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*Publication date:*  
2012

*Document Version*  
Early version, also known as pre-print

[Link to publication from Aalborg University](#)

### *Citation for published version (APA):*

Kristiansen, R., Nguyen, H. T. T., Saunders, A. M., Nielsen, J. L., Wimmer, R., Le, V., Petrovski, S., McIlroy, S. J., Seviour, R., Calteau, A., Nielsen, K. L., & Nielsen, P. H. (2012). *Metabolic model for an unusual and versatile Tetrasphaera involved in enhanced biological phosphorus removal based on whole genome sequencing*. Abstract from 14th International Symposium on Microbial Ecology, Copenhagen, Denmark.

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## **Metabolic model for an unusual and versatile *Tetrasphaera* involved in enhanced biological phosphorus removal based on whole genome sequencing**

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Enhanced biological phosphorus removal wastewater treatment systems are designed to remove inorganic phosphorus from wastewater by selecting for microorganisms, collectively known as the polyphosphate-accumulating organisms. These organisms store inorganic phosphorus as intracellular polyphosphate in excess of their growth requirements. This is essentially achieved by the cycling of the biomass through a carbon source rich anaerobic phase and carbon deficient aerobic phase. The polyphosphate stores provide the energy for anaerobic carbon assimilation and storage which can be utilized for growth and maintenance in the subsequent aerobic phase. Recently, microorganisms belonging to the actinobacterial genus *Tetrasphaera* have been identified as putative polyphosphate-accumulating organisms and found to be diverse and abundant in full-scale enhanced biological phosphorus removal plants. Knowledge about the physiology of *Tetrasphaera* is limited, but its ecological niche appears to be different from that of “*Candidatus Accumulibacter phosphatis*” which is considered the model for the polyphosphate-accumulating organism phenotype. To elucidate the physiology of the *Tetrasphaera*, the genomes of four activated sludge isolates (*T. australiensis*, *T. japonica*, *T. elongata*, and *T. jenkinsii*) were sequenced using the Illumina platform and annotated with the MaGe annotation software. The central aspects of carbon, phosphorus and nitrogen metabolism were investigated in detail. A metabolic model was then constructed to explain the physiology of the *Tetrasphaera* that allows them to be so successful under the dynamic conditions of enhanced biological phosphorus removal systems. Key features of this model were validated experimentally. We propose that *Tetrasphaera*-related polyphosphate-accumulating organisms can anaerobically assimilate a variety of carbon compounds, including glucose which is either fermented to a number of different compounds (acetate, alanine, lactate and succinate) or stored as glycogen. The energy required for anaerobic glycogen production is provided from the degradation of stored polyphosphate and the fermentation of glucose. During the subsequent aerobic phase, where the organisms are starved of an external carbon source, the stored glycogen is catabolised to provide energy for growth and to replenish their intracellular polyphosphate reserves needed for subsequent anaerobic metabolism. Additionally, *Tetrasphaera* can utilise nitrate and nitrite as electron acceptors in the absence of oxygen. The proposed model is considerably different to those previously developed for the polyphosphate-accumulating organisms, where the carbon source is stored as polyhydroxyalkanoates, with reducing equivalents sourced from the anaerobic hydrolysis of glycogen, which is utilised aerobically to replenish these glycogen stores. The findings described here have shown that the *Tetrasphaera* in enhanced biological phosphorus removal exhibit an unusual and versatile ‘polyphosphate-accumulating organism type’ metabolism. This study also provides a novel alternate metabolic strategy for survival of organisms with the ‘phosphate accumulating’ phenotype in these systems.