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New insights into the key microbial phylotypes of anaerobic sludge digesters under different operational conditions

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ABSTRACT

Analyses on bacterial, archaeal communities at family level and methane-production metabolism were conducted in thirteen full-scale and pilot-scale anaerobic sludge digesters. These digesters were operated at different conditions regarding solids concentration, sludge retention time, organic loading rate and feedstock composition, seeking to optimize digester capacity. Correlations between process parameters and identified microbial phylotypes were evaluated based on relative abundance of these phylotypes determined by Quantitative PCR and 16S rDNA amplicon sequencing. Results showed that, Total Solids concentration (TS), among the evaluated operational parameters, demonstrated the most positive correlation with chemical parameters (including NH_3 and VFAs) and significant impact on the abundance of key microbial phylotypes regardless of other factors. Digesters were grouped into 'Higher-TS' with higher stress (TS > 44 g/L, NH_3 > 90 mg/L, VFAs > 300 mg/L) and 'Lower-TS' under easier status (TS ≤ 44 g/L, NH_3 < 120 mg/L, VFAs < 525 mg/L) in this study. We identified the key microbial phylotypes, i.e. the most abundant and discriminating populations, in 'Higher-TS' digesters with high biogas production rate, which were the class Clostridia, the family Methanosarcinaceae and the order Methanobacteriales. Thermoanaerobacteraceae and Syntrophomonadaceae were identified as key families of Clostridia. Methane was produced both from acetoclastic and hydrogenotrophic methanogenesis. By contrast, in 'Higher-TS' digesters with low biogas production rate, the classes Alpha-, Beta- and Gamma-proteobacteria were detected in higher percentages, of which Rhodobacteraceae, Comamonadaceae and Xanthomonadaceae were the most abundant families respectively, and Methanomicrobiales was the prevailing methanogen order. Consistently, hydrogenotrophic pathway was predominant for methanogenesis, indicating existence of syntrophic acetate oxidation in such 'high-stress', low biogas production rate digesters. These microbial phylotypes were therefore considered to be associated to 'Higher-TS' operation. In 'Lower-TS' digesters, the abundance of the class Delta-proteobacteria, the families Anaerolineaceae, Rikenellaceae, Candidatus Cloacamonas and Methanosaetaceae was obviously higher compared with those in 'Higher-TS' digesters, which were thus considered to be marker phylotypes of easy status. The influence of TS and NH_3 on the microbiome should be considered when a 'TS-increasing' strategy is applied to increase digester capacity.

1. Introduction

The Anaerobic Digestion (AD) process has been used for years in sewage sludge treatment and represents an attractive technique for sludge reduction along with energy production *via* biogas (Rivière et al., 2009). This biological process relies on a very delicate balance between four functional groups of microorganisms through a food web: hydrolytic bacteria decompose insoluble macromolecules to soluble molecules, acidogens and acetogens further

Abbreviations: AD, anaerobic digestion; AM, acetoclastic methanogenesis; HM, hydrogenotrophic methanogenesis; SAO, syntrophic acetate oxidation; OLR, organic loading rate; SRT, sludge retention time; TS, total solids; VS, volatile solids; PSS, primary sewage sludge; BSS, biological sewage sludge; HyBSS, hydrolyzed biological sewage sludge.

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degrade them into smaller intermediates and methanogens convert these smaller substrates into methane and carbon dioxide through Acetoclastic (AM) and Hydrogenotrophic (HM) pathways. This microbiome is highly complex in terms of functionality and community diversity.

Due to insufficient knowledge on the microbial community ecology and function, the AD process has limited performance, below its optimum capacity (Regueiro et al., 2015; Rivière et al., 2009; Couras et al., 2014). Since anaerobic digesters are costly to build and operate, many possible solutions are considered to increase their capacity without affecting biogas yield and solids reduction: for instance, to increase the Organic Loading Rate (OLR) by increasing the flow rate, which then induces a substantial decrease of Sludge Retention Time (SRT) (this approach is named 'hydraulic increase'). Too short SRT can however lead to the washout of microorganisms and impair the digester performance (Regueiro et al., 2015). Thus, an 'organic increase' solution, which consists in raising the concentration of Total (TS) and Volatile Solids (VS) in the feedstock, can be considered in order to circumvent the short SRT problem induced by only hydraulic increase (Regueiro et al., 2015; Couras et al., 2014; Mcleod et al., 2015). Other solutions include pretreating the feeding sludge by processes like thermal hydrolysis to improve its dewaterability and biodegradability (Carrère et al., 2010; Gagliano et al., 2015). During these attempts to optimize the digester capacity, the delicate balance between the four microbial groups can easily suffer inhibition from ammonia, sulphides, metals, pH, fatty acids and other organic compounds (Chen et al., 2008), or even wash out of microbes at low SRT. The sensitivity of anaerobic microorganisms to operational and environmental factors may lead to decreased digestion efficiency under increased capacity, or in extreme cases to complete failure. Therefore, it becomes important to identify the key microbial phylotypes under different operational strategies (De Vrieze et al., 2012) and to get an in-depth understanding of their functionalities, which may help to achieve higher digester capacity (Vanwonterghem et al., 2014).

Operational conditions of the digester, such as OLR, SRT, solids concentration (in the influent and effluent), temperature and reactor configuration have been reported to determine the microbial community composition to a large extent, which in turn influenced the digestion efficiency (De Vrieze et al., 2015). Several attempts have already been carried out to correlate the microbial community composition with the reactor operating conditions and performance in AD (De Vrieze et al., 2015; Abendroth et al., 2015; Regueiro et al., 2015; Karakashev et al., 2005, 2006; Lerm et al., 2012). During these attempts, little information is available yet concerning the relationship between operational strategies, digestion efficiency and bacterial populations of lower taxonomic levels (below class level), especially in sewage sludge AD process. That is however essential to understand the potential functionalities of the microbial communities and their interaction with environmental factors (Narihiro and Sekiguchi, 2007; Ariesyady et al., 2007).

Additionally, the microbial community structure varies a lot depending on the inoculum (Wilkins et al., 2015), feedstock composition and feeding pattern (Sundberg et al., 2013; Abendroth et al., 2015; De Vrieze et al., 2015). As a major feedstock of AD, sewage sludge itself is prone to natural and unavoidable variation caused by numerous aspects such as type and source (Mcleod et al., 2015), which lead to highly diverse and specific structured microbial communities. This further increases the difficulty of investigating the correlations between microbial populations and physical-chemical parameters, and accessing to the common rules that may uncover the mechanisms driving differentiation of microbial communities under different operational conditions.

To better understand the interactions between the key microbial phylotypes and operational factors in anaerobic digestion of sewage sludge, we investigated the archaeal and bacterial communities in 13 digesters working under different operational conditions including TS, SRT, OLR and substrate compositions. Relative abundance of archaeal and bacterial phylotypes was analyzed at the family level and their correlations with operational factors and methane-production efficiency and pathways were evaluated by statistical tools.

2. Materials and methods

2.1. Description of digesters and samples

Two full-scale, five pilot-scale and six laboratory-scale anaerobic digesters were investigated, which have been operated for >150 days after being set up and inoculated with anaerobic sludge originating from five different sources (Table S7). In these digesters, three types of sewage sludge from different sources were used as feedstock, including Primary Sewage Sludge (PSS), Biological Sewage Sludge (BSS) and Hydrolyzed Biological Sewage Sludge (HyBSS), which were produced in wastewater treatment plants located in three sites named "A", "B" and "C". A wide range of operational conditions were set among the different installations, and in some digesters, operational conditions (mainly OLR) changed periodically.

For microbiological analyses, 200 mL of sludge from full- and pilot-scale digesters or 50–100 mL from laboratory-scale digesters were taken from the recirculation loop to obtain representative samples, which were then divided into several aliquots, snap-frozen in dry ice and stored at −80 °C for further analyses. From these 13 digesters, 21 samples were collected for Quantitative PCR (QPCR) analyses, each from one specific operational condition of one or different digesters, during a relatively steady-state period with constant biogas production and stable VFA concentrations. Among them, 10 representative samples from distinct digesters were used for amplicon sequencing.

Samples were named according to the digester's scale and the substrate's characteristics: L-, P-, F- for Lab-, Pilot- and Full-scale respectively; A-, B-, C- for source of substrate; B-, BP-, hyBP- for BSS, mix of BSS and PSS, and mix of hyBSS and PSS. The following first number expressed TS concentration of the substrate. The last part indicated: the operational period with a number, for example, P-C-hyBP45_**2** represented the second period of digester P-C-hyBP45; or distinguished digesters of the same type with a letter, for instance, F-C-BP42-**F** represented the **F**irst digester of type F-C-BP42. Information concerning characteristics of liquid and biogas in the effluent (except stable isotopic composition), OLR, SRT, temperature, reactor type and volume, and composition of influent stream for different digesters was obtained directly from the digester operators.

2.2. DNA extraction and QPCR

Sludge was thawed on ice and centrifuged at 18,000 *g*, 4 °C for 5 min to obtain pellets. DNA was extracted from 0.20 to 0.25 g pellets using the PowerSoil DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer's instructions.

QPCR analysis for methanogenic members of Methanobacteriales, Methanomicrobiales, Methanosarcinaceae and Methanosaetaceae was performed on a BIO-RAD CFX96 Touch™ Real-Time PCR Detection System (Carlifornia, USA) by the Taqman method. The order Methanococcales was neither detected in a first QPCR test (unpublished data) nor in 16S rDNA sequencing results