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Monitoring methanogenic population dynamics in a full-scale anaerobic digester to facilitate operational management

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HIGHLIGHTS

- Monitoring methanogenic populations facilitated digester management.
- Decreasing methanogenic populations corresponded with increasing VFAs and instability.
- Ammonia >156 mg/L caused a shift from aceticlastic to hydrogenotrophic methanogens.
- Addition of trace elements and alkalinity stimulated microbial populations.
- Methanogens from the family *Methanosaetaceae* dominated when underfeeding.

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ABSTRACT

Microbial populations in a full-scale anaerobic digester fed on food waste were monitored over an 18-month period using qPCR. The digester exhibited a highly dynamic environment in which methanogenic populations changed constantly in response to availability of substrates and inhibitors. The methanogenic population in the digester was dominated by *Methanosaetaceae*, suggesting that aceticlastic methanogenesis was the main route for the production of methane. Sudden losses (69%) in *Methanosaetaceae* were followed by a build-up of VFAs which were subsequently consumed when populations recovered. A build up of ammonium inhibited *Methanosaetaceae* and resulted in shifts from acetate to hydrogen utilization. Addition of trace elements and alkalinity when propionate levels were high stimulated microbial growth. Routine monitoring of microbial populations and VFAs provided valuable insights into the complex processes occurring within the digester and could be used to predict digester stability and facilitate digester optimization.

1. Introduction

Anaerobic digestion (AD) is a method of biological waste treatment which diverts waste from landfill and generates a valuable renewable energy source in the form of biogas. In Europe, increasing restrictions on sending organic waste to landfill combined with subsidies for renewable energy, have improved the economics of the process, resulting in a rapid deployment of this technology in a number of countries. Although AD is a well proven technology, digesters can be susceptible to process instability (Chen et al., 2008, 2012; Kleyböcker et al., 2012) for example during start-up or periods of stress due to inhibitory substances or sudden changes in organic loading.

Significant understanding of the biochemical interactions occurring during anaerobic digestion has been developed in the last few decades. However, the process is delivered by complex and dynamic systems where mechanical, microbiological and physicochemical aspects are closely linked and influence process performance. Process stability is dependent on the critical balance between the symbiotic growth rates of the principal groups of bacteria and archaea, i.e., acid forming bacteria, acetogens and methanogens (Schink, 1997). The fact that digesters operate with different substrates and operating conditions (e.g., retained or suspended bacterial culture, organic loading rates, retention times and temperature) and are populated by mixed bacterial and archaeal cultures with a competitive nature makes digesters performance difficult to predict and control. A more in-depth understanding of the complex biochemical interactions that determine digester stability and promote enhanced performance remains a significant challenge facing the AD industry today.

Routine monitoring of the AD process is critical to ensure intermediates such as hydrogen, acetate and propionate do not accumulate resulting in acidification and process failure. However,

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strategies employed for the routine monitoring and control of digesters are variable and depend on the individual plant. Madsen et al. (2011) has reported that many plants are operated based mainly on ex situ analysis and only sensors such as pH, redox potential and gas production rates are being employed in situ or in-line. Measurements such as alkalinity and VFA concentrations are also used at some plants to provide warning of an imbalance in the digester. These analyses however are not frequent and feedstocks are not always characterised.

Methanogens play an important role in waste mineralization as a result of their hydrogen and acetate consuming activities. However, despite of their importance, they have not been routinely monitored in full-scale anaerobic digesters. In addition, methanogens are difficult to culture because they are strict anaerobes and in many cases their growth requirements are unknown. Culture-independent molecular techniques such as real-time polymerase chain reaction (qPCR), denaturing gradient gel electrophoresis (DGGE) and sequencing have been developed and proven to be valuable tools for studying the biodiversity of complex microbial communities such as those in anaerobic digesters (Liu et al., 2002; Roest et al., 2005; Hori et al., 2006; Nettmann et al., 2008; Shin et al., 2008; Cardinali-Rezende et al., 2012). Yu et al. (2005) designed primer and probe sets for the detection and quantification of order-level (*Methanococcales*, *Methanobacteriales* and *Methanosarcinales*) and family-level (*Methanosarcinaceae* and *Methanosaetaceae*) methanogenic groups using qPCR based on the 16S rRNA gene. These group specific primer sets have been used to monitor population dynamics and community structures in anaerobic processes (Yu et al., 2006; Lee et al., 2009; Bialek et al., 2011). The combination of molecular tools with other methodologies such as activity measurements further enhanced the degree of understanding of these complex environments (Regueiro et al., 2012).

Only a few laboratory studies have investigated the impact of operational parameters like organic loading on the composition of methanogenic communities in anaerobic reactors (McMahon et al., 2004; Blume et al., 2010). Substrate overloading can lead to process imbalance, resulting in accumulation of acids and cessation of gas production (Leitão et al., 2006). However, in some studies the microbial populations have been shown to respond to substrate overloading or changing feeding regimes, overcoming the perturbation without process disruption (Chen et al., 2012; Vrieze et al., 2013).

The different responses observed from these studies emphasises the complexities present in individual systems and the difficulties in drawing conclusions or generalizations about other systems.

Whilst controlled lab scale studies have provided valuable insights into the AD process, it is also important to obtain an understanding of the dynamic processes occurring in full-scale digesters in which the inputs of feedstocks are not so tightly controlled and microbial populations are constantly being challenged due in many cases to multiple variations in volumetric and organic loading, trace elements and inhibitory substances such as ammonium and hydrogen sulphide. Knowledge is limited on the methanogenic communities present in full-scale anaerobic digesters. A few studies have investigated the diversity of the archaeal community in samples taken from different digesters (Leclerc et al., 2004; Karakashev et al., 2005; Regueiro et al., 2012). Collectively, these studies have shown marked differences in the diversity and dominance of the methanogenic communities between different full-scale digesters. However, as operating conditions are constantly changing, it is necessary to follow community changes over time within the same digester. This study is the first report of long-term monitoring of the diversity of methanogenic archaea in a full-scale digester treating food waste, which in conjunction with monitoring of the volatile fatty acid (VFA) intermediates and alkalinity has supported operational control and digester optimization by moderating organic loading, addition of trace elements and alkalinity.

Table 1
Typical solid composition of feedstocks.

Feedstock	TS%	$VS\%_{ww}$	$VS\%_{dw}$
DAF sludge	6.97	6.27	91.32
Depackaged food waste	9.34	8.86	94.50
Potato waste	4.37	3.89	92.21
Delactosed whey waste	54.20	47.20	87.10
Rendering waste	9.63	8.31	86.29

2. Methods

2.1. Operational parameters of the digester and sampling

The digester (Insource Energy AD Plant at Rogerstone, South Wales) was a continuously stirred tank reactor (CSTR) with a capacity of 3090 m^3, operated at mesophilic temperature (38 °C). The digester was seeded with approximately 1600 m^3 of sewage sludge from a waste-water treatment plant in February 2011 (Time 0). The digester was fed throughout with approximately 30–50 t wet weight d^{-1} food waste (waste potato, potato sludge, depackaged food from ready meals and sludge from a dissolved air flotation (DAF) unit treating wastewater) arising from an adjacent factory. The typical solid compositions of the food wastes fed into the digester is shown in Table 1. The digester was initially fed at an organic loading rate (OLR) of about 0.45 kg VS m^{-3} d^{-1}. The OLR was then gradually increased over time to 1.0 kg VS m^{-3} d^{-1}. From day 343 onwards, additional waste materials were also co-digested, i.e., liquid waste from a rendering plant and delactosed whey. The hydraulic retention time (HRT) of the substrate was 60–100 days and the pH was between 7.4 and 7.55. 1000 L of EnVital mineral and trace elements (EnviTec Biogas) and 1000 L Kalic liquid lime (Tarmac Ltd.) was added to the digester on day 272 to improve digester stability.

Biogas produced in the digester was utilised in a combined heat and power (CHP) engine to generate electricity. The quantities of feed added (kg), CH_4 (%) in the biogas, temperature and CHP output (kW) were continuously monitored using the plant's SCADA system. Data was averaged weekly for graphical representation.

Weekly samples (500 ml) were collected from the full-scale anaerobic digester over an 18-month period. During periods when VFAs were shown to be accumulating, samples were obtained daily from the digester. Samples were normally processed within 2 h (or stored overnight at 4 °C) for solid, pH and alkalinity determinations. Sub samples were also frozen within 2 h at −20 °C for subsequent DNA extraction, cation and volatile fatty acid analysis.

2.2. Analytical methods

Analyses of total and volatile solids (TS and VS) in the digester were performed according to American Public Health Association standard methods (APHA, 2005). Partial alkalinity (PA), intermediate alkalinity (IA) and the Ripley ratio were determined according to Ripley et al. (1986). Soluble cations (ammonium, potassium and sodium) were determined by ion chromatography using a Dionex ICS3000, equipped with an IonPac CS12A column (Dionex, Camberley, UK). Digestate samples (1.5 ml) were centrifuged (10,000 rpm, 5 min) and the supernatant was filtered through 0.45 μm pore size filters. The Dionex instrument was calibrated using a range of standard solutions prior to the analysis of samples.