**Title:**Unraveling the role of non-culturable microorganisms in wastewater treatment systems using metagenomics

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**Summary:**

Uncultured microorganims are frequently found in wastewater treatment systems using DNA-based tools. In particular we detect high abundance of unculturable microorganisms from the Chloroflexota phylum in different systems. However, their role and relevance to the processes is not fully understood. They are considered part of the "microbial dark matter" since they are detected through their DNA/RNA but very little is known about their ecology. The main problem is that they are very difficult to isolate in pure culture, which makes it difficult to analyze their physiological properties.

They have been detected in both anaerobic and aerobic biological wastewater treatment systems and even in nitrogen removal systems. Most have filamentous morphology and it is postulated that they may be essential to form microbial aggregates that retain biomass within the reactors, achieving adequate system operation. It has also been proposed that they could have a role as "scavengers" degrading cellular debris. On the other hand, its overgrowth has been reported as bulking episodes agent. In these episodes, the overgrowth of filamentous bacteria causes the biomass to float, reducing its sediment ability, causing serious operational problems. Our group has studied these microorganisms in various systems in our country using different methodologies (16S rRNA amplicon sequencing, FISH, qPCR, metagenomics and genome assembly).

Although we have advanced in our knowledge, we have not yet been able to fully elucidate the role of these microorganisms. Now, we are using a different approach combining the operation of laboratory reactors with molecular analysis. The operation of laboratory reactors will allow us to confirm or discard the hypotheses previously raised. New molecular tools including metatranscriptomic are applied to determine the role of this unknown microorganisms. Novel information are obtained about a group of unknown microorganisms that probably play a fundamental role as guardians of biological ecosystems for wastewater treatment.