

Metagenomic insights into bio-functionality of 21 anaerobic biogas systems

Y. Tao^{1,2}, D.W. Gao¹, H.Y. Wang^{1,2}, X.D. Zhang², G. Liu^{2,3}, C.H. Chen¹, D. Ghasimi², H. Ozgun^{2,4}, M.E. Ersahin^{2,4}, Z.B. Zhou^{2,5}, M. F. Temudo⁶, J. Kloek⁶, H. Spanjers², M. de Kreuk², N.Q. Ren¹, J.B. van Lier²

1, State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology, Harbin 150090, China; 2, Department of Water management, Section Sanitary Engineering, Delft University of Technology, PO Box 5048, 2600 GA Delft, The Netherlands; 3, OASEN Water Company, PO BOX 122, 2800 AC, Gouda, The Netherlands; 4, Istanbul Technical University, Civil Engineering Faculty, Environmental Engineering Department, Ayazaga Campus, Maslak, 34469, Istanbul, Turkey; 5, SYSU-HKUST Research Center for Innovative Environmental Technology (SHRCIET), School of Environmental Science and Engineering, Sun Yat-sen University, Guangzhou, 510275, China; 6, DSM Biotechnology Centre, P.O. Box 1, 2600 MA Delft, The Netherlands

Background and aims

Although abundant operational experiences has been accumulated to lead a well-functioned anaerobic digestion (AD), the microbial reasons behind a robust/failing AD system and the community dynamics in response to complex operational changes are still lack of insights. A balanced bacterial-archaeal community is undoubtedly a key to successful AD. In this study, we comprehensively investigate bacterial and archaeal communities in 21 lab-scale AD reactors using real-time qPCR and next generation sequencing. Our purpose is to link community differences to reactor configurations, and eventually supply with some metagenomic insights into the bio-functionality of AD reactors.

Methods

The working volume of all 21 reactors are in a range of 3.8 - 9.0 L. All reactors were inoculated with mature sludge from pilot or full-scale AD reactors. Different substrates were fed to reactors, including enzymatically hydrolysed brewer's spent grain, pig manure, fine sieve fraction, brackish recirculation wastes and synthetic organic medium. A total of 149 biomass samples were harvested for DNA extraction. A pair of new universal primers (U515F and U1071R) were applied for 454 pyrosequencing (Roche 454 GS-FLX system). Another two pairs primers, ARC787f – ARC1059r; BAC516f – BAC805r, were used for real-time qPCR of archaea and bacteria.

Results

The universal primers for pyrosequencing cover most bacterial species (92.0%) and methanogens (95.2%). A total of 652,975 sequences were retrieved for downstream analysis. Some key discoveries are:

- Top seven phyla (*Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Synergistetes*, *Chloroflexi*, *OP9* and *Euryarchaeota*) account for 92.6% among total 50 phyla.
- *Bacteroides* and *Clostridium* are most dominant genera (average abundance 28% and 14% of 149 samples) in all reactors despite of different inocula and substrates, indicating AD reactors share similar saccharolytic/fermenting members.
- Organic substrate availability (in kg-COD/(kg-VSS • d)) can impact both methanogen abundance and real quantity. A high availability led to a high abundance and vice versa. This indicates that substrate availability determines the ecological position of methanogens in AD reactors.
- EGSB communities have the most diverse microbial diversity (calculated in Shannon index, Chao1, observed species number and phylogenetic distance). EGSBs also have the highest methanogen abundance (Ave. 6.4%) and amount (~ 10¹¹ gene copies per gram dry biomass), which are about 10 times higher than the other reactors.

Accordingly, EGSB can bare the highest organic loading rate (Approx. 21 kg-COD/m³d), which is 7-10 times higher than the others.

- It is intriguing to find that endospore-forming bacteria prevail in digester systems while biofilm-based reactors are dominated by non-endospore-forming bacteria. This indicates different proliferous strategies in response to various environmental stresses inside AD reactors.

Conclusion and perspective

By applying next generation sequencing and real-time qPCR on 149 samples out of 21 reactors, we captured some prevalent phyla and genera who are dominant in almost every anaerobic biogas system. Meanwhile, it is critically necessary to link AD system's functionality to its microbial community, both of which response to key operational/environmental factors such as organic loading (and its increasing rate), F/M ratio, salinity, temperature and aggregation forms. More results will be discussed during ISME 15.