Bioresource Technology 228 (2017) 368–373

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Short Communication

Identifying methanogen community structures and their correlations with performance parameters in four full-scale anaerobic sludge digesters

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HIGHLIGHTS

- [ARC] and [ARC]/[BAC] positively correlated with process performance parameters.
- Most abundant methanogens did not show clear correlation with performance parameters.
- *Methanoculleus* was potentially a promising biomarker for positive performance.
- *Methanoregula* was abundant in samples with poor performance.

ARTICLE INFO

Article history:
Received 15 November 2016
Received in revised form 29 December 2016
Accepted 31 December 2016
Available online 4 January 2017

Keywords:
Anaerobic digestion
454 pyrosequencing
Real-time quantitative PCR
Archaea-to-bacteria ratio
Methanoculleus
Methanoregula
Biomarker

ABSTRACT

Four full-scale mesophilic anaerobic digesters treating waste sludge were monitored to characterize methanogen communities and their relationship with process parameters. The performance of the four digesters were dissimilar with the average chemical oxygen demand removal efficiencies between 24 and 45% and differing pH. Real-time quantitative PCR showed that archaeal 16S rRNA gene concentration ([ARC]) and, more pronouncedly, its ratio to bacterial counterpart ([ARC]/[BAC]) correlated positively with the performance parameters, including the lipid removal efficiency. Pyrosequencing identified 12 methanogen genera, of which *Methanolinea*, *Methansaeta*, and *Methanospirillum* collectively accounted for 79.2% of total archaeal reads. However, *Methanoculleus*, a numerically minor (1.9 ± 2.6%) taxa, was the most promising biomarker for positive performance, while *Methanoregula* was abundant in samples with poor performance. These results could be useful for the control and management of anaerobic sludge digestion.

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1. Introduction

The activated sludge process is widely used to treat municipal wastewater. This process generates a large volume of sludge, which requires appropriate management. The cost of sludge processing and disposal accounts for up to the half of the total operating cost of a municipal wastewater treatment plant (Shin et al., 2016). Therefore, development and application of an inexpensive method to treat excess sludge is desirable. This excess sludge consists mainly of degradable organic matter and thus has been regarded as a feedstock that can be converted by anaerobic digestion (AD) to a fuel such as methane gas (CH_4).

AD of organic matter is a sequential biochemical reaction performed by various interacting microorganisms, which can be broadly categorized as acidogens and methanogens. Acidogens are mostly bacteria that first hydrolyze and ferment complex organic materials to H_2 and organic acids. Methanogens are a unique group of archaea that produce CH_4 from the acidogenesis products. Although all known methanogens are classified under one phylum, Euryarchaeota, they are an extremely diverse group that includes more than 150 species within 34 genera. Due to their overall slow growth rates and susceptibility to environmental changes and inhibitors, methanogens have been claimed as the key microbial group for the stable operation of AD. Although

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http://dx.doi.org/10.1016/j.biortech.2016.12.118
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methanogen community structure shifts dynamically along with process parameters during stable and instable operations of the AD system (Williams et al., 2013), it is not clear if the total amount of methanogens is the key parameter, or are some specific species or groups, or both.

Waste sludge consists of undefined particles such as debris and concentrated aerobic microbial cells, so the environmental conditions for methanogens can vary widely in AD of sludge. Therefore, a long-term monitoring of how variations in environmental parameters affect the structure of methanogen communities can help develop operation strategies for AD of waste sludge. Although the information is growing in the literature on the methanogen community structure in different AD processes (Kim et al., 2013; Zhang et al., 2015), the relationship between methanogens and different process parameters is not yet fully understood in full-scale AD of waste sludge.

The objective of this study was, therefore, to investigate the methanogen community structure in full-scale anaerobic digesters treating waste sludge, and to find correlations against process parameters. Four full-scale digesters under similar operation conditions but with dissimilar performance were sampled seasonally for one year to this end. Real-time quantitative PCR (qPCR) and 454 pyrosequencing were conducted to assess the methanogen community.

2. Materials and methods

2.1. Sampling of full-scale anaerobic digesters

The four sludge digesters were located in Seoul (digester A), Daegu (B), Incheon (C), and Asan (D), respectively, in South Korea. The details of their operation parameters and the sampling campaigns were described previously (Shin et al., 2016). The digesters were commonly fed with waste sludge from local municipal wastewater treatment plants, at hydraulic retention time of 28.7–40 d. All the digesters were mesophilic (35 °C) and were continuously stirred. Four samples were collected from each digester seasonally (i.e., 3 months interval) between October 2010 and March 2012 and were designated as A1–A4, B1–B4, etc. The input substrate fluctuated slightly over the sampling periods, by no major change occurred before sampling. The influent samples were taken from pipes conveying waste sludge into the digester, and the digester samples were taken from digestate circulating through heat exchangers. Approximately 200 mL of sample was collected in sterile plastic containers, stored in a mobile refrigerator at 4 °C, and transported to the laboratory within 24 h.

2.2. Physicochemical analyses

The pH was measured using a benchtop pH meter (Cole Parmer, Vernon Hills, IL). Chemical oxygen demand (COD) was measured using the close reflux colorimetric method. Carbohydrate concentration was determined as previously described using the phenol–sulfuric acid method (Shin et al., 2016). Protein concentration was estimated using the Kjeldahl method as previously reported (Shin et al., 2016). Lipid concentration was analyzed as previously described using gravimetry after extraction of lipids by solvent (chloroform: methanol, 1:2 *v*/*v*) (Shin et al., 2016). Cations (Na^+ and NH_4^+) were quantified using an ion chromatograph (Personal 790 IC, Metrohm, Switzerland) equipped with a Metrosep Cation 1-2 column (Metrohm). Volatile fatty acids (VFAs; C_2–C_6) were measured using a gas chromatograph (6890 Plus, Agilent, Palo Alto, CA) equipped with an Innowax capillary column (Agilent).

2.3. Molecular methods

DNA was extracted using an automated nucleic acid extractor (Magtration System 6GC, Precision System Science, Chiba, Japan). The presences of possible PCR inhibitors and DNA from cell debris were minimized by removing the residual medium twice after centrifugation (5 min at 15,339*g*). The purified DNA was eluted with nuclease-free water and stored at −20 °C until use. All DNAs were extracted and analyzed in duplicate (i.e., duplicate analysis for qPCR and duplicate DNA use for PCR for pyrosequencing).

The qPCR analysis was conducted using a LightCycler 480 (Roche, Mannheim, Germany) with total Bacteria and Archaea primer–probe sets as previously described (Shin et al., 2010) with modifications. The 20 μL reaction mixture contained 10 μL of the master mix in LightCycler 480 Probes Master kit (Roche), 5 μL of PCR-grade water, 1 μL each of primer (final concentration 500 nM), 1 μL of the TaqMan probe (final concentration 100 nM), and 2 μL of template DNA.

The V5–V9 hypervariable regions of the 16S rRNA gene were amplified using primers 787f (ATTAGATACCCNGGTAG) and 1492r (GNTACCTTGTTACGACTT) modified with adapters and barcodes, gel-purified, and pooled for equal PCR products. The 454 pyrosequencing was performed by commercial sequencing service (Macrogen, Seoul, South Korea) according to the manufacturer's instructions. Low-quality reads (<Q20), short sequences (<270-bp), and potential chimeras were removed and the resulting sequences were clustered using CD-HIT-OTU (http://weizhongli-lab.org/cd-hit-otu/). Operational taxonomic units (OTUs) were defined at 97% sequence-identity cutoff. Taxonomic assignment was conducted using the QIIME pipeline and the SILVA database. The sequences identified as bacteria were reported previously (Shin et al., 2016). The nucleotide sequences obtained were deposited in National Center for Biotechnology Information (NCBI)'s sequence read archive (PRJNA315957).

2.4. Statistical test

The statistical calculations were conducted using R software package with the following libraries: gclus, optparse, and vegan. Pearson correlation coefficients were calculated from pairs of variables within and between process and microbial data. Detrended correspondence analysis (DCA), redundancy analysis (RDA), and canonical correspondence analysis (CCA) were performed using Canoco program version 4.5 (Biometrics, Wageningen, the Netherlands).

3. Results and discussion

3.1. Process parameters for full-scale anaerobic digesters

The four full-scale anaerobic digesters showed dissimilar performance (i.e., organic removal efficiencies) during the one-year period of sampling (Fig. 1a). The average COD removal efficiencies of the four digesters were between 24 and 45%: 45 ± 8% for A, 40 ± 8% for B, 30 ± 13% for C, and 24 ± 17% for D. The carbohydrate removal efficiency showed a clearly separated pattern to the COD removal efficiency (Fig. 1a), with a Pearson correlation coefficient *r* of 0.16 and the corresponding *p* value of 0.54. In contrast, the protein and lipid removal efficiencies agreed well with the COD counterpart (Fig. 1a), with respective *r* values of 0.76 and 0.73 ($p < 0.01$). Protein and lipid respectively contributed 48.7 and 37.8% equivalents of the influent COD, and 51.0 and 39.3% equivalents of the digester COD in this study, while carbohydrate only accounted for <12% equivalent of the influent and the digester COD values (data not shown).

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