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A Metabolic Model for the 0092 Morphotype Associated with Filamentous Bulking Problems in Wastewater Treatment Plants

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BACKGROUND
Overgrowth of filamentous bacteria in activated sludge wastewater treatment plants (WWTPs) leads to impaired sludge settleability, a condition known as bulking, which is a common operational problem worldwide. The B45 genus-level-taxon, exhibiting the Eikelboom 0092 filament morphotype, is among the most abundant members of the phylum Chloroflexi in activated sludge (Fig. 1), yet nothing is known about their metabolic characteristics. In this study, we constructed a genome based metabolic model, that describes in detail the physiology of the organism in WWTPs, which was partly validated in situ with single cell methods.

METAGENOMIC ANALYSES
A metagenome was prepared from a WWTP suffering from severe 0092 morphotype associated bulking. A closed genome was assembled from the metagenome using differential coverage binning (Fig. 2) (Albertsen et al., 2013 Nature Biotech. 31. 533-538)

The assembled genome was annotated with the Microscope platform (Vallenet et al., 2009 Database. bap021).

STUDY PERSPECTIVES
In this study metagenomics and in situ methods were applied to construct a metabolic model for members of the B45 phylotype – revealing their role as abundant fermenters in activated sludge treatment systems. For more detail see McIlroy et al., (2016) ISME J. 10. 2223-2234.

The genome obtained in this study provides the foundation for gene expression studies for more detailed insights into the in situ physiology of the B45 in WWTPs.

The multifaceted approach of this study provides a model for the systematic characterisation of the abundant organisms in full-scale systems.

METABOLIC MODEL CONSTRUCTION
Based on the annotated genome and in situ analyses the metabolic model (Fig. 3) predicts:
- The B45 ferment sugars, when carbon is available under anaerobic conditions, with glycogen storage possible.
- Acetate, lactate, acetoin, ethanol and H₂ are potential by-products of sugar metabolism.
- O₂, NO₃ and N₂O are potential electron acceptors, indicating activity over a range of conditions.

IN SITU MODEL VALIDATION
Fluorescence in situ hybridisation (FISH) was combined with microautoradiography (MAR) and staining to confirm key aspects of the model in situ. A fermentative metabolism was supported by anaerobic glucose uptake (Fig. 4c.)