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Aerobic granular sludge formation and microbial community structure is affected by reactor feed rates

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Manuscript Classifications:	68.2: Linking microbial community structure to the functionality of specific aspects of water treatment; 68.4: Microbial population dynamics in various reactor environments (suspended cultures, flocs, biofilms, granules, membrane systems); 68.11: Full-scale applications combining engineering and microbiological approaches

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Aerobic granular sludge formation and microbial community structure is affected by reactor feed rates.

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Abstract: Laboratory scale reactors treating domestic wastewater were operated for selection of aerobic granules. We found the formation, structure, biological performance and microbial community structures of aerobic granules were affected by the rates of the influent wastewater feed. In a reactor with slow feed (R1), poor granulation occurred during the 190 day operation. However, good granulation occurred in the fast feed reactor (R2) after 85 days of operation. Analysis of microbial community shifts indicated that granulation in R2 correlated with increasing abundance of *Gammaproteobacteria* (uncultured *Competibacter sp.*). In contrast, the formation of loosely structured, large aggregates and then later small granules in R1 correlated with high abundance of *Flavobacterium* and *Commamonas* spp. The results suggest that slow reactor feed rates are detrimental to granule formation, which is important to consider in the full-scale application of the technology.

Key words: Aerobic granule formation; feed strategy; microbial community structure.

Introduction.

Aerobic granular activated sludge is a developing and potentially advantageous technology for wastewater treatment (Adav et al. 2008). Laboratory scale investigations apply rapid feed of wastewater to reactors, typically in a few minutes; that would be unrealistic in full scale application of aerobic granules. We suspect that different feeding rates will select for different types of microorganisms and affect granulation. The delivery of low concentration organic substrate, such as occurring in a continuously feed system treating low strength domestic wastewater, may present particular challenges for aerobic granular formation. This study compared the performance, granule formation, and microbial community compositions in reactors operating to treat domestic wastewater and form aerobic granules.

Material and Methods.

Two lab-scale sequencing batch reactors (SBRs) were operated for biological nutrient removal and inoculated with a mixture of floccular sludge (70%) and crushed granules (30%). Two different wastewater feeding strategies were applied to the reactor cycles (total of 6 hours), with R1 having slow feed (60 min) and R2 a fast feed (6 min). Particle size was determined using the Malvern Mastersizer 2000. Microbial community compositions were characterized by terminal restriction fragment length polymorphism (T-RFLP) and pyro-tag-sequencing of 16S rRNA genes. Data was processed using GeneMarker® and Mothur (v.1.23.1) respectively. Fluorescence in situ hybridisation (FISH) was performed to detect *Accumulibacter* spp., and *Competibacter* spp. Statistical analysis of community composition was conducted using PRIMER and PERMANOVA (PRIMER-E Ltd., Plymouth, UK).

Results and Conclusions.

Both SBRs achieved BNR in the end of the 190 day operation, with average COD, N and P removals rate of 89%, 73% and 82%, respectively, for R1. The corresponding rates for R2 were 88%, 88% and 92%, respectively. Significant biomass loss occurred from the SBR start up. The MLVSS in R1 dropped from about 2 g/L at day 60 to 0.45 g/L at day 140, which then recovered to 1.6 g/L at day 165. In contrast, in R2, the biomass lowered to 1.5 g/L MLVSS (day 96), and then increased to 5 g/L by day 179. The BNR performance of R1 was least stable during the occurrence of low biomass, whilst the granule formation was poor. By day 150, large particles had formed. However, they were loose filamentous aggregates rather than granules. By day 180, only small

granules formed. In contrast, large dense granules formed in R2 by day 95 and were stable throughout the operation.

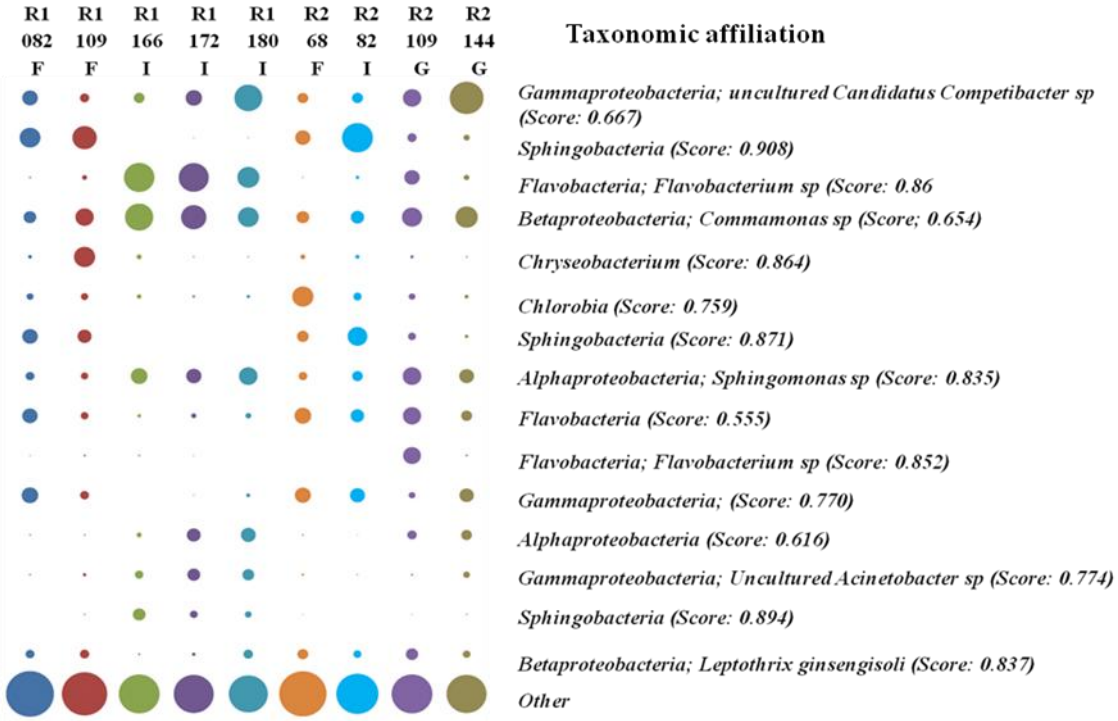

Figure 1. Abundance of the top 15 OTUs detected by pyrosequencing of R1 (day 82-180) and R2 (day 68-144) samples (according to the sum of the relative abundance across all samples). Floccular stage is shown by F, intermediate stage (I) and granular stage (G). Phylogenetic distance cutoff for OTU generation is 0.03. The size of the circle reflects the relative abundance of an OTU in a sample. The closer the score (in parentheses) to 1.0, the more confidence the sequence are.

Analysis of microbial community structure (Fig. 1) indicated that granulation in R2 correlated with increasing abundance of *Gammaproteobacteria* (uncultured *Competibacter sp.*). High abundance of *Competibacter* during formation of aerobic granules was confirmed by statistical analysis (dbRDA) and FISH of R2. The formation of loosely structured, large aggregates in R1 correlated with high abundance of *Flavobacterium* and *Commamonas spp.*. When R1 altered to produce small granules the abundances of uncultured *Competibacter sp.* increased while *Chryseobacterium sp*. decreased.

Competibacter sp. have been detected previously in aerobic granules and their physiology may be well suited to the feast/famine conditions in fast feed systems, such as in SBRs operating for BNR. Recently, Competibacter are suggested to produce an expolysaccharide that is considered an important structural component of aerobic granules (Seviour et al. 2011). From our studies, it appeared that granulation was favoured by fast feeding of influent wastewater, and this correlated with abundance levels of certain bacteria. The possible detrimental affects of slowly fed wastewater on granulation needs to be considered in the full-scale application of aerobic granules.

References:

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