

Lublin, 12.10.2026 r.

Dr hab. Anna Matuszewska, prof. UMCS  
Department of Biochemistry and Biotechnology  
Institute of Biological Sciences  
Faculty of Biology and Biotechnology  
Maria Curie-Skłodowska University in Lublin

**Re-review of the doctoral dissertation  
of M.Sc.Eng. Sunday Samson, entitled:  
“Cyanobacterial biomass in biocatalysis and bioaccumulation processes”,  
prepared under the supervision of  
Prof. dr hab. inż. Ewa Żymańczyk-Duda**

The formal basis for preparing the re-review of the doctoral dissertation of M.Sc. Eng. Sunday Samson is the letter from the Chair of the Discipline Council for Chemical Sciences at Wrocław University of Science and Technology, Dr hab. inż. Robert Góra, University Professor, dated 29 July 2026. The legal basis is Resolution No. 225/13/RDND10/2024-2028 of the Discipline Council for Chemical Sciences of 29 October 2025 on the appointment of reviewers in the proceedings for the conferment of the doctoral degree in the field of exact and natural sciences in the discipline of chemical sciences to M.Sc. Eng. Sunday Samson.

**Formal assessment of the doctoral dissertation**

M.Sc. Eng. Sunday Samson submitted a monographic doctoral dissertation entitled “Cyanobacterial biomass in biocatalysis and bioaccumulation processes”, comprising a total of 149 pages, in a layout typical for PhD theses. The text is divided into 5 chapters and a bibliography (Chapter one: **Introduction** – 5 pages, Chapter two: **Literature review** – 45 pages, Chapter three: **Materials and methods** – 24 pages, Chapter four: **Results and discussion** – 39 pages, Chapter five: **Conclusion** – 3 pages). At the beginning of the thesis, the Author also included a table of contents, a list of abbreviations used in the thesis, a list of tables, a list of



figures, acknowledgements, an academic CV, and abstracts in English and Polish. The work is enriched with 29 figures and 23 tables.

To sum up the formal assessment, the thesis is written in correct scientific language and displays high scientific value. It has a carefully prepared, clear and transparent structure, which facilitates analysis and interpretation of the results.

### **Significance of the research topic**

A sustainable circular bioeconomy is a direction of key importance both for environmental protection and for economic development. It is linked to a broad spectrum of activities – from biotechnology and waste management to the development of digital tools and regional strategies. The basis of all these activities is full utilisation of biomass and side-stream resources. One of the pillars of these innovations are modern biological technologies using, among others, biocatalysis and bioaccumulation, which constitute the foundation of the green transition – not only in laboratories, but also in industrial plants and on farms. The biomass of living organisms fits perfectly into the circular economy, offering the possibility of converting organic waste and wastewater into value-added products (e.g. biofuels, feed, fertilisers, cosmetics), which reduces the environmental footprint, recovers resources, supports the bioeconomy and creates innovative supply chains based on the principles of cascading use and industrial symbiosis.

The doctoral thesis by M.Sc. Eng. Sunday Samson, entitled “Cyanobacterial biomass in biocatalysis and bioaccumulation processes”, prepared under the supervision of Prof. dr hab. inż. Ewa Żymańczyk-Duda and Dr. inż. Monika Serafin-Lewańczuk, fits very well into this research trend.

Doctoral Student addresses an important topic concerning the potential of selected cyanobacterial strains – *Synechococcus bigranulatus* CCALA 187, *Nostoc cf-muscorum* CCALA 129, *Limnospira indica* PCC 8005, *Limnospira maxima* CCALA 27, *Leptolyngbya foveolarum* CCALA 76, *Kamptenema animale* CCALA 138 and *Nodularia moravica* CCALA 114 – as whole-cell biocatalysts and bioaccumulators that can form elements of strategies for bioremediation and resource recovery.

### **Analysis of the doctoral dissertation**

The **Introduction** chapter presents the background and aim of the doctoral work, concerning the use of cyanobacteria in biotechnology and environmental chemistry. In the revised work, the main objectives were verified to ensure consistency with the achieved results.

The thesis deals with the integrated use of selected cyanobacterial strains (including *Synechococcus bigranulatus* CCALA 187, *Nostoc cf-muscorum* CCALA 129, *Limnospira indica* PCC 8005, *Limnospira maxima* CCALA 27, *Leptolyngbya foveolarum* CCALA 76, *Kamptenema animale* CCALA 138, and *Nodularia moravica* CCALA 114) as whole-cell



photobiocatalysts and bioaccumulators of rare earth elements (Gd, Eu). In the revised work, the primary objectives were adjusted to align with the achieved results. The newly defined research objectives include:

- Assessing the ability of selected cyanobacterial strains to catalyze photobiotransformation reactions.
- Evaluating substrate stability and optimizing culture medium composition to balance biomass production yield with substrate durability; determining conversion selectivity and efficiency for selected cyanobacterial strains in biotransformation processes.
- Conducting preliminary studies on the antimicrobial activity of the resulting bioconversion products.
- Identifying the most promising cyanobacterial strains for laboratory-scale studies.
- Investigating the bioaccumulation potential of cyanobacterial biomass (before and after biotransformation) for the recovery of rare-earth elements ( $\text{Gd}^{3+}$  and  $\text{Eu}^{3+}$ ) using inductively coupled plasma mass spectrometry (ICP-MS).

The Author removed the research hypotheses (containing 6 points/hypotheses) that had been presented in the earlier version of the paper from the Introduction.

The **Literature Review** chapter provides an overview of the literature on biocatalysis, microbial cell factories, and cyanobacterial metabolites and bioaccumulation capacity, building the theoretical foundations for the subsequent experimental research in the thesis. The structure of this chapter has been revised and simplified; it now consists of nine main subsections (2.1 through 2.9). Subsections that previously appeared under separate headings - such as the advantages and limitations in sections 2.3 and 2.4, or "cell factories" in section 2.7 - have been incorporated directly into the main text under consolidated headings.

The chapter is logically divided into subsections from 2.1 to 2.9, providing a smooth transition from the general concepts of biocatalysis to the specifics of cyanobacterial photobiocatalysis and metal-binding mechanisms. The author discussed both classes of biocatalysts (purified enzymes, lysates, whole cells) and specific reaction types (reductions, hydrolyses, isomer synthesis). The inclusion of a section dedicated to rare earth elements (REE) and synthetic biology tools directly justifies the research objectives set out in the first chapter.

The text is richly illustrated with reaction schemes (e.g., deracemization, OYE mechanisms, redox cycles) and presented in clear tables (Tables 1–5), which facilitates the comprehension of complex biochemical knowledge.

In the new version of the work, the Author introduced additional diagrams and tables, thereby supplementing the chapter with elements essential to the analyses conducted:



- **Figure 3:** Schematic illustration comparing classical kinetic resolution (KR) and dynamic kinetic resolution (DKR).
- **Figure 4:** Representative biocatalytic strategies for enzymatic racemization and deracemization of chiral amines and alcohols.
- **Figure 5:** Mechanistic overview and substrate scope of flavin-dependent ene-reductases (Old Yellow Enzyme family).
- **Figure 6:** Mechanism of stereoinversion catalyzed by mandelate racemase.
- **Figure 8:** Schematic illustration of cofactor regeneration by alcohol dehydrogenases using sacrificial alcohols and representative examples of lipase catalytic promiscuity.
- **Figure 9:** Enantioselective kinetic resolution of racemic ibuprofen esters catalyzed by lipase.
- **Figure 10:** Penicillin G acylase-catalyzed hydrolysis of penicillin G to 6-APA.
- **Table 2:** Selected comparative parameters of biocatalytic and non-biological catalytic systems (expanding significantly on the broad comparison).
- **Table 3:** Detailed table summarizing major limitations of biocatalysis and representative mitigation strategies.
- **Table 5:** Comparative summary of heterotrophic whole-cell biocatalysts and cyanobacterial photobiocatalytic systems.

During a detailed reading of the chapter, a few stylistic and editorial errors were noted. In some places, inconsistency appears in the formatting of chemical symbols and fonts, e.g., NADPH and NAD(P)H.

The second chapter is a very good, thorough, and comprehensive literature review. It demonstrates the author's research maturity, as well as their deep understanding of the fields of biocatalysis, organic chemistry, and microalgal biotechnology.

The third chapter of the revised doctoral dissertation entitled **Materials and Methods** has been thoroughly reorganized, refined, and adapted to the final scope of research presented in the thesis. Compared to the original version of the document, the new edition exhibits a number of significant structural, substantive, and editorial improvements that significantly enhance the methodological rigor of the entire study. It has been divided into five subsections: 3.1. Reagents and materials; 3.2. Cyanobacterial strains and microorganisms; 3.3. Growth media and cyanobacterial cultivation; 3.4. Photobiocatalytic transformations method; and 3.5. Bioaccumulation process.



In the original version of the thesis, the chapter dedicated to reagents and methods described procedures related to the biotransformation of unsaturated fatty acids (e.g., 7-octenoic, 6-heptenoic, 10-undecenoic, or 4-pentenoic acid), even though—as shown in the discussion—these processes proved to be irreproducible or ineffective. In the revised version, these substances were eliminated from the table of reagents and methodological descriptions, focusing exclusively on the actually conducted and verified processes (acetic acid esters and organophosphonates). As a result, the chapter has become consistent with the actual results presented in the fourth chapter.

The arrangement of sub-sections concerning media preparation and cultivation was changed. In the revised thesis, sub-section 3.3 ("Growth media and cyanobacterial cultivation") logically and sequentially integrates the preparation of growth media (*Spirulina* Medium, BG-11/BG-110), their modifications for *Limnospira* sp., and the cultivation conditions. A more precise division was also introduced in the section on biotransformation methods (3.4), separating the esterase-dependent hydrolysis of 1-phenylethyl acetate from the transformation of organophosphonates.

In the new version, the descriptions of analytical procedures have been refined, including sample preparation for ICP-MS analysis (determination of rare earth elements – REE) and microbiological tests. Furthermore, the role of the chemical synthesis of DMHPP as a structural reference standard was described in greater detail.

The removal of methodological threads concerning experiments that did not yield positive results or were abandoned ensures that the chapter does not mislead the reader and serves as an accurate reflection of the research pathway described in the results section. The methodology is described in sufficient detail to guarantee the complete reproducibility of the experiments (which is crucial, among other things, for patent procedures carried out as part of the PhD, such as patent applications for the strains *Synechococcus bigranulatus*, *Nodularia moravica*, or *Leptolyngbya foveolarum*).

In conclusion, the modifications introduced in Chapter 3 of the revised doctoral dissertation deserve a very high assessment, as they eliminate inconsistencies from the original manuscript and impart complete professionalism to this section of the monograph.

In the revised version of the thesis, the structure of the subsections in the bioconversion section of the **Results and Discussion** chapter has been modified. Section 4.2 has been divided more precisely into, among others: 4.2.1. Dimethyl vinylphosphonate (DMVP) and diethyl vinylphosphonate (DEVP) bioconversions (78–82), 4.2.2. Bioconversion of epoxymethyl dimethyl phosphonate (82–84), 4.2.3. Conventional transformation of epoxymethyl dimethyl phosphonate (84–90), 4.2.4. Antibacterial plate testing of EMDP and DMHPP (90–93), and 4.2.5. Bioconversion of 1-phenylethyl acetate (93–100).

In the revised version, the broad discussion of unsaturated fatty acids as a primary element of bioconversion was omitted in favor of focusing on esters and phosphonate



compounds (including the chemical synthesis of the DMHPP standard and a detailed discussion of DMVP, DEVP, and EMDP).

In the revised version of the thesis, the Author introduced better-described spectra and reaction schemes (e.g., separate NMR spectra for DMVP and DEVP, a chemical synthesis scheme for DMHPP, and GC calibration curves for 1-phenylethyl acetate and derivative products). He also updated the literature references and the numbers of tables and figures throughout the chapter, adapting them to the modified table of contents (e.g., the table comparing the biosorptive capacities of microorganisms as Table 22 in the revised version).

**Chapter Four** in the revised version of the dissertation constitutes a logical, coherent, and comprehensive continuation of the methodological part. The presented results are arranged in a sequential manner: from the evaluation of substrate stability and medium selection, through a detailed analysis of photobiocatalytic processes (divided into individual groups of phosphonate and ester compounds) and antibacterial tests, to a comprehensive assessment of bioaccumulation capacities with respect to rare earth elements (REE). The improvement of the subsection structure (especially the separation of distinct sections for vinylphosphonates and the chemical synthesis of the standard) significantly enhances the clarity of the scientific argumentation.

The Author has demonstrated high professionalism in interpreting complex instrumental data. The application of complementary analytical techniques (NMR spectroscopy, gas chromatography GC with a chiral column, liquid chromatography HPLC, high-resolution mass spectrometry HRMS, and ICP-MS) ensures the full reliability and reproducibility of the put-forward conclusions. Particularly valuable is the thorough explanation of the disparity between the high substrate conversion rate (EMDP) and the low initial recovery of the pure product (DMHPP), which the author linked to biomass-product interactions (structural similarity to undecaprenyl phosphate). The author demonstrated that cyanobacteria serve as efficient, multifunctional whole-cell biocatalysts. They enable the simultaneous execution of precise organic synthesis reactions (photobiocatalysis) and the recovery of critical elements from wastewater/aqueous environments (bioaccumulation), perfectly aligning with the principles of a circular bioeconomy.

In the discussion section, the author does not limit themselves to a simple description of the charts, but actively confronts their own results with the current state of scientific knowledge, citing the latest reports in the fields of biocatalysis, cyanobacterial physiology, and environmental engineering. The insightful analysis of the hormesis phenomenon in ecotoxicological tests with REE and the discussion of metal transport and sorption mechanisms demonstrate the doctoral candidate's scientific maturity.



The final chapter, **Conclusion**, constitutes a synthesis of the most important research results and conclusions achieved within the framework of the doctoral dissertation. The research demonstrated that cyanobacteria (blue-green algae) can play a multifunctional role as multi-tasking whole-cell biocatalysts, combining the ability to:

1. Conduct selective photobiotransformation of organophosphorus compounds and acetate esters.
2. Efficiently bioaccumulate rare earth elements (REE), in particular gadolinium (Gd) and europium (Eu).

This chapter was enriched with a table presenting the conclusions in a condensed manner (Table 23).

The Author stresses that the combining photobiocatalysis (for the synthesis of valuable chemical and pharmaceutical products) and subsequent bioaccumulation (for the recovery of critical metals and bioremediation) within a single microalgae system represents an eco-friendly, highly efficient alternative to conventional chemical syntheses and metallurgical processes.

The bibliography is very extensive and includes a total of 261 references. The reference list has been prepared with great care and contains works relevant to the subject of the dissertation. The Author is familiar with key publications related to the topic and cites them in a conscious and appropriate manner.

Regarding the substantive content of the doctoral dissertation submitted by Sunday Samson, M.Sc. Eng., it must be noted that the revisions have improved the quality of the work, resulting in greater coherence. The Author demonstrated a thorough understanding of the research subject. He designed and conducted experiments in a well-conceived manner, displaying proficiency in operating a wide range of modern research equipment and obtaining valuable results. The use of cyanobacterial biomass for the recovery of rare-earth elements not only mitigates environmental pollution but also offers a means of securing resources essential for the supply of strategic materials. Consequently, the results obtained hold significant scientific value and offer potential for practical application in the fields of green chemistry and the recovery of critical raw materials.

### Final conclusion

In summary, I state that the doctoral dissertation submitted for review by M.Sc. Eng. Sunday Samson, entitled "*Cyanobacterial biomass in biocatalysis and bioaccumulation processes*", prepared under the supervision of Prof. dr hab. inż. Ewa Żyłańczyk-Duda, meets



the requirements of Article 187, paragraph 1–2 of the Act of 20 July 2018 – Law on Higher Education and Science (Prawo o szkolnictwie wyższym i nauce (t.j. Dz. U. z 2024 r. poz. 1571).

In view of the above, I hereby submit to the esteemed Discipline Council for Chemical Sciences of Wrocław University of Science and Technology a motion to admit PhD Candidate M.Sc. Eng. Sunday Samson to the further stages of the doctoral procedure.

