Correlating the microbial community composition of anaerobic digesters to process parameters

Rasmus H. Kirkegaard*, Simon J McIlroy, Poul Larsen, Søren M. Karst, Mads Albertsen and Per H. Nielsen

*Center for Microbial Communities, Department of Chemistry and Bioscience, Aalborg University, Fredrik Bajers Vej 7H, 9220 Aalborg, Denmark
(E-mail: *rhk@bio.aau.dk)*

Abstract
Anaerobic digestion is an important biological process driven by a mixed community of microbes. Different microbial groups carry out the different steps in the sequential conversion of complex biomass into carbon dioxide and methane. Here we present a microbial survey of 18 Danish full-scale wastewater treatment plants performing anaerobic digestion with a total of 32 reactors sampled during a period of four years. We have paired sets of samples with the feed activated sludge and digester samples to identify the effect of feed microbiota. In addition, we have matching sets of foam samples whenever the plants have experienced problems with foam formation to identify foam formers. The comprehensive survey is based on high throughput sequencing of 16S rRNA gene amplicons providing identity and abundance of all the microbes in each of the 250+ samples. The microbial community analysis revealed that thermophilic and mesophilic digesters contain very distinct microbial communities. Among the list of "well-known" bacteria, some of the abundant microorganisms turned out to be completely novel even at the phylum level. Furthermore, the analysis revealed a list of bacteria tightly linked to foam formation promising a potential for microbiology based informed process control in the near future.

Keywords
Anaerobic digestion; amplicon sequencing; full-scale plant survey; microbial community analysis; microbial ecology; 16S rRNA gene;

BACKGROUND
A thorough understanding of the microbial processes in anaerobic digestion is important for process optimisation and trouble shooting of operational problems. The present change from fossil fuel to sustainable energy production such as biogas production, that takes place in many countries, relies on a better understanding of microbial processes and a link to their influence on the operation of the plants. However, few studies have dealt with the microbial composition of multiple full-scale plants and related this to process parameters (Sundberg et al. 2013; Regueiro et al. 2012; Rivière et al. 2009; Lee et al. 2012).

AIM
To gather information about the microbial diversity of a variety of full-scale anaerobic digesters, their process performance, and investigate how this relates to the underlying microbial composition in the reactors and the influent. In addition, to generate a list of the process-detrimental bacteria that contribute to foam formation, and to characterise their physiology to aid in the development of strategies for their control.

METHODS
A sampling guide with a sheet for metadata reporting, and labelled sample tubes were shipped to each of the 18 anaerobic digester plants. The sludge samples were shipped and stored at -20 °C until analysed.

DNA was extracted using an optimised version of the FastDNA® SPIN Kit for soil; extending the bead beating step to four times the recommended duration. The 16S rRNA gene was PCR amplified

with Illumina compatible adaptors and the 16S rRNA gene amplicons were sequenced on the Illumina MiSeq. The reads were clustered and classified using QIIME (Caporaso et al. 2010) and a curated version of the SILVA taxonomy (Quast et al. 2013). The microbial composition was subsequently investigated along with the metadata using multivariate data analysis tools in R (R Core Team 2014).

RESULTS AND DISCUSSION

More than 250 digester samples were collected during a four-year period representing 18 different digesters (32 reactors) on wastewater treatment plants. In addition, activated sludge samples were collected from the feed surplus activated sludge from all 18 wastewater treatments plants at two of the sample events. To each digester sample, a set of corresponding metadata was collected. The metadata contained information such as temperature, pH, SS, ammonium, VFA, alkalinity, pre-treatments, feed composition, sludge retention time, biogas composition, and reactor configuration.

The reactors represent a broad variety of configurations including thermophilic and mesophilic reactors, some of which have pre-treatments in terms of thermal hydrolysis. Mixing is carried out using diverse strategies such as stirrers, gas mixing, and recirculation. Some plants have large capacity (>400,000 PE) while others are small (<30,000 PE); some plants have a parallel configuration while others are connected in series.

The samples were subjected to microbial composition analysis using high throughput DNA sequencing of 16S rRNA gene amplicons. This analysis provided the identity and relative abundance of bacteria in all digesters and activated sludge plants. The results showed that many of the incoming bacteria from the surplus sludge could be detected in the digesters. This means that the community was strongly affected by the source of activated sludge. However, whether they were active is still to be investigated.

The analysis also showed that the microbial community was strongly influenced by the mode of operation, resulting in separate clusters for mesophilic reactors, thermophilic reactors and reactors with the CAMBI pre-treatment process installed (see PCA plot in Figure 2). Specific groups of bacteria were linked to process critical problems such as foam formation while others were clearly process type specific.

The microbial survey also supports that anaerobic digesters contain a number of abundant microbes that are undescribed in the literature. These microorganisms have no closely related cultured representatives and no representative genomes in the databases. Following this survey, metagenomes were established to retrieve complete genomes of some of these key species using advanced bioinformatics (Albertsen et al. 2013).
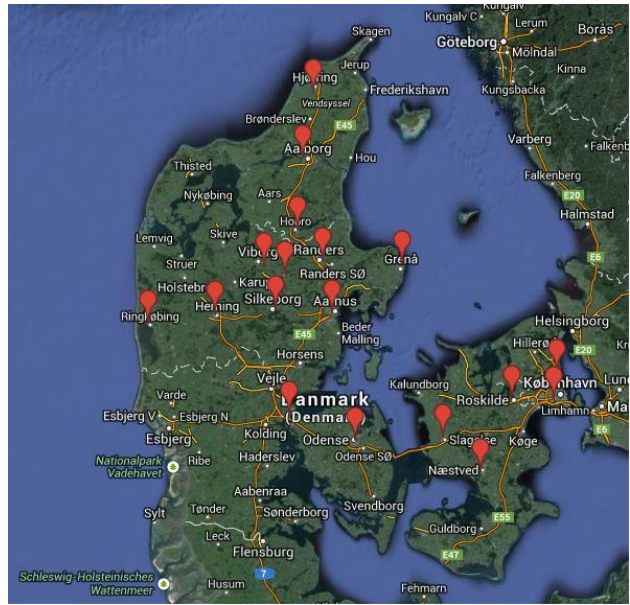
Figure 1 Geographical distribution of the 18 Danish plants.
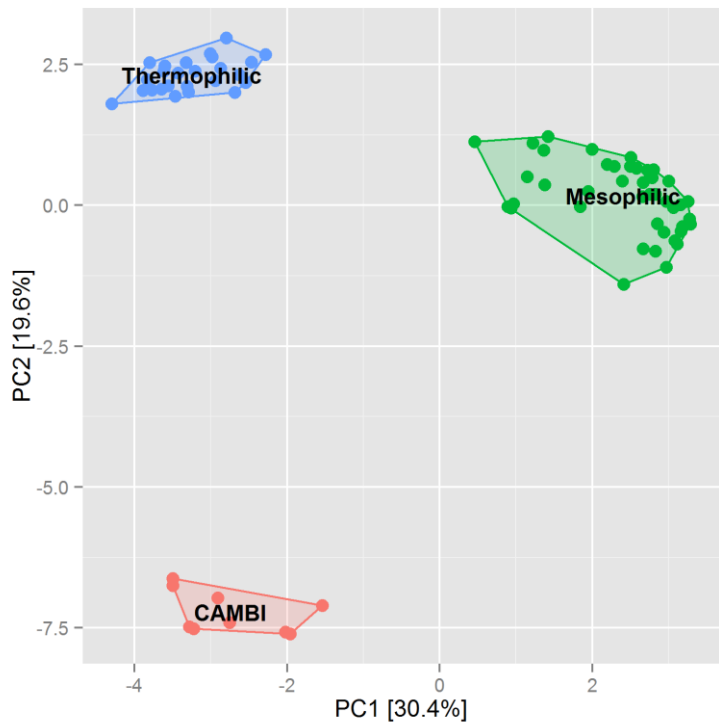

Figure 2. PCA analysis of Bacteria in digesters. Each of the dots represent the microbial composition of a sample. PC1 explains 30.4 % of the variation while PC2 explains 19.6% of the variation.. The samples are coloured using plant type overlay: Thermophilic (blue), Mesophilic (green), and CAMBI (red).

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