time to settle the score: retrieving the genome of a novel uncultured chloroflexi associated with bulking in activated sludge

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Wastewater treatment traditionally relies on the separation of the sludge and water phase before leading the effluent water into the environment. The so-called settleability of the sludge depends on the microbial community, which forms granules of varying sizes. Filamentous bacteria can impair settleability, which is known as bulking. Bacteria belonging to the phylum Chloroflexi are filamentous and often associated with bulking and foaming in activated sludge. An amplicon survey of 24 Danish wastewater treatment plants found several OTUs associated with uncultured members of a novel class in high relative abundance in several plants, suggesting a likely role in bulking problems. As this genus was only known by its 16S rRNA gene, this could not be confirmed. In this study, a genome representing the C10\_SB1A phylotype was assembled from a full-scale activated sludge metagenome and the bacterium visualized using fluorescent *in situ* hybridization.

Three samples, in which the target Chloroflexi was in different relative abundances, were paired-end sequenced on an Illumina MiSeq. The bin of interest was extracted using differential coverage and paired-end information. The genome was reassembled and scaffolded with Nanopore reads, producing the first complete genome in the novel class. A fluorescent *in situ* hybridization probe, designed to target the phylotype on a genus-level, confirmed the validity of our findings.

The assembly of this genome will help shed light on the ecology of an uncultured Chloroflexi species, which eventually can lead to a better understanding of bulking in activated sludge.