Microbial community composition and dynamics within two-stage anaerobic digestion of wheat straw

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Abstract
The bacterial and archaeal communities and their dynamics from start-up to stable biogas formation were investigated using a novel anaerobic digester system designed for efficient digestion of lignocellulosic biomass. The lab-scale reactor systems each consisted of an up-flow anaerobic solid-state reactor (UASS) connected to an anaerobic filter (AF) with recirculation of the liquid phase. Mesophilic and thermophilic digestion was carried out in parallel systems. Both reactor systems were fed with wheat straw as sole substrate at an organic loading rate of 2.5 g_{VS} L^{-1} d^{-1} over a time period of 218 days.
Samples were taken from the effluent of UASS and AF and of digestates. Additionally, at the last sampling day, biofilm carriers from the AF were analyzed. Terminal restriction fragment length polymorphism (T-RFLP) of PCR-amplified 16S rRNA genes was applied to analyze changes in the biocoenosis structure over time. Furthermore, for the samples of the last sampling, 16S rRNA gene libraries were constructed to obtain detailed insights into the taxonomical composition of the microbial communities responsible for biogas formation at stable process conditions.
The results offer a considerable higher taxonomic variety of Bacteria compared to Archaea, whereas the mesophilic communities were much more diverse than the thermophilic. Furthermore the mesophilic and thermophilic communities were entirely different – only five common OTUs were found at both temperature regimes. The fingerprinting pattern showed a clear alteration during and even after establishment of a steady-state biogas formation process.

Keywords
16S rRNA gene libraries, T-RFLP, microbial communities, microbial dynamics, two-stage reactor system

INTRODUCTION

The anaerobic conversion of biomass to methane and carbon dioxide is carried out by a complex microbial community of acid-forming and methane-forming microorganisms. These different functional groups have special demands in regard to various factors such as temperature and pH. The reactor system used in this study consists of the novel upflow anaerobic solid-state reactor (UASS) introduced by Mumme et al. (2010) in combination with an anaerobic filter (AF). In this two-phase biogas reactor system optimized environmental conditions for hydrolysis and methanogenesis can be provided.

The aim of this study was to monitor the dynamics of the microbial communities from start-up to the development of a steady-state process performance and to identify the bacterial and archaeal members involved in a stable biogas formation process at mesophilic and thermophilic conditions.

MATERIALS & METHODS

Reactor Performance and Sampling

The two analyzed lab-scale biogas reactors each consisted of a 39 liter UASS reactor combined with a 30 liter AF (Figure 1). The AFs were filled with polyethylene biofilm carriers commonly used in anaerobic waste water treatment for biofilm establishment. Over a period of 218 days the reactors were operated at thermophilic (55°C) and mesophilic (37°C) conditions in parallel. After an initial phase (until day 33) they were fed for five days a week at an OLR of 2.5 g_{VS} L^{-1} d^{-1} with

chopped wheat straw as sole substrate. A stable process performance was reached after approximately 140 days for both analyzed reactor systems indicated by stable biogas production.

Samples for molecular biological analysis were taken after 25, 67, 87, 108, 142 and 163 days of operation. At each sampling point, effluent process liquor from UASS and AF as well as solid digestate was taken. Additionally, after 163 days of fermentation one biofilm carrier from the middle of each AF was sampled. Subsequently, the biofilm carriers were washed with sterile 1x PBS buffer and the biofilm was detached using a sterile scalpel.

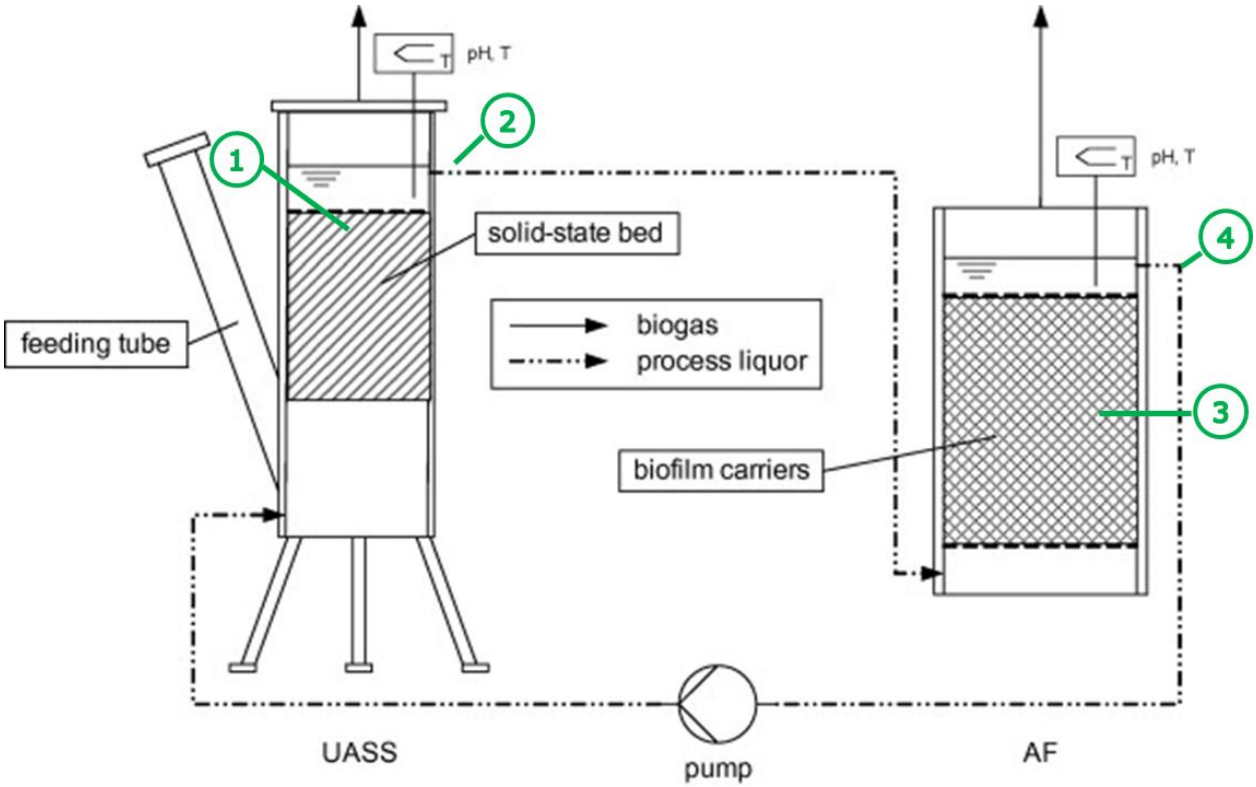

Figure 1. Overview about the experimental setup (UASS, up-flow anaerobic solid-state reactor; AF, anaerobic filter) and the collected samples (1 digestate; 2 effluent UASS; 3 biofilm carrier; 4 effluent AF) (modified after Pohl et al. 2012).

Molecular Biological Analysis

Genomic DNA was isolated using a FastDNA ® SPIN Kit for Soil (MP Biomedicals, Germany) according to the manufacturer's guidelines. Genes coding for the 16S rRNA were amplified with the PCR primers 27f (5´-AGAGTTTGATCMTGGCTCAG-3´) and 926MRr (5´-CCGTCAATTCMCTTRAGT-3´) for Bacteria and 63f (5´-YGAYTAAGCCATGCRAGT-3´) and ARC934r (5´-TGCTCCCCCGCCAATTCCT-3´) for Archaea. In PCR reactions assigned for T-RFLP analysis the forward primers were labeled with Cy5 at the 5' end. Terminal restriction fragment length polymorphism and the construction of 16S rRNA gene libraries for samples taken on day 163 were carried out according to Rademacher et al. (2012).

RESULTS & DISCUSSION

Microbial Dynamics

The detailed and concluding examination of the fingerprinting data is still in progress. First results showed a remarkable dynamic within both bacterial and archaeal communities over time.

As representing example the bacterial community dynamics within the UASS effluent of the thermophilic reactor system from day 25 to day 163 is displayed in Figure 2. For this analyzed sample a shift from a few dominant representatives at the beginning to a higher diversity at steady-state situations occurred. Alterations in the relative abundance of the main T-RFs appeared despite a steady-state biogas formation was achieved until day 142. This indicates that the adjustment of an optimal microbial community in the biogas reactor occurred very slowly and was still not completed even though a stable biogas production already established.

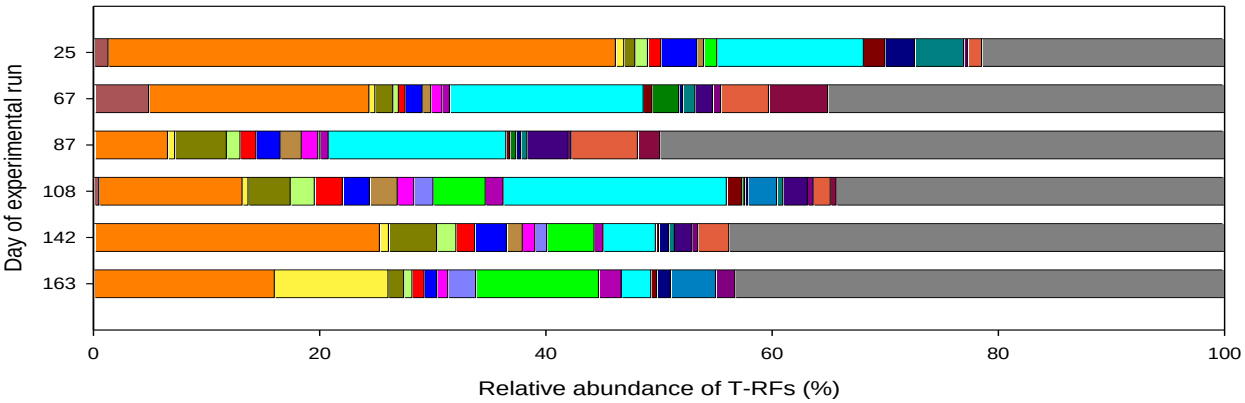

Figure 2. Dynamics of the bacterial community structure within the UASS effluent of the thermophilic reactor system. Illustrated are T-RFs with a relative abundance above 1.5% of the total fluorescence intensity in at least one profile.

Composition of Microbial Communities

With the samples of the last sampling day 16S rRNA gene libraries for Bacteria and Archaea were prepared. By means of their calculated coverage between 75 and 100% it can be assumed that the majority of all taxonomic members were captured.

In all bacterial 16S rRNA gene libraries a total of 224 OTUs within the mesophilic and 135 OTUs within the thermophilic libraries were detected. Only five of these OTUs were commonly found at both temperature regimes. As illustrated in Figure 3, members of the phylum Firmicutes clearly dominated in the communities at both temperatures. In the mesophilic system also representatives of the phylum Bacteroidetes were prevalently detected. In the effluent of the thermophilic reactor system sequences affiliated to a member of the phylum Thermotogae were frequently found.

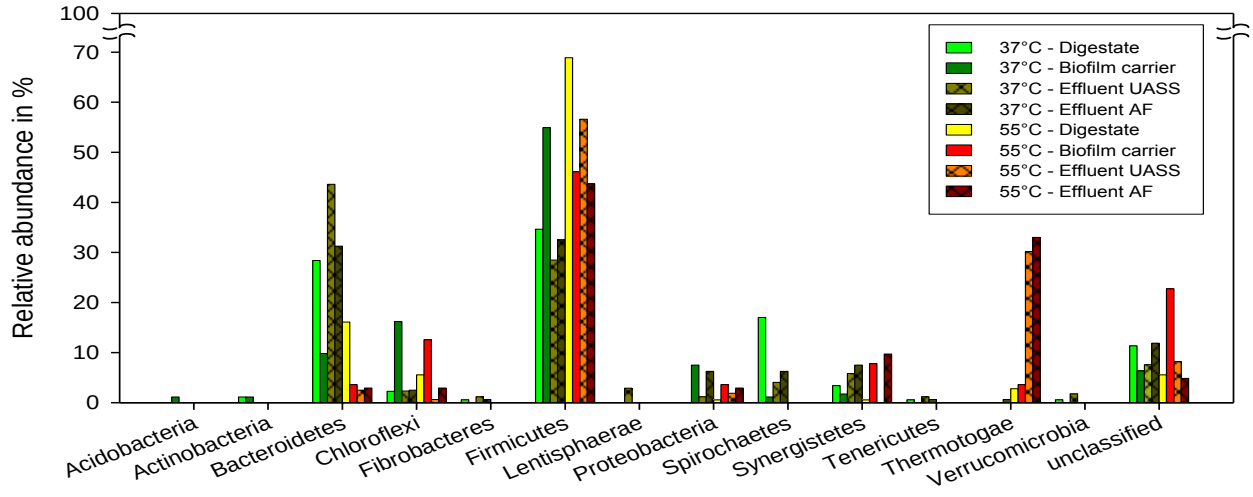

Figure 3. Taxonomic composition of bacterial communities at stable process conditions.

Within the thermophilic archaeal 16S rRNA gene libraries five different OTUs were obtained (see Figure 4). All of these five and eight further OTUs were found in the mesophilic reactor system. At both temperatures, the community composition of the solid and liquid phases was generally dissimilar. Within the biofilms at the solid compartments mainly acetoclastic methanogenesis performed by members of the order *Methanosarcinales* was occured. More precisely, a strict aceticlastic specis of *Methanosaeta* predominated in the mesophilic system, wheareas a mixotrophic *Methanosarcina* sp. dominated in the biofilms within the thermophilic reactor. The process liquors were mainly composed of hydrolytic Archaea belonging to the order Methanobacteriales and Methanomicrobiales. In the libraries of the thermophilic effluent the high proportion of sequences related to a member of the bacterial phylum Thermotogae (see Figure 3) combined with the high abundance of *Methanothermobacter* sp. is remarkable since this two groups are known to grow in a syntrophic relationship (Balk et al. 2002).

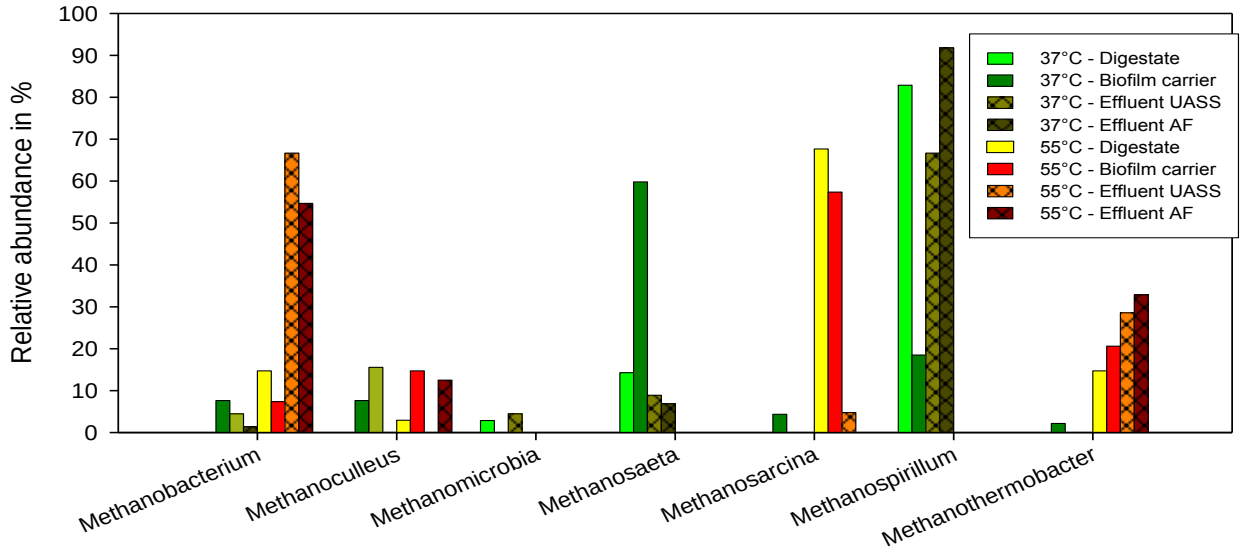

Figure 4. Taxonomic composition of archaeal communities at stable process conditions.

As conclusion, a high dynamic of the microbial communities from start-up to formation of a steady state biogas production was demonstrated. Depending on the process temperature, completely different microbial communities established in the mesophilic and thermophilic reactor systems although the same inoculum was used. Generally, there was a higher frequency of different OTUs at mesophilic conditions, indicating a higher diversity compared to the community present at the thermophilic temperature. In addition, the community composition varies between the different compartments within one reactor system apparently according to the metabolic skills of the respective microorganisms. In consequence, the separation of the hydrolytic and methanogenic biogas formation steps within the investigated two-phase reactor systems might be proven.

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