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Review of the doctoral dissertation by Mr Kamran Ayaz, MSc, entitled "Application of pure and mixed cultures of bacterial strains in synthetic dyes removal processes – mechanisms and optimisation of process conditions"

This review is based on the Resolution of the Discipline Council for Environmental Engineering, Mining and Power Engineering at the Silesian University of Technology dated 19 March 2026 and letter number RIE-BD.512.17.2026 dated 1 April 2026 from the Chair of the Discipline Council for Environmental Engineering, Mining and Power Engineering at the Silesian University of Technology, Prof. Krzysztof Labus, PhD, Eng.

Assessment of the research topic

The reviewed doctoral dissertation addresses the issue of the possibility of biological removal of synthetic dyes from industrial wastewater, with particular emphasis on the poorly degradable, sulphonated di-azo dye [called] Evans Blue (Direct Blue 53). The azo dyes selected by the PhD candidate for his research constitute the largest group in terms of both diversity and the quantities used in the textile industry. They very often pose a significant challenge in terms of their removal from wastewater during the treatment process. The breakdown of the azo bond causes colour loss (decolourisation), which does not guarantee complete decomposition of the molecule and may lead to the formation of aromatic, toxic by-products that may persist in the liquid or require additional processes, such as aerobic oxidation, to reduce their toxicity. Therefore, treatment must go beyond the mere effect of decolourisation to ensure a genuine chemical transformation, control of the resulting by-products, and has to operate across various microbial systems and reactors to achieve the complete removal of the compound and its derivatives.

This study fits aligns with the current trend of research into bioelectrochemical methods for the treatment of textile wastewater and constitutes a significant contribution to the disciplines of environmental engineering, mining and energy. In view of the above, I consider the research issue undertaken by the PhD candidate to be topical and of interest from both a cognitive and practical perspective.

Characteristics and structure of the doctoral dissertation

The doctoral dissertation submitted for assessment by Mr Kamran Ayaz, MSc, entitled "Application of pure and mixed cultures of bacterial strains in synthetic dyes removal processes – mechanisms and optimisation of process conditions" consists of 198 pages, is divided into six main parts, including chapters and subchapters, and is supplemented by a list of abbreviations and symbols used, as well as a bibliography of over 300 entries.

Following the abstract, there is a theoretical section in which the PhD candidate presents issues that concern the characteristics of the dyes used, methods and possibilities for their removal, their biodegradation processes, mechanisms of wastewater decolourisation, as well as the characteristics of bioelectrochemical systems and microbial fuel cells. The scope of the literature review presented in the doctoral dissertation is relevant to the research topics addressed; it is comprehensive, up-to-date and supported by numerous citations.

In the subsequent part of the dissertation, Mr Kamran Ayaz, MSc, presented the aim and scope of the research, which were clearly defined and encompass both cognitive and utilitarian aspects.

The PhD candidate then discussed the research methodology, which was described in detail. It includes both a description of analytical and statistical methods, as well as a description of the experimental test stands.

At the end of the doctoral dissertation, the author included conclusions, which were concisely formulated and accurately summarise the achievements of the work. Furthermore, future research directions were presented.

The structure of the entire work meets the requirements for doctoral dissertations. The arrangement of chapters and numbering are clear, and the technical language is correct and precise. The extensive bibliography demonstrates the author's sound familiarity with the international literature. The literature review is thorough and aptly linked to the research objective.

The scientific value of the work is high. The doctoral dissertation demonstrates that Mr Kamran Ayaz, MSc, possesses a sound theoretical grounding. This reviewed dissertation constitutes a valuable and coherent scientific contribution to the field of bioelectrochemical treatment of dye-containing wastewater. The author has demonstrated the ability to conduct high-quality experimental research, analyse it critically, and consistently develop his research concept.

The dissertation stands out above all for its rigorous approach to verification of the results – the author does not rely solely on measurements such as spectrophotometric analyses, but consistently employs chemical verification of biotransformation products. Another aspect worthy of recognition is the logical progression throughout the research, where each subsequent stage addresses the questions raised by the limitations of the previous stage. The dissertation combines microbiological, electrochemical and engineering aspects into a coherent whole, which is relatively rare in studies of this type. The research focuses on the transition from simple systems with immobilised actinomycete biomass to advanced microbial fuel cells.

Main achievement resulting from the doctoral dissertation
In my opinion, the PhD candidate's development of a comprehensive, multi-stage research strategy is worthy of note. The PhD candidate gradually deepened his knowledge and expanded the scope of his work, validating the results obtained (from pure cultures, through mixed microbial consortia, to optimised bioelectrochemical reactors). Each stage logically followed from the limitations of the previous one, which demonstrates methodological maturity. One of the key achievements of the work is the demonstration that UV-Vis spectrophotometry alone is insufficient for a reliable assessment of dye biodegradation. Apparent decolourisation may result from adsorption, biosorption or partial decomposition of the chromophore, rather than actual biotransformation. Hence, the PhD student verified the chemical transformation using HPLC chromatography to track the disappearance of the parent dye form, and employed GC-MS to identify the products of its transformation. The introduction of such a multi-analytical approach (UV-Vis + HPLC + GC-MS) constitutes a methodological innovation of great practical value.

It is particularly worth emphasising that Chapter 3 is not merely a presentation of results, but clearly outlines the decision-making/analytical process and the evolution of the research approach. The author does not shy away from presenting the limitations of the results obtained, which enhances the credibility of the work.

The PhD student also demonstrated the ability of isolated *Actinomycetes* strains to remove dyes. In this part of the research, the PhD student screened *Actinomycetes* on solid media for the decolourisation of dyes, including di-azo Evans Blue (Direct Blue 53), triphenylmethane brilliant green and crystal violet. During the study, the PhD candidate conducted the immobilisation of biomass on, amongst others, coniferous wood chips, straw and deciduous wood chips. Only on straw did he observe visible growth of microorganisms in preliminary tests, which is why he selected it for the main experiment. Strain 1K1 achieved 80% removal of Evans Blue dye, while strain EGK2 achieved 73%, with an incubation time of 14 days. The PhD student concluded that although these microorganisms demonstrate the ability to remove dyes, this process is highly dependent on the strain, incubation time and the involvement of physical phenomena such as sorption. He also concluded that there are fundamental limitations/difficulties in distinguishing between biodegradation and physical adsorption on solid carriers, which justified the transition to a more controlled model system. It is particularly commendable that the PhD student consciously chose not to pursue this line of research further, justifying the decision on the grounds of ambiguous results and limited practical applicability. Such critical self-reflection is rare and should be viewed very positively.

The application of the statistical Response Surface Methodology (RSM) enabled the PhD candidate to identify a statistically significant operating window for the biotransformation process of Evans Blue dye (rather than a single process parameter). The PhD candidate demonstrated that temperature and time are the dominant factors, while an excess of carbon source may act as an inhibitor. Mr Kamran Ayaz, MSc, achieved a decolourisation rate of over 99% within 3 days (having shortened the process from 14 days). The RSM statistical model showed a good fit (as indicated by the statistical measure determining how well the regression model fits the empirical data – R^2= 0.79; adjusted R^2= 0.74), and determined the optimal conditions as: time 53.65 h, dye concentration 100 mg/l, carbon concentration 250 mg/l, temperature 37°C. A preliminary biotransformation pathway for Evans Blue dye was also formulated. Furthermore, the PhD student demonstrated that a pure culture *of Shewanella oneidensis* MR-1 is an effective biocatalyst for the removal of Evans Blue dye and that a pure culture of this bacterium is capable of completely mineralising the dye under fully optimised process and growth conditions. At the same time, the author maintained a critical perspective, identifying the limitations of the pure culture approach – primarily the relatively long duration of the process at higher dye concentrations.

Another valuable aspect of the work is the transition to bioelectrochemical systems – a microbial fuel cell (MFC) with mixed microbial cultures – and the integration of anaerobic and aerobic stages. At this stage, the work clearly goes beyond classical microbiological research and falls within the scope of modern environmental engineering. The author demonstrated that the use of a DCMFC system in combination with an aerobic stage allows the process time to be reduced to approximately 20–24 hours at dye concentrations of 100–200 mg/l. This is a very good result and significant from the point of view of potential applications. The combination of the anaerobic stage in the MFC with the aerobic process using *Actinomycetes* (SK-2, SK-3) enabled complete mineralisation – the formation of 2-Methylbutanoic acid and 3-Methylbutanoic acid as products of further biotransformation was confirmed. This is significant evidence that the process went beyond mere decolourisation.

In addition to confirming the transformation of intermediate compounds, the PhD student proposed a degradation pathway, which demonstrates his high level of mechanistic analysis. The author also assessed the composition of the microbial community with the use of 16S rRNA gene sequencing. This revealed the presence *of Pseudomonadota* (45.5% in cultures adapted to the dye and 32% in inoculum cultures), with key genera including *Actinomarinicola* (13.75%), *Thermochromatium* (4.82%) and *Geobacter* (4.52%). These studies highlight the potential of an integrated DCMFC-aerobic system, utilising mixed strains of actinobacteria in the aerobic stage, which could in future be applied to the effective treatment of industrial dye wastewater, while also offering environmental and bioenergy benefits.

The results concerning anode modification, which lead to a further reduction in process time, are also of particular interest. The author addressed the significant issue of kinetic and transport limitations in bioelectrochemical systems, focusing on accelerating biofilm formation and improving the efficiency of extracellular electron transfer (EET). To this end, he proposed a multilayer modification of the carbon anode electrode using polyaniline (PANI; conductive layer), glucose and gelatine, which constitutes an interesting and well-founded material approach and the technological culmination of the work. During the research, he also utilised electrical impedance spectroscopy (EIS) to assess the resistance of the anode and the DCMFC.

The PhD student investigated the effect of these modifications on biofilm formation, charge transfer and the viability of microorganisms with the use of a pure culture *of Shewanella oneidensis* MR-1. Very good results were obtained: 96–97% decolourisation at 150 mg/l Evans Blue dye in just 13 hours, 90–91% at a concentration of 300 mg/l, and 73% at 450 mg/l – in a simplified, single-chamber MFC reactor. These results demonstrate a reduction of the process time from 3 days (pure culture, 100 mg/l) to 13 hours at significantly higher dye concentrations. This clearly indicates that the kinetic limitations of the process are strongly linked to the biofilm-electrode interface and can be effectively reduced through materials engineering, meaning that modification of the anode can lead to a significant improvement in the bioelectrochemical properties of the system. These results clearly indicate that the modified anode surface improved the adhesion of the pure bacterial culture and allowed the toxic effect of the high initial dye concentration to be eliminated, thanks to the well-developed biofilm on the modified electrode. The increase in biofilm coverage and its longevity was also particularly pronounced, which the author attributed to the presence of a local carbon source and the improvement in the surface properties of the electrode. Also worthy of note is the detailed material characterisation (FTIR, XPS, SEM), which confirms the effectiveness of the stepwise functionalisation of the electrode surface. The high consistency of the results obtained by these methods attests to a well-designed research methodology and a coherent interpretation of the data. The solution remains simple, utilises standard carbon fabric and is scalable for faster treatment. This stage of the work should be regarded as one of the PhD candidate's most significant scientific contributions.

An equally important achievement of the author demonstrates that the use of a multilayer anode leads to a significant reduction in charge transfer resistance and an increase in charge capacity, which directly translates into improved cell performance parameters. The PhD student accurately identifies the complementary roles of the individual layers: polyaniline is responsible for improving conductivity and redox properties; whereas the glucose and gelatine layers regulate bacterial adhesion and local substrate availability. This approach demonstrates a good understanding of the complex interactions occurring at the biofilm-electrode interface.

The author demonstrates that optimisation of process conditions and modifications to the anode surface allow for a significant acceleration of decolourisation and the complete mineralisation of toxic compounds. A key element of the work is the verification of the actual chemical degradation of dyes using analytical techniques such as HPLC and GC-MS. The results indicate that the use of pure cultures, such as *Shewanelta oneidensis* MR-1, in optimised reactors effectively eliminates the problem of industrial wastewater toxicity. The work as a whole constitutes a comprehensive study aimed at developing efficient and controllable wastewater treatment technologies.

In some sections of the work, the interpretation of the results precedes their full experimental confirmation. This observation applies in particular to the determination of the role of electron transfer (EET), which is postulated as a significant mechanism but has not been directly verified experimentally. Although the author does note this fact, it undoubtedly points to the need for further supplementary research. In the case of GC-MS analyses, the author rightly notes that this method allows only for the detection of volatile compounds or those analysable without derivatisation, which means that a significant proportion of potential products, particularly polar aromatic amines, may not have been detected. Consequently, it cannot be unequivocally stated whether the process leads to the complete mineralisation of the contaminant or merely to its partial transformation. The author points out this limitation, yet the research section lacks any attempts to experimentally demonstrate it, e.g. through the use of LC-MS/MS techniques. Naturally, this research may be further explored in the PhD candidate's further academic career.

In the future, efforts should also be intensified to extend the duration of the experiments and to analyse the stability of the system, which will enable an assessment of the long-term durability of the coating and the biofilm's resistance to operating conditions. At this point, a question also arises regarding the mechanical stability of the gelatine layer and its behaviour under flow conditions, which is of key importance from the perspective of potential industrial applications.

From the perspective of interpreting the results, a research gap is revealed in the lack of quantitative analysis of glucose release from the surface layer. The mechanism of controlled substrate delivery is one of the central elements of the research concept; however, its confirmation is based mainly on indirect evidence (FTIR, biofilm observations), without direct measurements of release kinetics.

It is also worth noting that the study was conducted under model conditions, using a single dye and a pure culture of microorganisms. This limits the possibility of directly extrapolating the results to real industrial wastewater, which is characterised by significantly greater compositional complexity and variability in conditions. Though the author suggests directions for further research, there is a lack of experimental verification of the proposed solutions in more complex systems.

Another limitation, which has, incidentally, been accurately identified by the author, is the decline in process efficiency at high dye concentrations and issues related to biofilm stability and mass transfer in MFC systems. This points to real implementation barriers that require further research. Despite certain interpretative and methodological limitations, the dissertation meets the requirements for a doctoral dissertation and contains elements constituting an original contribution to the development of the discipline of environmental engineering, mining and power engineering. The dissertation constitutes a valuable and well-developed contribution to the development of bioelectrochemical wastewater treatment methods; it stands out for the coherence of its concepts, methodological soundness, and skilful integration of issues from the fields of chemistry, electrochemistry, materials engineering, microbiology and environmental engineering. Despite the noted "shortcomings", the results obtained are of significant cognitive importance and point to a promising direction for further research, particularly in the field of bioanode surface engineering.

Issues requiring clarification (points for discussion)

1. How did you clearly distinguish between biodegradation, biosorption and chemical reduction occurring in the system?
2. You analysed dye concentrations such as 100 mg/l and 200 mg/l. Why these concentrations, and at what level does this dye occur in actual textile wastewater from the dyeing process?
3. To what extent is the proposed degradation pathway complete, and to what extent does it constitute a hypothesis based on GC-MS data?
4. Would it be easy and feasible to launch a pilot installation on a larger scale than a laboratory-scale one within a short timeframe?
5. From an economic perspective, what would be the costs of implementing the solution on an industrial scale? Compared to competing technologies, would this be cost-effective and justified?
6. Lack of a full toxicity assessment of the products – the products were identified (GC-MS), but no ecotoxicity tests were conducted.
7. What is the direct evidence for the involvement of the extracellular electron transfer (EET) mechanism in the process?
8. Does the increase in efficiency following anode modification result from improved EET, or rather from better biofilm adhesion?
9. Were the following analysed: the costs of electrode modification, the durability of the materials, and the long-term stability of the biofilm?

10. What other electrode modifications could further increase CSC efficiency?
11. Why was *Shewanella oneidensis* MR-1 specifically chosen as the model organism?
12. How do the results for pure culture relate to real-world environmental systems, such as activated sludge?
13. In the *Shewanella oneidensis* MR-1 system, no classic mutagenic aromatic amines, such as benzidine or aniline, were detected, suggesting a more environmentally safe degradation process. Why were they absent?

Conclusions

This reviewed dissertation constitutes a valuable and coherent scientific contribution to the disciplines of environmental engineering, mining and power engineering, particularly in the field of bioelectrochemical treatment of dye-containing wastewater. The author has demonstrated the ability to conduct high-level experimental research, analyse it critically, and consistently develop a research concept. The integration of various approaches – from microbiology, through statistical analysis, to bioelectrochemistry and materials engineering – deserves particular praise. The PhD candidate's main achievement is the development of an integrated, scalable approach combining the optimisation of process conditions with electrode-biofilm interface engineering, which has enabled the biodegradation time of Evans Blue dye to be reduced several-fold, while maintaining high process efficiency and its chemical verification. The proposed directions for further research are realistic and well-justified by the results obtained.

The doctoral dissertation submitted for assessment by Mr Kamran Ayaz, MSc, entitled "Application of pure and mixed cultures of bacterial strains in synthetic dyes removal processes – mechanisms and optimisation of process conditions" meets the requirements defined in Article 187 of the Higher Education Act of 20 July 2018 (Journal of Laws 2024, item 1571, as amended).

Mr Kamran Ayaz's dissertation constitutes an original and valuable scientific study, making a significant contribution to research into the effectiveness of textile wastewater treatment. The dissertation meets all the criteria for a doctoral dissertation in the field of engineering and technical sciences, and the PhD candidate has demonstrated independent research, proficiency in experimental and statistical methods, the ability to formulate and verify hypotheses, and the capacity to apply research findings in practice. The dissertation submitted for assessment has cognitive, applied and constructive value.

The PhD candidate possesses the appropriate knowledge and skills to conduct scientific research in the scientific disciplines of environmental engineering, mining and power engineering. The comments presented in the review are of a discursive nature and do not adversely affect the substantive value of the doctoral dissertation submitted for assessment. The dissertation demonstrates a high standard of experimental research, which is well-documented and scientifically sound. It constitutes a valuable contribution to the development of bioelectrochemical wastewater treatment methods.

In light of the above, I move that Mr Kamran Ayaz's doctoral dissertation, entitled "Application of pure and mixed cultures of bacterial strains in synthetic dyes removal processes – mechanisms and optimisation of process conditions" be accepted and admitted for a public defence.

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