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Review of the doctoral dissertation by Ruchi Manishkumar Upadhyay, MSc entitled 'Determination of the mechanism and optimisation of the conditions of the process of removing colored aromatic compounds by selected Basidiomycota"

(title in Polish: "Określenie mechanizmu i optymalizacja warunków procesu usuwania barwnych związków aromatycznych przez wybrane grzyby podstawkowe")

Supervisor: Prof. Wioletta Przystaś, PhD, Eng.

This review is based on a letter from Prof. Krzysztof Labus, Chair of the Discipline Council for Environmental Engineering, Mining and Power Engineering at the Silesian University of Technology in Gliwice, dated 6 March 2026 (RIE-BD.512.14.2026) concerning the preparation of an assessment of the aforementioned doctoral dissertation.
At the outset of this review, I would like to note that the doctoral dissertation was written in English.

Dyes are widely used in many industries, including the textile, paper and tanning industries, as well as in the manufacture of plastics, and are a part of our everyday lives. In 2023, approximately 1.1 million tonnes of synthetic dyes (and around 800,000 tonnes of synthetic pigments) were produced worldwide, and it is estimated that the production of dyes and pigments will continue to grow until 2032. Depending on the dyeing technology, a significant proportion – up to 60% of the initial dye mass – may go unused and end up in wastewater. Although scientists have been working on the removal of dyes from wastewater for many decades, the chemical diversity of dyes and their high consumption on the one hand, and the development of analytical methods, including bioinformatics, on the other, mean that this remains an interesting topic from both a practical and scientific perspective. I therefore consider the choice of topic for Ms Ruchi Upadhyay's doctoral dissertation to be appropriate and justified on scientific grounds, as well as by the potential for practical application of the research results obtained in the dissertation.

The main objective of the doctoral dissertation under review was, broadly speaking, to use white rot fungi to remove synthetic dyes from water or wastewater; these dyes, based on their chemical structure, fall into three main groups, namely azo dyes (Evans' blue (EB) and Congo red (CR)), triphenylmethane dyes (Brilliant Green (BG) and Cyanine Violet (CV)), and anthraquinone dyes (Brilliant Blue Remazol R (RBBR)). In total, the PhD student investigated five different synthetic dyes and used two species of basidiomycetes for their removal, namely *Trametes versicolor* (strain CB8) and *Pleurotus ostreatus* (strain BWPH). Both fungal strains were sourced from the Fungal Strain Collection of the Department of Environmental Biotechnology at the Silesian University of Technology in Gliwice. The scope of the doctoral dissertation research covers the optimisation of decolourisation processes, the assessment of the toxicity of decolourisation products, the characterisation of biosorption and biodegradation of dyes involving white rot fungi, as well as the application of omics techniques, namely transcriptomic and proteomic analysis, the determination of the activity of selected enzymes, in order to elucidate the mechanisms of dye biodegradation. I believe that the scope of the work undertaken by the PhD candidate is exceptionally broad and deserves recognition. It represents a comprehensive and innovative approach to solving the scientifically recognised problem of wastewater decolourisation, particularly in the section concerning the application of multi-omics analyses.

In Chapter 3, the PhD student accurately identified key research gaps and, based on these, formulated a comprehensive research hypothesis. The author posits that the selected strains of *Trametes versicolor* (strain CB8) and *Pleurotus ostreatus* (strain BWPH) will be capable of removing dyes through a synergy between biosorption and enzymatic degradation. Improving the efficiency of these processes and their potential application in decentralised wastewater treatment plants will be possible through the optimisation of process parameters. The mechanisms of dye removal via mycoremediation will be elucidated through the use of omics techniques, which may enable the prediction and modelling of decolourisation processes for other dyes in the future. The PhD candidate also assumes that the product resulting from mycoremediation will be

ecotoxicologically safe. In my opinion, though this hypothesis is correct in substance, it could be more concise or clearly divided into two or three parts. This would make it easier to read. Most importantly, however, the candidate has successfully verified the hypothesis by conducting extensive research, analysing the results in depth and drawing appropriate conclusions.

The doctoral dissertation is divided into 7 chapters, preceded by abstracts and keywords in English and Polish, a list of the candidate's own publications on the subject of the doctoral dissertation, and a list of abbreviations used in the dissertation; the candidate has also provided the sources of research funding. The list of abbreviations could be more comprehensive (e.g., KEGG, GO and MEA are missing), but I understand that with such a large number of analytical and statistical methods used, it is almost impossible not to omit something. I would also like to add that the PhD candidate generally explained the abbreviations and symbols used in the body of the dissertation, which made the text easier to understand. In the final section of the doctoral dissertation, following Chapter 7, there is a list of cited publications, appendices, and a list of figures and tables. The proposed structure of the doctoral dissertation is logical and appropriate for this type of academic work.

Before proceeding with further assessment of the doctoral dissertation, I would like to emphasise that Ms Ruchi Upadhyay is the co-author of four academic articles on the subject of her doctoral dissertation. All of these articles were published in journals with an impact factor (IF) between 2022 and 2025.

The doctoral dissertation under review contains 63 figures and 19 tables, not including the two figures and five tables included in the appendix. In my opinion, the PhD candidate has well documented the results of the research conducted and the statistical analysis of the data. All tables are clear and fully comprehensible. The figures have also been prepared with great care and, with the possible exception of two (Fig. 4 and Fig. 36), which in my view require minor editorial correction, they visualise the research conducted and its results very well. The tables and figures prepared by the PhD candidate demonstrate the author's great commitment and her maturity in conveying scientific knowledge. I would like to commend the PhD candidate in particular for the figures included in Chapter 4, which are clear, aesthetically pleasing and effectively illustrate the research methodology employed in the dissertation. The captions for the figures and tables have been edited correctly. References in the text to the figures and tables are correct and placed in the appropriate locations, ensuring that the text and visual elements form an integral whole. This also makes the doctoral dissertation easier to read.

In my opinion, the language used by the author in the dissertation is correct. It is academic language, appropriate for a doctoral dissertation. There are very few linguistic, stylistic or punctuation errors. On a few occasions, I had doubts regarding the use of the article 'the' or some unusual expressions, but I find it difficult to assess this definitively and classify it as an error, as I am not a native speaker. I consider that, as a whole, the doctoral dissertation was written in a language that was comprehensible, substantive and academic.

In terms of editing, the doctoral dissertation is very well prepared. I found no errors in the numbering of figures or tables, nor in the captions or references, although, as I have already mentioned, the work is richly illustrated. The numbering of chapters, subsections and equations is, in my view, also correct. Abbreviations and symbols are explained repeatedly in the text, which aids in reading the dissertation. It must be emphasised here that keeping track of a large number of abbreviations and symbols – which, according to my estimates, amounts to over 100 – is a very difficult task. Nevertheless, the PhD candidate has coped with this very well. She has endeavoured to ensure that, despite the broad scope and great diversity of the research methods employed, everything is presented clearly and precisely. In formal terms, I rate the dissertation very highly.

In the literature review, which constitutes the second chapter of the doctoral dissertation, the author characterises dyes, and in particular synthetic dyes; she then briefly describes the impact of dyes on ecosystems and human health, and in the following section lists and concisely discusses physical, chemical and biological methods for dye removal. The PhD candidate devoted relatively the most space to a review of publications concerning the use of white rot fungi for the degradation of dyes. In my opinion, this is the correct approach, as the literature review is intended to help identify research gaps and issues related to mycoremediation that have not yet been sufficiently clarified. In my view, the selection of topics and literature sources was appropriate and enabled the PhD candidate to accurately identify the gaps in the current state

of knowledge regarding the removal of synthetic dyes using basidiomycetes, which in turn led to the formulation of substantively sound objectives, scope and research hypothesis of her doctoral dissertation.

In total, the PhD candidate drew on an impressive 304 English-language literature sources throughout the dissertation. These were almost exclusively scientific articles. Nearly half (47%) of the cited works were published between 2020 and 2025. The candidate has therefore drawn primarily on the latest, up-to-date scientific achievements related to the subject of her dissertation. I consider the selection of literature sources to be comprehensive, appropriate and up-to-date; it indicates the ongoing interest of many research teams worldwide in the topic of removing synthetic dyes from the natural environment.

In Chapter 4 of the doctoral dissertation under review, the author discusses in great detail the research materials and methods employed in the study. This chapter comprises 16 sections. In these, the doctoral candidate describes, amongst other things, the synthetic dyes selected for the study, the basidiomycete strains used, the conditions under which they were cultured, the immobilisation of mycelium on two carriers, methods for determining the activity of three enzymes (laccase, manganese peroxidase and lignin peroxidase), transcriptomic and proteomic analysis for *Trametes versicolor*, and statistical methods. The length of this chapter stems from the broad range of research and the application of numerous analytical methods, as well as the advanced processing of data using various statistical methods, including one- and two-way analysis of variance (ANOVA), Tukey's post-hoc test, and principal component analysis (PCA). To apply these statistical methods, the PhD student had to master the use of several computer programmes. In addition to MS Excel, these included Design-Expert (Stat-Ease) and OriginPro Learning Edition (OriginLab). For the analysis of genes and proteins, it was essential to be proficient in the use of bioinformatics tools, including, above all, KEGG (Kyoto Encyclopedia of Genes and Genomes) and GO (Gene Ontology). The use of such a wide variety of research, analytical and statistical methods, ranging from relatively simple decolourisation tests to advanced bioinformatics tools, required knowledge and very good preparation, including in the fields of microbiology, chemistry, mathematics, engineering, and statistics, as well as a solid scientific foundation from the outset of the research, which the PhD student refined throughout the course of her work.

The research findings, their analysis and discussion are presented together in the fifth and most extensive chapter of the doctoral dissertation under review. In my opinion, combining the presentation of the results with their discussion is entirely justified, and relating the findings to existing, published data makes the work easier for the reader to follow and demonstrates what the PhD candidate has achieved against the background of current knowledge. The results are presented in tables and figures, arranged logically and discussed in detail. The author begins this chapter with an assessment of the growth of two selected fungal strains and the feasibility of using two different carriers (polypropylene and polyurethane) for mycelium immobilisation, as well as a presentation of the results of preliminary/screening decolourisation tests conducted for five dyes. Subsequently, the influence of several factors, such as agitation, carbon source, nitrogen source in the medium, and immobilisation, on the efficiency of dye removal are discussed. These studies, which can be described as optimising fungal culture conditions, were conducted for three dyes, namely Brilliant Blue Remazol R (RBBR), Evans Blue (EB) and Crystal Violet (CV), which were selected on the basis of preliminary decolourisation tests. The next subsection is devoted to the enzymatic activity of laccase, manganese peroxidase and lignin peroxidase, as determined for two fungal strains exposed to one of the three synthetic dyes mentioned above. The PhD student then devoted considerable space to the results of transcriptomic and proteomic analyses. These analyses were carried out on a single fungal strain selected on the basis of previous studies, namely *Trametes versicolor* exposed to one of two dyes (brilliant blue Remazol R (RBBR) or Evans' blue (EB)) in the case of transcriptomic analysis, or one of three dyes: brilliant blue Remazol R (RBBR), Evans blue (EB) or crystal violet (CV) in the case of proteomic analysis. The PhD student also conducted ecotoxicity tests using *Daphnia magna* and *Spirodela polyrhiza* to assess the toxicity of the three dyes (RBBR, EB and CV) and the products formed following their degradation and removal by white rot fungi. The PhD student generally observed a reduction in toxicity following mycoremediation processes involving both fungal strains studied. The last three sub-sections in the chapter 'Results and Discussion' are devoted to the influence of lighting conditions on dye degradation, dye biosorption and the use of bioreactors for wastewater decolourisation. This third sub-section is very brief and leaves the reader feeling somewhat unsatisfied.

In the conclusions (Chapter 6), the author first addressed the research hypothesis. She confirmed that it had been successfully verified thanks to meticulously conducted research and an analysis of the results. The doctoral student then outlined the key achievements of her dissertation in a series of key points. In my view, it was done in a clear and convincing manner, demonstrating the originality of the research conducted and the author's own contribution to the development of environmental engineering. Importantly, the PhD candidate did not stop at the conclusions, but also identified further directions for research into the decolourisation of wastewater using white rot fungi. In my opinion, these have been accurately identified. It would certainly be worthwhile to expand the bioreactor studies and conduct them on a larger scale, using not only culture medium but also actual wastewater containing synthetic dyes as the substrate.

I have three comments, or rather substantive questions, regarding the reviewed work, which the PhD candidate should address during the defence of her doctoral dissertation.

The first concerns the studies conducted in bioreactors: What were the total and working volumes of the bioreactors? How were they equipped? For example, I would ask for details on the method of mixing, pH control and dissolved oxygen concentration.

The second question concerns the omics studies. The exposure time of *Trametes versicolor* mycelium to the dye was 24 hours for the transcriptomic studies and 48 hours for the proteomic analysis. Why were these times chosen, and what is the reason for the difference between them? Why was the dye concentration 250 mg/l in these studies?

Another point I would like to ask about concerns the interpretation of the results of enzyme activity assays, specifically for laccase and peroxidase. What are the reasons why the activity of enzymes, such as laccase, was in some series with dyes (e.g. EB or CV on a polyurethaneol carrier) at a level similar to or lower than in the control series?

Despite the comments and questions raised above, I consider the reviewed work as a whole to be a doctoral dissertation of exceptionally high quality. As I have already mentioned, the dissertation stands out in terms of the scope of the research (two fungal strains, five synthetic dyes in the initial stages of the research, then three of them), the variety of techniques employed (including decolourisation tests, sorption tests, ecotoxicity tests, and omics techniques) and the thoroughness of the description of the results obtained.

Among the PhD candidate's most significant achievements, which expand knowledge in the field of environmental engineering and biotechnology, I would include the demonstration that two strains of white rot fungi, *Trametes versicolor* and *Pleurotus ostreatus*, can be utilised in the biodegradation of certain synthetic dyes, and that the resulting products are no more toxic to the biotic components of aquatic ecosystems than a pure dye solution. Furthermore, the PhD student demonstrated that optimising the biological removal of these dyes using fungi requires the selection of appropriate experimental conditions, in particular the mixing intensity and nitrogen source, which were found to have the greatest impact on the effectiveness of the decolourisation process. In my view, a significant achievement of this dissertation is the identification, using omics techniques, of relationships between the biochemical and degradative activity of the mycelium and the expression of genes associated with oxidative metabolism or intracellular detoxification. The PhD student demonstrated that in the case of *Tremetes versicolor* treated with the dyes Brilliant Blue Remazol R or Evans Blue, the genes showing increased expression compared to the control included cytochrome P450 monooxygenases, NAD(P)H-dependent oxidoreductases, ketoreductases and aldehyde dehydrogenases, as well as genes associated with transport.

In conclusion, I find that the doctoral dissertation by Ms Ruchi Upadhyay, MSc, meets the requirements defined in the relevant provisions of the Act of 20 July 2018 - Law on Higher Education and Science (Journal of Laws of 2024, item 1571, as amended). I therefore request the Discipline Council for Environmental Engineering, Mining and Power Engineering at the Silesian University of Technology in Gliwice to admit Ms Ruchi Upadhyay, MSc, to the subsequent stages of the doctoral procedure as provided for by the regulations. At the same time, I submit a request for this doctoral dissertation to be awarded a distinction. I justify this request primarily on the basis of the very high quality of the research conducted, its interdisciplinary nature, and the variety of modern analytical techniques employed, which resulted in the acquisition of comprehensive data on the mycoremediation of certain synthetic dyes.

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