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Short Communication

Reorganisation of a mesophilic biogas microbiome as response to a stepwise increase of ammonium nitrogen induced by poultry manure supply

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HIGHLIGHTS

- Process-risk feedstocks such as poultry manure have to be used with caution.
- Low amounts of NH_4^+-N and VFA favour a *Bacteroidetes–Methanosaetaceae* microbiome.
- The addition of 50% poultry manure led to a reconstruction of the microbiome.
- The functional redundant microbiome was *Clostridiales–Methanobacteriaceae*-dominated.
- A natural-regulated microbial diversity management was recorded.

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ABSTRACT

An anaerobic digestion experiment was investigated to evaluate the impact of increasing amounts of ammonium nitrogen due to poultry manure addition on the reactor performance, especially on the microbiome response. The microbial community structure was assessed by using a 16S rRNA gene approach, which was further correlated with the prevalent environmental conditions by using statistical analyses. The addition of 50% poultry manure led to a process disturbance indicated by a high VFA content (almost 10 $g_{HAc\text{-}Eq}\,L^{-1}$) in combination with elevated concentrations of ammonium nitrogen (5.9 g NH_4^+-N kg_{FM}^{-1}) and free ammonia (0.5 g NH_3 kg_{FM}^{-1}). Simultaneously the microbiome, changed from a *Bacteroidetes*-dominated to a *Clostridiales*-dominated community accompanied by a shift from the acetoclastic to the hydrogenotrophic pathway. The "new" microbial community was functional redundant as the overall process rates were similar to the former one. A further increase of poultry manure resulted in a complete process failure.

1. Introduction

The implementation of anaerobic digestion (AD) on animal waste has become a promising alternative treatment technology as it reduces the negative environmental impacts and offers a sustainable renewable energy resource. However, applying AD on animal manure is related to the risk of ammonium nitrogen (NH_4^+-N) accumulation, whereby the undissociated form (free ammonia, NH_3), is considered to be toxic for the microbial community, especially for the acetoclastic methanogens, which may lead to process disturbances/failures (Chen et al., 2008; Lv et al., 2014). It has also been shown that a process imbalance can naturally be prevented by a shift from acetoclastic to hydrogenotrophic methanogenesis in combination with syntrophic acetate oxidation (Schnürer and Nordberg, 2008). Hence one of the most important factors to ensure a stable biogas production is a highly efficient microbiome which is resilient against process disturbances (Theuerl et al., 2015). Consequently, the scientific challenge is to expand the knowledge of the complex ecological network within the biogas reactor.

In order to investigate the process microbiology, a broad range of methods is available, whereby it is recommended to use a combination of different methods and link the community structure information to its role in its respective habitat (Cabezas et al., 2015; Carballa et al., 2011; Verstraete et al., 2007). So far only a

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few long-term studies are available dealing with the effects of a gradual increase of NH_4^+-N due to the addition of poultry manure on the reactor performance, especially under consideration of the structure and dynamic variations of the microbiome. Therefore, the aim of the present study was to investigate how much poultry manure, respectively how much NH_4^+-N can be tolerated by the AD microbiome without any negative effects on the overall process performance.

2. Methods

2.1. Experimental setup, biogas reactor operation and chemical analyses

To investigate the response of the microbial community due to an increasing concentration of NH_4^+-N caused by addition of poultry manure (PM), two mesophilic (37 °C) laboratory-scale (eight litre working volume) continuously stirred tank reactors (CSTRs) were operated at an organic loading rate (OLR) of 3.0 $g_{VS}\ L^{-1}\ d^{-1}$. Over the experimental phase (EP), the control reactor (CR) was operated with cattle slurry (CS) as sole feedstock, whilst the experimental reactor (ER) was fed with an increasing amount of PM (based on volatile solids, VS) as followed: EP1 = 75% CS and 25% PM for 143 days with a hydraulic retention time (HRT) of 30 days; EP2 = 50% CS and 50% PM for 165 days (HRT = 37 days); EP3 = 25% CS and 75% PM for 171 days (HRT = 52 days); and finally EP4 = 100% PM for 22 days (HRT = 134 days). Over the entire trail period, the produced amount of biogas was measured continuously, whilst the gas composition was analysed twice a week. Digestate samples were analysed regarding their main chemical characteristics (total Kjeldahl nitrogen (TKN), NH_4^+-N content, volatile fatty acids (VFA) in terms of acetate, propionate, *iso*- and *n*-butyrate, *iso*- and *n*-valerate, and capronate) according to Schönberg and Linke, (2012).

2.2. Microbial community analyses

To investigate the process microbiology 14 different time points (depending on the reactor performance) were chosen from the ER (day 98, 137, 155, 185, 207, 230, 274, 305, 319, 337, 372, 479, 490, 514) and three from the CR (day 98, 207, 479). Genomic DNA was extracted using the FastDNA® SPIN Kit for soil (MP Biomedicals, Heidelberg, Germany) according to the manufacturer's guidelines.

To characterise the microbial community structure and its dynamic variation the genomic fingerprinting method terminal restriction fragment length polymorphism (TRFLP) targeting either the bacterial or the archaeal 16S rRNA gene was used as described by Rademacher et al. (2012) and Klang et al. (2015). Bioinformatic evaluation of the obtained data was performed as published by Klang et al. (2015) using the software package BioNumerics 7.1 (Applied Maths, Belgium).

Additionally, 16S rRNA gene sequence libraries were constructed from samples ER-98, ER-137, ER-185, ER-305, ER-372, ER-514, and CR-98, covering all putative community changes. Cloning of PCR products was performed according to Rademacher et al. (2012), followed by DNA sequencing (GATC Biotech AG, Konstanz, Germany). The obtained sequences were processed, grouped into operational taxonomic units (OTUs) and virtually cut according to Klang et al. (2015) using the software package BioNumerics 7.1 (Applied Maths, Belgium). Subsequently, OTUs were taxonomically assigned using the Ribosomal Database Project RDP Version 2.6 (Wang et al., 2007). All new sequences obtained in this study have been deposited in the European Molecular Biology Laboratory (EMBL) database under accession numbers LN849462–LN849688 (*Bacteria*) and LN874153–LN874209 (*Archaea*).

In order to get information about the microbial system ecology, the community organisation expressed by the Gini coefficients was calculated (Carballa et al., 2011; Klang et al., 2015; Theuerl et al., 2015; Verstraete et al., 2007). Additionally, the software package of PC Ord Version 6 (McCune and Mefford, 2011) was used to perform a non-metric multidimensional scaling (NMS) (Clarke, 1993) to conduct a correlation amongst and between the operational and microbiological parameters.

3. Results and discussion

3.1. Performance of anaerobic co-digestion of cattle slurry with stepwise increased addition of poultry manure

During the experimental period, the control reactor (CR) showed no significant changes neither in the produced biogas (376 ± 72 $L_N\ kg_{VS}^{-1}$ with a CH_4 content of 62 ± 2%; Fig. 1A) nor in the main chemical parameters (NH_4^+-N = 1.8 ± 0.2 g kg_{FM}^{-1}, NH_3 = 0.07 ± 0.02 g kg_{FM}^{-1} and VFA concentration ⩽ 0.5 g L^{-1}).

The increasing NH_4^+-N concentration caused by the addition of PM showed no significant effects on the process parameters until 4.2 g kg_{FM}^{-1} were reached in EP2; a values which exceeded the reported inhibition levels of the AD, especially of the acetoclastic methanogenesis (Drosg, 2013; Schnürer and Nordberg, 2008). Subsequently, a serious process imbalance occurred as indicated by a VFA accumulation of almost 10 $g_{HAc\text{-}Eq}\ L^{-1}$ (mainly acetic acid), and a drop in the biogas production (Fig. 1). After a certain time (without active counteracting) the system recovered symbolized by a decrease in the VFA concentration and a subsequent increase of the biogas, respectively methane contents. Hence, it can be supposed, that an adaption from the acetoclastic to hydrogenotrophic pathway of methane formation took place.

A further increase of PM (75% in EP3, respectively 100% in EP4) and, hence, a continuous increase of NH_4^+-N of up to 9.6 g kg_{FM}^{-1} resulted in a complete process failure indicated by a constant increase in the VFA concentration accompanied by a strong decrease of the biogas amount and methane content (Fig. 1).

3.2. The response of the microbiome to increased amounts of poultry manure

The microbiome of the three samples from the CR consisted mainly of members from the phylum *Bacteroidetes* (35%) followed by *Firmicutes* (17%), and the WWE1 candidate division (10%) at the bacterial level and members of the genus *Methanosaeta* (phylum *Euryarchaeota*) with an abundance of around 60% at the archaeal level. The Gini coefficients in the CR were 0.41 (*Bacteria*), respectively 0.69 (*Archaea*), values which are reported to symbolise well-established communities (Theuerl et al., 2015). Hence, it can be assumed that the methane formation was mainly carried out by the acetoclastic pathway which in turn indicated a good performing reactor system as previously reported by Regueiro et al. (2012).

Although no significant changes in the reactor performance were observed during EP1 (see Section 3.1), the microbial community, especially the archaeal community was affected by changes in the feedstock supply and, therefor, the nutrient availability as the abundance of members belonging to the family *Methanobacteriaceae* increased slightly. This led to a more even distributed community organisation combined with an increasing importance of the hydrogenotrophic pathway of methane formation.

Compared to EP1, the sample ER-155 taken 11 days after increasing the PM amount to 50% (EP2), showed significant differences in the bacterial community structure whereby members from the family *Porphyromonadaceae* (phylum *Bacteroidetes*)