Bioresource Technology 151 (2014) 397–401

Contents lists available at ScienceDirect

Bioresource Technology

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

Short Communication

Substrate type drives variation in reactor microbiomes of anaerobic digesters

Wei Zhang [a], Jeffrey J. Werner [a,b], Matthew T. Agler [a], Largus T. Angenent [a,*]

[a] Department of Biological and Environmental Engineering, Cornell University, Ithaca, NY 14853, USA
[b] Chemistry Department, SUNY-Cortland, Cortland, NY 13045, USA

HIGHLIGHTS

- Substrate type drives variation of reactor microbiomes.
- Continuum exists and co-digestion microbiomes fall between two mixed substrate types.
- To link other environmental factors, many more samples must be included.

ARTICLE INFO

Article history:
Received 16 May 2013
Received in revised form 2 July 2013
Accepted 1 October 2013
Available online 10 October 2013

Keywords:
Anaerobic digestion
Reactor microbiomes
16S rRNA characterization
Sanger sequencing
Beta-diversity

ABSTRACT

The goal of this study was to obtain causative information about beta-diversity (differentiation between microbiomes) by comparing sequencing information between studies rather than just knowledge about alpha-diversity (microbiome richness). Here, published sequencing data were merged representing 78 anaerobic digester samples originating from 28 different studies for an overall comparison of beta-diversity (measured using unweighted UniFrac). It was found that digester microbiomes based on bacterial sequences clustered by substrate type, independent of the study of origin, and that this clustering could be attributed to distinct bacterial lineages.

1. Introduction

Anaerobic digestion is advantageous due to its combined benefits of treating organic waste and producing bioenergy (Angenent et al., 2004; Lettinga, 1995). The open culture of complex, undefined microbial communities (referred to here as reactor microbiomes) of anaerobic digesters are necessary for robust performance (Kleerebezem and van Loosdrecht, 2007). Some work has been performed on the effect of external factors on anaerobic digestion performance and reactor microbiome structure. These external factors include the operating conditions: reactor configuration (Werner et al., 2011), staging (Angenent et al., 2002), mixing (Hoffmann et al., 2008), and organic loading rate (Chelliapan et al., 2011). However, a comprehensive analysis of how operating factors influence microbial community structure requires many sequencing samples and beta-diversity analysis (differentiation between microbiomes) (Werner et al., 2011). This also requires going beyond just reporting alpha-diversity (microbiome richness). Here, 78 digester samples of full-length bacterial 16S rRNA clone libraries were surveyed by collecting sequences from published digester studies, representing a wide range of substrate types, and merged them to generate an overall comparison of the variation in reactor microbiomes.

2. Methods

2.1. Utilized sequences

19,674 Bacterial 16S rRNA gene sequences were collected from 28 studies from 1998 to present (Table 1). The sequences representing the bacterial portion of the reactor microbiome were focused on, because it has generally been observed to be more diverse and complex than archaeal populations in anaerobic digesters (Fernández et al., 1999). Studies were chosen based on the criteria that random clone libraries were picked and sequenced to produce near-full-length reads. This excludes the sequences that were generated by high-throughput sequencing platforms, such as 454 pyrosequencing and Illumina platforms, and therefore this

* Corresponding author. Tel.: +1 607 255 2480; fax: +1 607 257 4449.
E-mail address: la249@cornell.edu (L.T. Angenent).

0960-8524/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2013.10.004

Table 1
Characteristics of the 78 anaerobic digester samples from 28 studies.

Number of samples	Number of reads	Substrate categories	Reactor types	Refs.
1	205	MW	Stirred	Chouari et al. (2005)
2	172	MW	CSTR	Zhang et al. (2009)
9	10,416	MW	Stirred	Rivière et al. (2009)
1	69	Manure	Stirred	Liu et al. (2009)
2	50	Household	Plugflow	Goberna et al. (2009)
2	32	Chemical	Upflow	Enright et al. (2007)
3	3167	Chemical	Upflow	Perkins et al. (2011)
1	47	Food	Stirred	Ariesyady et al. (2007)
3	38	Co-digestion	CSTR	Wang et al. (2009)
2	42	Household	Stirred	Levén et al. (2007)
2	76	Manure	Stirred	Cheon et al. (2008)
1	68	Household	Plugflow	Cheon et al. (2008)
1	37	Household	Stirred	Cheon et al. (2008)
1	102	MW	CSTR	Cheon et al. (2008)
1	67	MW	Plugflow	Cheon et al. (2008)
2	43	VFA	Others	Tatara et al. (2008)
1	29	VFA	Upflow	Satoh et al. (2007)
1	28	Food	Stirred	Sasaki et al. (2007)
1	20	Chemical	Others	Chen et al. (2009)
3	21	VFA	CSTR	Shigematsu et al. (2006)
1	56	Food	CSTR	Klockea et al. (2007)
2	33	VFA	CSTR	Tan et al. (2007)
3	47	VFA	Upflow	McKeown et al. (2009)
1	114	Food	Stirred	Feng et al. (2009)
1	39	VFA	Stirred	Weiss et al. (2009)
12	479	Beverage	Upflow	Narihiro et al. (2009)
2	57	Beverage	Upflow	Sekiguchi et al. (1998)
16	4120	Manure	ASBR	GenBank accession numbers GQ132191–GQ135228 and GQ138118–GQ139199 (Werner et al. submitted)
Total 78	19,674			

MW = municipal waste; VFA = volatile fatty acids.

work encompasses the work that has been performed with Sanger sequencing during the last one to two decades.

2.2. Sequence analysis

Sequences were aligned to the Greengenes core alignment (DeSantis et al., 2006) and operational taxonomic units (OTUs) were picked at 97% identity using UCLUST (Edgar, 2010) in QIIME 1.3.0 (Caporaso et al., 2010). To account for the variation in the number of sequences per sample, 100 rarefactions of 50 sequences per sample were performed on the samples for which raw reads were available (i.e., samples for which all reads and not just OTU representatives were available on GenBank). For samples in which only OTU representative sequences were publicly available, rarefaction was not possible, and OTU content were combined for these samples with each of the rarefied-sample OTU tables after rarefaction and before analysis of beta-diversity. Phylogenetic distances between samples were calculated for each rarefaction using the unweighted UniFrac metric (Lozupone and Knight, 2005), and the resulting distances were averaged. Unweighted UniFrac was chosen as the beta-diversity metric because it ignores relative abundances and compares samples solely based on the evolutionary histories of their OTUs. This also removes some of the PCR biases that would be different between studies. The unweighted UniFrac distances were analyzed by principal coordinates analysis (PCoA).

3. Results and discussion

The variation in unweighted UniFrac distances between all samples are shown in Fig. 1, and graphed as the first three axes from PCoA. This PCoA plot visualizes distances between samples by including as much variation as possible in lower-numbered axes, with each axis representing a different component of the variation in between-sample distances. The first axis, which is the PC1 (7.2% of overall variation), primarily represents the bacterial community differences between samples of digesters that treated livestock wastes (referred to here as manure) and samples of all other digesters (Fig. 1A). Although the emphasis of the clustering along PC1 is in part weighted by the fact that there were a high number of swine manure digester samples (Table 1), the ordination along this axis demonstrated an important relationship among other samples containing manure. Three of the points labeled as manure came from other studies (Liu et al., 2009; Cheon et al., 2008) (see Fig. 2A for samples graphed by study of origin). In addition, the three samples labeled as "co-digestion" that also contained manure (Wang et al., 2009) were placed between the manure cluster and the remainder of the samples (Fig. 1A). This indicates that not only do manure digesters have distinctly different microbial communities from digesters treating other substrates but that there is a continuum of possible phylogenetic structures, depending on the specific substrate composition. Communities performing co-digestion lie along that continuum, sharing some phylogenetic components with both livestock manure digesters and others (e.g., food wastes).

The variation represented by PC2 (5.4% variation) and PC3 (4.1% variation) shows that there were unique phylogenetic structure characteristics of digesters treating municipal biosolids (referred to here as municipal waste) (Fig. 1B). Also observed along these axes was that the digesters fed food waste and beverage waste clustered together with some overlap, and communities treating chemical waste were similar to each other. The clustering was not simply a confounding effect due to samples from within a study (Fig. 2B). The most striking variation along PC2, however, was the wide spread of samples fed substrates that were categorized as volatile fatty acids (VFAs). This included lab-scale reactors fed synthetic substrates composed of acetate, propionate, and *n*-