CORE
Metadata, citation and similar papers at core.ac.uk
Provided by Ghent University Academic Bibliography

Operational conditions determine the anaerobic digestion microbiome

Jo De Vrieze[1], Aaron M. Saunders[2], Ying He[3], Willy Verstraete[1] and Nico Boon[1]

[1] Laboratory of Microbial Ecology and Technology (LabMET), Ghent University, Coupure Links 653, B-9000 Gent, Belgium.
[2] Department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University, Sohngårdsholmsvej 49, 9000 Aalborg, Denmark.
[3] Shanghai Jiao Tong University, Shanghai, China.

Anaerobic digestion (AD) can be considered a key environmental technology in the future bio-based economy, as it allows the conversion of a wide diversity of organic downstream products into biogas. The microbial consortium carrying out the AD process is considered to be quite complex and several attempts already have been carried out to determine the key microbial communities [1, 2]. However, an understanding of the key differences in the microbial communities in different AD process configurations and the environmental/process parameters that drive these differences are unknown. Arumugam et al. [3] identified 3 functionally distinct "enterotypes", or conformations of the community in the human gut microbiome.
In this research, we hypothesized that differences in operational parameters might also lead to particular conformations of microbial communities in full-scale AD installations. If so, it will inform which are the main parameters driving the overall microbial community composition.

A total of 38 samples were collected from different full-scale AD installations. Digestate samples were analyzed for total ammonia nitrogen (TAN) and free ammonia (FA), pH, volatile fatty acids (VFA), conductivity, volatile solids (VS) and total solids (TS). Information concerning the organic loading rate (OLR), biogas production and composition, temperature, reactor type and volume and influent stream composition of the different digesters was obtained directly from the plant operators.
Samples for microbial community analysis were stored directly at -20°C until further analysis. PCR amplification, 16S rRNA gene amplicon sequencing and analysis were carried out using the Illumina HiSeq platform at the University of Aalborg. Real-time PCR (qPCR) was performed on total bacteria and the methanogenic populations *Methanobacteriales*, *Methanomicrobiales, Methanosarcinaceae* and *Methanosaetaceae*.

In-depth analysis of the operational parameters of the 38 samples revealed a wide range of values for the different operational parameters, with values ranging between 128 and 6400 mg TAN L^{-1}, 2 and 1460 mg FA L^{-1}, 0 and 36.8 g VFA L^{-1} and pH values between 7.10 and 8.52. This indicates that depending on the OLR and influent stream composition different levels of steady state can be obtained.

Real-time PCR results showed an overall dominance of *Methanosaetaceae* in all the samples of the full-scale installation, with a minimum value of 3.2×10^{6} and a maximum value of 1.5×10^{10} copies g^{-1} sludge. *Methanosarcinaceae* on the other hand where far less dominant and remained below detection limit in 17 of the 38 samples, whilst *Methanosaetaceae* were present in all samples.
Amplicon sequencing results revealed an overall dominance of the bacterial phyla *Firmicutes* and *Bacteroidetes* in all samples. This was to be expected, as several *Clostridia sp.*, belonging to the *Firmicutes* phylum, form syntrophic interactions with hydrogenotrophic methanogens and the *Bacteroidetes* phylum contains several fermenting species [1].

Principle component analysis (PCA) revealed the presence of three distinct clusters with a distinct microbial community (Figure 1). The three clusters could be distinguished based on the operational conditions. Samples belonging to Cluster 1 were originating from digesters at "easy" conditions, i.e. low TAN, FA and VFA concentrations at mesophilic conditions. Cluster 2 contained samples from digesters at more "harsh" conditions, i.e. higher TAN, FA and VFA concentrations at mesophilic conditions, whereas Cluster 3 consisted of samples from thermophilic digesters at even more "harsh" conditions.

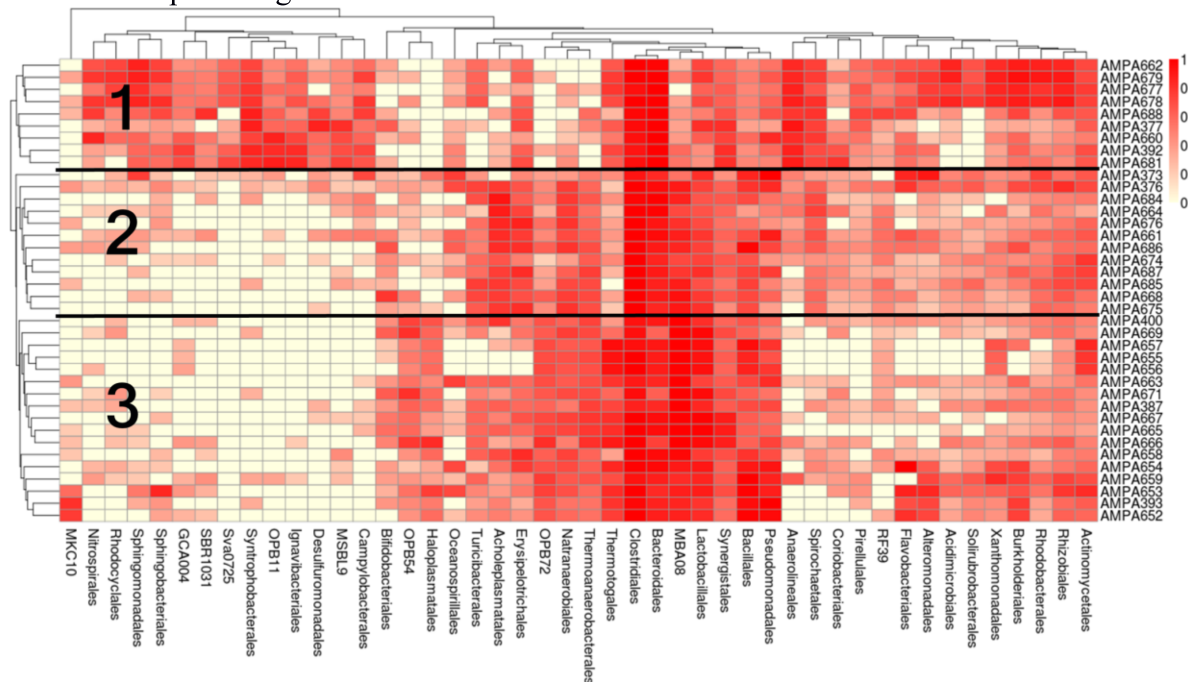

Figure 1 Heatmap representing all microbial orders present at a relative abundance ≥ 5% in at least one of the samples. Hierarchical clustering separated the samples into 3 distinct groups (numbered).

In conclusion, in this research three different "AD ecotypes" (clusters) were distinguished, depending on the operational conditions in the digester. *Methanosaeta sp.* appeared to be the dominant acetoclastic methanogens, clearly surpassing *Methanosarcina sp.*, irrespective of the operational conditions.

References

1. Vanwonterghem, I.; Jensen, P. D.; Ho, D. P.; Batstone, D. J.; Tyson, G. W., Linking microbial community structure, interactions and function in anaerobic digesters using new molecular techniques. *Curr. Opin. Biotechnol.* **2014,** *27*, (0), 55-64.

2. Angenent, L. T.; Karim, K.; Al-Dahhan, M. H.; Domiguez-Espinosa, R., Production of bioenergy and biochemicals from industrial and agricultural wastewater. *Trends Biotechnol.* **2004,** *22*, (9), 477-485.

3. Arumugam, M.; Raes, J.; Pelletier, E.; Le Paslier, D.; Yamada, T.; Mende, D. R.; Fernandes, G. R.; Tap, J.; Bruls, T.; Batto, J. M.; Bertalan, M.; Borruel, N.; Casellas, F.; Fernandez, L.; Gautier, L.; Hansen, T.; Hattori, M.; Hayashi, T.; Kleerebezem, M.; Kurokawa, K.; Leclerc, M.; Levenez, F.; Manichanh, C.; Nielsen, H. B.; Nielsen, T.; Pons, N.; Poulain, J.; Qin, J. J.; Sicheritz-Ponten, T.; Tims, S.; Torrents, D.; Ugarte, E.; Zoetendal, E. G.; Wang, J.; Guarner, F.; Pedersen, O.; de Vos, W. M.; Brunak, S.; Dore, J.; Weissenbach, J.; Ehrlich, S. D.; Bork, P.; Meta, H. I. T. C., Enterotypes of the human gut microbiome. *Nature* **2011,** *473*, (7346), 174-180.