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Differences in microbial key players in anaerobic degradation between biogas and sewage treatment plants

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ABSTRACT

Biogas production in biogas plants (BPs) and sewage treatment plants (STPs) of industrial and municipal waste, respectively, is principally based on the same metabolic processes. Process parameters, bacterial and archaeal community composition of 16 BPs and 10 STPs with large-scale biogas production were analyzed by 16S rRNA gene amplicon sequencing to assess a common core microbiome. Both community compositions differed between plant types, and those of BPs were significantly affected by substrate, whereas STP community compositions were governed by pH. Operational taxonomic unit (OTU) richness and diversity were significantly higher in STPs compared to BPs. Microbial network analysis of the overall bacterial and archaeal community composition identified 39 generalistic OTUs for BPs and only 11 for STPs, respectively. Our findings indicate that BPs were less complex with various common key players in all plants whereas STPs were composed by a more plant-specific microbial network. Assignment of each generalistic OTU to one or more steps of anaerobic degradation corresponded to well-known syntrophic interactions as well as to potential but unknown interactions.

1. Introduction

Rising energy costs and discussions about long-term sustainability shift the economics towards power generation by renewable resources (RR). In this context, biogas production from waste, residues and energy crops is of emerging interest (Weiland, 2010). Governmental support and regulations in German energy market and in other countries facilitated construction of plants as well as basic research in biogas production. The majority of studies focused on the microbial community composition in biogas plants (BPs) mainly supplied with energy crops such as maize (Cirne et al. 2007; Kampmann et al. 2012; Merlino et al. 2012; Rademacher et al. 2012; Stolze et al. 2015). Due to reduction of governmental subsidies many plant operators are interested in feeding their plants with cost-effective, alternative RR substrates like food residues or municipal waste. Likewise operators of sewage treatment plants (STPs) are interested to optimize biogas production and to reduce the amount of fermentation residues. Co-digestion of sewage sludge with other substrates, such as organic wastes from landfill (Park et al. 2016) or food wastes (Naran et al. 2016), might be a promising approach for future applications.

Same microbial processes of anaerobic degradation and CH_4 production are principally present in both plant types. Anaerobic degradation of organic substrates is a complex microbial process involving the steps hydrolysis, acidogenesis, acetogenesis and methanogenesis (Conrad, 1999; Demirel and Scherer, 2008). During these steps, complex organic material is stepwise degraded to substances of lower complexity. Finally, C1-compounds, such as formate or methanol can be directly consumed by mainly acetoclastic or hydrogenotrophic methanogens and converted to CH_4 and CO_2 (Ferry, 1992, 1992; Conrad, 1999; Conrad et al. 2009).

As majority of process-relevant microorganisms in BPs were not cultured so far, many culture-independent methods mainly based on 16S rRNA gene level have been applied to characterize microbial communities in small-scale biogas reactors (Delbes et al. 2000; Ye et al. 2012; Li et al. 2013) and in few large-scale BPs (Sundberg et al. 2013; Bremges et al. 2015; Theuerl et al. 2015; Goux et al. 2016). A common primer set for such amplicon-sequencing studies was the primer pair 515F and 806R (Theuerl et al. 2015; Stolze et al. 2016; Alcantara-Hernandez et al. 2017), as this primer combination is known to cover a great diversity of members of the kingdoms *Bacteria* and *Archaea* (Klindworth et al. 2013).

Most previous studies dealing with microbial diversity in different environments are strictly focused on the description of species composition and abundances. However, the role and interactions of different species in the corresponding microbial network could be more important than just abundance (Montoya et al. 2006; Zhou et al. 2010). Microbial network analyses have therefore been applied for microbial communities in different environments such as marine (Wang et al.

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2016), soil (Zhou et al. 2011) or rhizosphere (Shi et al. 2016) to investigate the microbial interaction patterns and key players. Modularity is another important property of networks. A module is defined as nodes that are highly connected among each other. According to their topological role, network nodes can generally be classified in specialists and generalists. Latter were subdivided into connectors, module hubs and network hubs (Olesen et al. 2007). Specialists have only few links within their own module and rarely any link to nodes of other modules. Connectors are linking nodes between different modules, while module hubs connect many different species within their module. Network hubs are connecting many species within and outside their module and are therefore named supergeneralists (Olesen et al. 2007).

The aim of this study is the phylogenetic and functional assessment of the microbial community composition obtained from many large-scale BPs (n = 16) and STPs (n = 10). All plants were in stable operation for at least two years. Documentation of plant parameters, sampling, 16S rRNA gene based amplicon sequencing of these plants and thereafter multivariate and network analyses were carried out to investigate key-players for robust biogas production in both plant types.

2. Materials and methods

2.1. Sampling

Samples from primary digesters of 16 BPs and digestions tanks of 10 STPs were taken in August 2016 in Northern Bavaria (see Tables 1 and 2 in Buettner and Noll, submitted). Before sampling, digester content was mixed thoroughly and all pipelines were flushed. Material of flushing was discarded. Approximately 300 mL of digester sludge were obtained of each plant and subsequently shock-frozen in liquid nitrogen. Samples were thereafter transferred in liquid nitrogen and stored at −80 °C for further investigations. Plant parameters were collected from the plant operators. Parameters from STPs were capacity, types of sewage, pH, chemical oxygen demand (COD), biological oxygen demand (BOD), process temperature (see Table 1 in Buettner and Noll, submitted), space-time-yield (STY), substrate pretreatment and mixing conditions, whereas parameters from BPs were process temperature, percentages of main and additional substrates, pH, fermentation type, substrate treatment and mixing conditions (see Table 2 in Buettner and Noll, submitted).

2.2. DNA-extraction

Different methods for extraction of nucleic acids were tested, which have been described previously for similar types of samples (Zhu et al. 2011; Kim et al. 2015; Theuerl et al. 2015; Pore et al. 2016) or were routinely applied in our lab (Griffiths et al. 2000; Noll et al. 2005). Following nucleic acid extraction kits and methods were evaluated: PowerSoil® DNA isolation kit (MO BIO Laboratories Inc., CA, USA) (Theuerl et al. 2015), E.Z.N.A™ Soil DNA Kit (Omega Bio-tek Inc., GA, USA) (Zhu et al. 2011), NucleoSpin® soil kit (Macherey-Nagel, Düren, Germany) (Kim et al. 2015), innuspeed soil DNA kit (Analytik Jena AG, Jena, Germany) (Pore et al. 2016), a modified phenol-chloroform extraction (Noll et al. 2005) and a method based on cetyltrimethylammonium bromide (CTAB) as described earlier (Griffiths et al. 2000). Nucleic acid extracts of each method were evaluated quantitatively (A_{260}) as well as qualitatively ($A_{260/280}$, $A_{260/230}$ and agarose gel electrophoresis) in triplicates as previously described (Weiss et al. 2007).

Best results for STPs were obtained using a modified phenol-chloroform extraction method as described earlier with minor modifications (Noll et al. 2005). Briefly, STP sludge samples were centrifuged and 500 mg of sludge pellet (wet weight) were mixed with 400 µL of cold TPM-buffer (50 mM Tris-HCl [pH 5.0], 20 mM $MgCl_2$ and 1.7% (w/v) polyvinylpyrrollidone K25 (PVP)), 200 µL of cold $NaPO_4$-buffer (200 mM, pH 5.6), 600 µL of pre-heated 20% (w/v) sodium dodecyl sulfate aquaphenol-mixture (Carl Roth GmbH, Karlsruhe, Germany) and 250 mg zirconia beads with a diameter of 0,7 µm (Carl Roth GmbH) or without beads. After 10 min of incubation at 65 °C, samples were homogenized for 0.5 min at 5.5 m s^{-1} (FastPrep®-24, MP Biomedicals, CA, USA) and subsequently centrifuged for 5 min at 20,000 g and 4 °C. 800 µL of the supernatant was transferred to a new tube and mixed with 800 µL of TPM-buffer. Samples were centrifuged at same conditions as mentioned above. 800 µL of phenol-chloroform-isoamylalcohol (25:24:1, Carl Roth GmbH, Karlsruhe, Germany) were added to 800 µL of the supernatant and centrifuged as mentioned before. 1300 µL of PEG-buffer (30% polytheylenglycol-6000 in 1,6 M NaCl-solution) and 2 µL of glycogen solution (VWR International, Darmstadt, Germany) were added to 650 µL of supernatant, followed by centrifugation for 30 min at 20,000 g and 4 °C. Resulting nucleic acid pellet were washed twice with ice-cold 70% (v/v) ethanol. Precipitated nucleic acid pellets were dissolved in 100 µL of Tris-Ethylenediaminetetraacetic acid (EDTA) buffer (10 mM Tris-HCl [pH 8.0], 1 mM EDTA, Carl Roth GmbH) and stored at −20 °C until further use. Final nucleic acid extracts with or without mechanical homogenization by beads, were mixed 1:1 as template for amplicon sequencing to circumvent underrepresentation of readily lysed microorganisms due to DNA fragmentation by bead beating. For BPs a CTAB based method was modified (Griffiths et al. 2000). CTAB extraction buffer was altered by the addition of 1% PVP. Furthermore, 5 µL β-mercaptoethanol were added prior to homogenization. Resulting pellets were dissolved in 50 µL Tris-EDTA buffer and stored at −20 °C. To avoid sequencing problems amplifiability of all nucleic acid extracts were tested using the same primer combination as for amplicon sequencing described by Klindworth et al. (2013), and PCR protocol published earlier (Gilbert et al. 2014).

2.3. Amplicon sequencing

As primer selection is crucial for 16S rRNA gene based amplicon sequencing different combinations of primer pairs suggested by Klindworth et al. (2013) were *in silico* tested by using the “probe match” function for the ribosomal database project (RDP) (Cole et al. 2014) and the “TestPrime” function for the SILVA database (Quast et al. 2013). Coverage of *Archaea* and *Bacteria* sequence diversity of V4-targeting primer pair 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′), allowing one mismatch, were 95% and 96% for SILVA database (SSU-database) and 71% and 79%, respectively, by RDP database (domains *Bacteria* and *Archaea*, respectively) verified at 21 march 2018.

Two-Step PCR libraries of each STP and BP plant sample were created with the primer set 515F and 806R by using a v2 500 cycles kit (Illumina, San Diego, CA, USA) as recommended by manufacturer instructions. Sequencing of the libraries was carried out by 300-bp paired-end sequencing on an Illumina MiSeq system. Raw data were de-multiplexed, quality filtered and adaptor trimmed using the Illumina real time analysis software and thereafter sequence quality of the reads was checked with the FastQC software, version 0.11.5 (Andrews, 2010). For trimming of the locus specific V4 adaptors the software cutadapt v.1.9.2.dev0 (Martin, 2011) was used. Reads that could not be trimmed were discarded. Merging of the trimmed forward and reverse reads, considering a minimum overlap of 15 bases, was done by using USEARCH version 8.1.1861 (Edgar, 2010). Merged sequences were filtered by allowing a maximum of one nucleotide mismatch per merged read. Reads containing ambiguous nucleotides were discarded. Clustering of OTUs with a 99% similarity level, discarding singletons and chimeras, was performed with USEARCH. Same software was used for the alignment of the OTUs against the Greengenes v13.8 database (DeSantis et al. 2006) and taxonomic assignment with an identity threshold of 0.7 (Wayne et al. 1987). Libraries, sequencing and data analysis described above were performed by Microsynth AG (Balgach, Switzerland).