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Review of the Doctoral Dissertation of Jeremiah Abong'o Otieno

"Contribution of denitrifying polyphosphate-accumulating organisms to the enhanced biological phosphorus removal process and nitrous oxide (N_2O) gas emissions"

This dissertation addresses an important problem in biological wastewater treatment: the role of denitrifying polyphosphate-accumulating organisms in enhanced biological phosphorus removal, and the extent to which operating conditions that support denitrifying phosphorus removal may also promote nitrous oxide accumulation. The topic is timely, relevant, and well worth studying. The central question is clear throughout the dissertation: whether glucose, relative to acetate, can serve as an effective carbon source for DPAO-driven EBPR in *Tetrasphaera*-rich sludge under nitrate- and nitrite-based anoxic conditions, and how those conditions shape phosphorus removal, denitrification, and N_2O behavior. The dissertation is organized in a conventional and coherent way, moving from introduction and literature review to methods, results, discussion, conclusions, and future work. That overall structure serves the work well.

My overall view is positive. I think this is a good dissertation and a worthwhile contribution. The author has identified a real gap in literature, designed a coherent set of experiments, and produced several findings that are technically useful and practically relevant. I found the acetate-versus-glucose comparison convincing, the nitrate-versus-nitrite contrast especially clear, and the modified anoxic experiments in Series II more thoughtful than one often sees in a dissertation of this kind. At the same time, the dissertation has several weaknesses that should be considered in its evaluation, including some interpretive overreach, a few internal inconsistencies, and uneven editorial polish. Some of the interpretation reaches a bit beyond what the data can directly support, a few of the conclusions need to be tightened for internal consistency, and the document still needs editorial cleanup before final deposit. None of that undermines the core scientific value of the work, but it does matter for the final version.

One of the stronger aspects of the dissertation is the way the study was put together. The author did not jump directly into a single batch experiment with a single plant and finished the story there. Instead, the dissertation begins with screening four full-scale WWTPs in the Pomeranian region, identifies the plant with the strongest apparent *Tetrasphaera* enrichment, and then uses that system as the basis for the more detailed experimental work. That gives the project a logical progression and makes the choice of the GD plant feel justified rather than arbitrary. I appreciated that. The experimental design then develops further in a useful way: first, a comparison of carbon sources and electron acceptors under more standard conditions, and then a second series of modified anoxic tests intended to probe the role of phosphate availability and intracellular storage compounds. That progression is one of the strengths of the dissertation.

The clearest substantive result, in my opinion, is the acetate-versus-glucose story. Across the dissertation, acetate consistently supports much stronger EBPR-relevant behavior than glucose. That pattern shows up in phosphorus release and uptake, in denitrification-linked behavior, and in the PHA results. The author reports, both in the abstract and later in the dissertation, that acetate can produce PRR values up to 25 times higher than glucose and that acetate paired with nitrate was the most favorable condition for DPAO activity. I found that conclusion well supported by the data presented. Whatever one may want to say about glucose as a potential substrate in some *Tetrasphaera*-rich systems, this dissertation makes a strong case that, in the biomass studied here and under the conditions tested, glucose did not perform nearly as well as acetate in supporting classical EBPR behavior. In my opinion that is a useful and convincing result.

The N_2O results are also one of the strongest parts of the dissertation. Here the dissertation is at its clearest. Under COD non-limiting conditions in Series I, nitrate produced essentially no N_2O accumulation, while nitrite produced clear and sustained N_2O accumulation, especially in the glucose-fed case. The difference between the acetate- NO_2^- and glucose- NO_2^- cases is particularly striking, with the glucose-fed system showing a much more prolonged buildup of N_2O. The later discussion and conclusion sections reinforce the same point: nitrate was comparatively less impactful from an N_2O standpoint, whereas nitrite was the main condition associated with elevated N_2O risk, and full-dose nitrite produced worse outcomes than pulse dosing. That is a strong operational message, and I think it is one of the most valuable takeaways of the dissertation.

I also think the Series II experiments add real value. The use of phosphate precipitation and PHA depletion to disturb the system and infer the importance of DPAO-linked metabolism is a thoughtful idea. Even if the resulting partitioning between DPAO-associated and ordinary heterotrophic denitrification remains indirect, the approach moves beyond simple rate comparison and adds a more mechanistic dimension to the work. The author's conclusion that PHA depletion more severely disrupts denitrification-linked phosphorus behavior than phosphate precipitation is especially interesting, because it highlights the importance of internal reserves in these systems. The estimated DPAO/OHO shares presented in the conclusions are useful as working interpretations of the tested conditions, even if they should not be read as definitive ecological fractions.

That said, I do have various reservations, and I think they should be addressed more clearly in the final version. The first one is generalizability. The dissertation does include an initial four-plant screening, which is helpful, but the core experimental claims are still based on batch tests using sludge from a single case-study plant. That is perfectly acceptable for a dissertation, but it does place limits on how broadly the findings should be stated. At a few points, the writing becomes slightly more general than the evidence really allows. I would encourage the author to frame the main conclusions more clearly as conclusions supported by a carefully chosen case-study system, rather than as general rules for all EBPR systems.

My second concern is the attribution of process behavior to particular microbial groups. This is probably the main scientific reservation I have. The dissertation often discusses results in terms of DPAOs versus OHOs, and at times in terms of *Tetrasphaera* versus *Accumulibacter*-type behavior. In principle that is reasonable, since the work is about DPAOs and the microbial characterization is an important part of the dissertation. Still, most of the organism-level interpretation remains indirect. The evidence comes from mixed-community batch tests, carbon-source responses, reserve manipulations, and community composition, not from direct activity-resolved measurements of which organisms were actually carrying out each step at each time. This matters especially in the N_2O discussion. I think the process-level conclusion, that glucose-fed, nitrite-stressed conditions were associated with worse N_2O behavior, is well supported. What I am less convinced by is the stronger implication that *Tetrasphaera* specifically was the dominant mechanistic reason for that outcome. For example, in the discussion the dissertation suggests that *Tetrasphaera*'s metabolic profile and ability to use glucose may explain the higher N_2O observed in glucose-fed NO_2^- systems. That is certainly plausible, but the dissertation does not demonstrate it directly, and I would like to see the wording made a little more cautious there. More broadly, the work supports a mixed-community process interpretation more strongly than a *Tetrasphaera*-specific mechanistic claim.

A related issue is that the dissertation sometimes treats *Tetrasphaera* abundance as though it were very close to *Tetrasphaera* functional dominance. I understand why the author does this, given the focus of the work and the way the case-study plant was selected, but those are not the same thing. Abundance data is useful and relevant, yet they do not prove that the later process behavior, especially glucose response and N_2O accumulation, was primarily driven by *Tetrasphaera* rather than by a broader mixed heterotrophic community. I would not remove those interpretations, but I would soften them.

Another issue that stood out to me is internal consistency, especially in the conclusion section. The main example is the COD:P interpretation. In one place the dissertation concludes that COD:P below 2.5 enhanced DPAO activity and reduced N_2O production, while in another place it concludes that non-limiting COD:P conditions provided sufficient carbon for complete denitrification and reduced N_2O accumulation. As written, those two claims sit uneasily together. I think the discussion chapter actually handles this better than the conclusions do. In the discussion chapter, the argument is more nuanced: lower COD:P may help stabilize EBPR and suppress unwanted competition, but overly low carbon can also limit complete denitrification. That is a more defensible interpretation, and I think the conclusions should be revised to reflect that nuance more closely. This is not a minor stylistic issue; it affects one of the central practical messages of the dissertation.

I also think the abundance narrative needs tightening. The dissertation uses several different abundance values in different contexts, the four-plant screening, the GD case-study community, and the final summary statements, but does not always distinguish clearly which number belongs to which dataset. For example, the four-plant screening identifies GD at 12.4%, while the GD case-study community discussion gives overall PAO/DPAO abundance as 9.4% ± 0.02%, and then the conclusions return to 12.4% as though it were the definitive overall number. These values may all be defensible in context, but the contexts need to be separated much more clearly. As written, the reader has to work too hard to keep them straight.

In addition to those scientific issues, the dissertation still needs a fair amount of editorial attention. There are unresolved placeholders in the front matter, including supervisor information and the defense date. Some terminology is unusual, especially the repeated use of "nitrous (iv) oxide," which is not standard phrasing for this field. The front matter also includes duplicated abbreviations, conflicting abbreviation definitions, and figure/table labeling problems. There is also some confusion early in the dissertation about whether community analysis was performed using PCR-DGGE or high-throughput 16S rDNA sequencing. These are not trivial matters in a final doctoral document. They do not detract from the scientific core of the work, but they do create the impression that the dissertation was submitted before the final round of careful proofreading and standardization.

Despite the concerns noted above, my overall assessment is positive. This dissertation addresses an important and timely topic, presents a coherent experimental program, and offers several findings that are relevant to EBPR research and practice. In particular, the acetate-versus-glucose comparison, the clear distinction between nitrate and nitrite with respect to N_2O accumulation, and the modified anoxic experiments in Series II represent worthwhile contributions.

My view is that the dissertation is suitable for doctoral examination. Its main weaknesses are the indirect nature of some organism-level interpretations, the need for greater internal consistency in the COD:P and abundance narratives, and the uneven editorial quality of the document. These issues do not outweigh the scientific value of the work, but they should be taken into account in the overall evaluation.

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