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A novel single-cell tool for absolute *in situ* quantification of intracellular poly-P and other storage polymers in wastewater systems key organisms

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Background and Aim

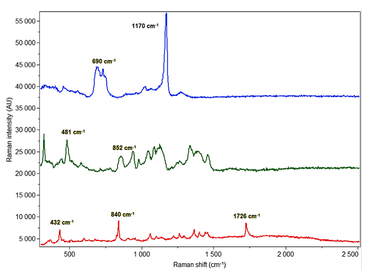
Enhanced Biological Phosphorus Removal (EBPR) is a process widely applied in wastewater treatment and relies on the ability of some microorganisms to store phosphate intracellularly. Among them, *Candidatus* Accumulibacter and *Tetrasphaera* are found worldwide, but more insights are needed on their individual contribution to the EBPR process. **This study aims** to develop a novel approach, which combines Fluorescence *in situ* *H*ybridization (FISH) and Raman microspectroscopy, to provide *in situ* and absolute quantification of intracellular storage compounds relevant for the EBPR process.

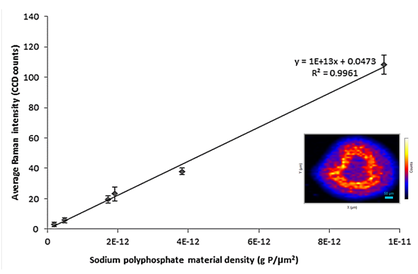
Conclusions

- *Ca.* Accumulibacter is capable to store up to 3 times more than *Tetrasphaera*. However, *Tetrasphaera* cells are more abundant in some Danish EBPR plants and therefore, both genera appear to be equally important for the EBPR process.
- This novel approach provides a powerful tool for microbial ecologists and can be applied to quantify storage compounds in other microbial systems.

Methods

Raman microspectroscopy and calibration of the instrument



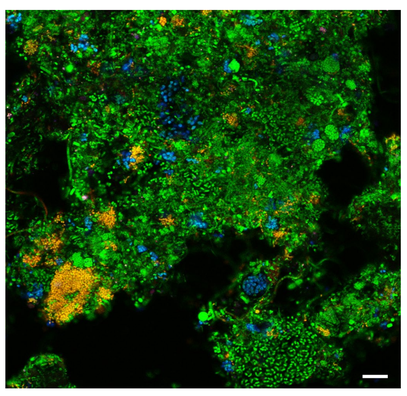


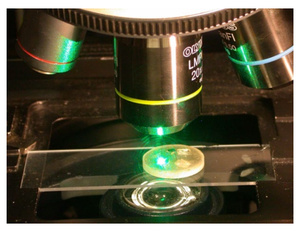
Validation of the quantification method → P-uptake/release experiment with *Tetrasphaera elongata* and activated sludge

Quantification *in situ* using FISH-Raman

Samples were obtained from 8 different EBPR plants at different process stages.

FISH was performed using the probes PAO651 and Actino658 for *Ca.* Accumulibacter and *Tetrasphaera,* respectively.



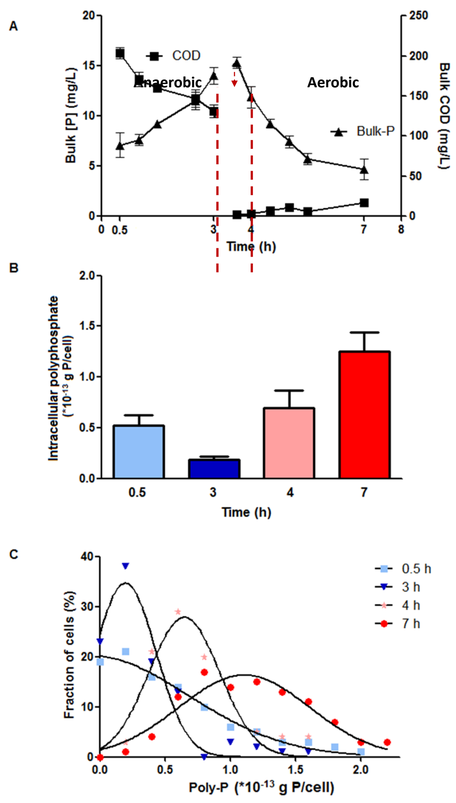


FISH-positive cells were bleached and Raman spectra were recorded from the target cells.

$$[\text{Poly-P}] = k^* \Sigma S^* A_{cell}$$

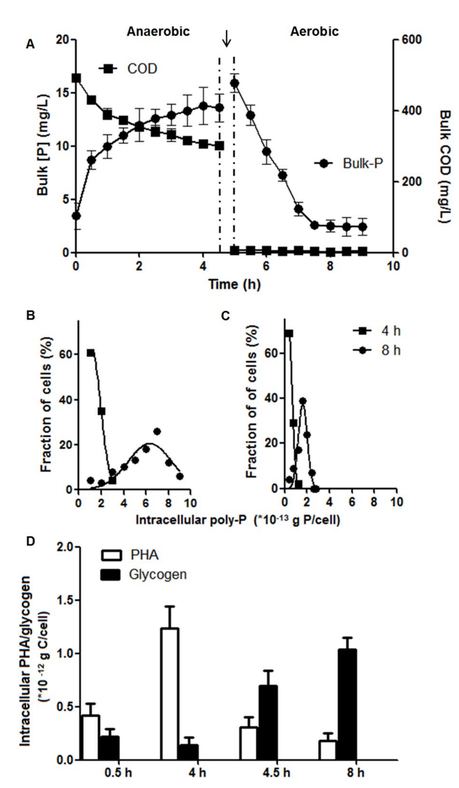
Lab-scale P-uptake/release experiment confirms dynamic intracellular-P behaviour

T. elongata



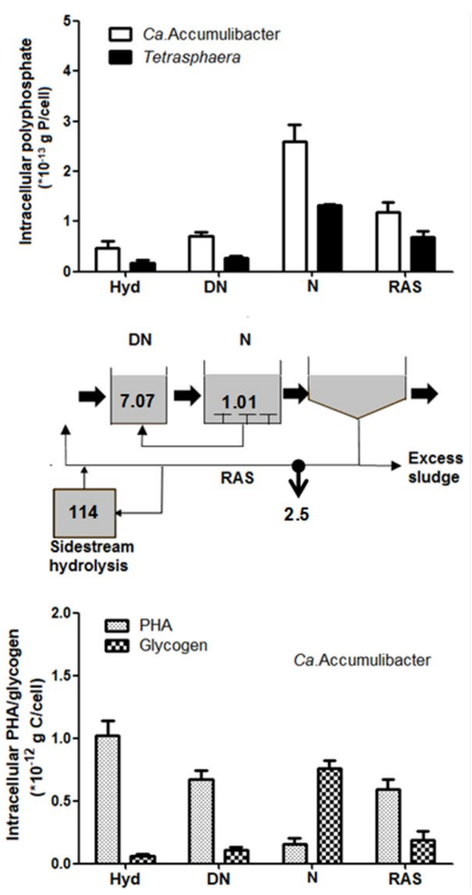
The fluctuations of ortho-P concentration in the bulk medium (A) reflected the Raman-based quantifications of intracellular poly-P content (B-C).

Activated sludge

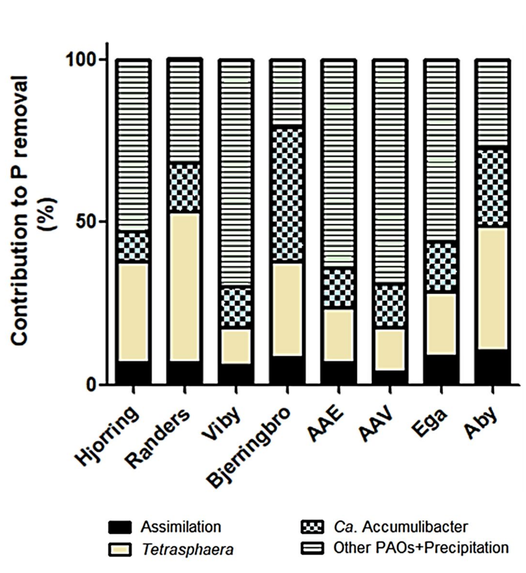


The dynamics of the feast-famine experiment with activated sludge (A) were corroborated by the changes in the storage polymers in *Ca.* Accumulibacter (B and D) and *Tetrasphaera* (C).

In situ quantification of storage compounds in full-scale EBPR plants



Surprisingly, no PHA or glycogen were found in *Tetrasphaera* and, in most of the plants, its contribution to the total P-removal was higher than that of *Ca.* Accumulibacter. However, they are both key organisms for the EBPR process, storing together up to 70% of the total P present.



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