Waste Management xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Waste Management

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

Monitoring of microbial communities in anaerobic digestion sludge for biogas optimisation

Jun Wei Lim [a], Tianshu Ge [b], Yen Wah Tong [a,c,*]

[a] NUS Environmental Research Institute, National University of Singapore, 5A Engineering Drive 1, Singapore 117411, Singapore
[b] Institute of Refrigeration and Cryogenics, Shanghai Jiao Tong University, Shanghai, China
[c] Department of Chemical and Biomolecular Engineering, National University of Singapore, 4 Engineering Drive 4, Singapore 117585, Singapore

ARTICLE INFO

Article history:
Received 27 June 2017
Revised 5 October 2017
Accepted 6 October 2017
Available online xxxx

Keywords:
Anaerobic digestion sludge
Clone library
Pyrosequencing
Terminal restriction fragment length polymorphism

ABSTRACT

This study characterised and compared the microbial communities of anaerobic digestion (AD) sludge using three different methods – (1) Clone library; (2) Pyrosequencing; and (3) Terminal restriction fragment length polymorphism (T-RFLP). Although high-throughput sequencing techniques are becoming increasingly popular and affordable, the reliance of such techniques for frequent monitoring of microbial communities may be a financial burden for some. Furthermore, the depth of microbial analysis revealed by high-throughput sequencing may not be required for monitoring purposes. This study aims to develop a rapid, reliable and economical approach for the monitoring of microbial communities in AD sludge. A combined approach where genetic information of sequences from clone library was used to assign phylogeny to T-RFs determined experimentally was developed in this study. In order to assess the effectiveness of the combined approach, microbial communities determined by the combined approach was compared to that characterised by pyrosequencing. Results showed that both pyrosequencing and clone library methods determined the dominant bacteria phyla to be *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, and *Thermotogae*. Both methods also found that sludge A and B were predominantly dominated by acetogenic methanogens followed by hydrogenotrophic methanogens. The number of OTUs detected by T-RFLP was significantly lesser than that detected by the clone library. In this study, T-RFLP analysis identified majority of the dominant species of the archaeal consortia. However, many of the more highly diverse bacteria consortia were missed. Nevertheless, the combined approach developed in this study where clone sequences from the clone library were used to assign phylogeny to T-RFs determined experimentally managed to accurately predict the same dominant microbial groups for both sludge A and sludge B, as compared to the pyrosequencing results. Results showed that the combined approach of clone library and T-RFLP accurately predicted the dominant microbial groups and thus is a reliable and more economical way to monitor the evolution of microbial systems in AD sludge.

© 2017 Elsevier Ltd. All rights reserved.

1. Introduction

The anaerobic digestion (AD) process produces biogas which is an environmentally friendly source of energy fuel and alternative to fossil fuels. Other than energy production, AD also play important roles in nutrients recycling and waste management. Because of these benefits to the environment, scientists and power industry companies have been interested in the AD of organic waste for more than 100 years (Ali Shah et al., 2014). Biogas arises from the activities and syntrophic interactions of a consortium of anaerobic bacteria and methanogens. Therefore, gaining a deeper understanding of microbial communities and their function is an important step in improving the efficiency and process stability of anaerobic digesters. Though possessing great potential as a source of renewable energy, the anaerobic treatment technology did not take off for decades since its introduction in the 1860s. Instead, the biological reactor has been considered as a "black box" due to the lack of appropriate microbiological techniques (Fang, 2011).

Following great advances in recent years, nucleic acid-based molecular methods are able to identify microorganisms by the DNA sequences of their ribosomal RNA (rRNA) genes, without the need to isolate the microorganisms. Direct amplification and sequencing of 16S rRNA genes from the environment have been practised since the 1990s for the characterisation of microbial consortia (Case et al., 2006). This procedure revolutionised microbial

* Corresponding author.
E-mail addresses: eriljw@nus.edu.sg (J.W. Lim), baby_wo@sjtu.edu.cn (T. Ge), chetyw@nus.edu.sg (Y.W. Tong).

https://doi.org/10.1016/j.wasman.2017.10.007
0956-053X/© 2017 Elsevier Ltd. All rights reserved.

ecology and led to insights in the highly diverse microbial species involved in AD. Certain molecular tools are now commonly used, such as sequence-based techniques – clone libraries and pyrosequencing, and fragment-based analysis – terminal fragment length polymorphism (T-RFLP). Each of these methods measures a different aspect of the community, but all of them generally utilize the 16S rRNA gene to differentiate operational taxonomic units (OTUs).

The cloning and sequencing of the gene coding for 16S rRNA is a widely used molecular technique in the field of microbial ecology (Sanz and Köchling, 2007). The cloning technique is capable of sequencing the whole 1500-bp length to provide much more exact genetic information as compared to pyrosequencing and T-RFLP. However, due to the laborious nature of cloning, the coverage of biodiversity is usually limited to numbers ranging from fifty to two hundred, which might not be representative of the whole microbial population. Though cloning techniques can yield full-length 16S rRNA gene sequences, they are less popular in the research of AD because it is extremely time consuming and laborious to obtain a complete picture of its bacterial habitat (Cobb and Zhao, 2012).

T-RFLP analysis is one of the most frequently used high-throughput fingerprinting methods for the comparison of microbial communities (Liu et al., 1997). Each T-RF is represented by a peak in the output chromatogram and corresponds to members of the community that share a given terminal fragment size. This method is rapid (very much less time consuming as compared to cloning), inexpensive and provides distinct profiles that reflect the taxonomic composition of sampled communities (Nocker et al., 2006). Although it has been used extensively for comparative purposes, a T-RFLP fingerprint alone does not allow for conclusive taxonomic identification of individual phylotypes because it is technically challenging to recover terminal fragments for direct sequencing.

In recent years, high-throughput sequencing technologies are prevalently applied in metagenomics to analyse community structures of AD habitats. Pyrosequencing is one of the more popular high-throughput sequencing technologies (Liu et al., 2012). By eliminating the need for the construction of a clone library, which can be technically challenging for novice in microbial analysis, pyrosequencing is a much easier technique for most to pick up. Being a high-throughput sequencing technique, pyrosequencing provides a relatively unbiased overview of the community structure when it is applied for whole-microbial-community DNA sequencing. Since pyrosequencing usually generates sequence lengths of around 400-bp, the detection of new species or minor microbial groups may be limited by this technique.

In this study, both sequence-based (clone library and pyrosequencing) and fragment-based (T-RFLP) approaches that target the 16S rRNA gene were employed to characterise the microbial consortia of AD sludge samples. The results were cross-compared for the purposes of evaluating the strengths as well as limitations of each method. This work also studied the methodology of a combined sequence-based and fragment-based approach where clone sequences from the clone library were used to assign phylogeny to T-RFs determined experimentally. The combined approach exhibits complimentary benefits as it taps on the strengths of both clone library and T-RFLP methods. The effectiveness of the combined approach was also accessed by comparing the predicted microbial consortia to that determined by pyrosequencing.

2. Experimental procedures

2.1. Two different sources of sludge analysed for their microbial consortia

Two different sources of anaerobic sludge were used in this study so that a more diverse group of microbial species could be analysed. Sludge A was collected from a digester treating activated sludge from a wastewater reclamation plant in Singapore (PUB, Singapore). The anaerobic digester was operated in continuous flow stirred-tank reactor (CSTR) mode, at mesophilic temperature and a hydraulic retention time (HRT) of 20–30 days. Approximately 120 N m^3/h CH_4 was generated with a biogas composition of 60–70% CH_4. Sludge B was collected from an anaerobic digester used to treat palm oil mill effluent in Johor, Malaysia (Keck Seng (M) Berhad). The anaerobic digester was operated in CSTR mode, at thermophilic temperature and a HRT of 18 days. 11,200 m^3/d CH_4 was generated with a biogas composition of 62.5% CH_4. The organic loading rate (OLR) of the digester was 400 m^3/d, and achieved COD removal rates of 83% (Tong and Jaafar, 2005). Sludge A had average pH level of 7.51 ± 0.01, total solids (TS) of 22.97 ± 0.57 g/L and volatile solids (VS) of 16.06 ± 0.36 g/L. On the other hand, sludge B had average pH level of 8.02 ± 0.01, TS of 8.36 ± 0.05 g/L and VS of 3.31 ± 0.07 g/L. TS and VS were measured according to the Standard Methods (APHA, 1998) while pH value was measured using a portable ExStik PH100 pH meter (EXTECH Instruments).

2.2. Cloning of 16S rRNA and construction of gene clone library

Genomic DNA was extracted from both sludge A and sludge B using soil DNA isolation kit (Norgen Biotek Corporation, Canada) and protocol as recommended by the manufacturer. Extracted DNA was used in the subsequent PCR amplification with a domain Bacteria-specific forward primer 8f (5′-AGAGTTTGATYMTGGCTC-3′) and a reverse primer 1490r (5′-GGTTACCTTGTTACGACTT-3′). Archaea 16S rRNA genes were amplified with a domain Archaea-specific forward primer 1f (5′-TCYGKTTGATCCYGSCRGAG-3′) and reverse primer 1100r (5′-TGGGTCTCGCTCGTTG-3′). The thermal program used for amplification of bacterial 16S rRNA gene was as follows: hotstart 94 °C for 3 min, 30 cycles of denaturation (30 s at 94 °C), annealing (30 s at 56 °C) and extension (45 s at 72 °C) and a final extension at 72 °C for 5 min. The thermal program used for the amplification of archaeal 16S rRNA gene was similar to that of bacterial 16S rRNA gene other than having 35 cycles of denaturation (30 s at 94 °C) and annealing for 30 s at 54 °C. TOPO TA cloning kit (Invitrogen, CA) was used for clone library construction according to the manufacturer's instructions. Approximately 100 clones and 50 clones each were randomly selected from sludge A and sludge B for the members in the domain Bacteria and Archaea, respectively. The amplified DNA insert was then PCR amplified with a vector-specific primer set M13F (5′-GTAAAACGACGGCCAG-3′) and M13R (5′-CAGGAAACAGC TATGAC-3′). Restriction fragment length polymorphism (RFLP) was used to screen the 16S rRNA gene fragments to further remove the possible redundant clones. The M13-PCR products were separately digested to completion with tetramer restriction enzymes (MspI and RsaI) (New England BioLabs, UK), and separated by electrophoresis in a 1.5% agaose gel. Gels were visualized using the G:BOX Chemi XX6 Gel Imaging (Syngene, Cambridge, UK) after staining with Gelred (Invitrogen, CA). Unique RFLP patterns were defined as a unique sequence type of OTU. The amplified PCR products were purified with GeneJET PCR purification kit (Thermo Scientific, United States).

The 16S rRNA genes of the representative clones with different RFLP patterns were sequenced by Axil Scientific (Singapore) to determine their phylogenetic affiliation. Nearly full-length (at least 1300 bp for bacterial and 1100 bp for archaeal) 16S rRNA gene sequences of representative clones were compared to available rRNA gene sequences in GenBank using the NCBI BLAST program. Chimeric artifacts were determined using DECIPHER (Wright et al., 2012) and phylogenetic trees were constructed with MEGA7 program using the remaining clone sequences (77, 72, 69 and 72

Download English Version:

https://daneshyari.com/en/article/8870248

Download Persian Version:

https://daneshyari.com/article/8870248

Daneshyari.com