

1.02. 238/W/11/2026



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DATE
25 March 2026

SUBJECT
PhD dissertation evaluation

Title of the dissertation: Contribution of denitrifying polyphosphate-accumulating organisms to the enhanced biological phosphorus removal process and nitrous (iv) oxide (N₂O) gas emissions.

Author of the dissertation: Jeremiah Abong'o Otieno

Name of the reviewer: Jan Dries, Faculty of Applied Engineering, University of Antwerp, Belgium

1. Short summary of the thesis

The thesis studies the role of denitrifying polyphosphate-accumulating organisms (DPAOs) in the enhanced biological phosphorus removal (EBPR) process and the associated nitrous oxide (N₂O) emissions. More specifically, the impact of two different external carbon sources (acetate vs. glucose) at different COD/P ratios is evaluated, using either nitrate (NO₃) or nitrite (NO₂) as terminal electron acceptors.

The main results are:

- DPAO activity was significantly higher with acetate as carbons source
- Both nitrate and nitrite could be used by DPAO for EBPR
- N₂O emissions were mostly associated with nitrite as electron acceptor

2. Importance and relevance of the thesis

The research topic is highly relevant to the field of environmental (bio)technology as the EBPR process is gaining global interest as a sustainable alternative to conventional chemical phosphorus removal. The thesis increases our knowledge of this fascinating microbial process by assessing the impact of alternative carbon sources. In addition, DPAO combine phosphate removal with denitrification, using storage compounds to drive nitrate (or nitrite) removal. The thesis contributes to our knowledge about the potential role of DPAO in N₂O formation and emission during biological nitrogen removal.

3. Evaluation

The thesis is well written and structured. There is a clear narrative, and the experiments are well designed. The tests using precipitated P and PHA depleted conditions, are very clever, and could help differentiate different mechanisms. Experimental results mostly support the conclusions.

The dissertation would profit from a deeper discussion on some of the mechanistic aspects (as indicated in the following sections), and a better motivation of some experimental settings.

4. Original contribution to the Field

The EBPR process potentially represents a sustainable alternative for current P-removal technologies. Full development of the biological P-removal process requires a deep understanding of the sustainability of this alternative technology. The focus of the dissertation is on the emission of greenhouse gases, especially nitrous oxide (N₂O) during simultaneous nitrogen and phosphorus removal during EBPR. The results of the work extend the current knowledge in this particular research field, and will contribute to defining potential nitrous oxide emission mitigation.

5. Structure and presentation of the thesis

As mentioned above, the thesis is well structured, and follows a clear and logical flow. The graphs, figures and tables are clearly presented, and support the discussion. There are a limited number of orthographic and style errors (see below).

P49, lines 1-3	Sentence is wrong
P62, lines 19-20	Something is missing in the sentence (presence of what ?)
P75, lines 5-7	Please correct: DO/NO ₂ /NO ₃ cannot compete with PAO (how can an electron acceptor compete with an organism?)
P77, lines 5-6	5 genera of what ?
P101, title 3.6.2.2.	The title does not correspond with the content
P118, table 4-2	NuiR should be NiUR
P121, Fig. 4-4	"glucose" is missing from the figure title
P171, lines 16-17	Something missing in the sentence

6. Critical remarks and questions

INTRODUCTION	
P35	Please explain how DPAO can reduce aeration costs and carbon limitations? "address 50% of carbon limitations": what does that mean?
P36	"N ₂ O as an intermediate during denitrification contributes to 3.3-10% of total N ₂ O emissions in WWTPs"; what are the main/other contributors?
P41	Two COD/P ratios are investigated: COD/P<2.5 and COD/P>2.5. it is unclear how these ratios are defined. Influent COD to influent P? please motivate this specific ratio. how does these ratios compare to the COD/P value of "20" generally defined as design value for EBPR?

LITERATURE REVIEW	
P47	Thermal treatment. What do you mean? For P-recovery?
P50	Fig. 2-4: sulphuric acid is dosed for sludge digestion, and then NaOH for struvite (by the way: not Na(OH) ₂): these pH correction make little sense? Please check
P51	"Anaerobic/aerobic increases costs related to energy consumption": compared to what?
P59	Fig. 2-7, and, in general for the thesis: how do you make the distinction between "regular" PAO and DPAO?
P61	"Tetrasphaera store glycogen and other energy reserves": which other reserves?
P63	First line: DPAO can mitigate this impact: please explain how
P65	"reduced ability of DPAO to metabolize intracellular carbon sources": please explain what you mean
P70	"high DO negatively impacts EPBR": please explain the mechanism
P72	Fig. 2-10: please explain what this figure shows
P74	Please explain the mechanism of nitrite inhibition
P75	Please explain the mechanism behind excessive aeration promoting GAO
P78-79	this section should discuss (1) the factors influencing N ₂ O emissions, and (2) the (potential) contribution of DPAO and DGAO to N ₂ O emissions

MATERIAL AND METHODS	
P82	How do you specifically detect DPAO using sequencing? Please explain in more detail
P91	Please motivate the COD/P ratio of 2.5
P91	All experiments used a pH of 7.0; does this not support GAO?
P94	Table 3-4: what do you mean with "equivalent mass", and why is it expressed as mg/L (that's a concentration, not a mass); what is the difference with the COD concentration in the last row?; a COD of 1501-198 mg/L and a PO4-P of 1.8-4.9 mg/L does not result in a COD/P < 2.5

RESULTS	
P118	Table 4-1; the table states "COD anaerobic" and gives "rates" (mg COD/gMLVSS.h); however, on
P119	P119, the same value (e.g. 32.1) is expressed as "%" ... please correct
P120	Fig. 4-3 and others: how did you calculate the rates (PUR, PRR, NUR...): over the entire period, or just the linear part?
P121	Fig. 4-4; during the anaerobic phase, the COD drops from "200" to "100" mg/L and less. What is the fate of glucose?
P124	What do you mean with the "COD removal coefficient"?
P124	Fig. 4-5; COD removal under anaerobic conditions is different in panel a vs. b: please explain
P125	Lines 3-5: "higher COD consumption rates account for up <4% compared to..." unclear what you want to say; in addition (last lines): please elaborate the "competition between HET and DPAO under glucose-rich conditions": competition for what? How?
P126	Fig 4-7... this is with acetate, right? (not with glucose)? Please correct
P131	Line 12: which "ratio" are you referring to?; how do you know PP is depleted?; I don't understand the sentence about GAO competition (lines 13-14)
P133	Unclear what you mean with "microbial share contribution to P-removal relative to denitrification rates"
P137	Fig. 4-12 & Fig. 4-13: the NO _x -rate does not change upon addition of glucose: please explain why
P140	not!
P138	If there is no DPAO activity (P is precipitated), how can DPAO be more "efficient"?
P143	How did you estimate the microbial group contribution? Where do we find the HET activity? Explain in more detail
P146	In these experiments, in what form is the carbon: stored PHA, acetate, glucose?
P146	The text mentions peaks at 2.9 mg N/L, but the figure (4-16b) does not go that high: please explain
P147	"the presence of NO ₂ and high C, may lead to incomplete denitrification": please explain the mechanism, because this seems very strange

Figs 4-15 & 4-16	Please show NH ₄ , NO ₂ and NO ₃ profiles as well to give a complete overview (also for other N ₂ O figures)
P150	Fig. 4-17a shows a rapid decline in N ₂ O... this seems like an experimental error?, if not: please explain
P153-158	The discussion requires NO _x data

DISCUSSION	
P164	Low abundance of Accumulibacter: the discussion should include sequencing bias
P169	How is glucose a substrate for GAO: what is the metabolism?
P170	About PO ₄ -P release in the anaerobic phase, under COD non-limiting conditions: in the presence of COD and NO _x , how can DPAO compete with HET? Line 9: the outcome seems to contradict this statement about high COD impairing DPAO carbon storage?
P171-172	These conclusions seem wrong? PO ₄ -precipitated conditions did not lead to increased NUR?!
P174	The PHA profiles show little change during the anoxic phases, please discuss in more detail (also in comparison to NO _x profiles)

7. Final conclusion

The dissertation deals with an important aspect of wastewater treatment and contributes to extending our knowledge of the EPBR process, especially regarding the role of DPAO in production and emission of the potent greenhouse gas nitrous oxide. This is done in a clear and well-structured way, with a solid research approach and results that support the conclusions. Although the discussion of the results could go deeper into more mechanistic aspects in some occasions, this is a solid dissertation.

Jan Dries



