# Evolution of microbial communities in aerobic granular sludge during changes in wastewater composition





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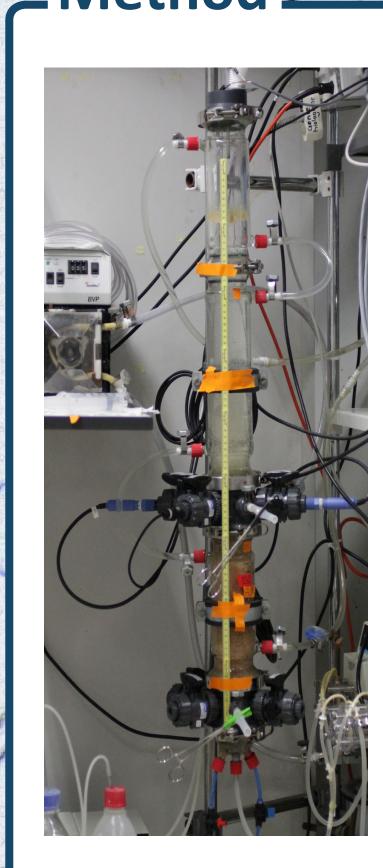
### Objectives

Understand the effect of hydrolyzable and fermentable compounds in the influent wastewater on the microbial communities, the settling characteristics and the nutrient removal performances of aerobic granular sludge (AGS).

#### Context

- AGS is a promising alternative wastewater treatment to the conventional activated sludge system.
- It presents various advantages such as high biomass concentration, enhanced settleability, presence of different redox conditions simultaneously in the granules, allowing substantial space, energy and chemical savings.
- Phosphate accumulating organisms (PAO) often found in high proportions in AGS enable biological phosphorus removal.
- Most studies on AGS are performed with simple synthetic wastewater containing volatil fatty acids as main carbon source, which is not the case of most municipal wastewater.

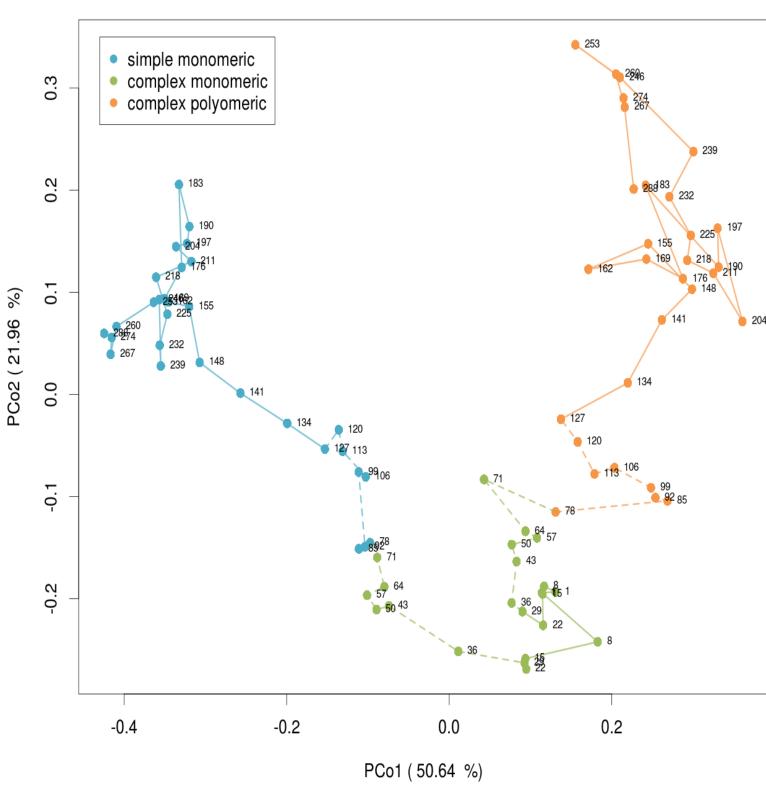
#### Method



- Two AGS sequencing batch-reactors were run in parallel for 18 months.
- The composition of the synthetic wastewater was progressively changed in two different directions.

1 reactor ← 2 reactors → 1 reactor Transition to AGS fed with Transition to simple complex complex monomeric polymeric monomeric wastewater wastewater wastewater VFA, glucose, VFA, glucose, amino acids, amino acids starch, peptone

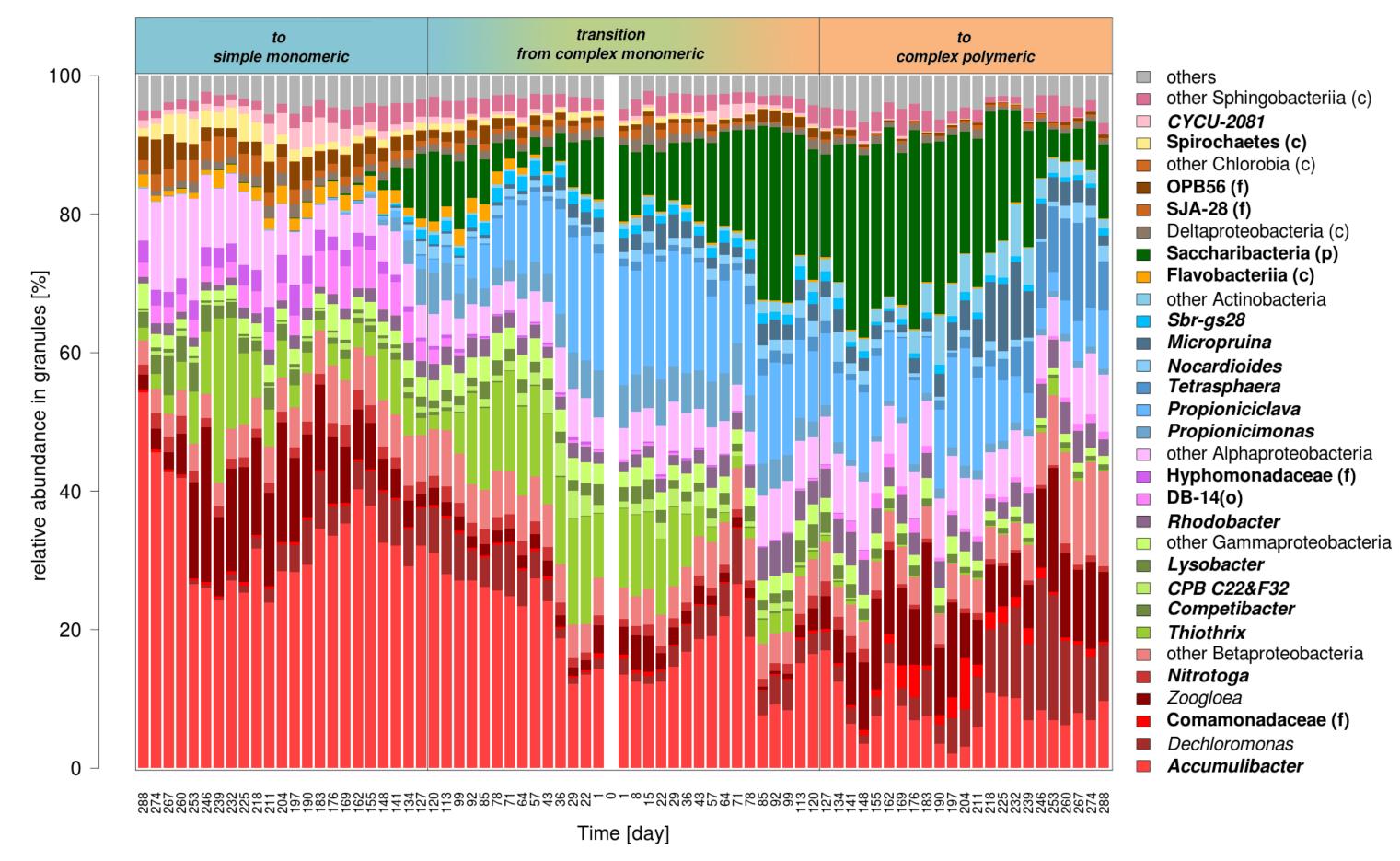
## Principal coordinate analysis



Principal coordinate analysis (PCoA) on the Bray-Curtis distance matrix of the bacterial genera abundances, showing 73% of the variability of the microbiological data (R, package vegan²).

- •The bacterial communities evolved in two different directions during the changes of influent wastewater from complex monomeric to complex polymeric or to simple monomeric.
- •Three weeks after the transition (day 126), the two communities seem to stabilize in two distinct bacterial "stable states".

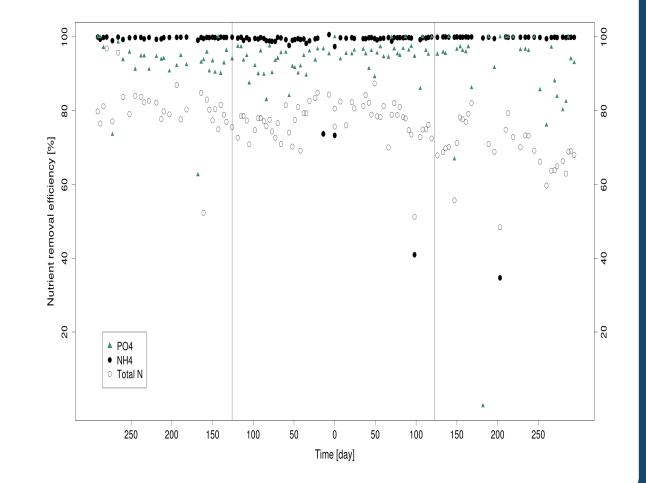
#### Microbial communities<sup>1</sup>



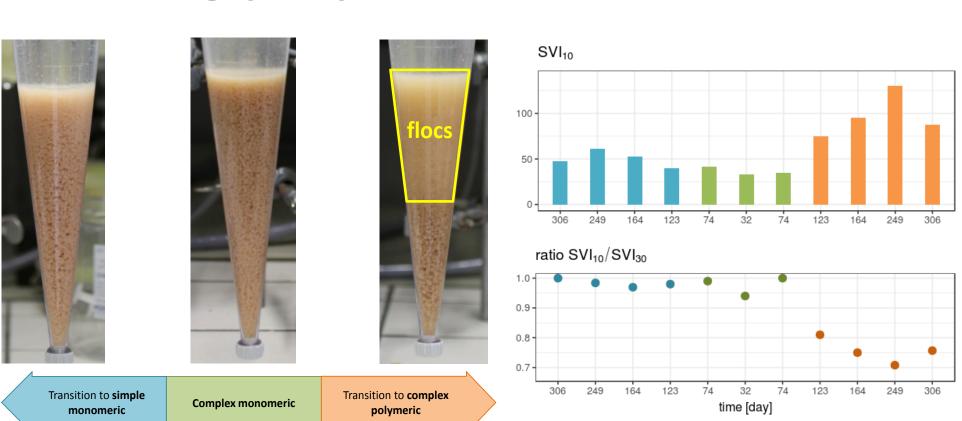
- •The barplot shows the evolution of the most abundant genera in AGS during the two changes of influent wastewater composition. The taxa for which the abundance is significantly different between the simple monomeric and the complex polymeric wastewater are indicated in bold.
- •The class of Actinobacteria is abundant in the sludge treating complex wastewater, but absent from the one treating simple wastewater.
- •The **PAO** *Accumulibacter* is the dominant genus in the reactor treating simple monomeric wastewater, whereas the **PAO** *Tetrasphaera* is only found in significant abundance in the reactor treating complex polymeric wastewater.

#### . Performances.

- •The ammonium (NH4) and Phosphate (PO4) -removal efficiencies stayed high during the two changes of wastwater composition.
- •The removal efficiency of the total nitrogen (Total N) slighly diminished with the addition of polymeric coumponds in the influent wastewater, due to an increased concentration of nitrate in the treated wastewater.



# Settling properties.



- The settling properties of the sludge were at the beginning impaired by the introduction of particulate compounds in the wastewater, with the appearance of an important proportion of flocs.
- Small granules appeared again with the polymeric wastewater around day 250.

#### Conclusion

- The changes of the wastewater composition induced clear shifts in the bacterial communities.
- The  $\beta$ -proteobacteria are dominant with the simple wastewater, the  $\gamma$ -proteobacteria were abundant with the monomeric wastewater and the Action bacteria with the complex synthetic wastewater.
- The PO4- and NH4-removal efficiencies were maintained during the change of influent wastewater
- The total N -removal and the settling characteristics diminished with the presence of polymeric compounds in the influent wastewater.

#### **Outlooks**

- Determine which organisms are responsibles for P- and N-removal with the polymeric substrate, by using metagenomic and metatranscriptomic analysis.
- Investigate de role of uncharacterized discriminant genera

AGS = Aerobic Granular Sludge

**Total N-removal** = Total nitrogen removal

AGS = Aerobic Granular Sludge

NH4-removal = Ammonium removal

PAO = Phosphate accumulating organism

P-removal = Phosphorus removal

(c) class

(f) family

(o) order

(p) phylum

<sup>1</sup> MiDAS taxonomy database version 2.0, McIlroy S.J., Kirkegaard R.H., McIlroy B., Nierychlo M., Kristensen J.M., Karst S.M., Albertsen M., Nielsen P.H. (2017) MiDAS 2.0: an ecosystem-specific taxonomy and online database for the organisms of wastewater treatment systems expanded for anaerobic digester groups. Database 2017

<sup>2</sup> Package Vegan, Oksanen J, Blanchet FG, Kindt R et al. Vegan: Community Ecology Package. R package version 2.0-10.