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REVIEW

of the doctoral dissertation of Sunday Ocholi Samson

entitled: “Cyanobacterial biomass in biocatalysis and bioaccumulation processes”

prepared at the request of the Council of the Discipline of Chemical Sciences

of Wrocław University of Technology, on the basis of Resolution No. 77/RDND10/2025 of 7 November 2025

The doctoral dissertation of Sunday Ocholi Samson was carried out at the Department of Biochemistry, Molecular Biology, and Biotechnology, in the Biotechnology Laboratory of the Faculty of Chemistry, Wrocław University of Technology, under the supervision of Professor Ewa Żymańczyk-Duda, an expert in the field of biotechnology and biocatalysis, with Assistant Professor Monika Serafin-Lewańczuk serving as the auxiliary supervisor. In general terms, the scope of the submitted work concerns the cultivation and application of cyanobacteria as valuable and efficient organisms, employed both as photobiocatalysts and as tools for improving environmental quality.

In assessing the submitted doctoral dissertation, primary consideration was given to the originality and innovative character of the conducted research, the current relevance of the research topic, the appropriateness of the selection and formulation of the research problem, the research methodology, the choice of applied methods and analytical techniques, as well as the correctness of the interpretation of the obtained results and the ensuing discussion, also in the context of the current state of knowledge. Attention was likewise paid to the effectiveness in achieving the stated research objectives. An important, though not decisive, element of the overall evaluation was also the scientific achievements of the doctoral candidate, including those extending beyond the direct scope of the submitted dissertation.

Cyanobacteria constitute one of the oldest and most versatile groups of photosynthetic microorganisms, whose application potential has attracted increasing scientific and technological interest in recent years. Particularly promising is their use in photobiocatalytic processes and in the removal and recovery of valuable resources from aquatic environments and industrial waste streams. These applications are well aligned with current trends in sustainable development, environmental biotechnology, and the circular economy.

In photobiocatalytic processes, cyanobacteria act as natural biocatalysts capable of converting light energy into chemical energy, which is subsequently utilized to drive redox reactions of synthetic and environmental relevance. These reactions are catalyzed by endogenous enzymes or by heterologously expressed biocatalysts.

In practice, this enables a wide range of biotransformations, including reductions, hydroxylations, and asymmetric syntheses of chiral compounds, without the need for external electron donors. Importantly, these processes can be carried out under mild conditions using solar light as the sole energy source, which significantly reduces their environmental footprint and improves process sustainability.

Another important area of application of cyanobacteria is their ability to remove and accumulate metals, including rare earth elements such as gadolinium, europium, lanthanum, cerium, etc. These elements are essential for modern technologies, including electronics, renewable energy systems, and the automotive industry, yet their conventional mining and processing are associated with substantial environmental burdens. Cyanobacteria exhibit a natural capacity for metal uptake through biosorption, which occurs via the binding of metal ions to functional groups present on the cell surface and within extracellular polymeric substances. In addition to surface binding, cyanobacteria may also internalize metals through bioaccumulation or promote biomineralization, further enhancing the efficiency of metal removal from aqueous solutions.

The integration of photobiocatalysis with resource recovery offers a compelling opportunity to develop multifunctional biotechnological systems. In such systems, cyanobacteria can simultaneously contribute to environmental remediation, the production of value-added chemicals, and the recovery of strategically important raw materials. This dual functionality not only improves the economic feasibility of bioprocesses but also supports sustainable resource management by reducing waste and reliance on environmentally damaging extraction methods.

The subject matter of the doctoral dissertation by Sunday Samson fits perfectly within this research stream, clearly demonstrating its significance, topical relevance, and the considerable potential of the solutions developed in the study. The primary objective of the work was to evaluate the capabilities of various cyanobacterial strains with respect to their ability to perform photobiocatalysis, followed by the removal of rare earth elements (REEs). Significant attention was devoted to evaluating substrate stability and optimizing growth media in order to balance biomass productivity with substrate integrity. Furthermore, the study aimed to determine stereoselectivity and conversion efficiency of cyanobacteria during enantioselective transformations, employing a range of advanced analytical techniques. Another important aspect of the research was the investigation of the antimicrobial activity of bioconversion products and the assessment of their potential application as precursors in biomedical or environmental fields. These investigations were followed by an in-depth exploration of the bioaccumulation capacity of cyanobacterial biomass, both before and after biotransformation, for the recovery of REEs. Additionally, the most promising cyanobacterial candidates were identified for downstream scale-up studies, based on catalytic performance, tolerance to elevated substrate concentrations, and efficiency of REE accumulation. Therefore, it can be concluded that the objectives of the dissertation were clearly defined and are supported by a series of well-formulated and compelling research hypotheses.

The doctoral dissertation by Sunday Samson comprises 149 pages, is written in English, and is illustrated with 29 figures and 23 tables. The thesis follows a classical structure and is divided into five main chapters, the most important of which include: Introduction (5 pages), Literature Review (45 pages), Materials and Methods (24 pages), Results and Discussion (40 pages), and Conclusions (5 pages). The dissertation is complemented by a

comprehensive reference list containing 261 citations, most of which were published within the last 20 years. The cited literature is diverse and appropriately selected, as the referenced examples effectively illustrate the issues addressed in the dissertation and facilitate both the introduction to the research topic and the interpretation of the obtained results. The thesis also includes abstracts in both Polish and English, a summary of the doctoral candidate's scientific achievements, a list of tables and figures, and a list of abbreviations used throughout the work.

The literature review section comprises 39 pages and is divided into nine subsections, in which the Author presents, in a concise and synthetic manner, the key issues related to the subject of the dissertation and the research conducted. General aspects of biocatalysis and biotransformation are introduced, and the advantages of these approaches over conventional chemical synthesis methods are clearly highlighted. The review also provides information on major classes of enzymes and identifies the key transformations applied in the synthesis of compounds with high application potential. Overall, the literature review is engaging and contains a substantial amount of valuable information, while the topics discussed are well-selected and appropriately aligned with the overall scope of the dissertation.

The next part of the thesis is the Experimental Section, in which the Author presents a detailed description of the applied research methodology. This includes procedures related to the cultivation of cyanobacteria, photobiocatalytic assays, and experiments focused on the removal of rare earth elements. The dissertation also provides a description of the analytical techniques used for the characterization of the synthesized systems, as well as the equations employed for calculation of the key parameters. All experimental procedures are described clearly and precisely, and, most importantly, in a manner that enables the reproducibility of the experiments.

The most extensive part of the dissertation is the Results and Discussion chapter. This section, divided into several subsections, presents the obtained results along with their discussion, explanation, and interpretation. It is well structured and clearly outlines the main stages of the research, which are focused on the application of cyanobacteria in photobiocatalytic processes and in the bioaccumulation of rare earth elements.

In the first stage of the research, the doctoral candidate focused on understanding the chemical stability of the substrates in biological media, which is a critical prerequisite for the successful design and interpretation of biotransformation studies. Three cultivation media (Spirulina Medium, BG-11, and BG-11 without sodium nitrate (named as BG-11₀) were employed to support cyanobacterial growth and to evaluate substrate compatibility with biologically relevant conditions. Stability tests in these media were conducted on phosphonate compounds, namely, epoxymethyl dimethyl phosphonate (EMDP) as a representative substrate for further bioconversions. The results demonstrated that EMDP was chemically stable in both BG-11 and modified BG-11 (BG-11₀) media, indicating that these environments are chemically inert with respect to this compound. In contrast, in the presence of Spirulina medium, EMDP exhibited a loss of structural integrity, pointing to its instability under these conditions. These compatibility studies were essential for the rational selection of experimental conditions and confirmed that BG-11 and BG-11₀ media constitute suitable platforms for evaluating cyanobacterial biocatalysis. By eliminating the risk of abiotic substrate degradation, the applied approach ensured that any observed transformations in subsequent experiments could be reliably attributed to biological activity.

In the second stage of the study, which in my opinion represents the most interesting and scientifically valuable part of the dissertation, the aim was to investigate the catalytic potential of selected cyanobacterial strains under photoautotrophic conditions to convert/degrade dimethyl vinylphosphonate, diethyl vinylphosphonate, epoxymethyl dimethyl phosphonate and 1-phenyl acetate and to follow potential enzymatic transformations and their products. At first, the research on substrate bioconversion involved use of dimethyl vinylphosphonate and diethyl vinylphosphonate as representative vinyl phosphonate compounds. Various cyanobacterial species were subjected to long-term bioconversion experiments lasting up to 30 days. However, after this incubation period, no enzymatic conversion or degradation of either compound by the tested cyanobacterial strains was observed. This lack of activity was explained by the genomic and physiological constraints, including the absence or limited expression of phosphonate transport and C–P bond cleavage genes as well as the tight regulation of photosynthetic electron transport and intracellular redox metabolism, which prioritize photoautotrophic growth over biotransformations.

In further experiments, epoxymethyl dimethyl phosphonate was subjected to bioconversion using *Synechococcus* sp. and *Limnospira* sp. The results indicated a probable two-step bioconversion pathway leading to the final product. Among tested strains, *Limnospira* sp. and *Synechococcus* sp. demonstrated the fastest and most complete bioconversion, achieving 99% substrate transformation within 24 h and 72 h, respectively, and maintaining this high efficiency throughout the experimental period. In contrast, *Leptolyngbya foveolarum* CCALA 76 and *Nodularia moravica* CCALA 114 showed moderate conversion efficiencies, reaching 96% and 75% conversion, respectively, by day seven, whereas *Nostoc cf. muscorum* CCALA 129 was the least effective, with only 14% conversion over the same timeframe. In this subsection of the dissertation, detailed mechanisms of substrate conversion were discussed. It was suggested by the Author that transformation of epoxymethyl dimethyl phosphonate into dimethyl (1E)-3-hydroxyprop-1-enylphosphonate by cyanobacterial strains is a two-step biochemical process concerning epoxide ring opening, yielding a vicinal diol intermediate, and a subsequent dehydration/elimination, resulting in the conjugated vinyl phosphonate product. It was further concluded that the ability of certain cyanobacterial strains to catalyze this reaction is related to species-specific differences in enzymatic equipment, gene regulation, and metabolic prioritization. At the final step of this research stage, antibacterial activity tests of the epoxymethyl dimethyl phosphonate, and the dimethyl (1E)-3-hydroxyprop-1-enylphosphonate were performed in order to assess their possible application as antibacterial agents. The results of the disk diffusion method analysis showed no antibacterial activity for both the substrate and the product, suggesting the need for structural modifications or its use in combination with other antimicrobial agents to enhance its potential efficacy in future applications.

At the final research stage of the study on microalgae-supported biocatalysis, 1-phenylethyl acetate was subjected to bioconversion under precisely controlled cultivation and reaction conditions using a phytotron system, which ensured high reproducibility and allowed for accurate comparison of the enantioselective activity of cyanobacteria. The application of the phytotron represents a significant methodological advancement compared to earlier studies conducted in manually controlled reactors. Under optimized conditions, enantioselective hydrolysis of 1-phenylethyl acetate led to the formation of optically enriched 1-phenylethanol as the major product, with a

conversion rate exceeding 99% when *Synechococcus* sp. was employed. After seven days of incubation, a minor but reproducible formation of acetophenone was also observed as a by-product, which was attributed to the oxidation of 1-phenylethanol by alcohol dehydrogenase.

After carrying out the bioconversion processes, in the final stage of the study, the *Cyanobacteria* biomass was applied for a bioaccumulation study of rare earth elements. Prior to bioaccumulation experiments, the morphology of the pre- and post-bioconversion biomass was examined, followed by examination of the toxicity of the gadolinium and europium. The microscopical images showed that cells retained structural integrity with no visible signs of lysis, aggregation, or abnormal pigmentation, indicating that the biotransformation processes did not induce significant cytotoxic effects under the applied conditions. However, the obtained results showed different responses to the presence of RRE. The highest growth inhibition was observed for *Limnospira* strains, and the increased sensitivity towards these elements was also shown by *Leptolyngbya* sp., both in post-bioconversion biomass. Likewise, *Synechococcus* sp. shown moderate tolerance towards both elements and no growth inhibition in its presence, indicating possible use of this strain in bioaccumulation. The bioaccumulation experiments shown that after 72 h pre-bioconversion, cyanobacterial samples treated with REEs exhibited a higher increase in biomass weight compared to controls. Further, it was observed that bioconversion significantly enhances the ability of cyanobacterial biomass to accumulate REEs, mainly due to structural and biochemical modifications in the biomass that contributed to improved metal sequestration. For instance, the most pronounced increases were observed in *S. bigranulatus*, where Eu accumulation raised from $32,257.0 \pm 1,114.9$ mg/g to $53,599.0 \pm 1,619.5$ mg/g in post-bioconversion biomass followed by increased Gd accumulation from $34,978.0 \pm 3,270.7$ mg/g to $54,197.0 \pm 2,753.1$ mg/g clearly showing that bioconversion plays a crucial role in improving REE sequestration across all tested strains.

In the context of the achievements discussed above, it should be emphasized that the reviewed dissertation addresses not only the application of cyanobacteria in biotransformation and bioaccumulation processes but also constitutes an in-depth study of the influence of process conditions on their efficiency, as well as on the mechanisms underlying the investigated transformations. The presented material, encompassing both theoretical and experimental aspects, is intellectually engaging and forms a conceptually coherent and self-contained body of work. The research objectives formulated in the dissertation have been successfully achieved, and the identified relationships introduce a significant element of scientific novelty, while also demonstrating considerable practical relevance.

In my opinion, the most significant achievements of the dissertation include:

- demonstrating that cyanobacterial biomass can be regarded as a multifunctional biological resource, capable of catalyzing structurally diverse chemical substrates while simultaneously enabling the recovery of rare earth elements (REEs);
- proving that the capacity for REE bioaccumulation increases in post-biotransformation biomass, indicating enhanced metal uptake following catalytic activity;
- establishing high selectivity and stereospecificity in the performed biotransformations, which highlights the applicability of the investigated systems in the production of chiral pharmaceutical intermediates.

However, it is also the duty of the reviewer to identify certain inconsistencies or imprecise formulations and to provide a substantive evaluation of the dissertation, indicating issues that require more detailed explanations or corrections. I therefore have several comments, remarks, and questions that arose during reading of the thesis, which I present with the aim of clarifying specific aspects of the work. My comments and questions are listed below:

- my concern is related to the fact that explanations provided in the study are in some cases insufficient for deep understanding of the presented phenomena. For instance, I missed deeper explanations why substrates are unstable in growth medium, why strains lost their activity over time in biotransformation processes, why produced compounds do not show antibacterial activity or what is the mechanism that govern REE accumulation, etc. I would like to ask Author to provide deeper explanations to these issues during PhD defence.
- in order to provide a more detailed explanation of the EMDP conversion mechanism and the differences in efficiency observed between the strains, did the author consider a comprehensive analysis to identify which specific enzymes are produced by the individual strains and in what quantities and activities? Such an analysis would facilitate a more accurate interpretation of the data.
- The conclusion that phosphate formation results from a non-enzymatic hydrolytic process appears insufficiently supported. What experimental controls allow enzymatic and abiotic processes to be distinguished?
- A major issue is the very large discrepancy between substrate conversion and isolated/detected DMHPP yield. Near-complete substrate disappearance accompanied by only a few percent product yield requires a mass-balance analysis. Were intracellular or biomass-associated fractions analyzed for DMHPP and possible intermediates or side-products? Without such data, substrate disappearance should not automatically be interpreted as selective conversion to DMHPP.
- The reliability of conclusions derived from duplicate experiments should be discussed, particularly considering the very large variability observed for several conditions. Statistical significance should not be implied where the dataset does not support formal statistical comparison. Further, why there is a lack of error bars in Fig. 25?
- The description of EMDP stability in modified BG-11 medium appears internally inconsistent. Please clarify whether significant degradation occurred in this medium or whether the compound remained substantially stable during the investigated period.
- There are some editorial and language issues in the dissertation. Several inconsistencies and language-related issues should be corrected throughout the text, including the inconsistent use of terms and abbreviations such as *EMDPP/EMDP* as well as inappropriate or incorrect expressions. The notation for enantiomeric excess should also be standardized throughout the manuscript (e.g., *ee* or *e.e.*, but not both), and species names should be presented consistently according to accepted scientific nomenclature. In addition, numerous awkward or grammatically unclear sentence constructions should be revised to improve readability and scientific clarity. Further, for example, in the ICP-MS section the text refers to Figure 23, although Figure 23 has already been assigned earlier to the biotransformation pathway of 1-phenylethyl acetate.
- There appears to be a numerical inconsistency in the comparative discussion of REE values. The authors state that the values obtained for *L. maxima* and *S. bigranulatus* "approach or exceed" the previously reported range

of 84.2–91.5 mg/g, whereas the highest value presented in the authors' own tables is approximately 54 mg/g. This statement is therefore not supported by the reported data and should be carefully revised.

The reviewed dissertation represents a good scientific standard, while simultaneously incorporating elements of scientific novelty with substantial practical relevance. The critical remarks and questions raised above do not diminish the positive overall evaluation of the work. Particular attention should be given to the holistic approach adopted in addressing the research topic, encompassing the synthesis and assessment of substrate stability in cultivation media, cyanobacterial growth, and applied photobiocatalytic and bioaccumulation assays. The Author has demonstrated the ability to conduct comprehensive literature studies, to develop appropriate research methodologies for addressing the formulated research problems, to plan experiments and critically evaluate empirical evidence, as well as to interpret research results and formulate sound conclusions.

Finally, it is also important to acknowledge the scientific output of Sunday Samson. He is a co-author of 13 scientific publications, including three directly related to the subject of the dissertation, as well as three patent applications, and a co-author of six national and international conference presentations. In addition, the doctoral candidate participated in the implementation of five research projects funded from various sources.

On the basis of the evaluation of the doctoral dissertation authored by Sunday Samson, entitled "Cyanobacterial Biomass in Biocatalysis and Bioaccumulation Processes", and the scientific activity presented therein, I unequivocally conclude that the dissertation meets the requirements set forth in Article 187(1)–(2) of the Act of 20 July 2018 – Law on Higher Education and Science (consolidated text: Journal of Laws of 2024, item 1571) and also meets all statutory and customary requirements for doctoral theses. I therefore submit a formal request to the Council of the Discipline of Chemical Sciences at Wrocław University of Science and Technology to accept the dissertation and to proceed with the subsequent stages of the doctoral degree procedure.

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