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**REVIEW**

**of DOCTORAL THESIS**

**by Bhautik Dave, MSc**

**entitled "Degradation of natural and anthropogenic pollutant in surface water in aspect of self-cleaning phenomenon"**

The reviewed doctoral dissertation was completed at the Faculty of Energy and Environmental Engineering at the Silesian University of Technology, within the Department of Water and Wastewater Engineering. The dissertation supervisor is Dr Ewa Łobos-Moysa, Professor at the Silesian University of Technology.

## FORMAL BASIS OF THE REVIEW

This review was prepared on the basis of:

- a resolution of the Discipline Council for Environmental Engineering, Mining and Power Engineering at the Silesian University of Technology dated 19 March 2026, and a letter from the Chairperson of the Discipline Council, Prof. Krzysztof Labus, PhD, dated 1 April 2026, and agreement UMC/1297/2026 dated 23 March 2026
- the attached copy of the dissertation in the form of a bound printout
- the Act "Law on Higher Education and Science" of 20 July 2018, as subsequently amended.

## CHARACTERISTICS OF THE STRUCTURE AND CONTENT OF THE THESIS

The reviewed dissertation entitled "Degradation of natural and anthropogenic pollutant in surface water in aspect of self-cleaning phenomenon" addresses the issue of removing selected natural and anthropogenic micropollutants from surface waters using specific species of fungi and bacteria. The research is situated within the context of micro-pollutant degradation processes occurring in natural aquatic ecosystems and in hydrophytic systems for wastewater treatment or final purification, which makes the topic interesting from a scientific point of view and important in terms of practical applications. Although the research described in the dissertation was conducted solely on synthetic wastewater under laboratory conditions, the results have the potential to be scaled up to an engineering level.

The work submitted for review comprises 232 typewritten pages, including the first 30 pages, which are numbered separately. The work contains 59 figures and 25 tables.

The dissertation is written in English. Formally, it is divided into eight numbered chapters, preceded by an abstract in English and Polish, a list of the author's publications, and an explanation of the abbreviations and symbols used by the PhD candidate. At the end of the work, there is a list of references and a list of all tables and figures.

The dissertation as a whole follows the typical structure of a scientific monograph, comprising, in sequence, a literature review—including the identified research gap, which serves as the starting point for the research topic—followed by the aim, scope and thesis of the work. The subsequent sections of the dissertation comprise a description of the research materials and methods, a presentation of the results and their discussion, finalised with the conclusions. This structure organises and accurately reflects the PhD candidate's intention: to document the current state of knowledge and the methodology employed to achieve the set objectives and prove the thesis.

In the introduction, "1. Introduction", the PhD candidate briefly, in just under two pages, introduces us to the subject matter of the work, highlighting the exceptional value of water to humans and the problem of

micropollutants being introduced into natural waters. He also addresses the phenomenon of self-purification, as well as the role which extensive systems, such as hydrobotanical treatment plants, can play.

Chapter two, “2. Literature Review”, provides an overview of the global literature on the subject of the dissertation. It is divided thematically into seven sub-chapters, which logically organise the current state of knowledge and form the theoretical foundation for the experimental part of the work. Section 2.1 provides a very concise description of the occurrence of micropollutants in surface waters and their circulation in the environment. The information provided partly overlaps with the content of Chapter 1. Section 2.2 is devoted to the characterisation of four selected pollutants: two natural ones – caffeine and nicotine – and two anthropogenic ones – methylparaben (MeP) and triclocarban (TCC), which are potent antibacterial and antifungal agents used in cosmetics, food and pharmaceuticals. At the end of this section, the author justifies the selection of these four substances for study as representatives of a broad group of micropollutants entering surface waters. The next, very brief sub-section is devoted to factors limiting the effectiveness of the self-purification process, where dilution and the widespread dispersion of pollutants at very low concentrations are identified as the main factors. In section 2.4, the PhD student outlined the mechanism by which micropollutants are degraded with the involvement of bacteria and fungi, as well as their mutual interactions. A very interesting addition to the descriptions is the graphical representation of this issue in Figure 2. In the remainder of the chapter, the author focused on hydrophytic treatment plants and the role played by the introduction of bacterial and fungal consortia adapted to remove selected micropollutants (section 2.5), as well as on the challenges arising during the transition from the laboratory scale to full-scale technical application (2.6). Section 2.7 summarises the literature review, in which the PhD candidate highlights a gap in the current state of knowledge (which he had already partially addressed at the end of section 2.6) regarding the biodegradation of micropollutants—represented by substances such as caffeine, nicotine, methylparaben and triclocarban, as well as in experiments concerning the transfer of results from the laboratory to full-scale systems. Furthermore, the author justifies the importance of the topic presented.

Chapter three, “3. Research Aim, Objectives, and Scope of the Study”, consists of five sub-sections. Section 3.1 presents the motivation and scientific rationale for the research undertaken. In this section, the PhD candidate emphasises that conventional wastewater treatment plants are not designed to remove micropollutants, and that while natural self-purification processes in aquatic ecosystems contribute to reducing the concentrations of substances polluting the environment, they are not easily amenable to deliberate intervention. In section 3.2, the author formulates the aim of the dissertation as: the development and experimental validation of a self-purification strategy utilising microorganisms, designed to intensify the biodegradation of anthropogenic micropollutants. To achieve this objective, the PhD student formulated six specific tasks (section 3.3): (1) selection and evaluation of bacteria and fungi capable of degrading micropollutants; (2) investigating the adaptation of selected microorganisms during the transition from a nutrient-rich environment to growth on synthetic wastewater containing micropollutants; (3) assessment of the kinetics and efficiency of the biodegradation and removal of selected micropollutants using advanced analytical techniques; (4) the design and operation of model hydrophytic treatment plants; (5) the assessment of synergies between fungi and bacteria, and the system’s efficiency under conditions where a mixture of various micropollutants is introduced; (6) the assessment of the transferability of results from static laboratory experiments to model hydrophytic treatment plants. Section 3.4 formulates a research hypothesis positing that the natural self-purification capacity of contaminated surface waters is generally insufficient for the effective removal of micropollutants under real-world environmental conditions, particularly when biogenic substances are scarce. This hypothesis is further elaborated by the assertion that the appropriate adaptation of relevant bacteria and fungi in hydrophyte systems enables an improvement in the efficiency of the biodegradation of these micropollutants in the aquatic environment. In the final section (3.5) the PhD candidate clearly defines the limitations of his experiments: these are restricted to four types of micropollutants, conducted on a laboratory scale using synthetic wastewater of a strictly defined composition, and deliberately omitting the identification of the cultured microorganisms—including molecular biology techniques—in favour of focusing on the selected micropollutants and their biodegradation.

Chapter four, “4. Research Methodology”, provides a detailed description of the PhD candidate’s research methodology. It is divided into three parts. Section 4.1 describes the methods for extracting nicotine and

caffeine, including methods for analysing the extracted substances, and the procedure for preparing anthropogenic substances (methylparaben and triclocarban) for the experimental part. Section 4.2 contains descriptions of the methods used to conduct laboratory experiments on the adaptation of fungi and bacteria to various conditions, the assessment of their growth and the efficiency of biodegradation of the analysed micropollutants, inoculation, and the preparation of samples for further analysis. Section 4.3 is devoted to model hydrophytic systems, which were used in the subsequent stage of the research. The author described the test facility, characterised the methods for assessing the adaptation and growth of microorganisms, the inoculation of the systems with fungi and bacteria, the method of monitoring the process in the model systems, and the collection and preparation of samples for analysis.

Chapter five, "5. Analytical Methods and Data Validation", which continues the description of the PhD student's research methodology, presents a selection of advanced analytical techniques required for this PhD dissertation (section 5.1) and a description of the individual research methods employed: Fourier-transform infrared spectroscopy (FTIR) (section 5.2), nuclear magnetic resonance (NMR) (section 5.3), thin-layer chromatography (HPTLC) (section 5.4) and scanning electron microscopy (SEM) (section 5.5).

Chapter six, "6. Results and Discussion", which contains a summary of the results and a detailed discussion thereof, is the most extensive section of the entire dissertation and has been divided into three main parts. In the first (6.1), the author presents the results of the laboratory phase of the study on the adaptation of bacteria and fungi, and the biodegradation kinetics of all four selected micropollutants, including an interpretation of these results.. The second section (6.2) describes the results and interpretation of the pilot studies in model hydrophytic systems. The third section (6.3) provides a comprehensive review of the entire study, including issues relating to upscaling and possible future changes to the design and role of hydrophytic systems.

Chapter seven, "7. General Conclusions and Future Perspectives", consists of three sections, which present, respectively: 7.1 conclusions from the preliminary laboratory experiments (7.1.1) and model experiments (7.1.2); 7.2 general conclusions from the entire study; and 7.3 the scientific contribution and significance of the research conducted.

In Chapter eight, "8. Conclusions", the PhD candidate has compiled what he considers to be the key specific conclusions from the entire thesis.

The list of cited literature ("References") demonstrates that the PhD candidate has conducted a thorough and insightful study of the available bibliographic sources. Formally, the list comprises 217 entries; however, the reviewer noted that some entries appear repeatedly under different numbers (22 and 34; 64 and 72; 85 and 86). All cited items are in English, with the exception of no. 123, which is likely a translation of the original English text (IWAPublishing, 2017, open access) into a language unknown to the reviewer. The vast majority of the sources used are articles and monographs from the last two decades, including the most recent works, dating from 2023–26.

In terms of editing, the PhD candidate has not managed to avoid certain errors and shortcomings. However, these are minor issues which do not affect the substantive assessment of the work, nor do they detract from the overall positive evaluation of the thesis.

In summarising my assessment of the thesis's structure and the content presented, I conclude that Mr Bhautika Dave's doctoral dissertation is written in a well-considered and logical manner, in accordance with the general principles of academic monograph writing. The structure of the dissertation effectively reflects both the author's vision and the consistent execution of the individual sub-tasks, as well as the manner in which the results obtained are interpreted to support the thesis.

**SUBSTANTIVE EVALUATION OF THE THESIS**

Bhautika Dave's doctoral dissertation is the result of in-depth literature reviews and carefully planned and executed experimental research aimed at achieving the set objective and verifying the thesis. In the substantive assessment of the thesis, key factors include the appeal and significance of the research problem addressed, the approach taken to resolve it, and the quality of the results obtained. The research topic addressed is one of many possible responses to increasingly stringent environmental protection regulations

and the ever-growing understanding of the harmful effects of many substances entering aquatic ecosystems, despite their trace quantities and concentrations measured in micro- and nanograms. There is currently a pressing need to develop technologies for the effective further treatment of wastewater as part of the so-called fourth stage of treatment and to support the natural self-purification processes occurring in surface waters, in particular extensive, low-cost methods. In this context, I consider the PhD candidate's choice of topic to be apt, highly topical and firmly rooted in the field of environmental engineering.

The theoretical and literature review section has been well structured by the author, making the dissertation a highly engaging read. The PhD candidate introduces us to the subject by outlining the general nature of the problem he intends to address. A minor drawback is that some of the information at the beginning of the chapter overlaps with the content of the introduction. In the first part of the review, the detailed characterisation of the four micropollutants selected for the study is particularly noteworthy. The PhD candidate has identified numerous publications describing both the characteristics of these substances and their recorded concentrations in aquatic environments and sewage systems, as well as their susceptibility to biodegradation. The section devoted to the mechanisms of pollutant degradation by fungi and bacteria leaves something to be desired. These issues are described very briefly, yet they concern a fundamental part of the thesis's experimental work. Furthermore, in the reviewer's opinion, the section describing hydrophytic systems could have been expanded further. Regardless of the critical comments presented above, however, I consider that both the content and the order of the individual points in the literature review, the selection of bibliographic sources, and the form and substantive scope of the presentation allow for a high assessment of both the literature review as a whole and – indirectly – the PhD candidate's knowledge and command of the thesis's subject matter.

The justification for the choice of the topic, which follows the literature review, is logical; it stems from the earlier presentation of the problem and reinforces the reader's positive assessment of the PhD candidate's work. Based on the preceding considerations, the author has set as his main objective the development and experimental validation of a model hydrophytic system utilising bacterial and fungal microorganisms to intensify the biodegradation of anthropogenic micropollutants. I also view the division of the research section into six specific tasks favourably. These logically reflect the successive stages of the experimental work. The thesis of the dissertation put forward for verification is correctly formulated, and the previously established research stages enable this verification to be carried out. This planning of the research as a whole demonstrates the PhD candidate's scientific maturity and independence. Having analysed the overall objective, the sub-tasks, the thesis put forward and the scope of the research, I conclude that Mr Bhautik Dave, MSc, has set himself an interesting scientific task with potential for future practical application, which he has fully accomplished.

The determination of various micropollutants remains a major challenge, despite enormous advances in analytical techniques, due to the very low concentrations of these substances and their complexity. This requires advanced research equipment and validated methods for determining individual compounds or groups of pollutants that yield reliable and reproducible results. In his work, the PhD candidate demonstrated the use of a wide range of advanced analytical techniques, including infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR), high-performance thin-layer chromatography (HPTLC) and scanning electron microscopy (SEM), which constitutes a major scientific strength of the thesis. The types of equipment selected by the author were well suited to the micro-contaminants under investigation, both of natural origin (caffeine and nicotine) and anthropogenic origin (methylparaben and triclocarban). I view favourably the choice of *Trametes versicolor* as the fungal species for the biodegradation of caffeine and nicotine, and the choice of *Rhodococcus* sp. and *Alcaligenes* sp. for the biodegradation of methylparaben and triclocarban. The PhD student drew on the work of other researchers, which he documented with appropriate citations. The appropriate selection of microorganisms was of significant importance for the results subsequently obtained. In summary, having analysed the research methods employed – including the selected instrumental analysis techniques, the methods for extracting naturally occurring micropollutants, and the design of the experiments – I highly commend Mr Bhautika Dave's scientific approach and the research methods he adopted.

In the section devoted to the presentation and interpretation of the results, the PhD student presents each stage of the research in detail and in chronological order. The results obtained are described in a logical and

clear manner and supplemented with clear tabular summaries and graphical material. Laboratory studies conducted in conical flasks confirmed the very high efficiency of caffeine degradation by the fungus *Trametes versicolor,* reaching 97–98% under conditions considered optimal: a temperature of 25°C in a slightly acidic environment, as well as the degradation of methylparaben (89.1%) and triclocarban (100%) by *Rhodococcus* and *Alcaligenes* bacteria under conditions of deficiency in typical nutrients. Pilot studies in constructed wetland fed with synthetic wastewater showed that, during a seven-week experiment, both caffeine – as a representative of naturally occurring micropollutants – and methylparaben – an anthropogenic pollutant – underwent complete degradation while the system maintained stable operation. To summarise his work, the PhD candidate presents four main conclusions in detail: (1) the synergy between microorganisms and their adaptation are crucial for the degradation of pollutants with a complex chemical structure; (2) environmental parameters – the author highlights temperature in particular – have a key influence on the efficiency of the process; (3) the microbiological degradation of micropollutants may be stimulated under the conditions in which real-world systems operate; (4) biomimetic pilot hydrophyte systems can be regarded as scalable wastewater treatment systems and a step towards further scaling up. Apart from the main conclusions listed above, the PhD candidate closes the dissertation with a chapter in which he concludes that it is possible to develop and validate a strategy enabling the intensification of the self-purification process of surface waters, in which caffeine, nicotine, methylparaben and triclocarban will be biodegraded. He also formulates nine specific conclusions which summarise what the author considers to be his most important achievements.

I view the presentation of the research results and the interpretation provided in a positive light, and I consider the most significant achievement of the dissertation to be the demonstration of a substantial increase in the efficiency of removing the studied micropollutants in hydrophytic systems supported by the combined use of appropriately selected bacteria and fungi, compared to conventional systems without such support; this was unequivocally demonstrated by the PhD candidate through the results of the experiments conducted.

The research conducted by Bhautika Dave, MSc, clearly highlights the practical significance of the results obtained, particularly in the context of increasing requirements for the protection of natural waters against micropollutants, including the quality of effluent from urban wastewater treatment plants. On the other hand, a key issue in the context of applying the results in engineering practice is the question of upscaling and the associated challenges, which the PhD candidate himself highlights in Chapters 2.6 and 6.3 of his work. The conducted research represents an important step towards implementation; however, it does not yet provide an answer to the question of how the proposed method for removing selected micropollutants would function once it moves beyond strictly controlled laboratory conditions to systems operating with actual wastewater inflows of variable composition and under dynamically changing environmental conditions.

The questions and critical comments on the dissertation are presented below. Some of these are of a debatable nature, but they do not detract from the overall high assessment of the reviewed dissertation.

- Following the completion of the laboratory experiments in the first stage of the research, the results were used to construct and commission pilot-scale plants. Some doubts are raised by the scale of these plants – 15 L of active volume and only a few small common reed cuttings – as a model for a hydrophyte treatment plant. To what extent do the experiments conducted in the laboratory in small model reactors, fed with synthetic effluent of a strictly defined composition and described as pilot studies, reflect the actual conditions that will prevail in field installations at demonstration scale or at full technical scale? Which similarities, when scaling up, would the PhD student consider to be the most significant?
- In his work, the PhD candidate deliberately chose not to conduct microorganism identification studies, including those using molecular biology techniques, as noted in section 3.5, presumably because he used identified bacterial and fungal cultures obtained from verified sources in his research. In the reviewer's opinion, such studies would certainly have enhanced the scientific value of the results obtained, particularly in the context of potential future publications in prestigious scientific journals. Could the PhD candidate provide a more detailed justification for the reasons behind his decision? Looking back at the research that has now been completed and the interpretation made, would he make the same decision today? In the PhD candidate's view, what

approach should be taken if the research were to be continued on a larger scale, using actual wastewater rather than synthetic wastewater?

- Figure 2 (p. 21) graphically illustrates the pathways of pollutant degradation by fungi and bacteria. Is this the PhD candidate's own work, or did he draw on the work of other authors (no references are provided)?
- On page xix, it is stated that the work consists of four monothematic publications, which are listed. This is a misleading statement, as a concise monograph was submitted for review, and the listed publications were not included with the dissertation submitted for review. All of them relate to the subject matter of the dissertation; however, the PhD candidate cites only three of them in his work. In the reviewer's opinion, this list should be renamed, for example, "Publications related to the subject of the dissertation".
- The Polish summary contains stylistic errors that make it difficult to understand, as well as incorrect technical terminology ("sztuczne mokradła").
- On page xxvii, there is a list entitled "Nomenclature" containing a number of commonly used physical units (such as °C, cm, g, mg, ng, h, min, Hz, MHz, L, etc.) and five symbols ($\delta$, $\lambda_{max}$, J, $R_f$, v/v). I consider that providing explanations of units for such basic quantities as degrees Celsius, centimetres, grams, etc., is unnecessary in a doctoral thesis. However, the explanation of symbols could be included in the "List of Abbreviations" (p. xxix) and its title changed to "List of Abbreviations and Symbols". Furthermore, not everything has been consistently included by the PhD candidate in the list of abbreviations; for example, the abbreviation AU (absorbance units, p. 123).
- In the reviewer's opinion, creating two distinct concluding chapters: "7. General Conclusions and Future Perspectives" and "8. Conclusions" makes it difficult for the reader to assess which conclusions are most important to the author, particularly as Chapter 7 contains Section 7.2, "Key Conclusions", which presents the most important general conclusions arising from the PhD candidate's work. It is also good practice to clearly distinguish between the scientific and practical conclusions of the dissertation.
- A significant editorial oversight is the lack of numbering of equations throughout the dissertation, which makes it difficult to refer to them within the text and in any subsequent discussions.

**In summary**, **the overall substantive assessment of the dissertation submitted for review is positive. Mr Bhautik Dave, MSc, has demonstrated extensive knowledge in the field of environmental engineering, as well as the ability to formulate a research problem and to plan and conduct the research necessary to address it. This demonstrates that the PhD candidate possesses sufficient maturity to conduct independent scientific research**. **The results obtained in the course of the work, and their interpretation, are very interesting, reliable and valuable both from a cognitive perspective and in terms of their potential practical application; they constitute an original solution to the scientific problem formulated by the author. They also contribute to the existing body of knowledge in the areas addressed by the PhD candidate.**

## FINAL CONCLUSION

The doctoral dissertation by Bhautik Dave, MA, entitled "Degradation of natural and anthropogenic pollutant on surface water in aspect of self-cleaning phenomenon" is a research-based work. It contains interesting and original findings which are primarily of a scientific nature, but also have practical application potential. Mr Bhautik Dave, MSc, has demonstrated extensive knowledge of the subject matter covered in the dissertation, as well as the ability to conduct research independently and interpret the results obtained.

In my opinion**, this dissertation meets the requirements for doctoral theses** as specified both in the currently applicable regulations (the Act on Higher Education and Science, Article 187 of the Act of 20 July 2018, as amended) and as is customarily expected of doctoral dissertations.

This authorises me to propose **that the doctoral dissertation of Mr Bhautik Dave be accepted by the Discipline Council for Environmental Engineering**, **Mining and Power Engineering at the Silesian University of Technology and that it be admitted to the subsequent stages of the doctoral procedure**.

[handwritten signature] Zbysław Dymaczewski