Effects of different operational conditions on the microbial community in laboratory-scale phosphorus biological removal reactors

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Introduction

Phosphorus (P) is a fundamental fertilizer in food production, but it also represents a major environmental problem as it is a main driver of water eutrophication. Therefore, it requires removal from wastewater prior to disposal. The enhanced biological phosphorus removal (EBPR) process based on the capability of certain bacteria to store large amounts of intracellular phosphate (polyphosphate accumulating organisms, PAO), has been widely applied in different countries to remove and recover P from wastewater. However, this technology has not yet been implemented in Uruguay. The aim of this study was to evaluate changes in the microbial community within lab-scale reactors for biological P removal, operated under different conditions: different activated sludge (AS) sources from Uruguayan industrial wastewater treatment plants (WWTP) as inoculum, different feeding strategies (synthetic or industrial effluent), bioaugmentation with *Tetrasphaera* and different durations of anaerobic/aerobic stages.

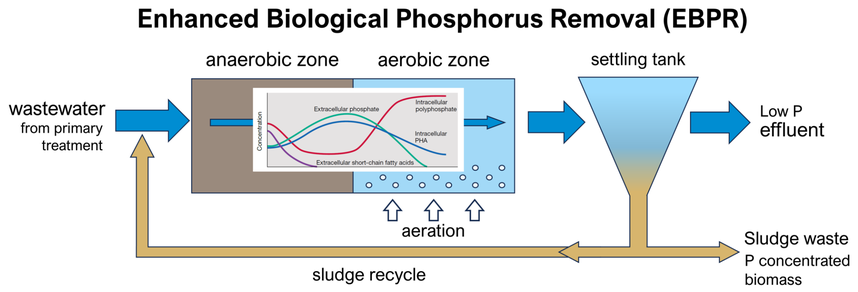


Figure 1. EBPR process. During the passage of wastewater through the reactor system, the microbial community transitions from anaerobic to aerobic growth. Anaerobic zone: short-chain fatty acids are taken up and internal stores of polyP are released as extracellular orthophosphate. Aerobic zone, the extracellular phosphate is reassimilated as polyP and the intracellular stores of polyhydroxyalkanoates (PHAs) are metabolized. High-P sludge is harvested for disposal.

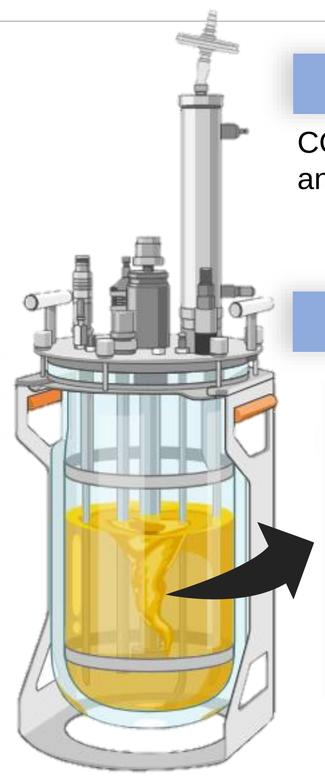
Materials and methods

SBR operation for biological phosphorus removal

Table 1. Conditions tested in the operations of lab-scale reactors

Condition	Inoculum	Feed	Bioaugmentation	Cycle
C1	AS_Mal1 / AS_AviF / AS_Ali1	AcGlu	no	short
C2	AS_Ali2	AcProp / E_AviF	no	short
C3	B_C2	E_AviF	Tet / NoTet	long
C4_1	AS_Ali2	AcProp	no	short
C4_2	AS_Ali2	AcProp	no	long
C4_3	AS_Mal2	AcProp	no	long

Inoculum - AS: activated sludge from industrial WWTPs. Mal: malting plant, AviF: poultry industry, Ali: food processing industry. B_C2: biomass obtained in C2. **Feed -** PT 20mg P/L (C:P = 20:1, 42% KH_2PO_4, 58% K_2HPO_4) and 40mg N/L (C:N = 10:1, NH_4Cl); AcGlu: COD 400mg/L, 75% Acetate + 25% Glucose; AcProp: COD 800 mg/L, 50% Acetate + 50% Propionate; E_AviF: poultry industry wastewater; **Cycle - Anaerobic (An) / Aerobic (A) -** short: total cycle of 6h, 2:10h An / 2:30h A; long: total cycle of 8h, 3:35h An / 3:15h A

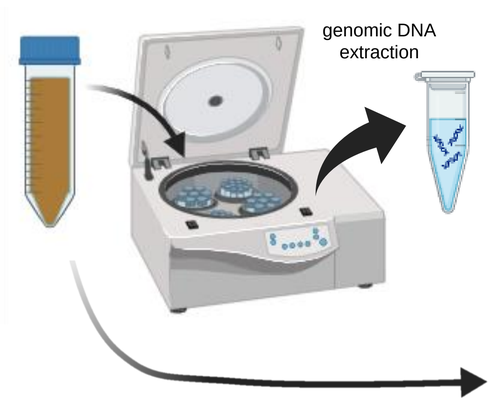


Physicochemical monitoring of reactors

COD, PO_4^{3-} and NH_4^+ in feed, beginning of An stage, beginning of A stage and discharge.

% P removal = [(P feed – P discharge) / P feed] x 100

Microbial communities monitoring of reactors



16S rRNA amplicon sequencing
Ion GeneStudio™ S5 System, IIBCE platform, V4 region, 520F/ 802R
Data analysis: QIIME2 2023.7, R with Rstudio, DADA2, taxonomic assignments with MiDAS 5.3

Confocal RAMAN Microscopy
Alpha 300 RA WITec Raman Microscope
λ = 532 nm excitation laser,
100X objective.

Results

Biological phosphorus removal, microbial community and putative PAOs

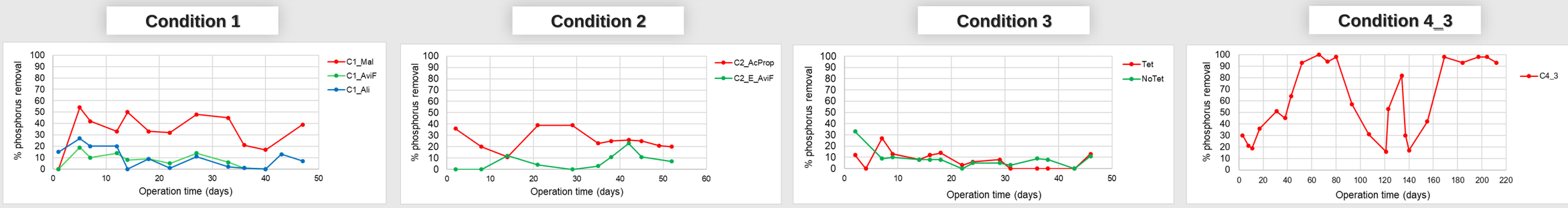


Figure 2. Percentages of biological phosphorus removal throughout the days of operation of the reactors in the different operating conditions

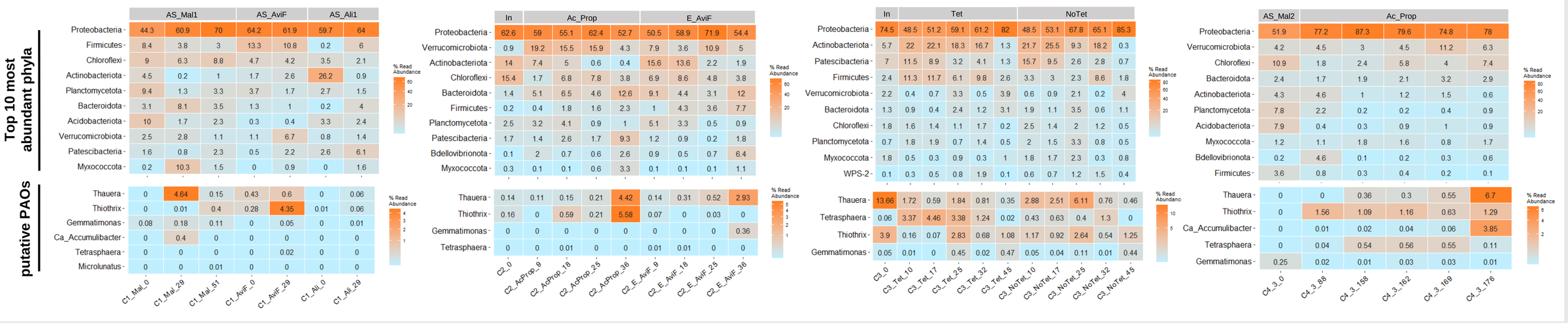


Figure 3. Heatmaps showing the evolution of relative abundances of the 10 most abundant phyla and putative PAOs at genus level in the reactors operated under different conditions

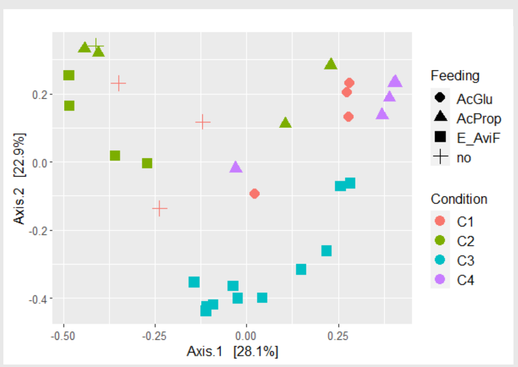


Figure 4. PCoA of the structure of the bacterial communities present in the reactors operated under different conditions.
Distance: bray.

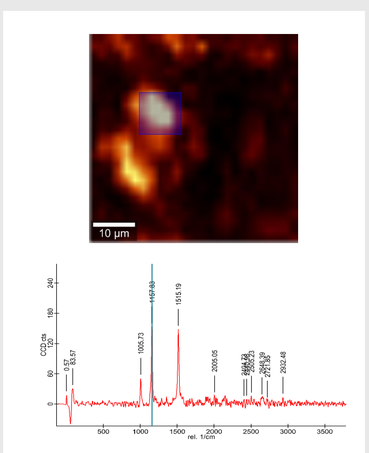


Figure 5. Raman image of the spatial distribution of polyP inclusions within individual cells of sample C4_3_176. The image was reconstructed from Raman intensities of polyP between 1140 and 1180 cm^{-1}, showing the polyP distribution in yellow. The spectrum below is representative of the yellow regions.

Conclusions

- The structure of the microbial communities established in the reactors changed with the operating conditions evaluated, particularly with variation in feed composition.
- Using AS from a malting industry WWTP as inoculum and synthetic feed with acetate and propionate (C4_3) it was possible to achieve a complete biological P removal, confirmed by RAMAN microspectroscopy detecting intracellular polyP accumulation. Within the putative PAOs, an increase in the abundance of *Ca*. Accumulibacter observed after 176 days of operation could explain this.
- This PAO-enriched biomass will allow us to conduct future assessments, thereby driving the local development of EBPR technology.