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Review of the PhD thesis of Zhila Honarmandrad
Entitled „Management of broth after biomass hydrolysis to improve biohydrogen production"

The issuance of the opinion on the doctoral dissertation of Ms. Zhila Honarmandrad, M.Sc., is hereby based on the letter of the Chair of the Council of the Discipline of Chemical Sciences, Associate Professor Marek Tobiszewski, D.Sc., Eng., dated 18 July 2025 (Ref. No. 181 WCh/Dz/2025).

M.Sc. Zhila Honarmandrad has prepared the presented doctoral dissertation under the supervision of dr hab. inż. Jacek Gębicki, prof. GUT at the Faculty of Chemistry, Gdańsk University of Technology.

This dissertation is devoted to the development and evaluation of advanced, environmentally sustainable processes for the efficient removal of inhibitory compounds—such as furfural, hydroxymethylfurfural, hydroquinone, and vanillin—that hinder microbial activity during biohydrogen production from lignocellulosic biomass. To address this challenge, the Doctoral Candidate proposed three original sorption-based extraction strategies: the use of hydrophobic magnetic deep eutectic solvents (HMDES), supramolecular deep eutectic solvents (SUPRADES), and metal–organic framework-based pseudo-deep eutectic solvents (MOF@pseudo-DES).

Hydrogen, and in particular biohydrogen, has attracted significant scientific attention due to its potential as a clean and sustainable energy carrier with high calorific value. Its production from renewable resources offers a promising alternative to fossil fuels, contributing to the reduction of greenhouse gas emissions and supporting the transition toward a low-carbon energy system. Moreover, studies on biohydrogen generation provide valuable insights into biological and catalytic mechanisms, fostering the development of efficient and environmentally friendly energy technologies. One of the challenges to overcome is the efficient removal of inhibitory compounds from hydrolysates prior to fermentation in order to maintain microbial activity and enhance hydrogen production. In view of this, the topic of the doctoral thesis is both timely and important.

The thesis is well written and contains a total of 131 pages and 220 references. The dissertation follows a traditional structure. It begins with an introduction. The literature section (Chapters 2–7) provides a review of the subject literature, followed by the statement of research objectives (Chapter 8). The experimental section (Chapter 9) presents the research methodology and the materials used. Subsequently, Chapters 10–14 describe the results obtained. The final part of the dissertation contains the findings, a comparison of the applied methods, a summary, an outline of further research directions, as well as lists of figures and tables, and the bibliography. Additionally, the dissertation includes summaries in both Polish and English, as well as the Doctoral Candidate's academic achievements.

The first part (Chapters 2–7) presents the state of the art in the field. Non-renewable and renewable fuels are described, including the environmental and health impacts of the former and the advantages of the latter. More detailed information is then provided on biomass, biohydrogen, production methods and feedstocks. These methods include fermentation as well as pretreatment of lignocellulosic biomass and techniques for removing inhibitory compounds. Finally, green solvents (e.g., deep eutectic solvents, supramolecular deep eutectic solvents) and new types of sorbents (e.g., MOFs) are described. This literature review is relatively short (~30 pages) and very general. In the individual chapters, coherence is lacking—the data are largely listed rather than systematically presented. Moreover, the cited information remains at a fairly general level. In a doctoral dissertation, a greater degree of detail and more data drawn from scientific articles would be expected.

Chapter 8 presents the aim and scope of the work. The Candidate identified the development and evaluation of advanced, green, and sustainable extraction methods for removing inhibitory compounds from fermentation broths derived from lignocellulosic biomass hydrolysates as the aim of the research. The scope of the work included: (i) synthesis of novel green solvents; (ii) physicochemical characterization of the developed systems; (iii) optimization of extraction conditions using statistical models; (iv) comparison of performance between developed solvents and conventional methods; (v) application of the optimized system to real fermentation broth; and (vi) evaluation of biohydrogen production under selected conditions.

Chapter 9, entitled *Materials and Methods*, provides a comprehensive overview of the methods and instrumentation employed (HPLC, FTIR, PXRD, SEM, BET surface area analysis, TGA, GC-TCD-FID). The chapter details the preparation methodologies for HDES, NH_2-UiO-66@pseudo-DES, and SUPRADES, as well as the protocols for sample preparation for inhibitor removal using the developed systems. It also outlines procedures for inoculum preparation, dark fermentation, and the implementation of sorption processes using HDES, NH_2-UiO-66@pseudo-DES, and SUPRADES. An inventory of the materials and reagents used is also included.

In Chapters 10–11, the Candidate discusses the influence of individual parameters—such as pH, solution temperature, initial inhibitor concentration, analyte volume, volume of hydrophobic magnetic deep eutectic solvent, stirring speed, and contact time—on the removal of inhibitors using HMDES. However, based on the methodology outlined in Section 9.2.8 and the results presented in Subsections 10.1–10.7, it is difficult to ascertain the additional conditions applied when evaluating the effect of individual parameters on process efficiency. Furthermore, without this information, the reported influence of analyte volume and HMDES volume remains unclear. There is also no indication of how many times each experiment was replicated, and Figures 15–21 do not include error bars. Although the effects of individual parameters are briefly discussed with reference to active and sorption sites, these aspects are not addressed in the discussion of the sorption mechanism using HMDES. Furthermore, in my opinion, the term "active sites" is rather used to describe catalytic rather than sorption processes. Finally, the developed method was applied to real samples obtained after hydrolysis and fermentation. Figure 30 presents removal efficiencies for four compounds across 11 different real samples. However, only RE values of 83.3, 71.42, 69.99, and 76.80% (for hydroquinone, hydroxymethylfurfural, furfural, and vanillin, respectively) appear in the discussion, which cannot be correlated with sample type based on the description in the dissertation.

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Chapters 12–13 present the results on the application of NH_2-UiO-66@pseudo-DES for the removal of inhibitory compounds. A Box–Behnken Design (BBD) was employed to evaluate the effects of four variables (vortex time, pH, analyte concentration, and MOF@pseudo-DES dosage) on removal efficiency. However, details regarding the BBD modelling are limited. While the data matrix and equations used in the BBD model are provided in a published paper (10.1016/j.molliq.2025.126845), they are not included in the thesis. The analysis revealed that sorbent dosage had a significant effect on removal efficiency. Furthermore, the identified optimal conditions were validated for removing inhibitors from real lignocellulosic hydrolysates and post-fermentation broths (14 different samples). These investigations confirmed the effectiveness of NH_2-UiO-66@pseudo-DES in removing inhibitory compounds from real matrices, with efficiencies ranging from approximately 35% to 65%. It was also observed that NH_2-UiO-66@pseudo-DES remained stable over four consecutive cycles, although its effectiveness declined with further reuse. Finally, based on FTIR analysis, a mechanism of interaction between adsorbates and adsorbent was proposed.

Chapters 14–15 present the key findings on the application of SUPRADES for the removal of inhibitory compounds. A Plackett–Burman statistical design was employed to optimize efficiency and to assess the influence of seven selected parameters on removal performance. Based on the Plackett–Burman matrix, twelve experimental runs were conducted and analyzed using standardized Pareto charts. The effect of the tested factors were evaluated in terms of the extraction efficiency of the selected substances. The results indicate that removal efficiency is governed by distinct factors: sample volume and cyclodextrin dosage for HQ extraction; sample volume for HMF extraction; inhibitor concentration for FF extraction; and both inhibitor concentration and sample volume for VAN extraction. The employed SUPRADES was further characterized with respect to viscosity, density, and surface tension, and subsequently applied to the extraction of inhibitory compounds from a real fermentation mixture, confirming its practical applicability. Comparative testing of SUPRADES and conventional DES revealed higher extraction efficiency with SUPRADES. The reusability of SUPRADES was also confirmed. Finally, evaluation of biohydrogen production yield during fermentation demonstrated the positive effect of SUPRADES treatment of hydrolysates on process efficiency.

Assessment of hydrophobic magnetic deep eutectic solvents (HMDES), supramolecular deep eutectic solvents (SUPRADES), and metal–organic framework-based pseudo-deep eutectic solvents (MOF@pseudo-DES), as well as the overall summary and conclusions, are presented in Chapters 16–18, while future research directions are outlined in Chapter 19.

It should also be mentioned that M.Sc. Zhila Honarmandrad appears as co-author of six papers (four original and two review). These works have been published in internationally recognized journals (e.g., *International Journal of Hydrogen Energy, Journal of Molecular Liquids, Journal of Environmental Chemical Engineering, BMC Chemistry,* etc.). Furthermore, she is listed as co-author of two conference presentations.

Below I would like to mention some of my minor remarks and questions:

1. My principal reservation concerns the overall construction of the dissertation. The content of the thesis cannot be fully comprehended without reference to the Candidate's earlier publications, which considerably diminishes its clarity and self-sufficiency. Given that the dissertation is not presented as

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a collection of thematically related published articles, it should, by academic convention, provide all essential information in a self-contained manner—an expectation that, in its current form, is not met.

2. There is no scheme of the system employed to carry out the fermentation process.
3. Why was NH_2-UiO-66 chosen for study among various MOFs? The thesis lacks data on the MOF properties that led to this choice. Why was it combined with choline chloride?
4. Figure 39 – according to the description, Figure 39 presents a comparison between the removal efficiency of inhibitors by NH_2-UiO-66 and NH_2-UiO-66@pseudo-DES. However, the legend in Figure 39 shows that all data refer to MOF@pseudo-DES. The difference between experimental and predicted data for MOF@pseudo-DES is not discussed. What does the dataset described as "Y MOF@pseudo-DES (Prediction)" represent?
5. Subchapter 12.5: Were the samples regenerated between each sorption cycle?
6. How could the process using MOF@pseudo-DES be implemented on an industrial scale?
7. In the summary (Chapter 17), it is stated that "the goal was not only to eliminate inhibitors but also to improve hydrogen yield, operation stability, and process scalability." Please explain during the defense how operational stability and process scalability were improved in your work.
8. In Chapter 18, the PhD Candidate concludes that NH_2-UiO-66@pseudo-DES demonstrated fast adsorption kinetics (11 min). Where in the dissertation are the data supporting this sorption kinetics claim?
9. Why was the Box-Behnken Desing method employed to optimize process for MOF@pseudo-DES, while other procedures were used for another systems?

In summary, a novel approach for the removal of four different fermentation inhibitors was systematically investigated using three sorbent systems: hydrophobic magnetic deep eutectic solvents (HMDES), supramolecular deep eutectic solvents (SUPRADES), and metal–organic framework-based pseudo-deep eutectic solvents (MOF@pseudo-DES). UiO-66@pseudo-DES and SUPRADES have been applied for the fist time to remove inhibitory compounds from the fermentation medium in this work. Thus, the solution presented in this doctoral dissertation constitutes an original contribution to addressing the scientific challenge of removing inhibitors generated during biomass fermentation. Concluding, the doctoral dissertation of Zhila Honarmandrad complies with the conditions set out in Art. 187 of the Law on Higher Education and Science in Poland of July 20, 2018 (in Polish: Prawo o szkolnictwie wyższym i nauce, Dz.U. z 2018 r. poz. 1668 ze zm.) In view of the above, I am applying for admission of Zhila Honarmandrad to the next stages of the doctoral dissertation defense.

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