Influence of wastewater composition on microbial communities of aerobic granules and their nutrient removal performances

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ABSTRACT
Basic understanding of aerobic granular sludge (AGS) processes has mainly been obtained in laboratory-scale studies with simple synthetic wastewaters. Two approaches were applied here to make a step toward the comprehension of AGS systems treating real municipal wastewater. One approach consisted in increasing the complexity of the influent composition of an AGS sequencing batch reactor (SBR) fed with volatile fatty acids, the other in starting up four AGS SBRs with four different wastewaters. Nutrient removal could be maintained in the first approach and indications for a change in the population responsible for biological phosphorous removal were obtained (P-removal). The four reactors started up with different wastewaters showed different granulation behaviour and P-removal was impaired in the reactors fed with municipal wastewater. More detailed investigations of the microbial communities will allow to elucidate the reasons behind the observations made in this preliminary study.

INTRODUCTION
Aerobic granular sludge (AGS) is a promising alternative wastewater treatment to the conventional activated sludge system. As AGS has enhanced settling abilities and provides different redox conditions across the granules at the same time, the processes based on AGS allow substantial savings in space, energy, and chemical products. Wastewater treatment plants using AGS at full-scale have already been successfully implemented (Giessen, 2013). Nevertheless, the basic understanding of AGS systems has mainly been obtained by running lab-scale sequencing batch reactors (SBRs) fed with synthetic wastewater containing volatile fatty acids (VFA) and relatively high phosphorous concentrations. These systems have been dominated by bacterial populations such as the phosphate-accumulating organism (PAO) *Accumulibacter* and its metabolic counterpart, the glycogen-accumulating organism (GAO) *Competibacter* (Weissbrodt, 2013). Reactors treating real wastewater, where granules often have a fluffy structure (Wagner, 2015) or flocs are present (Derlon, 2016), have been much less investigated concerning their microbial community structures.

In order to make a step toward the comprehension of AGS systems treating municipal wastewater, two approaches were applied to study the impact of wastewater composition on the AGS microbial communities and their nutrient removal performances. Two main research questions were addressed: (i) which are the main bacterial populations likely to be involved in phosphorus (P-) and nitrogen (N-) removal depending on the wastewater composition and (ii) can nutrient removal performance be correlated with the complexity of wastewater?

METHODS
The first approach was to run an AGS SBR with VFA (acetate and propionate) as main carbon source

and progressively changing the wastewater composition by introducing fermentable compounds (glucose and amino acids) in a first phase and hydrolysable compounds (starch and peptones) in a second phase. The second approach was to start-up in parallel four SBRs with activated sludge as inoculum and fed with four distinct wastewaters. The inoculum sludge was taken from a wastewater treatment plant carrying out enhanced biological phosphorous removal. Two reactors were fed with synthetic wastewaters that contained VFA only or a mixture of VFA, fermentable compounds and hydrolysable compounds. The other two reactors were fed with either primary clarifier effluent of a municipal wastewater treatment plant or raw influent from the same plant. All SBRs were operated with variable lengths of the anaerobic and aerobic phases to allow full consumption of chemical oxygen demand (COD) during the first phase and full nitrification during the latter with changing wastewater composition.

P- and N-removal were determined by measuring phosphate, ammonium, nitrate and nitrite concentrations in the influent and effluent of the reactors and microbial community structures by 16S rRNA gene amplicon sequencing of weekly biomass samples. The AGS was characterized by determining two parameters, the sludge volume index after 10 and 30 minutes (SVI10, SVI30) and the ratio of the two (SVI30/SVI10), and the particle size distribution by sieving the sludge.

RESULTS AND DISCUSSION

Approach 1: Transition from VFA to a more complex substrate mixture

By increasing the length of the anaerobic phase from about 1 to 2 hours, full removal of dissolved COD was achieved with all different media applied. By increasing the length of the aerobic phase from 2 hours with full aeration up to 5 hours with intermittent aeration, nitrification efficiency remained, with some exceptions, larger than 90% despite the change of wastewater composition (Fig. 1). Total N-removal was around 65-70% with media containing 450 mg O_2/L COD and increased to 80-90% when COD was increased to 600 mg O_2/L (Fig.1) indicating that denitrification was COD-limited. P-removal experienced a first severe drop from day 85 to 129 but recovered again, probably due to the increase in total COD provided (Fig. 1). A second less severe drop was observed around day 200 for which no explanation has yet been found, but P-removal recovered and was quite stable with approximately 90% efficiency until the end of the experiment.

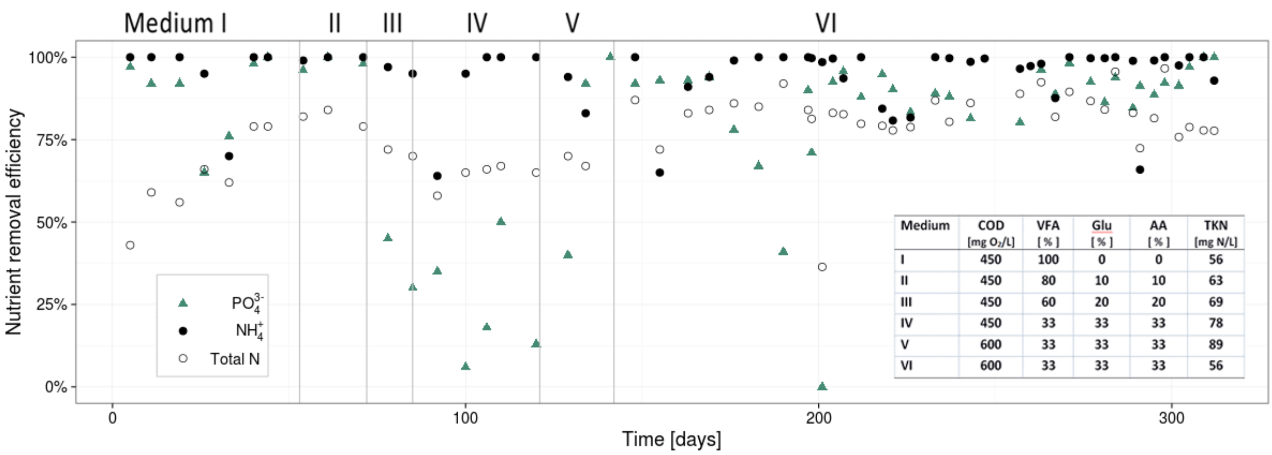
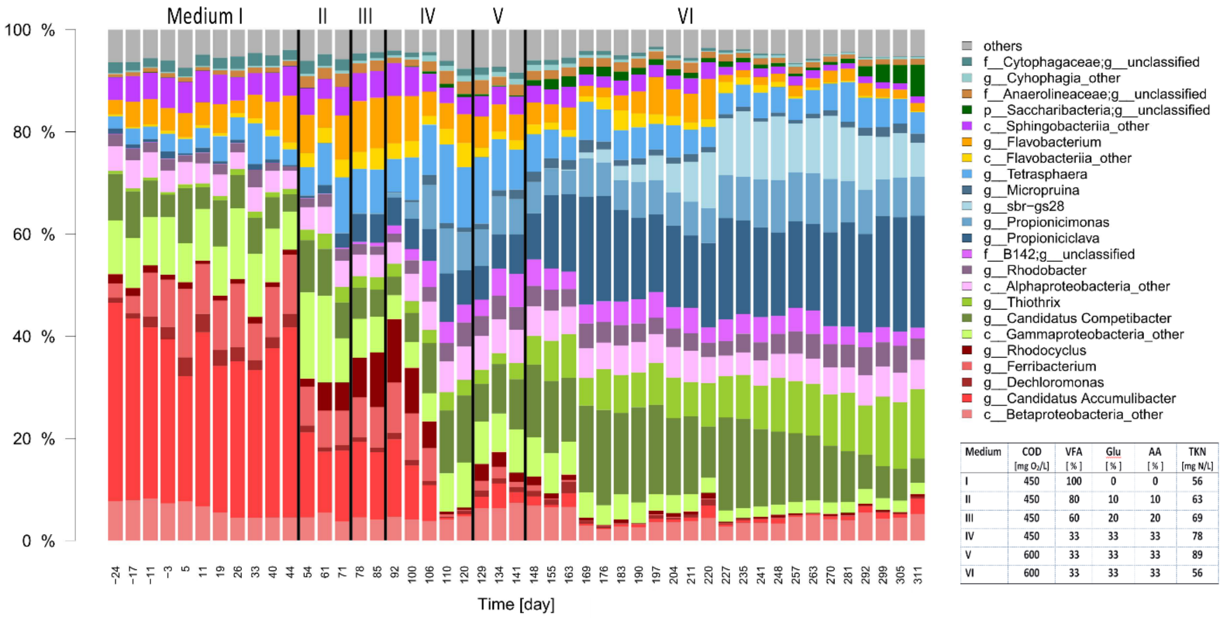


Figure 1. P- and N-removal efficiencies in AGS-SBR during transition from VFA to a more complex substrate mixture containing VFA, glucose, and amino acids.

The microbial community structure shifted from mainly beta-Proteobacteria to Actinobacteria and gamma-Proteobacteria during the replacement of part of the VFA by fermentable compounds (Fig. 2). The abundance of the initially dominant PAO *Accumulibacter* decreased drastically and the GAO *Competibacter* increased. The observed high P-removal efficiencies were likely due to the presence of the PAO *Tetrasphera*, which is able to ferment carbon sources like glucose and amino acids, and became more abundant upon substrate change. The possible roles of the other populations that became abundant upon substrate modification remain to be elucidated.



Medium	COD [mg O_2/L]	VFA [%]	Glu [%]	AA [%]	TKN [mg N/L]
I	450	100	0	0	56
II	450	80	10	10	63
III	450	60	20	20	69
IV	450	33	33	33	78
V	600	33	33	33	89
VI	600	33	33	33	56

Figure 2. Evolution of the most abundant genera in AGS sampled during transition from VFA to more complex substrate mixture. Glucose and amino acids were progressively introduced in the synthetic wastewater from day 44 and reached 2/3 of the total COD from day 92.

Approach 2: Start-up of four AGS-SBR fed with four different wastewaters

Similar to the operation mode of the AGS-SBR described above, the length of the anaerobic and aerobic phase was adapted to maintain good treatment performances. In addition, very low selective pressure for rapid settling biomass was applied in the beginning and increased with higher degree of granulation in order to achieve a high SRT of about 20 days on all size fractions and to avoid too high washout. Nitrification could indeed be maintained in all four reactors while N-removal was dependent on wastewater composition and was the highest with synthetic wastewater (Fig. 3). P-removal efficiency decreased quite rapidly with municipal wastewater but could be maintained in the two SBRs fed with synthetic wastewater.

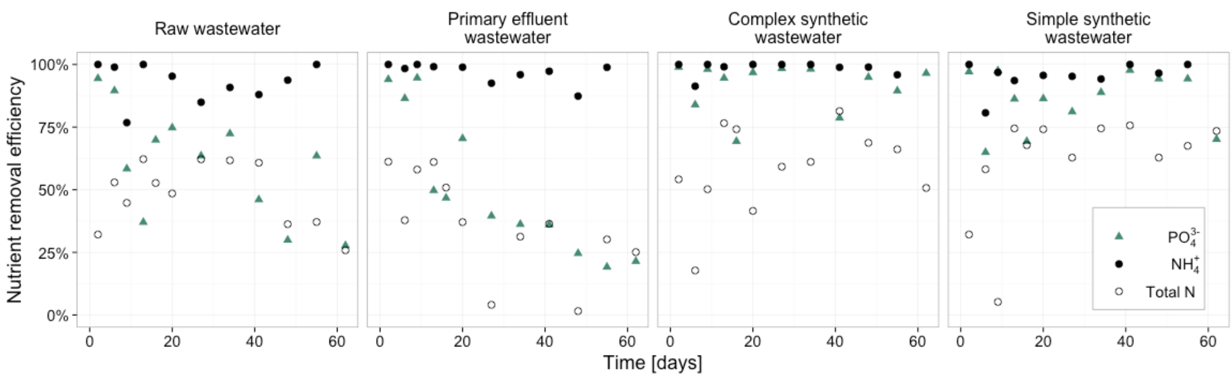


Figure 3. P- and N-removal efficiencies in four parallel AGS-SBR during start-up fed with four different wastewaters and inoculated with activated sludge from an EBPR plant.

The ratio SVI30/SVI10 showed that the sludge of the two reactors fed with synthetic wastewater had already very good settling properties two weeks after start-up (Fig. 4). This tendency was less pronounced in the two reactors fed with municipal wastewater since the sludge bed still compacted within the additional 20 minutes of settling. The evolution of the particle size distribution was quite similar in the four reactors with a tendency of larger particle formation in the reactors fed with synthetic wastewater (Fig. 4). Especially the biomass fed with only VFA formed the largest aggregates, a fact that was also confirmed by microscopic observations. Very preliminary analysis of the microbial communities of these four reactors showed completely different evolution of their structures depending on the wastewater composition.

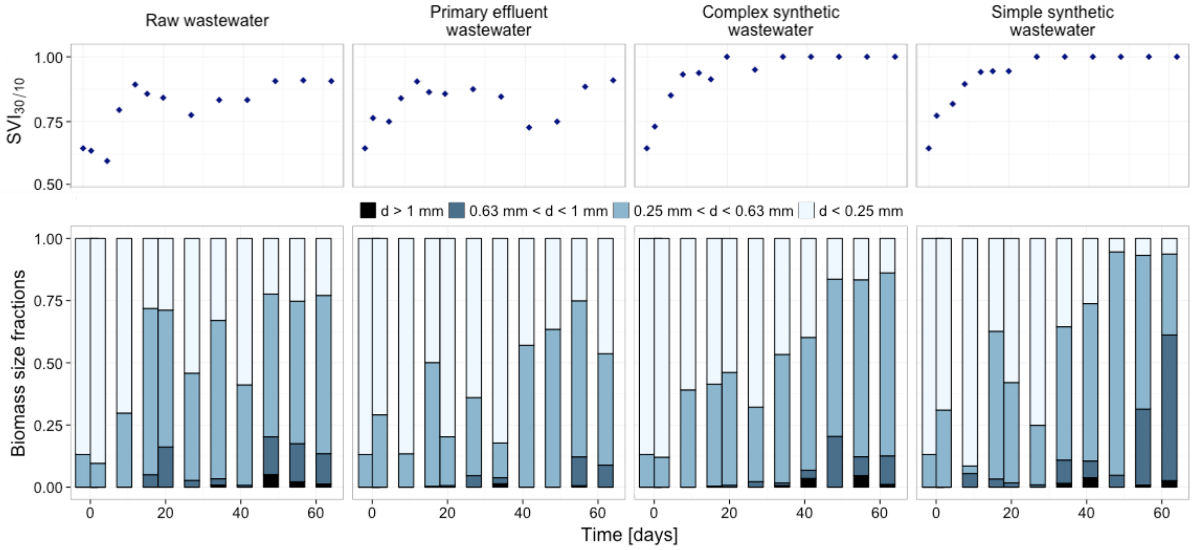


Figure 4. Sludge characteristics determined during start-up in the four AGS-SBR operated in parallel. The upper graph shows the ratio of the SVI after 30 and 10 minutes, the lower the particle size distribution of the sludge.

CONCLUSIONS AND PERSPECTIVES

The main conclusion of this preliminary study is that nutrient removal efficiencies can be achieved with more complex wastewaters if the reactors are operated in such a way that little dissolved carbon substrate is present in the aerobic starvation phase. However, the start-up of AGS SBRs with municipal wastewater did not yet allow maintaining efficient biological P-removal. Microbial community structures changed significantly with different influent compositions, an observation that was expected. In future work, more detailed investigations of the microbial communities will be carried out to better understand the reasons for the observations made in the preliminary study presented here and to identify the bacterial populations involved in N- and P-removal.

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