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Review of the Doctoral Thesis

titled

Contribution of denitrifying Poliphosphate Acumulating Organisms to the Enhanced Biological Phosphorous Removal Process and Nitrous (iv)oxide Gas Emissions

written by mgr inż. Jeremiah Abong'o Otieno

I. The characteristic of the thesis

The thesis composition is typical of experimental works and includes all the required chapters for this type of work. It begins with an Introduction, followed by a Literature Review, Materials and Methods, Results, and Conclusions. The thesis also includes the following necessary components as an Abstract and a Summary, both in Polish and English, as well as a List of Figures, Tables, Symbols, and Abbreviations. At the end of the thesis, the References and Appendices are placed.

II. The thesis subject and its importance

Enhanced biological phosphorus removal (EBPR) is the subject of this thesis. This process is widely used in treatment plants to remove phosphorus from treated wastewater. Its effectiveness is vital for protecting water bodies from eutrophication. Although the EBPR process has long been known and applied in practice, its microbial and biochemical aspects remain incompletely understood. Mr Jeremiah Otieno, in his thesis, focused on one of the bacterial groups involved in the EBPR process, which can remove phosphorus and nitrogen simultaneously. This group of bacteria is particularly interesting because it facilitates phosphorus removal during denitrification, thereby reducing the need for aeration and, consequently, the costs of wastewater treatment. In the thesis, the author paid particular attention to *Tetrasphaera* bacteria, which are denitrifiers capable of taking up phosphorus. As the aim of the thesis, the author decided to verify the usefulness of glucose as a substrate and N_2O production during an anoxic phosphorus uptake by *Tetrasphaera*-rich activated sludge.

As the costs of aeration continue to rise at wastewater treatment plants, research focused on bacteria capable of taking up phosphorus and, in parallel, reducing nitrogen oxides to N_2 is justified. They can result not only in new knowledge about bacterial metabolism but also in improved energy efficiency and economic results of wastewater treatment. Therefore, I consider the thesis topic valid and essential to scientific and engineering practice.

III. Values of the thesis

The knowledge about *Tetrasphaera* bacteria behavior within the enhanced biological phosphorus removal is not fully understood. Therefore, the research on EBPR process in the *Tetrasphaera*-rich activated sludge is valuable. In his literature review, Mr Jeremiah Otieno presented the latest research on microorganisms active in the EBPR process, with

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particular attention to those capable of denitrification and simultaneous phosphorus uptake. He thoroughly analysed the impact of technological parameters, including temperature, pH, microbial respiration conditions, and the quality of the treated wastewater. He also considered the quality of the treated wastewater, the efficiency of the EBPR process, and nitrous oxide emissions. This demonstrated his thorough understanding of the current state of knowledge regarding the EBPR process.

Mr Jeremiah Otieno decided to examine the influence of different substrates – acetate, glucose, nitrates and nitrites on EBPR process effectiveness. The special stress has been put on N_2O emission from the *Tetrasphaera*-rich activated sludge during the simultaneous enhanced phosphorus uptake and denitrification. This aspect of the research presented in the PhD thesis is especially worth underlining.

IV. Comments

The reviewed thesis has been written clearly. The thesis objectives and results have been presented logically, supporting the conclusion that the Candidate is well prepared to conduct the research independently. However, the content of the work raises certain observations and comments, which are presented below in the order in which they appear in the work:

Introduction

1.1 Background, page 39, Figure 1-1 (A) – The scheme of the conventional metabolic pathway in EBPR is misleading. It suggests PO_4^{3-} release in oxygen conditions.

2. Literature review

The literature review section lacks even a brief description of currently used integrated C, N, and P removal systems, for example, those protecting the anaerobic zone from nitrates present in recirculated sludge. Especially since the Author frequently writes about the dependence of P removal efficiency on technological parameters and the technological system used.

2.3.2 Mechanism of EBPR, page 55, Figure 2-8 – The wrong electron acceptor is presented for OHO in the aerobic conditions.

3. Material and Methods

3.1 Preliminary study and selection of case study WWTP

Why was the abundance of DPAOs examined only in relation to ordinary heterotrophic microorganisms (OHOs), not in relation to all PAOs?

3.2 Dębogórze WWTP – case study facility

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On page 82 the Author identified Dębogórze WWTP as employing the Johannesburg (JHB) process but on page 84 BARDENPHO process is mentioned. It needs to be clarified.

3.5 Experimental series I

What was the place of activated sludge sampling at WWTP? Was the concentration of nitrate nitrogen measured in the activated sludge samples taken for the tests?

Page 93, Table 3-4 – The COD limiting conditions (COD:P < 2.5) in Scenario 1.1 should be clarified. Table 3-4 shows much higher COD:P ratio than 2.5. COD = 195 mg/L and P = 3.9 mg/L give ratio equal to 50. COD:P > 2.5 is also in Table 4-1 presented in the chapter Results (page 118).

3.6.1 The anaerobic phase test in the CCT

Was soluble PO_4^{3-} measured after the overnight mixing? A further PO_4^{3-} release can be expected in these circumstances.

3.6.2.2 Scenario 2.2: PO_4^{3-} precipitated anoxic conditions

The title does not fit the text of this section. Details describing the phosphates' precipitation are missing. Scenario 2.2 and 2.3 presented on pages 101 and 102 do not fit the scheme presented on page 110.

4. Results

The calculations of the NUR and NiUR correlations should be explained. What is the meaning of these correlations for the Author?

4.2.1 Scenario 1.1 – COD-limiting conditions

Page 120 – The comments for Figure 4-3 in comparison with Figure 4-5 are obvious. If the intention is to promote anoxic P uptake, the anaerobic period should be long enough to ensure maximal COD uptake, thereby preventing uptake during the anoxic phase by ordinary denitrifiers and by aerobic PAOs responsible for further P release.

4.2.2.1 Phosphate release and uptake rates

Page 125 – When commenting on COD consumption during the anoxic phase, aerobic PAOs should also be considered. During the first 3.5 or 5.5 hours of the anoxic phase, P release is observed to some extent. Aerobic PAOs may be responsible for this phenomenon because they can take up COD, and their metabolism is blocked.

4.3.2.2 Scenario II: PO_4-P anoxic precipitated condition

Page 139 – The conclusion that NO_2^- is a more effective electron acceptor than NO_3^- can't be accepted. NO_2^- isn't a more effective electron acceptor than NO_3^- because NO_2^- can take only 3 electrons and NO_3^- can accept 5 electrons.

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Moreover, the conclusions drawn so far can't be based on a single test. The results of the trial ST2 have not been discussed at all.

4.4.1 N_2O production under COD non-limiting conditions: Series I

Page 145 - Analysing the results presented in Figure 4-15, the Author concluded that nitrates, as electron acceptors, ensure complete denitrification. However, the concentration of nitrites wasn't discussed in this case. Was the concentration of nitrites measured within the test?

Less significant flaws and mistakes, such as discrepancies between the data cited in the text and that presented in the Tables and Figures, as well as incorrect numbers of Tables and Figures, have been marked directly in the thesis for the Author.

V. Final Conclusion

In his thesis, Mr Jeremiah Abong'o Otieno demonstrated his ability to formulate scientific problems and solve them using proper methodology. His research deepened the understanding of the *Tetrasphaera*-rich activated sludge's ability to denitrify and simultaneously remove biological phosphorus. Therefore, in my opinion the reviewed thesis titled *"Contribution of denitrifying Poliphosphate Acumulating Organisms to the Enhanced Biological Phosphorous Removal Process and Nitrous (iv)oxide Gas Emissions* fulfills the requirements of *the Law on higher education and science* dated 20th of July 2018 (Journal of Laws 2024, item 1571, as amended) and concluding I propose to admit it to public defense.

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