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Understanding microbial ecology can help improve biogas production in AD

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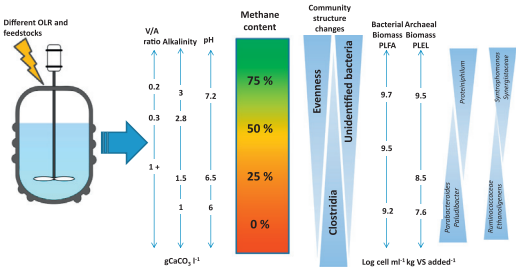
HIGHLIGHTS

- For all feedstocks, bacterial community composition was governed by methane content.
- Reactors with >60% methane had a more even distribution of bacterial diversity.
- Methane < 30% correlated to a 50% increase in Firmicutes (Ruminococcaceae).
- Methane > 60% correlated to unidentified OTUs and Synergistaceae.

GRAPHICAL ABSTRACT



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ABSTRACT

454-Pyrosequencing and lipid fingerprinting were used to link anaerobic digestion (AD) process parameters (pH, alkalinity, volatile fatty acids (VFAs), biogas production and methane content) with the reactor microbial community structure and composition. AD microbial communities underwent stress conditions after changes in organic loading rate and digestion substrates. 454-Pyrosequencing analysis showed that, irrespectively of the substrate digested, methane content and pH were always significantly, and positively, correlated with community evenness. In AD, microbial communities with more even distributions of diversity are able to use parallel metabolic pathways and have greater functional stability; hence, they are capable of adapting and responding to disturbances. In all reactors, a decrease in methane content to <30% was always correlated with a 50% increase of Firmicutes sequences (particularly in operational taxonomic units (OTUs) related to Ruminococcaceae and Veillonellaceae). Whereas digesters producing higher methane content (above 60%), contained a high number of sequences related to Synergistetes and unidentified bacterial OTUs. Finally, lipid fingerprinting demonstrated that, under stress, the decrease in archaeal biomass was higher than the bacterial one, and that archaeal Phospholipid etherlipids (PLEL) levels were correlated to reactor performances. These results demonstrate that, across a number of parameters such as lipids, alpha and beta diversity, and OTUs, knowledge of the microbial community structure can be used to predict, monitor, or optimise AD performance.

1. Introduction

Anaerobic digestion (AD) is a widely implemented technology for the treatment of wastewater and organic mixed solid wastes. Notwithstanding this, poor anaerobic digester performance and system failure are still common issues. Most of these problems originate from inadequate operational and process control and a lack of understanding of the dynamics of the microbial processes taking place in the digesters (Leitao et al., 2006). Plant management is mainly achieved through the monitoring of physicochemical parameters rather than biological ones. However, there is a general consensus

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among the scientific community that in-depth understanding of the AD microbial communities and their ecology is vital to optimise and adequately manage the process (Ferguson et al., 2014; Rittmann et al., 2006). Developments in culture independent molecular methods have led to a number of studies analysing the microbial communities in AD reactors, both at laboratory (Ferguson et al., 2016; Goux et al., 2015; Vanwonterghem et al., 2015) and at full-scale (Werner et al., 2012a, 2012b; Valentin-Vargas et al., 2012). Most of these studies demonstrated that the microbial ecology of AD is highly diverse and dynamic. Unstable communities have been observed in digesters with stable performance and functional redundancy renders it difficult to formulate any generic trends/relationships between microbial community response and digester performance (Fernández et al., 1999; Goux et al., 2015; Wang et al., 2011). Recently, studies of the microbial ecology of wastewater anaerobic digesters showed that it was possible to link digester performance with fundamental ecological parameters such as community evenness, as well as ecological theories such as the biogeography model, the species-area relationships and the taxa-time relationships (Wells et al., 2011; Valentin-Vargas et al., 2012; Werner et al., 2012a, 2012b). Therefore, as discussed by a number of authors, the possibility of integrating the engineering of anaerobic digesters with microbial ecological theory is now a genuine prospect (Rittmann et al., 2006; Vanwonterghem et al., 2014a, 2014b).

In this context, there are still a number of key problems that need to be addressed; in particular and the relationship between AD performance and microbial community structure (alpha and beta diversity) and the consistency of these correlations. In short, to what extent do deterministic or stochastic processes determine the structure of microbial communities in AD. If stochastic processes dominate then unique functionally redundant microbial communities will exist in different digesters, making prediction of AD based on microbial community impossible. However, if deterministic processes dominate it will be possible predict species turnover and hence use this to monitor and predict AD performance (Måren et al., 2018). The syntrophic relationships involved in methanogenic degradation of most organic substrates in AD mean that species co-occurrence will be relatively even, and that species with similar ecological requirements will respond in similar ways (Schink, 2002). This means that it is probable that monitoring based on the presence of certain phylogenetic or functional groups should be possible after a deeper understanding of the AD microbial community.

Molecular based lipid fingerprinting and PCR-based 454-pyrosequencing analyses were carried out to investigate the microbial community structure, biomass and dynamics in digesters running under different conditions (with varying co-digestion substrates and changing organic loading rate (OLR)). Molecular based lipid fingerprinting analysis provided insights into the microbial biomass changes and microbial community structure in the digesters. Whereas, 454-pyrosequencing was used to gain detailed phylogenetic information on both the dominant and minor important members of the microbial community.

2. Methods

2.1. Digester operational parameters

Laboratory-scale semi-continuous digesters consisted of 1-L borosilicate glass bottles with a 700 ml working volume and 5-L bottles with a 4.5-L working volume maintained at 38 °C using a water bath. All reactors were seeded with digested sludge from a commercial Sewage Treatment digester (in a ratio of 30:70%) and fed with autoclaved primary sludge three times a week to achieve a retention time of 7 days and an organic loading rate (OLR) of 1.4 kg VS m^{-3} d^{-1}. A different organic waste (glycerol or fat rich – FOG waste collected from a restaurant grease trap) was used to induce periods of unstable performance in the digesters (see Table 1 for details of feedstocks). Glycerol or FOG was added to the autoclaved primary sludge to increase the OLR from 1.4 kg VS m^{-3} d^{-1} to 2.9 for one hydraulic retention time (HRT = 7 days) and then returned to 1.4 kg VS m^{-3} d^{-1}. These OLRs were selected as they were known to cause digester failure based on our preliminary work. All the reactors were run for >130 days (18–20 HRT) depending on the substrate. The effects of one or two sequential changes in OLR were investigated using the same feedstock (glycerol - glycerol) or with a different feedstock (glycerol - FOG waste). Feedstock and feeding regimes are those reported previously (Ferguson et al., 2016).

2.2. Biogas production, methane concentration and physicochemical characterisation

Gas production was measured daily by water displacement in a glass column (150 × 5 cm) and volumes corrected to standard atmospheric conditions. Methane content was measured using a SERVOPRO1400 CH_4 gas analyser (Servomex, UK) according to manufacturer recommendations. pH and alkalinity were measured according to standard APHA methods (APHA, 1989).

2.3. Volatile fatty acids analysis

A 40 ml aliquot of the digestate was centrifuged at 5000*g* for 5 min and the supernatant was filtered to <0.45 μm with a syringe filter (Eduok et al., 2017). 5 μl of 97% sulphuric acid was added (to avoid acid degradation during storage) and the sample was stored at −20 °C until analysis. 100 μl of the sample was injected into a HPLC (535 Kontron, Bio-TEK, UK) equipped with a Bio-Rad fermentation column (Cat 125-0115) 300 × 7.8 mm maintained at 65 °C, and a UV detector at 210 nm. The mobile phase was 0.001 M sulphuric acid in HPLC grade water with a flow rate of 0.8 ml/min. Acetic, propionic, *n*-butyric, isobutyric and lactic acids were quantified using an external multilevel calibration ranging from 0.1 g l^{-1} to 5 g l^{-1}. The % error in the repeatability of measurements for each acid was <4%.

Table 1
Summary of seed, primary sludge and co-digestion feedstock composition. Triplicate average error bars show standard deviation.

Characteristic	Unit	Seed	Primary sludge	Co-digestion		
Co-digestion substrate				Glycerol waste + PS		FOGs waste + PS
Co-digestant concentration	g l^{-1}			30	50	1.5
pH		7.73 ± 0.005	7.09 ± 0	7.40 ± 0.04	7.43 ± 0.03	7.53 ± 0.1
TS*	%	4.59 ± 0.46	1.46 ± 0.56	2.34 ± 0.43	2.53 ± 1.65	2.33 ± 1.36
VS**	% of TS	63.17 ± 0.04	65.93 ± 0.13	88.38 ± 2.31	91.66 ± 3.42	97/82 ± 1
sCOD***	g l^{-1}	237 ± 0.65	43.0 ± 1.45	84.46 ± 0.97	115.65 ± 0.62	141.43 ± 3
Alkalinity	g l^{-1} $CaCO_3$	5.5 ± 0.5	2.5 ± 0.7	2.3 ± 0.1	2.4 ± 0.6	2.1 ± 1.2

* TS = total solids.
** VS = volatile solids.
*** sCOD soluble chemical oxygen demand.