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Ammonia and temperature determine potential clustering in the anaerobic digestion microbiome

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ABSTRACT

Anaerobic digestion is regarded as a key environmental technology in the present and future bio-based economy. The microbial community completing the anaerobic digestion process is considered complex, and several attempts already have been carried out to determine the key microbial populations. However, the key differences in the anaerobic digestion microbiomes, and the environmental/process parameters that drive these differences, remain poorly understood. In this research, we hypothesized that differences in operational parameters lead to a particular composition and organization of microbial communities in full-scale installations. A total of 38 samples were collected from 29 different full-scale anaerobic digestion installations, showing constant biogas production in function of time. Microbial community analysis was carried out by means of amplicon sequencing and real-time PCR. The bacterial community in all samples was dominated by representatives of the Firmicutes, Bacteroidetes and Proteobacteria, covering 86.1 ± 10.7% of the total bacterial community. Acetoclastic methanogenesis was dominated by Methanosaetaceae, yet, only the hydrogenotrophic Methanobacteriales correlated with biogas production, confirming their importance in high-rate anaerobic digestion systems. In-depth analysis of operational and environmental parameters and bacterial community structure indicated the presence of three potential clusters in anaerobic digestion. These clusters were determined by total ammonia concentration, free ammonia concentration and temperature, and characterized by an increased relative abundance of Bacteroidales, Clostridiales and Lactobacillales, respectively. None of the methanogenic populations, however, could be significantly attributed to any of the three clusters. Nonetheless, further experimental research will be required to validate the existence of these different clusters, and to which extent the presence of these clusters relates to stable or sub-optimal anaerobic digestion.

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1. Introduction

Anaerobic digestion (AD) can be considered one of the most prominent technologies in the field of renewable energy production. This microbial technology has been applied at full scale for the treatment of organic waste for several decades (Angenent et al., 2004; Appels et al., 2011; Holm-Nielsen et al., 2009; Verstraete et al., 2005). The amount of organic waste that is treated by means of AD still increases, with a yearly global growth of almost 25% (Appels et al., 2011), as new industrial organic waste streams are constantly being generated in the emerging bio-refineries (Menardo and Balsari, 2012; Ryan et al., 2009; Verstraete, 2010). Unlike energy consuming aerobic treatment technologies, AD leads to the formation of biogas that can be used as a renewable energy source, and a nutrient-rich digestate, that can be used as a fertilizer (Appels et al., 2011; Holm-Nielsen et al., 2009).

The microbial community completing the AD processes has a high complexity in terms of functionality and community diversity, and several attempts already have been carried out to determine the key microbial populations, as summarized by Vanwonterghem et al. (2014). This resulted in the well-known AD food web, consisting of 4 steps, i.e. hydrolysis, acidogenesis, acetogenesis and methanogenesis, with each step carried out by specific micro-organisms (Angenent et al., 2004; Vanwonterghem et al., 2014). The first three steps are performed by bacteria, while the methanogenesis is completed by a specific branch of archaea. Methanogenesis can take place via the hydrogenotrophic or acetoclastic pathway. Stable and continuous methane production, starting from complex organic substrates, requires a substrate-specific and close interaction between the micro-organisms carrying out the different steps (McInerney et al., 2009; Stams and Plugge, 2009). These interactions can take place through successive metabolite or (in)direct electron transfer (McInerney et al., 2009; Rotaru et al., 2014; Stams and Plugge, 2009). In specific cases, due to energy limitations, the partnership between two micro-organisms becomes necessary to maintain the metabolic activity that they are unable to achieve on their own. This interaction is called syntrophy (Schink, 1997; Schink and Stams, 2013).

Syntrophic interactions, however, require specific partners to perform particular processes. Syntrophic acetate oxidation requires partnership of specific bacteria, of which most are representatives of the Clostridia class, with hydrogenotrophic methanogens (Hattori et al., 2000; Schnurer et al., 1996; Westerholm et al., 2011b). The presence or absence of specific microbial taxa, as well as the occurrence of certain (syntrophic) pathways, depends on several factors. First, the substrate composition determines to a great extent the microbial community composition and organization. Indeed, it not only defines reactor conditions, but also provides the introduction of new species that are present in the substrate matrix, as, for instance, is the case for manure and waste activated sludge (Sundberg et al., 2013; Zhang et al., 2014b). For example, nitrogen-rich substrates lead to high total ammonia concentrations, which in several cases initiated a shift from acetoclastic methanogenesis to syntrophic acetate oxidation, coupled to hydrogenotrophic methanogenesis (Karakashev et al., 2006; Schnurer and Nordberg, 2008; Schnurer et al., 1999; Sundberg et al., 2013). Second, the feeding pattern of the substrate may also influence the microbial community (Conklin et al., 2006; De Vrieze et al., 2013; Xing et al., 1997). Finally, other operational parameters of the digester, such as temperature, organic loading rate, sludge retention time, and reactor configuration, also determine the microbial community to a large extent (Carballa et al., 2011; Leitao et al., 2005).

This high degree of potential variables makes it difficult to determine the main selecting factors for microbial community composition and organization. Evaluation of the microbial community of in total 51 full-scale AD plants, and 28 full-scale aerobic wastewater treatment plants led to the identification of a core microbial community in both cases. However, no clear significant correlation with operational parameters or plant design could be determined (Leclerc et al., 2004; Mielczarek et al., 2012, 2013; Riviere et al., 2009).

In this study, an extensive molecular analysis by means of 16S rRNA amplicon sequencing and real-time PCR was carried out on the microbial community of 38 samples from 29 full-scale AD plants. It was hypothesized that differences in operational parameters might lead to particular configurations of microbial communities in full-scale AD installations. Potential clustering of the samples was investigated, and environmental and operational parameters driving the overall microbial community composition and organization were identified.

2. Materials and methods

2.1. Sample and data collection

In total, 38 samples were collected from 29 different full-scale AD installations. Those samples with the same name and a different number originate from the same anaerobic digestion plant at a different time point. Samples of at least 1 L were taken directly from the reactor suspension, and transferred to the laboratory in air-tight recipients, upon which a direct measurement of the pH was carried out. Samples for total ammonia, conductivity, volatile solids and total solids analysis were stored at 4 °C until further analysis. Samples for volatile fatty acids and microbial community analysis were stored directly at −20 °C, prior to analysis. Information concerning the organic loading rate, sludge retention time, biogas production and composition, temperature, reactor type and volume, and influent stream composition of the different digesters was obtained directly from the plant operator. For further analysis of the different parameters, only those samples for which a value was obtained were included in the analysis.

2.2. 16S rRNA gene amplicon sequencing

Total DNA extraction from the digestate samples was carried out by means of the FastDNA® SPIN Kit for Soil (MP Biomedicals, Solon, OH, USA), following the manufacturer's instructions. Prior to extraction, the samples were thawed, and homogenized by means of a Vortex-Genie® 2T (Scientiis