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Biogas production and methanogenic archaeal community in mesophilic and thermophilic anaerobic co-digestion processes

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ABSTRACT

Over 258 Mt of solid waste are generated annually in Europe, a large fraction of which is biowaste. Sewage sludge is another major waste fraction. In this study, biowaste and sewage sludge were co-digested in an anaerobic digestion reactor (30% and 70% of total wet weight, respectively). The purpose was to investigate the biogas production and methanogenic archaeal community composition in the anaerobic digestion reactor under meso- (35–37 °C) and thermophilic (55–57 °C) processes and an increasing organic loading rate (OLR, 1–10 kg VS m^{-3} d^{-1}), and also to find a feasible compromise between waste treatment capacity and biogas production without causing process instability. In summary, more biogas was produced with all OLRs by the thermophilic process. Both processes showed a limited diversity of the methanogenic archaeal community which was dominated by *Methanobacteriales* and *Methanosarcinales* (e.g. *Methanosarcina*) in both meso- and thermophilic processes. *Methanothermobacter* was detected as an additional dominant genus in the thermophilic process. In addition to operating temperatures, the OLRs, the acetate concentration, and the presence of key substrates like propionate also affected the methanogenic archaeal community composition. A bacterial cell count 6.25 times higher than archaeal cell count was observed throughout the thermophilic process, while the cell count ratio varied between 0.2 and 8.5 in the mesophilic process. This suggests that the thermophilic process is more stable, but also that the relative abundance between bacteria and archaea can vary without seriously affecting biogas production.

1. Introduction

1.1. Anaerobic digestion

In Europe, municipalities produce in excess of 258 Mt of solid waste annually (Montejo et al., 2010), a large fraction of which is biowaste. Sewage sludge, an insoluble residue produced during wastewater treatment and subsequent sludge stabilization, is another major waste fraction (Arthurson, 2008). Anaerobic digestion is an established and sustainable treatment option for biowaste and sewage sludge, giving that according to the European Council Regulation (EC) No. 1774/2002 the process residues can potentially be used as a biofertiliser in agriculture (Bagge et al., 2005; Arthurson, 2008; Lozano et al., 2009; Goberna et al., 2010). The biogas produced by anaerobic digestion processes is a valid substitute for fossil fuels in a myriad of technical applications, the actual application determining the quality requirements of the gas produced (Bagge et al., 2005; Kymäläinen et al., 2012). Anaerobic digestion produces methane, carbon dioxide, a number of trace gases, some heat, and an end product of stabilised sludge. A typical organic loading rate (OLR) for fully mixed anaerobic digesters lies between 1 and 5 kg COD m^{-3} d^{-1} (Tchobanoglous et al., 2003). There are four stages in anaerobic digestion — hydrolysis, acidogenesis, acetogenesis and methanogenesis. Bacterial groups are responsible for acetate, hydrogen and carbon dioxide production in the first three stages. In the last stage, methanogenic archaea produce methane from acetate, or alternatively from hydrogen and carbon dioxide (Griffin et al., 1998; Liu et al., 2004; Bouallagui et al., 2005; Kotsyurbenko, 2005; Lozano et al., 2009; Pycke et al., 2011; Ritari et al., 2012).

The most common problematic organic wastes are those that are rich in lipids, cellulose and proteins. Previous studies have demonstrated that combining different organic wastes for anaerobic co-digestion results in a substrate better balanced and more

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efficiently degradable, leading to a significant increase in biogas production (Esposito et al., 2012). Wang (2009) and Wu et al. (2010) reported significant biogas production increases in the co-digestion process by combining carbon rich agricultural residues with swine manure.

1.2. Microorganisms in anaerobic digestion

Microbial communities in anaerobic co-digestion processes respond easily to changes in substrate composition, OLR, reactor design and operating temperatures (Tang et al., 2011; Dohrmann et al., 2011; Levén et al., 2007; McHugh et al., 2004). Previously, only a few studies have focused on the effects of temperature on bacterial and methanogenic archaeal communities in anaerobic bioreactors (Pycke et al., 2011; Levén et al., 2007; Pender et al., 2004; Hernon et al., 2006; Sekiguchi et al., 1998, 2002). Anaerobic digestion reactors have commonly been operated at mesophilic (30–40 °C) and thermophilic (50–60 °C) temperatures. In general, higher bacterial and archaeal diversities are found at mesophilic temperatures (Levén et al., 2007; Pycke et al., 2011). Bacterial communities appear to be considerably more diverse and dynamic than archaeal communities at any temperature (McHugh et al., 2004; Ritari et al., 2012). Despite lower diversity, digestion at thermophilic temperatures results in higher organic matter degradation efficiency (Zabranska et al., 2000; Fernández-Rodríguez et al., 2013), more total biogas produced (McHugh et al., 2004; Levén et al., 2007; Goberna et al., 2010; Siddique et al., 2014), and superior feed substrate hygienization (Zabranska et al., 2000; Bagge et al., 2005; Arthurson, 2008).

The aim of the research was to understand the link between the microbial communities co-digesting biowaste and sewage sludge and the key methanogenesis intermediates at both meso- and thermophilic temperatures. The aim was also to find a functional compromise between waste treatment capacity, biogas production, and a stable microbial community. To the best of our knowledge this concept has not been previously documented. Specifically the objectives were a) to identify major methanogens in the mesophilic (35–37 °C) and thermophilic (55–57 °C) anaerobic co-digestion processes, b) to study the effects of incrementally rising OLRs on biogas production and methanogenic archaeal community composition, and c) to study the effects of elevated loading rates on the relative abundance of microbial types and production of key methanogenesis intermediates. The hypothesis was that clear changes in dominating methanogenic groups would be observed with increasing temperatures and OLRs.

2. Material and methods

2.1. Anaerobic digester and gas analysis

A semi-continuously operated anaerobic digestion reactor (fed once per day) with an operating volume of 150 L was used for two consecutive production cycles under differing temperature conditions; the mesophilic digestion process was held at 35–37 °C for 19 weeks (September 2007–February 2008), and the following thermophilic digestion process was held at 55–57 °C for 20 weeks (April–September 2008). The feed mixture of finely minced, homogenised, and hygienized biowaste and sewage sludge (30% and 70% of total wet weight, respectively) was diluted with water before loading into the anaerobic digester. The reactor was stirred (ca. 160 rpm) for 30 min every 2 h and the OLR was increased incrementally from 1 to 10 kg VS m^{-3} d^{-1} (kg volatile solids per reactor volume per day). The dry solids content of the feed mixture was kept constant (ca. 8%) and increased amount of this mixture was fed. Thus, the hydraulic retention time was decreased stepwise from 58 days to 8 days.

Online reaction monitoring of the total volume of produced biogas was measured with a KIMMON SK35 gas metre, and the methane fraction was measured with a Simrad GD10 IR gas detector. The biogas flowed out freely from the reactor to the gas metre. The overpressure in the digestion reactor was continuously measured (<5 mbar), therefore the pressure in the gas metre was expected to be close to 1 bar. Major gas components — methane and carbon dioxide — as well as those of ammonia and nitrous oxides, were measured by FT-IR analysis (Gasmet, Temet Instruments), while the quantity of key trace compounds such as siloxanes, sulphur compounds, and volatile organic compounds (VOCs) were measured with gas chromatography (Voyager Perkin Elmer) (Arnold and Kajolinna, 2008). Biogas production (mesophilic versus thermophilic process at each OLR) was analysed with paired-samples *t*-tests at each time point (IBM SPSS 21, IBM Inc, Armonk, NY). The assumptions of the analyses were met.

2.2. DNA extraction and quantification

In order to study the microbial communities, total DNA was extracted from 0.25 ml of the reactor's output sludge at OLRs of 1–10 kg VS m^{-3} d^{-1} in both meso- and thermophilic treatments using a FastDNA® SPIN Kit for Soil (Qbiogene Inc., Carlsbad, USA) according to the manufacturer's instructions. The DNA concentration was measured fluorometrically using PicoGreen® dsDNA Quantitation Reagent and Kits (Molecular Probes Inc., Eugene, OR, USA).

2.3. PCR, DGGE and cloning analyses

Methyl-coenzyme M reductase (MCR) is the catalyst for the methane-forming step in methanogenic archaea metabolism, and the *mcrA* gene is a functional marker present in all methanogens (Friedrich, 2005). The methanogen-specific primers were obtained from TAGC (Copenhagen, Denmark). Primer sets of *mcrA-F* (5′-GGT GGT GTM GGA TTC ACA CAR TAY GCW ACA GC-3′) and *mcrA-R* (5′-TTC ATT GCR TAG TTW GGR TAG TT-3′) by Luton et al. (2002) were used for PCR amplification. A 41-bp GC-rich sequence (5′-CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG CCC CCG CCC GC-3′) was attached to the 5′ end of the *mcrA-R* for DGGE analysis. The PCR reaction mixture was composed of 3 μl of crude DNA extract, 1 μl of each primer (10 μM); 5 μl of 10× DyNAzyme Buffer, 1 μl of dNTP (10 mM), 1 μl of DyNAzymes II DNA polymerase (2 U μl^{-1}) from Finnzymes, Thermo Scientific, Finland; and 1 μl of Bovine Serum Albumin (BSA) (20 mg ml^{-1}) from Fermentas, Thermo Scientific, Finland. Sterile water was added to reach a final volume of 50 μl. The PCR amplification of 3 μl of crude DNA extract was done in a PTC-100 Thermo Cycler (MJ Research Inc., Waltham, MA, USA). The initial denaturation step was set to 94 °C for 7 min followed by 40 cycles of 45 s at 94 °C, 45 s at 55 °C, 1 min at 72 °C, and a final elongation step of 10 min at 72 °C. The yield of PCR products was estimated with agarose gel electrophoresis stained with EtBr.

The methanogen community composition was examined by utilising DGGE and direct clone library analysis on PCR products obtained with *mcrA*-GC and *mcrA* primers. The PCR products with a GC-clamp (20–25 μl) were separated using DGGE as described by Kurola et al. (2005) with modifications in the denaturant gradient (30–60%) and acrylamide-bisacrylamide concentration (9%). The gels were run for 17.5 h at 80 V. After electrophoresis, the gels were stained with SYBR® Gold nucleic acid gel stain and photographed as described by Kurola et al. (2005). The PCR fragments without a GC-clamp were cloned into commercial plasmid vectors as described by Partanen et al. (2010).