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Review

of the doctoral thesis by *MSc Zhila Honarmandrad*, *„Management of broth after biomass hydrolysis to improve biohydrogen production"*

Assessment of research topic selection

Ms. Zhila Honarmandrad completed her doctoral thesis under the supervision of Professor Jacek Gębicki at the Department of Process Engineering and Chemical Technology, Faculty of Chemistry, Gdańsk University of Technology. In my opinion, the topic of the thesis and studies is very timely. Renewable energy sources are critical, especially now, at a time of growing climate crisis, which is no longer in doubt. Research into renewable fuels is very important, and its significance for the future of the environment and human life is beyond doubt. These fuels take advantage of sunlight, wind, water, biomass, and certain waste materials. Biomass is particularly interesting because it is a source of biohydrogen. Even though the production of the latter poses many challenges (low efficiency, development of dark fermentation, photofermentation), research into biohydrogen is currently one of the most rapidly developing areas, especially in increasing scalability or decreasing the activity of inhibitors. Therefore, it is necessary to search for new methods and new materials enabling the removal of inhibitors that reduce the efficiency of biohydrogen production from biomass. The doctoral thesis fits perfectly into these trends. Despite studies conducted worldwide in this field, the topic remains relevant due to the practical aspects of using new types of extraction media.

Formal and substantive evaluation of the thesis

From a formal point of view, the PhD thesis consists of 123 pages of typescript and takes the form of a classic dissertation. It comprises seven basic parts, including: abstract, literature review, objectives, methodology, results of own research with discussion, conclusions, and bibliography. The work is illustrated with graphs, diagrams, and tables. The high quality of the presentation of the results is particularly noteworthy. The theoretical part is presented at only 32 pages, while the experimental part at 77 pages.

In the literature review, PhD candidate briefly discussed non-renewable fuels, emphasizing the negative effects of their use. The information presented is a good introduction to the next chapter, which is devoted to renewable fuels, with a brief description of biomass, solar and wind energy, hydropower, geothermal and ocean energy, and finally energy from hydrogen. The table summarizing the advantages and disadvantages of renewable and non-renewable energy is a very good summary worthy of recognition. In the next part of the thesis by MSc Zhila Honarmandrad presented issues related to biomass with particular emphasis on biofuels, which are products of its decomposition. The author paid special attention to biohydrogen because increasing its

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production efficiency by reducing the proportion of inhibitors was one of the objectives of the doctoral thesis. For this reason, the review of scientific literature devoted to methods of biohydrogen production in thermochemical and biological processes is justified. The thesis was expanded to include a discussion of feedstock for biohydrogen production. MSc Zhila Honarmandrad has comprehensively presented lignocellulose as a source of plant biomass. Its decomposition leads to the production of biohydrogen after appropriate pretreatment. Fermentation can be used for this purpose, but its efficiency is largely dependent on the inhibitors formed during the process. This problem is well known and described both in scientific literature and in doctoral theses. What is more, PhD student presented physicochemical and biological methods for removing inhibitory compounds after hydrolysis of lignocellulosic biomass. The next part of PhD thesis is devoted to green solvents, such as deep eutectic solvents (DES), hydrophobic magnetic deep eutectic solvents (HMDES), and supramolecular deep eutectic solvents (SUPRADES). This chapter, although crucial for experimental research, is only five pages long and, in my opinion, is far too short, similar to the next chapter on new types of adsorbents, including metal-organic frameworks (MOF) and the integration of MOFs with DES.

To sum up, I would like to emphasize that the theoretical part of the doctoral thesis is good introduction to the goal of experimental research. Literature review provides and explains the reasons that inspired PhD candidate to choose this research topic. The main goal was to develop and characterise new, innovative, and sustainable extraction methods for removing inhibitors from the fermentation broth resulting from lignocellulosic biomass. These studies are important for increasing the yield of biohydrogen production. I appreciate the effort of developing environmentally friendly removal strategies.

The next chapter of the thesis describes the reagents, methods, techniques, and equipment used during the research. It also presents the procedures for synthesizing the new materials, preparing real samples, and the procedures used during adsorption tests. Next, the PhD candidate described the research with an interpretation of the results. The experiments were presented in a proper manner, and the research was conducted comprehensively, thus providing an excellent basis for discussion and conclusions. The author researched the synthesis, characteristics, and use of HMDES, MOF@PSEUDO-DES, and SUPRADES for removing inhibitor compounds. In each of these cases, the workflow was very similar.

The sorption efficiency parameters have been carefully optimized by studying temperature, stirring speed, volume of material, sample volume, pH, initial concentration of inhibitors, and contact time. Unfortunately, none of the design of experiments attempts have been applied for HMDES, contrary to MOF@PSEUDO-DES and SUPRADES, where optimization was more complex through the application of Box-Behnken and Plackett-Burman designs. Moreover, the MOF@PSEUDO-DES characterization was conducted and presented in great detail to demonstrate and prove the integration of both groups of compounds and the formation of a new material. Unfortunately, the doctoral thesis lacked detailed information on the characteristics of HMDES.

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I greatly appreciate the research into the regeneration and reusability of each of the three extraction mediums. It clearly shows that they can be efficiently regenerated and reused several times without significant degradation in performance (from three times for SUPRADES to six times for HMDES). It is extremely important from a sustainability perspective and proves their usefulness with low costs. However, it should be noted that SUPRADES effectiveness diminishes with extended use and is suitable manly for short-term applications (where high removal efficiency is critically required). MSc Zhila Honarmandrad attempted to study the extraction mechanism for all prepared adsorption media. To this end, she used FTIR spectroscopy to record the spectrum before and after adsorption of the inhibitory compounds. Results suggest that hydrogen bonding serves as the primary extraction mechanism for HMDES, while the same type of interactions, together with electrostatic attraction, π-π stacking, and dipole-based interactions, remain critical for two other extraction media. Generally, the contribution of these interactions to adsorption strongly depends on inhibitor structure, proving another advantage of the developed procedures, namely the possibility of selectivity tuning.

The effectiveness of the three developed materials was successfully compared with their unmodified analogues, i.e., HMDES with DES (without hydrophobic modification of magnetic nanoparticles), MOF@PSEUDO-DES to MOF, and SUPRADES to unmodified DES. This was undoubtedly an essential part of the experimental research aimed at demonstrating the superiority of the new solvents and adsorbents synthesized by the PhD candidate. She was successful in this field, as the results of the research proved that they have greater adsorption efficiency for inhibitory compounds.

The most valuable research is related to the application of the developed procedures to real samples; hydrolysates derived from lignocellulosic biomass. The wide variety or samples were used, namely hardwood blend, starch and lignocellulose hydrolysate from potato, corn cobs. For two of synthesized extraction (adsorption) media, lower removal efficiency of inhibitor compounds was observed compared to the standard solutions. This is a typical and expected effect (matrix interferences and competitive interactions), but the most important thing is that these decreases were not high. Removing efficiency confirms the robustness of HMDES, MOF@PSEUDO-DES in a complex hydrolysate matrix. This is a valuable result, as it shows that developed procedures can find practical application in the production of biohydrogen. Regarding SUPRADES, PhD candidate applied it during the hydrogen production across four fermentation cycles in two bioreactors with different feedstocks. Addition of this extraction medium caused constant growth of microbial performance and hydrogen yield by reducing inhibitors and preserving fermentable substrates. Utilization of SUPRADES is a promising strategy for large-scale hydrogen fermentation processes.

In comparison with conventional solvent-based techniques HMDES, MOF@PSEUDO-DES, and SUPRADES showed superior operational advantages: rapid action (11-15 minutes) at room temperature, eco-friendly composition (biodegradable), reusability, operational simplicity and high affinity for multiple inhibitory compounds.

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Taken together, these findings make them a promising, scalable, and sustainable solution for fermentation inhibitor removal. In comparison with HMDES and SUPRADES, MOF@PSEUDO-DES provided greater selectivity and operational simplicity, despite lower removal efficiency. On the other hand, SUPRADES showed the highest adsorption of inhibitory compounds but required longer contact time and alkaline pH, contrary to two HMDES (notable for its magnetic separability).

MSc Zhila Honarmandrad conducted her research in a very skilful manner with a high degree of complexity and systematicity, which is an unquestionable advantage of the dissertation. She conducts the discussion of results in an interesting and clear manner, and her findings contain a lot of novelty. PhD candidate has already published all results included in the doctoral dissertation. They are the subject of three publications in international renowned journals: *Journal of Environmental Chemical Engineering*, *Journal of Molecular Liquids*, *International Journal of Hydrogen Energy*. The total IF of these papers equals 20.7, which is very good result considering the scientific achievements for a doctoral dissertation. PhD candidate is the first author in all of them proving her leading role. Additionally, Ms Zhila Honarmandrad is the co-author of thirteen other publications (Scopus, 11.09.2025). Worth noting is high citation number of her papers (519 according to Scopus, h index equals 10) which indicates the high level of interest among other scientists. PhD student presented her scientific results at scientific national and international conferences. To sum up, scientific achievements of MSc Zhila Honarmandrad allow to conclude that she is an active researcher with high potential for further scientific development.

I consider the following to be the most important achievements of the doctoral dissertation:

- Development new, three sorption methods for the removal of inhibitors from fermentation broth. This approach has not yet been explored in the scientific literature, making it a significant contribution to the field.
- Proving that synthesized adsorption media provide efficient, sustainable, and customizable solutions for the removal of inhibitory compounds from fermentation broth and increasing biohydrogen yields. Moreover, the proposed green solvents outperformed conventional ones.
- Demonstrating that HMDES is best suited for rapid, reusable, and scalable operations, SUPRADES for biohydrogen-oriented pretreatment, and MOF@PSEUDO-DES for eco-compatible selective detoxification under mild conditions.

Specific comments

MSc Zhila Honarmandrad made some minor linguistic and editorial errors, but they are insignificant to the substance of the thesis. However, a detailed reading of the doctoral thesis raised several other questions and doubts, which I have listed below for a discussion during doctoral defense:

- Were the figures in the theoretical part designed and prepared by the author (none of them have a reference to the literature)

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- Is the HMDES suspension stable in solution? If so, for how long? Is it necessary to use ultrasound to disperse HMDES particles in solution?
- The initial concentration (c_i) is used in the removal efficiency formula (Eq. 6). How was this concentration determined for real samples?
- There are no standard deviations on any of the bar charts presenting results. Why?
- I would like to request an HPLC chromatogram for the mixture of standards and several different real samples (hydrolysis and fermentation of lignocellulosic biomass). I want to verify to what extent the inhibitory compounds were separated from (presumably many) other compounds in such a rich matrix.
- Has the determination of the sorption capacity of the synthesized materials/eutectic solvents been considered?
- Figure 19: I would like to ask for a more complete interpretation of the effect of reducing RE with an increase HMDES volume (than is provided in the doctoral thesis). Did the amount of HMDES increase with the increase in volume?
- The experiments conducted by PhD candidate are small-scale. How does she assess the possibility of changing and using the developed extraction media on a large, industrial scale (e.g., the need to use very large magnets, high synthesis costs of adsorption medium, possible differences in efficiency between different scales)?
- PhD candidate state that more pronounced peak shifts (FT IR) observed for different inhibitor compounds indicate stronger hydrogen bonding interactions between these inhibitors and HMDES. I'm not convinced that peak shifts can be indicator of interaction strength.

Despite the above comments and questions, most of which are entirely debatable, I believe that PhD thesis is on a very topical subject and to a high standard.

Concluding statement

To sum up my review, I conclude that the goals of MSc Zhila Honarmandrad research have been achieved, the results presented in the doctoral dissertation are very valuable and new. The work meets the requirements for doctoral dissertations in accordance with the Act of July 20, 2018 (Law on Higher Education and Science, Article 187(1) and (2)). In view of the above, I request that the Chemical Sciences Discipline Council at the Gdańsk University of Technology, MSc Zhila Honarmandrad, to the next stages of the doctoral procedure.

Signature Not Verified

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