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Case Study

Temperature-dependent transformation of biogas-producing microbial communities points to the increased importance of hydrogenotrophic methanogenesis under thermophilic operation

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ABSTRACT

Stability of biogas production is highly dependent on the microbial community composition of the bioreactors. This composition is basically determined by the nature of biomass substrate and the physical–chemical parameters of the anaerobic digestion. Operational temperature is a major factor in the determination of the anaerobic degradation process. Next-generation sequencing (NGS)-based metagenomic approach was used to monitor the organization and operation of the microbial community throughout an experiment where mesophilic reactors (37 °C) were gradually switched to thermophilic (55 °C) operation. Temperature adaptation resulted in a clearly thermophilic community having a generally decreased complexity compared to the mesophilic system. A temporary destabilization of the system was observed, indicating a lag phase in the community development in response to temperature stress. Increased role of hydrogenotrophic methanogens under thermophilic conditions was shown, as well as considerably elevated levels of Fe-hydrogenases and hydrogen producer bacteria were observed in the thermophilic system.

1. Introduction

Biogas generation is based on the decomposition of organic materials carried out by complex microbial communities under anaerobic conditions. The coordinated interactions between bacterial and archaeal groups are responsible for the gradual biodegradation of complex polymers such as polysaccharides, proteins and lipids into a mixture of CH_4 and CO_2. The initial processes involve the hydrolytic activities of bacterial participants decomposing the polymers to oligomers which are further degraded during acidogenesis and acetogenesis. The final step is the methanogenesis where the previously formed acetate, H_2 and CO_2 are converted into biogas by aceticlastic and hydrogenotrophic methanogenic archaeal consortia (Wong et al., 2013). Aceticlastic methanogens produce methane via acetate degradation, while hydrogenotrophic methanogens catalyze CO_2 reduction into methane in the presence of H_2. Temperature is one of the crucial factors in shaping the microbial community structure during the anaerobic digestion (beside substrate type, OLR (organic loading rate), VFA (volatile fatty acids) composition, ammonium concentration, pH of the digested sludge, alkalinity, mixing and the geometry of the anaerobic digester) (Levén et al., 2007; Tukacs-Hájos et al., 2014). Elevated operation temperature enhances the efficacy of the enzymatic processes and initiates faster growth rate of the methanogens, thereby ensure that besides lower hydraulic retention time (HRT) microbes remain in optimal concentration within the fermentor (Weiland, 2010; Yadvika et al., 2004).

However, higher temperature is also correlated to a general decrease of the microbial diversity including the complexity of the methanogenic community (Karakashev et al., 2005; Tiago

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et al., 2004). Higher ammonia and VFA levels were observed at higher temperature compared to those in mesophilic systems. Elevated ammonia level and consequent VFA accumulation are associated with higher risk of process failure due to the possible inhibition of methanogens, especially the aceticlastic methanogens are sensitive to the acid level and composition (Angelidaki and Ahring, 1993; Boe et al., 2010; Demirel and Scherer, 2008; Schnürer et al., 1999; Shigematsu et al., 2004). Furthermore high ammonium (and VFA) concentration can result in a dynamic transition from aceticlastic methanogenesis to syntrophic acetate oxidation, which is mostly coupled to hydrogenotrophic methanogenesis (SAO) (Schnürer and Nordberg, 2008). The syntrophic acetate oxidation becomes dominant at elevated ammonium concentration due to the ammonium tolerance of the syntrophic acetate oxidizing bacteria (SAOB) (Schnürer and Nordberg, 2008). SAOB generally produce acetate via the Wood-Ljungdahl pathway (Müller et al., 2013). Beside acetate oxidation further syntrophic pathways can be linked to methanogenesis, e.g. propionate, butyrate, lactate, ethanol, amino acids and glyoxylic acid can be degraded to acetate, formate and H_2 by various syntrophic oxidizing bacteria, the resulting metabolites are converted to methane by hydrogenotrophic or aceticlastic methanogenesis (Sieber et al., 2012). SAO requires low hydrogen pressure which is maintained by the hydrogenotrophic methanogens effectively consuming the H_2 in exergonic reactions (Hattori, 2008).

Continuous hydrogen transfer occurs between hydrogen producing and consuming microbes, thus it is essential to briefly overview the relevant aspects of hydrogen metabolism, especially the roles of various hydrogenase enzymes. Hydrogenases are grouped into families based on the metal content of the active site: Fe-hydrogenases (alternative names: FeFe-hydrogenases, Fe-only hydrogenases), NiFe-hydrogenases, the methylenetetrahydromethanopterin dehydrogenase family (Hmd) initially called metal-free hydrogenases and Ech hydrogenases representing a distinct group of membrane-bound NiFe-hydrogenases (Vignais et al., 2001). Fe-hydrogenases are mostly involved in hydrogen evolution processes, while NiFe-hydrogenases rather function in the hydrogen uptake direction (Tard and Pickett, 2009).

Next-generation sequencing (NGS)-based metagenomic approach was applied to monitor the alterations in the microbial communities of the biogas reactors in response to temperature adaptation. The use of sequencing-based techniques is justified by the fact that the highly complex microbial consortia are mostly composed of uncultivable microorganisms. Furthermore this approach has the capability to assess and compare functional profiles of microbial communities by mapping the sequences (especially those of mRNA origin) to the coding regions of selected metabolic pathways. These analyses are becoming more reliable by the rapid expansion of whole genome, draft genome and metagenome databases. Recently, 454 pyrosequencing and SOLiD next-generation sequencing methods were employed for high-throughput sequencing-based metagenomic characterization of various biogas-producing communities (Kovács et al., 2013; Sundberg et al., 2013; Wirth et al., 2012). Here we applied the Ion Torrent PGM technique for metagenomic study, this system provides sequence data rapidly and for a significantly lower cost compared to other NGS instruments (Liu et al., 2012).

The primary aim was to follow the transformation of the microbial ecosystems during anaerobic digestion in response to temperature adaptation. Furthermore community-level taxonomic and functional changes were correlated with selected parameters of the biogas generation process such as biogas volume and VFA level of the reactor medium. Special attention was paid on the role of hydrogenase enzymes in the anaerobic digestion, another important aim was the direct demonstration of the increased significance of hydrogen metabolism at elevated operational temperature.

2. Methods

2.1. Anaerobic digestion conditions

The anaerobic digestion experiments were performed in 15-l, continuously stirred lab-scale reactors in fed-batch mode using a working volume of 10 l. The fermenters were designed and constructed by Nawaro Ltd, Hungary and installed at the Department of Hydrotechnology, Politehnica University in Timisoara, Romania. The constant volume was maintained by daily substrate feeding followed by sludge removal (around 250–300 mL). HRT was about 32–36 days. Three parallel reactors and one reference reactor were fed periodically with maize silage of 34% VS (volatile solids content) and 37% TS (total solids content) to sustain an average of 7–9% reactor medium TS during the fermentation experiment (reactor medium TS was analyzed once a week). TS and VS were determined according to the standard method (Kovács et al., 2013). The substrate was diluted with distilled water, a fixed daily volume of 300 mL was introduced into each reactor. In order to keep the medium TS in the 7–9% range, a changing organic loading rate (OLR) was applied for the three reactors, OLR of 5 g VS L^{-1} d^{-1} (150 g substrate in 300 mL volume) was applied at stable mesophilic and stable thermophilic operation, while decreased rates were used between day 12 and day 42 (Supplementary Table 1). The control mesophilic reactor was running at an OLR of 5 g VS L^{-1} d^{-1} throughout the experiment. Heating was maintained constant for 15 days at 37 ± 1.0 °C prior to the adaptation experiment (day 1 represent the first day of temperature increase). Temperature adaptation was achieved by gradually increasing the temperature in three reactors at an average ratio of 0.9 ± 0.3 °C day^{-1} up to 55 ± 1.0 °C, then was maintained constant at this final temperature (control reactor was kept at 37 ± 1.0 °C during the whole experiment). The evolved gas volume was measured with thermal mass flow meters attached to the gas exit ports and data were normalized to standard ambient temperature and pressure (25 °C and 100 kPa). The pH was continuously measured using an AD-132 Professional pH measuring instrument. Mixing speed was controlled and maintained at 100 rpm throughout the experiment.

2.2. Sampling protocol and storage

Sludge samples were collected from all fermentors (including the control reactor) into 50 mL sterile tubes every day. Three time points (day 1, day 20 and day 80) were selected for metagenomics analysis. Half of these selected samples were processed immediately for total microbial DNA isolation, the remaining were stored at −20 °C.

2.3. VFA determination

Sludge VFA was determined every day. Samples were diluted fivefold using 10 mM potassium phosphate buffer, pH 7.0 then 15 mL was centrifuged (15,000g) at room temperature for 30 min. A two-step filtration was applied on the supernatant, filtration on a 0.45 μm filter was followed by a filter of 0.22 μm pore size. The filtered supernatant was applied to high-performance liquid chromatography (HPLC) measurement using an UV detector at 210 nm. A Hitachi HPLC instrument was used equipped with ICSep ICE-COREGEL column using the following parameters: 0.05 M H_2SO_4 as solvent, a flow rate of 1.0 mL min^{-1} (isocratic solvent flow), a column temperature of 50 °C, and detector temperature of 40 °C. One sample was taken from each reactor every day, in these 3 samples acetic acid, butyric acid and propionic acid were measured, the VFA values represent the summarized averages of the specific acids.