# [[1]](#footnote-1)Intracellular accumulation of acetate in *Tetrasphaera elongata* and activated sludge during dynamic anaerobic-aerobic conditions

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Introduction.

It has now become clear from various analyses that *Tetrasphaera*-related polyphosphate accumulating organisms (PAOs) play important roles in Enhanced Biological Phosphorus Removal (EBPR) plants. They are present in very high abundances (up to 30% of the total bacterial biovolume, Kong et al., 2005; Nguyen et al., 2011), and have a physiology that is markedly different from the model PAOs, *Ca*. Accumulibacter. PHA is not an important storage polymer for *Tetrasphaera*-related PAOs. Instead, glycogen and monomeric substrates such as glycine are probably significant storage compounds. The metabolic model details that *Tetrasphaera*-related PAOs take up glucose anaerobically and ferment this to succinate and other components. They also synthesize glycogen as a storage polymer, using energy generated from substrate fermentation and degradation of stored polyphosphate. During the aerobic phase, the stored glycogen is catabolized to provide energy for growth and to replenish the intracellular polyphosphate reserves needed for subsequent anaerobic metabolism (Kristiansen et al., 2013). Glycine was recently found to accumulate intracellularly when *Tetrasphaera*-related PAOs biomass was fed with glycine under anaerobic conditions. Subsequently, in the aerobic phase without addition of external substrate, the stored glycine was consumed. The uptake of glycine and oxidation of intracellular metabolites took place along a release and uptake of orthophosphate, respectively (Nguyen et al., 2015).

In situ ecophysiological studies have shown that *Tetrasphaera* can assimilate a range of substrates under anaerobic conditions (glucose, acetate and amino acids) (Kong et al., 2005; Nguyen et al., 2011). Genomic investigation of four *Tetrasphaera* isolates showed that they had the potential to take up acetate in addition to glucose and amino acid, but the metabolic models were described and validated only for glucose (Kristiansen et al., 2013) and glycine (Nguyen et al., 2015). The aim of this study was to identify potential storage products and metabolic pathways when pure cultures of *Tetrasphaera elongata* or biomass from full-scale EBPR wastewater treatment plants were exposed to acetate under anaerobic conditions.

Materials and methods

Pure cultures of *Tetrasphaera elongata* was grown in R2A broth without starch and sodium pyruvate, to generate enough biomass for biochemical studies. Biomass from 21 full-scale EBPR wastewater treatment plants were collected from the aeration tank, stored at 40C for < 24 h to carry out all experiments. Intracellular storage products were analyzed using NMR (Nguyen et al., 2015). 16S rRNA gene amplicon sequencing was used to investigate the microbial community structure in the 21 WWTPs.

Results and conclusions

The ability to take up and accumulate acetate of *T.* *elongata* was investigated during dynamic anaerobic/aerobic conditions. 13C-labeled substrates and Nuclear Magnetic Resonance (NMR) was applied to investigate the potential storage products and important metabolic pathways when *T. elongata* was fed with acetate anaerobically. *T. elongata* stored intracellular acetate, glutamate and other soluble compounds under anaerobic conditions along a release of phosphate. Subsequently, in the aerobic phase, the stored compounds were consumed and phosphate taken up (Figure 1). Similar experiments were conducted on activated sludge from 21 WWTPs containing many *Tetrasphaera* (up to 30% read abundance from 16S rRNA amplicon sequencing). Uptake of acetate and accumulation of intracellular acetate and glutamate was observed, confirming that this capability also was present in activated sludge. The range of intracellular acetate concentration ranged in the 21 WWTPs from 0.37 to 3.7 mg/g biomass. This study demonstrates an additional strategy for growth under dynamic fest-famine conditions in *Tetrasphaera*: uptake and intracellular accumulation of soluble substrate along with a release of orthophosphate under anaerobic conditions for subsequent use as carbon source in the aerobic phase.

Figure 1. Intracellular metabolites with acetate as sole carbon source in *T. elongata* under dynamic anaerobic/aerobic conditions. The average concentrations from duplicate experiments are shown.

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