

Paweł Popielski, Ph.D., D.Sc., Eng., Professor at the Warsaw University of Technology
Warsaw University of Technology
Faculty of Environmental Engineering
Hydro Engineering and Hydraulics Department

Review

of the doctoral dissertation by Nadella Marchelina, MSc
entitled: "EXPERIMENTAL STUDIES OF MICROBIOLOGICALLY INDUCED
IMPROVEMENT OF FINE SANDS"
„BADANIA EKSPERYMENTALNE MIKROBIOLOGICZNEGO WZMACNIANIA
DROBNYCH PIASKÓW”

1 Formal basis

1.1 Resolution of the Discipline Council

In a letter (RDNILGIT - 17/2025) sent by the Scientific Council of the Discipline of Civil Engineering, Geodesy and Transport at the Lublin University of Technology on 12 November 2025, I was informed that the Scientific Council of the Discipline of Civil Engineering, Geodesy and Transport at the Lublin University of Technology adopted Resolution No. 2025/X/01 appointing me as a reviewer of the doctoral dissertation specified in the title. The supervisor of the doctoral thesis submitted to me for review is: Małgorzata Franus, Ph.D., D.Sc., Eng., Prof. of the university, and the assistant supervisor is: Joanna Fronczyk, Ph.D., Eng.

1.2 Procedure

The proceedings for awarding the degree of Doctor to the candidate, Ms Nadella Marchelina, MSc, are conducted based on the provisions of the Act of 14 March 2003 *on academic degrees and titles and degrees and titles in the field of art*, (Journal of Laws No. 65, item 595, as amended), hereinafter referred to as the Act, and *the Regulation of the Minister of Science and Higher Education of 19 January 2018 on the detailed procedure and conditions for conducting activities in doctoral proceedings, habilitation proceedings and proceedings for the award of the title of professor* (Journal of Laws of 19 January 2018, item 261), hereinafter referred to as the Regulation.

1.3 Formal requirements

Pursuant to Article 13 of the Act, a doctoral dissertation should constitute an original solution to a scientific problem and demonstrate the candidate's general theoretical knowledge in a given scientific discipline, as well as the ability to conduct independent scientific work. Pursuant to § 4(1)

of the Regulation, the review of the doctoral dissertation shall contain a detailed and reasoned assessment of whether the dissertation meets the conditions specified in Article 13 of the Act.

The materials provided include a copy of the candidate's diploma certifying her master's degree and her curriculum vitae. The candidate obtained her master's degree on 7 May 2021, completing her second-cycle studies in Civil Engineering at the Faculty of Civil and Environmental Engineering of the Warsaw University of Life Sciences. Based on the information contained in her CV, it can be concluded that the candidate has not previously applied for a doctoral degree.

2 Review content

2.1 General description of the dissertation

The dissertation comprises 210 pages, including a bibliography, a summary in Polish and English, a list of figures and tables, and a list of symbols used in the work with definitions. The very extensive bibliography (203 records) contains ASTM International standards cited in the thesis and publications with their netography, enabling direct access from the electronic version of the thesis. Unfortunately, not all publications are currently available in readable form; however, the bibliography format is entirely correct. It is worth noting that over 150 of the cited publications were produced within the last 10 years.

The thesis also includes fourteen appendices containing the results of tests and analyses.

The thesis consists of nine parts, divided into chapters, as well as a summary, directions for further research, conclusions, and a bibliography. The thesis contains a large number of tables, drawings, photographs, and graphs. Each type of graphic illustration is numbered separately in each chapter.

The main parts of the thesis are:

Chapter 1 - **Introduction**, which emphasises that microbiologically induced carbonate precipitation (MICP) is a promising soil biocementation technique. It involves biological and chemical processes that facilitate carbonate precipitation, thereby improving soil properties by binding grains and filling pore space. It is stated that there are two different approaches to MICP: bioaugmentation, which involves introducing foreign bacteria directly into the soil, and biostimulation, which optimises the growth environment for native bacteria. Both processes are currently the subject of intensive research.

Chapter 2 – **Microbially induced carbonate precipitation (MICP) – literature review**. This chapter describes MICP application methods, including bioaugmentation and biostimulation. The potential of MICP for ureolytic and non-ureolytic processes is presented. Factors affecting MICP effectiveness are discussed, including soil type and grain size, bacterial strains, and cementation sources. Methods and possibilities of application are also presented, including injection, spraying, or

percolation, and a mixed method. Mineral precipitation, the impact of cementation on improving soil parameters, and the methods used for testing are discussed. A separate subsection characterised the development of MICP research in various countries. The graph presented in the chapter did not include China, a country that is actively working on biocementation. A significant number of publications on MICP come from China, and the author herself cites many Chinese researchers in her work. In summary, the review has been carefully prepared, and the author has discussed many aspects of biocementation processes, analysing them thoroughly based on an extensive literature review. However, there is a certain feeling of incompleteness, as, among other things, no analysis of the impact of water on biocemented soil is presented.

In subsection 2.4.3, Table 2.1, p. 27, the name of *Bacillus pasteurii* strain is given, which corresponds to the previous taxonomy of the strain currently classified as *Sporosarcina pasteurii*.

Subsection 2.6.1. on page 34, there is an analysis of shear strength. The text states that "the phenomenon caused by the effects of precipitated carbonate binding contributes to the increase in strength of materials subjected to MICP (DeJong et al., 2010). Although MICP treatment increases soil cohesion through carbonate precipitation, it does not always have a significant effect on soil particle friction, which contributes to shear strength." Increased cohesion is mentioned, but Table 2.4 does not include an analysis of this parameter.

In section 2.7. Bonding mechanisms in MICP, p. 38 – It is stated: "As mentioned earlier, the precipitation of CaCO_3 / MgCO_3 in the MICP process creates bonds between particles in sandy soil. This causes significant changes in the mechanical properties of the soil by creating bonds between sand particles, which can increase strength, stiffness, and erosion resistance." - With an increase in strength (in terms of shear strength), the cohesion of the soil also increases, and this is one of the parameters used to characterise the soil.

Chapter 3 - **Research thesis, objectives, and research plan.** The thesis formulates three main hypotheses and proposes dividing the work into four main research areas. The introduction of three hypotheses already suggests a large, if not huge, area of research at the outset. One main thesis would be sufficient, as having three hypotheses introduces a lack of clarity as to the importance of each thesis. Nevertheless, the three hypotheses are well integrated into the work plan, and Chapter 3 is coherent and logically structured.

Chapter 4 – **Materials and Methods.** This chapter discusses the materials used in the study and the research methods applied. It examines the characteristics of the soils, the bacterial cultures used, and the various chemicals used to prepare the cementing medium. Various chemicals containing urea and calcium lactate as sources of dissolved inorganic carbon were used to prepare the biocement solutions. Calcium chloride and magnesium chloride were used as sources of calcium and magnesium. The chapter discusses sample preparation and optimisation of MICP solutions for soil

modification through bioaugmentation, biostimulation, and soil injection conditions in columns. The original wind erosion resistance test site is discussed. Methods of assessment and their results are presented using pH and electrical conductivity values, compressive strength tests, wave velocity measurements, and penetration tests. Environmental resistance tests were also carried out for freezing and thawing resistance and wind erosion.

A microstructural analysis was performed using an optical and a scanning microscope and X-ray diffraction. At the end of the chapter, a statistical analysis of the results was conducted.

Chapter 5 - Optimisation of chemical and bacterial densities for bioaugmentation based on MICP covers the analysis and optimisation of the content of biological and chemical components for bioaugmentation based on MICP. The chapter analyses the possibility of optimising the application using a ureolytic approach with non-ureolytic support. The development of bacteria and the concentration of chemical compounds were analysed, as well as the microstructures of non-ureolytic samples.

Chapter 6 – MICP application via biostimulation method covers the presentation of the possibilities of applying MICP using the biostimulation method. The possibility of optimisation using a ureolytic approach with non-ureolytic support was analysed. Changes in pH and electrical conductivity during the MICP process were evaluated, and compressive strength tests and microstructure analysis were performed. The homogeneity of the samples after biostimulation was assessed.

Chapter 7 - Optimisation of injection procedures under sand column conditions with the compressive strength evaluation - contains analyses concerning the optimisation of injection procedures for samples in the form of a sand column with the evaluation of compressive strength. The chapter presents assumptions and results on the impact of flow direction on the compressive strength of cemented samples, as well as on the effects of the incubation period and injection flow rate. The results obtained for the flow rate varied slightly. This indicates that, in this study, the flow rate did not cause any significant difference in the results. Further studies analysed the effect of bacterial density (*Bacillus subtilis*) on the distribution of carbonate precipitation along the sample. They examined the relationship between the number of injection cycles and soil compressive strength. It was found that the increase in the strength of soils treated with MICP was influenced by the cumulative effects of multiple injection cycles. Each cycle allowed for additional precipitation of carbonates, which filled the empty pore spaces and increased the number of contacts between particles. This effect was demonstrated using SEM images. The degree of cementation obtained also influenced the damage mechanisms observed in the samples. After compression and sample destruction, various types of damage were observed, as described in the literature, including delamination, internal damage, and mixed damage. The last group of experiments described in the

chapter was the durability analysis (resistance to freezing and thawing). In summary, the chapter concluded that increasing the number of biocementing solution cycles increased the amount of precipitated carbonate, thereby improving the strength of the tested samples. The injection direction also influenced the uniaxial compressive strength (UCS) values obtained. The incubation time also played a key role – a 2-day incubation period yielded the greatest increase in precipitation, reaching over 80%.

Chapter 8 – **Lab-scale assessment analysis** – contains analyses and assessments at the laboratory sample level. Surface-modified soil samples were subjected to wind erosion and then assessed for strength, thickness of the consolidated surface layer, erosion depth, and carbonate content. The connections – bonds between grains and changes resulting from wind erosion – were analysed. Further tests were laboratory studies of the effect of injection on the change in sample stiffness measured with a penetrometer and changes in wave velocity. In this series of tests, SEM analysis of microstructures was also performed in the context of grain-grain bonds.

The tests confirmed the possibility of using all MICP variants in the actual improvement of soil parameters, taking into account their advantages and limitations. Among the methods evaluated, ureolytic bioaugmentation with non-ureolytic support proved to be the most effective in terms of time efficiency and mechanical performance.

Chapter 9 - **Conclusions and further research** - contains conclusions and future research. The chapter emphasises that the study's hypotheses were confirmed by demonstrating the effectiveness and wide applicability of MICP in soil. The experiments carried out made it possible to achieve the research objectives set out in the study and led to the presentation of a plan for further work.

Further work will include continuing research on the impact of selected combinations of bacterial strains and application procedures on the physical and mechanical properties of soils, including the large-scale use of MICP in the field for slope stabilisation and the assessment of the long-term impact of biological and chemical agents on native microorganisms.

2.2 Detailed justification for meeting the conditions of Article 13 of the Act

2.2.1 Originality of the solution to the scientific problem

In her doctoral thesis, the candidate conducted research related to microbiologically induced carbonate precipitation (MICP) for soil biocementation. The work covers biological and chemical processes that facilitate carbonate precipitation, thereby improving properties by binding grains in the soil matrix. There are two different ways to induce MICP in soil. The first, bioaugmentation, involves introducing foreign bacteria directly into the soil, while the second, biostimulation, optimises the growth environment for bacteria found in native soils. The effectiveness of microbiologically induced carbonate precipitation is influenced by several factors, including soil grain type and size, bacterial strain, optical density, and the source and concentration of metal ions (calcium/magnesium). In addition, the method of applying bacteria and ions plays a significant role in determining the overall success of the biocementation process. The MICP process can be divided into ureolytic and non-ureolytic, depending on the ability of bacteria to break down urea or use other metabolic pathways to produce carbonates. The aim of this study was to investigate the potential of MICP in various geotechnical applications. To optimise bacterial density, chemical composition, and methods for introducing bacteria into the soil, studies were conducted to determine the maximum mechanical properties of biocemented soils. Statistical analyses were performed to evaluate the impact of individual MICP-inducing and stimulating activities on soil strength and carbonate precipitation. Key research methods included microstructure analysis, shear wave velocity (V_s) measurements, and uniaxial compressive strength (UCS) testing, all of which aimed to quantitatively assess the improvements in these parameters achieved through MICP. Various strains of bacteria were used in the studies, as well as solutions containing urea, calcium chloride, and magnesium chloride. The studies were conducted in several ways and with different research plans. Optimisation studies were conducted to determine how changes in the concentration of chemicals and bacteria affect carbonate precipitation, focusing on improving the cementation process through bioaugmentation and biostimulation methods of the ureolytic plan using non-ureolytic support and non-ureolytic solutions. The potential advantages of both techniques and pathways, in particular biostimulation, were investigated in the context of improving the overall efficiency of biocementation.

Another objective of the research was to assess MICP's ability to reduce wind erosion by forming a protective biocemented layer on the soil surface. The study measured parameters such as carbonate precipitation, erosion depth, and layer thickness to assess the effectiveness of this method. Experiments were also conducted on the environmental impact in terms of resistance to freezing and thawing.

The innovative and original nature of the research described in the paper lies in comparing the ureolytic method, supported by non-ureolytic mechanisms, with the non-ureolytic method, tested

both in biostimulation and bioaugmentation approaches, which provides a new perspective on the solutions used and the effectiveness of soil biocementation methods. The results indicate that MICP, implemented using both methods, has the potential to serve as a sustainable, flexible technology for soil improvement, erosion control, and other geotechnical engineering applications.

When scaling up processes for larger applications, such as infrastructure projects, slope stabilisation, and erosion prevention, the technologies, materials, and methods presented and discussed in the paper should be critically analysed, taking into account the results of the studies.

The thesis reviews the basic principles of the MICP method and the impact of several factors on the effectiveness of microbiologically induced carbonate precipitation. The thesis contains original empirical results obtained during the research.

I conclude that the discussed solution is original and meets the requirements of industrial scientific research defined in Article 2(3)(c) of the Act of 30 April 2010 on the principles of financing science (Journal of Laws 2010.95.615, as amended).

2.2.2 Demonstration of general knowledge of the candidate

The paper comprehensively presents the scope of application and wide use of the microbiologically induced carbonate precipitation (MICP) method for soil biocementation. The research methods used effectively enabled the determination of optimal concentrations of chemicals and bacteria to improve the effectiveness of the MICP process and assess the impact of the process on soil parameters.

The paper draws on the author's experience gained in numerous courses related to the research used in the paper, including: Training in XRD (X-ray Diffraction Analysis) and SEM (Scanning Electron Microscopy), Fukuoka, Japan (Kyushu University 2024); Training in Triaxial Apparatus, Lublin, Poland (Lublin University of Technology 2022–2023); Training in microbiology, Warsaw, Poland (Warsaw University 2021-2023)

The candidate's general knowledge allowed her to design a research plan and conduct research on how changes in the concentration of the biocement solution affect the efficiency of MICP and to develop alternative techniques based on biostimulation for the use of the biocement solution by optimising the composition of nutrients targeted at both ureolytic methods with ureolytic carriers and non-ureolytic carriers, as well as non-ureolytic methods. This included an assessment of the impact of environmental factors, in particular pH and electrical conductivity. Another area of work focused on optimising injection procedures for the biocementation process to increase the strength of treated sands. This included investigating how flow directions, injection cycles, incubation time, flow rate, and bacterial density affect the strength and homogeneity of biocemented samples. Another area of general knowledge involved tasks related to evaluating the effectiveness of MICP procedures on

laboratory-scale samples, with particular emphasis on the role of MICP in controlling the effects of wind erosion. The impact of wind erosion on cemented samples was assessed by measuring carbonate precipitation, erosion depth, and composite layer thickness.

The statistical analyses presented indicate the ability to use statistics in scientific work and to evaluate the obtained results.

Of the 203 publications cited in the bibliography, over 150 were published in the last 10 years, indicating that the candidate remains abreast of the latest developments in this field. The entire work is richly illustrated with examples, drawings, tables, photographs, and graphs.

I conclude that the candidate has demonstrated general knowledge of the use of methods for determining biological and chemical composition, microstructure analysis, and changes in the mechanical properties of soil through the formation of bonds between sand grains as a result of MICP. The planning and execution of research to compare the ureolytic method, supported by non-ureolytic mechanisms, with the non-ureolytic method, tested both in a biostimulation and bioaugmentation approach, provides a new perspective on the application and effectiveness of soil biocementation methods, which is a current and important field of science in the area of technical sciences.

2.2.3 Demonstration of the Candidate's ability to conduct independent scientific work

The work focuses on the analysis and wide use of the method of microbiologically induced carbonate precipitation (MICP) for soil biocementation.

The dissertation is both experimental and analytical. To achieve the dissertation's objective, the doctoral student conducted extensive literature reviews and comprehensive, well-documented laboratory research. The research required the creation of dedicated research stations and the use of specialised research equipment. The research plan was primarily based on the assumptions and recommendations of ASTM International and Eurocode standards. The research results were statistically analysed. The analysis of the research results allowed the doctoral student to provide practical recommendations regarding MICP using the ureolytic method, supported by non-ureolytic mechanisms, and the non-ureolytic method, tested both within the framework of the biostimulation and bioaugmentation approaches to the process.

The research methods used effectively allowed the determination of the optimal concentrations of chemicals and bacteria to improve the effectiveness of the MICP process, as well as to determine the impact of the process on the parameters of the soil medium.

By citing 203 sources in her dissertation, the candidate demonstrated her ability to search for relevant scientific literature.

The clarity and conciseness of the thesis chapters demonstrate that the candidate can convey her knowledge.

I believe that the candidate has demonstrated her ability to conduct independent scientific work in her dissertation.

2.2.4 Questions and critical comments

Regardless of the many substantive merits of the dissertation, which I have assessed positively, the candidate has not avoided various shortcomings. I have several questions for the doctoral student, to which I expect answers during the public defence of this work.

There are several minor errors in the thesis, some of which are easy to interpret correctly and do not affect the analysis of the thesis, but some require explanation and comment.

The first question concerns the title of the thesis, which differs in Polish and English. Was this difference intentional or accidental? There are also some imperfections in the Polish and English abstracts. In the Polish version of the abstract, the word "gleba" is used repeatedly, which, according to the reviewer, means "soil". In my opinion, a better term would be "soil", as the doctoral thesis concerns sands and largely belongs to the field of soil mechanics. Another example of imperfection is the literal translation (linguistic calque) of the name of the UCS test, "unconfined compressive strength tests", as "badania wytrzymałości na ściskanie bez ograniczeń (UCS)" (unconfined compressive strength tests). In Polish, this test is known as "badanie wytrzymałości na ściskanie jednoosiowe".

This raises another question: Uniaxial compressive strength testing is not recommended for natural sands because it is not possible to form a sample for this test from natural sands. It can therefore be concluded that the initial parameters of the material selected for testing were not checked until after MICP application. Was this effect achieved in all biocementations carried out, allowing uniaxial compression testing to be performed? Was the sand used for testing sterilised before testing?

The next question concerns shear strength tests. What was their configuration? Were only three samples used in the shear strength tests? Would it have been more appropriate to use a minimum of five?

A critical issue is the place where the samples were formed and the method and manner of placing them in the direct shear apparatus. Please provide information on this subject. Were the parameters of the natural sands used for the tests controlled? Were their parameters, such as cohesion and internal friction angle, determined in their natural state and after the biocementation process? This is particularly important when planning to use biocementation for slope stabilisation, as we need these parameter values to calculate slope stability.

The characteristics of the sands used have been well described, and the distinctive features of the tested material have been presented: grain size distribution curve, density, and minimum and maximum bulk density. But why was the shape of the grains not characterised? For example, based

on an image from an optical microscope or a high-resolution magnified photograph. The author herself notes in the literature review that the shape of the grains influences the formation of the crystal structure.

The thesis lacks information on how statistical calculations were performed, whether manually or using software, and if so, which software. There is no information about the software in the bibliography.

Please supplement this information during the public defence.

2.2.5 Other comments

Comments that do not affect the final assessment of the doctoral dissertation will be communicated to the candidate orally.

2.3 Conclusion

I hereby declare that the thesis by Ms Nadella Marchelina, MSc, entitled: "EXPERIMENTAL STUDIES OF MICROBIOLOGICALLY INDUCED IMPROVEMENT OF FINE SANDS" "EXPERIMENTAL STUDIES OF MICROBIOLOGICAL IMPROVEMENT OF FINE SANDS" meets the requirements of Article 13 of the Act of 14 March 2003 *on academic degrees and titles and degrees and titles in the field of art*, (Journal of Laws No. 65, item 595, as amended).

The doctoral thesis of Ms Nadella Marchelina, MSc, fully meets the conditions set for applicants for the degree of Doctor of Technical Sciences in *the Discipline of Civil Engineering, Geodesy and Transport*, and I hereby request that it be admitted for public defence.



Pawel Popielski