

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

*By Marieska Verawaty*



## 5<sup>th</sup> Quadrennial IWA Specialty Conference on Microbial Ecology and Water Engineering

University of Michigan, Ann Arbor, MI USA  
July 7-10, 2013

### Conference Keynote and Plenary Speakers

**Lutgarde Raskin**  
University of  
Michigan, USA

**Akihiko Terada**  
Tokyo University of  
Agriculture &  
Technology, Japan

**Per Halkjær Nielsen**  
Aalborg University,  
Denmark

**David Stahl**  
University of  
Washington, USA

**Holger Daims**  
University of Vienna,  
Austria

**Maria Reis**  
Universidade  
Nova de Lisboa,  
Portugal

**Charles Bott**  
Hampton Roads  
Sanitation District,  
USA



**Sunday, July 7, 2013 – Special Workshops**

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Continental breakfast, breaks, and lunch will be served each day

Workshops are held in the Rackham Building, 915 E Washington Street.

- 7:30-11:30** Registration
- 9:00-17:15** **Workshop 1: Dynamics of Antimicrobial Resistance Genes and Collateral Effects of Antibiotics in Wastewater Treatment Systems**  
**Organizers:** Dominic Frigon and Timothy M. LaPara - McGill University (Canada), University of Minnesota (USA)
- Workshop 2: Principles, Potential, and Limitations of Novel Molecular Methods in Water Engineering; from Amplicon Sequencing to -omics Methods**  
**Organizers:** Per Halkjær Nielsen, Trina McMahon, Mads Albertsen\*, and Aaron Saunders - Aalborg University (Denmark) and University of Wisconsin-Madison (USA)
- 19:00-21:00** **Welcome Reception** - at the University of Michigan Museum of Art, 525 South State Street, Ann Arbor, Michigan, <http://www.umma.umich.edu>

**Monday, July 8, 2013 – Main Conference Day 1 - Rackham Building, 915 E. Washington St.**

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Continental breakfast, breaks, and lunch will be served each day

- 7:30-11:30** Registration
- 8:30-8:40** **Opening:** Nancy Love, University of Michigan (USA)
- 8:40-8:50** **Comments:** IWA President **Glen Daigger**, CH2M Hill (USA)
- 8:50- 9:45** **Plenary:** Per Halkjær Nielsen, Aalborg University (Denmark), **Microbial Ecology of Wastewater Treatment: A Journey from "Black Box Understanding" to "Systems Microbiology."**
- 9:45-10:30** **Keynote 1:** Holger Daims, University of Vienna (Austria), **Surprising Diversity and Unexpected Functions: Features of Nitrite-Oxidizing Bacteria Revealed by "Omics" and In Situ Tools**
- 10:30-11:00** Break
- 11:00-12:00** **Nitrogen Transformations (Chairs: Dan Noguera and Lauren Stadler\*)**  
**Hongkeun Park\***, Yiwei Ma, Suneethi Sundar, Kartik Chandran - Columbia University (USA), Anna University (India) (Abstract 12875)  
Niche Differentiation in Microbial Population and Activity Dynamics and the Impact of Inorganic Carbon Limitation on a CANON (Completely Autotrophic Nitrogen-removal Over Nitrite) Process
- Micol Bellucci**, Tomoko Yamamoto, Shohei Riya, Shohei Mizuma, Ryo Kanai, Kohei Kamimura, Keisuke Hojo, Thomas Peter Curtis, Masaki Hosomi, Akihiko Terada - Tokyo University of Agriculture & Technology (Japan), Newcastle University (UK), INRA, LBE (France) (Abstract 12779)  
Nitrification in Extremely Low Dissolved Oxygen Conditions
- Samik Bagchi**, Shan Sun, Regina Lamendella, Mark C.M. van Loosdrecht, Pascal E. Saikaly – King Abdullah University of Science and Technology (Saudi Arabia), Von Liebig Center for Science (USA), Delft University of Technology (The Netherlands) (Abstract 12895)  
r-RNA Subtracted m-RNA Metatranscriptomics of Granular Anammox Process
- Satoshi Tsuneda**, Yuki Kagawa, Naohiro Kishida, Shinya Matsumoto, Cristian Picioreanu, Mark C.M. van Loosdrecht - Waseda University (Japan), National Institute of Public Health (Japan), Delft University of Technology (The Netherlands) (Abstract 12915)  
Mathematical Modeling for the Nutrient Removal Process with Growth of Microbial Granules
- 12:00-12:15** Discussion
- 12:15-13:15** Lunch

\*Registered Student Delegate

- 9:15-10:15** **Attached Growth** (Chairs: Josh Shroust and Maria Cristina Gagliano\*)  
Elena Brei Jevric, D. Grant Allen, **Steven N. Liss** – University of Toronto (Canada), Queen’s University (Canada) (Abstract 12848)  
Bacterial Adhesin Gene Expression Associated with Microbial Flocs in Activated Sludge  
**David Gregory Weissbrodt\***, Noam Shani, **Christof Holliger** - École Polytechnique Fédérale de Lausanne (Switzerland) (Abstract 12822)  
Linking Microbial Population Dynamics and Nutrient Removal During Wastewater Treatment  
Marieska Verawaty, Lu Fan, Frances Slater, Zhiguo Yuan, **Philip Lindsay Bond** – The University of Queensland (Australia) (Abstract 12853)  
Aerobic Granular Sludge Formation and Microbial Community Structure is Affected by Reactor Feed Rates  
**Kelly Martin**, Cristian Picioreanu, Robert Nerenberg - University of Notre Dame (USA), Delft University of Technology (The Netherlands) (Abstract 12854)  
Modeling Denitrification, Sulfate-Reduction, and Methanogenesis in a Hydrogen-Based Membrane Biofilm Reactor
- 10:15-10:30** Discussion
- 10:30-11:00** Break
- 11:00-12:00** **Emerging and Trace Contaminants** (Chairs: Cyndee Gruden and Krassimira Hristova)  
**Nicole Fahrenfeld**, W. Cully Hession, Katharine F. Knowlton, Amy Pruden - Virginia Tech (USA) (Abstract 12797)  
Watershed Processes Governing Dissemination of Antibiotic Resistance Genes  
**Celia Manaia**, Ana Varela, Sandra André, Olga Nunes – Universidade Católica Portuguesa (Portugal), Universidade do Porto (Portugal) (Abstract 12852)  
Bacterial Communities and Antibiotic Resistance in Hospital and Municipal Effluents  
**Dong Li**, Mazahirali Alidina, Julia Regnery, Josh O. Sharp, Jörg E. Drewes - Colorado School of Mines (USA), King Abdullah University of Science and Technology (Saudi Arabia) (Abstract 12846)  
Microbial Community Characteristics in Managed Aquifer Recharge Systems and Relationship with Trace Organic Compounds Removal  
Xinzhu Yi, Wenchao Cheng, Qing Wei, **Zhi Zhou** – National University of Singapore (Singapore) (Abstract 12934)  
Evaluation of Antimicrobial Resistance in Urban Environments in Singapore
- 12:00-12:15** Discussion
- 12:15-13:15** Lunch
- 13:15-14:00** **Keynote 4: Maria Reis**, Universidade Nova de Lisboa (Portugal), Microbial ecology in dynamic PHA producing reactors: linking reactor performance and population identity
- 14:00-14:45** **Phosphorus Removal** (Chairs: April Gu and Fabrizio Sabba\*)  
Ricardo Marques, **Adrian Oehmen**, Hien Nguyen, Jorge Santos, Gilda Carvalho, Per H. Nielsen, Maria A. M. Reis - Universidade Nova de Lisboa (Portugal), Aalborg University (Denmark) (Abstract 12866)  
Characterisation of the ecological niches in enhanced biological phosphorus removal processes with *Tetrasphaera* and *Accumulibacter*  
**Aaron Marc Saunders**, Mads Albertsen\*, Artur Tomasz Mielczarek, Per H. Nielsen – Aalborg University (Denmark) (Abstract 12897)  
Wastewater Treatment Plants with Biological Phosphorus Removal from Around the World Have a Common Core Microbial Community

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

Marieska Verawaty\*, Lu Fan, Frances R. Slater, Zhiguo Yuan, Philip L. Bond.

\* The University of Queensland, Advanced Water Management Centre (AWMC), QLD 4072, Australia.

**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 R1 the formation of loosely structured, large aggregates and then later small granules 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, Di Iaconi et al. 2007). Laboratory scale investigations apply rapid feed of wastewater to reactors, typically 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 sludge systems. This study compared the performance, granule formation, and microbial community compositions in reactors operating to treat domestic wastewater and form aerobic granules.

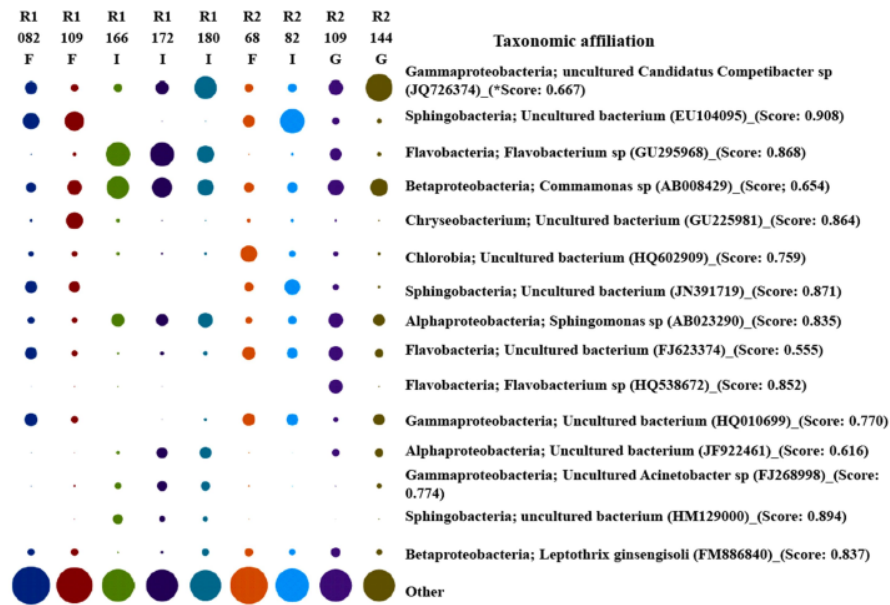
### Material and Methods.

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<sup>®</sup> 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 were achieving BNR during the 190 day operation, with average COD, N and P removals for R1 were 89%, 73% and 82% respectively, while that 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. In R1, the slow feed SBR, granule formation was poor. By day 150 large particles had formed, however, these were loose filamentous aggregates and were not considered to be granules. By day 180 only small granules were seen to form. 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 similar the sequence are.

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

*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 exopolysaccharide 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:

- Adav, S.S., Lee, D.J., Show, K.Y. and Tay, J.H., 2008. Aerobic granular sludge: Recent advances. *Biotechnology Advances* 26, 411–423.
- Seviour, T.W., Lambert, L.K., Pijuan, M. and Yuan, Z., 2011. Selectively inducing the synthesis of a key structural exopolysaccharide in aerobic granules by enriching for *Candidatus "Competibacter phosphatis"*. *Applied Microbiology and Biotechnology* 92, 1297-1305.

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