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An 'omics' approach towards the characterisation of laboratory scale anaerobic digesters treating municipal sewage sludge

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ABSTRACT

In this study, laboratory scale digesters were operated to simulate potential shocks to the Anaerobic Digestion (AD) process at a 350 ML/day wastewater treatment plant. The shocks included high (42 °C) and low (32 °C) temperature (either side of mesophilic 37 °C) and a 20% loading of fats, oil and grease (FOG; 20% w:v). These variables were explored at two sludge retention times (12 and 20 days) and two organic loading rates (2.0 and 2.5 kgTS/m^{3}day OLR). Metagenomic and metabolomic approaches were then used to characterise the impact of operational shocks in regard to temperature and FOG addition, as determined through monitoring of biogas production, the microbial profile and their metabolism. Results showed that AD performance was not greatly affected by temperature shocks, with the biggest impact being a reduction in biogas production at 42 °C that persisted for 32 ± 1 days. The average biogas production across all digesters at the completion of the experiment was 264.1 ± 76.5 mL/day, with FOG addition observed to significantly promote biogas production (+87.8 mL/day). Metagenomic and metabolomic analyses of the digesters indicated that methanogens and methane oxidising bacteria (MOB) were low in relative abundance, and that the ratio of oxidising bacteria (methane, sulphide and sulphate) with respect to sulphate reducing bacteria (SRB) had a noticeable influence on biogas production. Furthermore, increased biogas production correlated with an increase in short chain fatty acids, a product of the addition of 20% FOG. This work demonstrates the application of metagenomics and metabolomics to characterise the microbiota and their metabolism in AD digesters, providing insight to the resilience of crucial microbial populations when exposed to operational shocks.

1. Introduction

Effective sludge handling and management forms a major component in the treatment of municipal wastewater and can contribute up to 50% of the entire costs of wastewater treatment (Appels et al., 2008). A common approach employed in a modern treatment facility comprises a biological treatment step using anaerobic digestion (AD). The AD process degrades the majority of the organic content in wastewater sludge, greatly reducing the total amount of solids produced. This process also stabilizes the sludge to control odours while also reducing pathogens (Smith et al., 2005). Furthermore, AD produces renewable energy in the form of biogas, typically comprised of 50–70% methane (Athanasoulia et al., 2012; Bolzonella et al., 2005; Herout et al., 2011), which can be used to offset a utility's carbon emissions (Beale et al., 2013a). AD is therefore considered as a cheap and low energy input process, with an overall positive energy output (Appels et al., 2008; Chen et al., 2008). Recent trends in the AD of municipal wastewater sludge have also demonstrated that co-digestion with food (*i.e.,* FOG) and other highly degradable feedstocks (*i.e.,* glycerol) can lead to significant increases in methane production, and therefore greater offsets against rising energy costs (US EPA, 2014).

In spite of all the advantages, AD suffers from some limitations

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and challenges, which still remain to be addressed in order to avoid failure and inefficient operation, which may arise due to biological and/or chemical imbalances within the digester. These limitations result from biological and non-biological inhibitors, sludge retention times (SRT), organic loading rates (OLR), temperature, aerobic or anaerobic conditions and pH, among numerous others (Chen et al., 2008; de la Rubia et al., 2006; Lee et al., 2011; Nges and Liu, 2010; Razaviarani et al., 2013).

Ammonia and sulphide formation by nitrate reducers and sulphate reducing bacteria (SRBs), respectively, form some of the major inhibitors of methanogenesis, with the latter being the dominant competitor of methanogens for electron donors and substrates (Brand et al., 2014; Moestedt et al., 2013). Additionally, they produce considerable quantities of H_2S, SO_2 and other sulphides, which not only inhibit the methane production but are also toxic towards a vast number of other fermenters involved in the wastewater treatment process. It has been observed that methanogens are one of the most sensitive anaerobes towards this toxicity (Appels et al., 2008; Chen et al., 2008; de Arespacochaga et al., 2014). In spite of its toxicity, ammonia concentrations below a threshold level of 0.2 g/L have been observed to be a growth promoter for numerous anaerobes (Liu and Sung, 2002).

With regards to FOG, long-chain fatty acids (LCFAs) are also known to inhibit methanogenesis (Fogarty and Tobin, 1996), and in particular, methane producing Gram-positive bacteria. LCFAs are the primary components of FOG and are degraded by anaerobes using the β-oxidation reaction pathway, primarily to generate acetate and H_2, followed by methanogenesis (Madigan et al., 2014). Whilst FOG has a higher methane potential than most digestion feedstocks, if overloaded, attention must be paid to controlling the inhibition caused by LCFAs. The theoretical yields of methane due to the addition of FOG have been estimated to be 1.4 L biogas/g of substrate. It follows that total methane production is increased by ca. 30% in digesters which have ca. 10–30% FOG (Long et al., 2012), with a recent observation describing a 137% methane yield (Wan et al., 2011).

Due to AD being a biological process, temperature is a key determinant of the composition of bacterial populations and their susceptibility to various inhibitions. AD is usually reported to occur in three stages; hydrolysis, acidogenesis and methanogenesis. Methanogenic organisms require temperatures of 30–38 °C and within this temperature range, a matching acidogenesis rate (and also acetogenesis of LCFAs) needs to be established to ensure that inhibition does not occur. Hydrolysis, on the other hand, is improved at high temperatures and is often encouraged using thermal hydrolysis pretreatments operating at 150–180 °C (600–1000 kPa pressure). These processes have been proven at full scale (Cano et al., 2015).

With these inhibition challenges in mind, a series of experiments were undertaken to study the effects of different temperatures (*i.e.,* high and low shocks) and the addition of 20% FOG compared to standard operating conditions, in order to investigate the total biogas generation from the activated sludge obtained from municipal wastewaters. Furthermore, the experiments included metagenomic and metabolomic analyses in order to characterise the microbial and metabolite distribution and variation in the activated sludge treated under varied conditions, thereby elucidating the effect on an overall microbial population responsible for the AD process.

The application of metagenomics to characterise microbial populations in anaerobic digesters is not new (Talbot et al., 2008; Vanwonterghem et al., 2014). For example, Tuan et al. (2014) used multiple approaches including denaturing gradient gel electrophoresis (DGGE), clone library and pyrosequencing techniques applied to a thermophilic anaerobic digester using swine manure as sole feedstock revealing that Clostridia from the phylum Firmicutes account for the most dominant Bacteria. Pap et al. (2015) used metagenomics to monitor the microbial community in mesophilic digesters (37 °C) that were gradually switched to thermophilic (55 °C) operation. In this study, temperature adaptation resulted in a clearly thermophilic community having a generally decreased complexity compared to the mesophilic system (Pap et al., 2015). Furthermore, Kovács et al. (2015) demonstrated that a protein-rich mono-substrates can lead to sustainable biogas production using metagenomics. Yang et al. (2014) conducted a metagenomics survey of anaerobic digestion sludge (ADS) from two wastewater treatment plants, and identified Proteobacteria (9.52–13.50%), Bacteroidetes (7.18%–10.65%) and Firmicutes (7.53%–9.46%) were the most abundant phyla. Furthermore, genera of Methanosaeta and Methanosarcina were the major methanogens (Yang et al., 2014). However, to the author's knowledge, the application of metagenomics and metabolomics applied to AD treating municipal wastewater sludge has not been reported. It is anticipated that through the metagenomic and metabolomic approaches, new insight on microbial populations in terms of diversity and activity when exposed to AD shocks and stress will be obtained. The entire set of methane generation experiments coupled with different 'omics' approaches was performed to illustrate strengths, weaknesses and robustness of AD of activated sludge in order to provide possible solutions to improve overall metabolic efficiencies of higher methane generation and effective wastewater treatment.

2. Methods and materials

2.1. Inoculum and substrate

Anaerobically digested sludge was acquired from the Eastern Treatment Plant (Melbourne Water, Bangholme, Victoria, Australia). The components considered for this study were Primary Sludge (PS) and Waste Activated Sludge (WAS). The samples were stored at 4 °C before applying further treatment, as suggested previously (Elefsiniotis and Oldham, 1994). As per the procedure suggested by the treatment plant operator, PS and WAS were mixed in a ratio of 0.9:1 (w/w). To obtain the appropriate solids concentrations for the varying OLR at different SRT's, the samples were first centrifuged at 2290 g for 8 min using an Eppendorf 5702 bench-top centrifuge (Eppendorf Vertrieb Deutschland GmbH, Wesseling-Berzdorf, Germany). The supernatant was then decanted and solids were re-mixed with the sludge mixture.

2.2. Digester set-up

The samples used in this paper were obtained from the laboratory scale digesters described in McLeod et al. (2015). Briefly, laboratory glass bottles of 500 mL volume (Duran Group GmbH, Wertheim/Main, Germany) were used as the digesters, each bottle was modified to include two GL 14 fittings that were added to the upper region of the laboratory bottle, which were sealed using Suba seal-13 septa (Sigma–Aldrich Pty. Ltd., Sydney, Australia). The digesters were then seeded with the inoculum (sludge mixture, 300 mL effective volume). The bottles were flushed with nitrogen gas (purity: 99.9%) for 2.0 min to create an anaerobic environment via one of two GL14 fittings. The digesters were then incubated in Infors-HT Multitron Standard incubation shakers (Infors AG, Bottmingen, Switzerland) at a constant rotational speed of 100 rpm. Each digester was operated in a semi-continuous manner with a daily feeding and wasting. Biogas generation was measured every 24 h using a liquid displacement gasometer (Walker et al., 2009). Post biogas removal, the digesters were re-flushed with nitrogen gas for about 1 min to restore anaerobic conditions. The sludge was