent a new approach to the problem and can be developed in the future at higher TRL levels.

Considering the above remarks, I conclude that the problems undertaken by the author of the dissertation are topical, and justified, it is a new and necessary direction of research, and further development of these issues can make a significant contribution to the development of low methane content biogas and polyhydroxyalkanoates production technologies. I believe, that it is confirmed by the National Science Centre, which supported the research project titled “Production of hydroxyalkanoate copolymers in gas fermentation of methane with mixed microbial consortia” - 2019/35/D/ST8/03530 grant agreement number.

4. Substantive characteristics of the work

The goal of this work was to investigate the conversion of CH₄ into selected PHAs by mixed-methanotrophic culture. The author wrote that “The primary research objective was to determine whether a co-feeding strategy of precisely timed pulses of CH₄ and secondary carbon source will result in the production of PHAs with defined composition in mixed methanotrophic microbial communities.” The author intended “to deepen the current understanding of phase accumulation under specific process conditions in a complex culture enriched with methanotrophic bacteria.”

The particular aims of the thesis were to:

- investigate the conditions that promote a stable community of PHB-producing methanotrophs,
- study the microbial composition and process conditions influence on properties of PHAs produced and the process efficiency,
- to determine whether the composition of the desired copolymer can be obtained via alternating the feeding regime between methane and a secondary carbon source,
- to compare the PHBV copolymer production by pure and mixed methanotrophic culture.

The dissertation is generally well structured. First, Aleksandra Gęsicka did excellent work studying the massive number of scientific papers and preparing the review paper summarizing the most recent state of the art in the field of biological oxidation of methane and production of

PHAs from this gas. It gave the basis for the conceptualization and design of the experiments. The first experiment (published) was dedicated to checking the influence of the methanotrophic culture inoculation by microorganisms sampled from various environments. Here, either CH₄ was used as a sole carbon source to produce PHB or was supplemented by valeric acid as a co-substrate to produce PHBV. Additionally, the influence of nitrogen form on the microbial culture composition and PHB production was evaluated. Varied CH₄:O₂ ratios were used. It has been shown that the mixed culture enriched from waste-activated sludge, gave the most promising results in PHAs production, which is not surprising, as activated sludge microorganisms consortia are well adapted to the accumulation of PHAs. The nitrogen supplementation and CH₄:O₂ ratio were optimized. As a consequence of the previous results, in the next manuscript, the influence of various alcohols and carboxylic acids, as co-substrates on PHAs production, was studied. The most effective co-substrate – valeric acid, was selected to investigate PHBV production. Various carbon supply and pH control strategies were then optimized. The next step was the optimization of the co-substrate feeding rate a fed-batch process influencing polymer production and composition. Pure and mixed methanotrophic cultures were compared to show the potential of using mixed cultures on a technical scale. At the end of the thesis Ph.D. student summarized her work providing the most important conclusions, and showing remarks and recommendations for the development of the studied technology. One of the most important remarks is that the results in all papers and manuscripts were very deeply and critically discussed. Reading this thesis, I was wondering if the author will discuss the low solubility of CH₄ in water. That aspect may be a bottleneck of the technology. I found that this issue was also discussed. Another aspect, which may also have an importance is the risk of the creation of an explosive mixture of methane and air, especially when the low content of methane in air was used. That aspect also was discussed. Good job.

Considering the above, I believe that the goal of the dissertation has been achieved.

I also believe that the dissertation presents the Ph.D.'s general theoretical knowledge at a high level in the field of study and in the discipline of environmental engineering, mining, and energy.

In my opinion, the dissertation demonstrates the Ph.D.'s ability to conduct scientific work independently. The experiments were designed correctly, including statistical evaluation of the results, however, more advanced regression analysis of the influence of pH, O₂, and other parameters on PHA production could be done.

I believe that the dissertation represents an original solution to a scientific problem in the area of producing PHAs from methane, having the potential for further development and final implementation in the industrial practice, which is important in engineering sciences.

5. Specific comments

Despite the relevance of the topic undertaken and the innovative approach, there are issues in the work, and formulations that raise questions and doubts. These comments are arranged in the chronological order of reading the work.

Page 7. The content of methane is 10%. It is not specified if it is a volumetric content or mass content. Especially, in the case of gases, it makes a difference. The comment concerns other places in the thesis having this unit.

Page 8. The author uses different units for the description of the mass yields of the products (g/L or mol%). It could be converted and unified for comparative purposes. The comment concerns other places in the thesis having this issue.

Page 56 (second paper). In my opinion, the inoculum source is not fully described. The type of activated sludge should be described, from what treatment reactor was it taken, and what was the capacity of the WWTP? What materials were used for the creation of the landfill's top cover? From which depths and places (spatial distribution of samples)? Were these covers planted by grass or other plants or left unplanted? The same concerns the biocompost. What was the feedstock for composting, and what was the duration of the composting? From which phase of composting the samples were taken? All these aspects should be described, as they may influence the results. One of the scientific paradigms is the repeatability of the experiments. Without these descriptions, other researchers cannot do similar experiments and also they cannot do a deep comparable discussion, as they cannot refer to the origin of samples.

Page 92. The author wrote, "It is important to note that the culture was transferred from a nitrogen-deficient reactor and the biomass already had PHB accumulated at the level of 57% PHB in DCW". What would happen if the different feedstock, e.g. landfill soil cover, were used?

Page 131 – conclusions. The author wrote, "10% CH₄ in the air (1:2 CH₄:O₂ ratio) is the most optimal for biomass growth and PHA production in mixed methanotrophic cultures among tested CH₄:O₂ ratios." What is the practical implication of it? How we can obtain such a ratio from real, low-quality landfill biogas on a technical scale when CO₂ also is present in the biogas?

Page 131 – conclusions. The author wrote, “PHBV can be produced from CH₄ and valeric acid under a feast-famine regime in long-term CSTR cultivation.” Is valeric acid a cheap source of carbon? How do we substitute it with waste materials?

Chapter 6.4. Perspective for CH₄-based PHA production. I have a technical question: What about the source of CH₄, how do we prepare the biogas for treatment, does it require any purification, CO₂ removal, H₂S removal, etc?

6. Conclusions

The topic of the dissertation is topical and important both from a scientific and practical point of view. The results obtained contribute to the development of the discipline of environmental engineering, mining, and energy in the development of PHAs production due to the biological oxidation of methane. The content of the dissertation is consistent with the title, the stated goal of the work has been fulfilled, and the hypotheses have been verified. The dissertation was edited correctly from the formal side.

The dissertation confirms the author's very good knowledge of general theoretical knowledge in the analyzed area of the discipline of environmental engineering, mining, and energy. The dissertation also confirmed the author's ability to conduct scientific work independently, apply her experimental solutions, and formulate conclusions, although the results obtained could have been subjected to statistical analysis.

The author's extensive previous experience and acquired knowledge allow me to believe that the research will be continued and developed to improve the technological readiness of the proposed solutions. This will allow, at a further stage of scientific development the Doctoral Student, to supplement the presented results with specific studies on a semi-technical and technical scale.

I conclude that the issues taken up by the author of the dissertation are justified, it is a new and current direction of research, and further development of these issues can make a significant contribution to the development of methods of PHAs production from methane.

When evaluating the doctoral dissertation of Aleksandra Gęsicka, MSc. Eng., the following were considered: the significance and originality of the subject matter undertaken, the correctness of the formulation of the objectives and research hypotheses, the design of the research methodology, the description and interpretation of the results obtained, the structure of the dissertation and its technical side. Considering the above criteria and the comments made in the review, I conclude that, despite the listed shortcomings and doubts, the doctoral dissertation I have evaluated entitled "Conversion of methane into selected

polyhydroxyalkanoates with the use of methanotrophic microorganisms" meets the requirements of the Law of July 20, 2018. - Law on Higher Education and Science (Journal of Laws 2018 item 1668, as amended) and I request that it be admitted to public defense. Due to the high quality of the research and the insightfulness of the conclusions, I am applying to the Discipline Council for Environmental Engineering, Mining, and Energy at Poznan University of Technology for a distinction for this doctoral dissertation.

Wrocław, 10.09.2024 r.

.....*Białowiec*.....

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