Influence of sludge pre-treatment on the microbial community structure in anaerobic digesters.

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Abstract
Anaerobic digestion plays a key role in the generation of sustainable energy carriers, in the form of biogas, from various organic waste streams. This paper aims at describing and discussing the effects of an ultrasound and a microwave pre-treatment on sludge COD, biogas production and microbial community dynamics in the digester. The microbial community was analyzed by 454 pyrosequencing. The microwave pre-treatment resulted in the lowest COD release, with a soluble COD concentration comparable to the blank, but the highest increase in biogas production. Furthermore, a clear shift in the microbial community during digestion was also observed. The results of the ultrasound treatment were the complete opposite: high soluble COD concentration, lowest biogas increment and the microbial community did not differ to that in the blank reactor. The feeding of disintegrated sludge to an anaerobic digester thus not necessarily induces a shift in microbial community composition.
Keywords
Anaerobic digestion, pre-treatment, microwave, ultrasound, microbial community

INTRODUCTION
Anaerobic digestion is generally considered as one of the most promising technologies for the energetic valorization of various types of biomass and solid organic wastes, and it is projected to play a crucial role in the future renewable energy production. Due to the complexity of the microbial and physicochemical reactions involved, anaerobic digestion has traditionally been treated as a black box system, and optimization is commonly based on experience or trial and error methods. However, with the advent of culture-independent molecular techniques (reviewed in Talbot *et al.*, 2008), an increasing number of studies have reported on the microbial community composition and dominant members in anaerobic digesters treating waste streams (e.g. Rivière *et al.*, 2009; Shin *et al.*, 2010a,b; Werner *et al.*, 2011), opening the door to engineer communities with superior functions. In this research, the influence of two pre-treatment methods, i.e., microwave and ultrasound treatment, on the biogas production and on the microbial community was investigated during a 3.5 month experiment. The microbial community was determined using 454 pyrosequencing, which enables to determine the community structure and composition in detail. To our knowledge, this is the first study of the microbial community in anaerobic digesters using specific primers targeting both Bacteria and Archaea. The study of Archaea is imperative, since it is thought that they play a major role in methanogenesis.

MATERIALS & METHODS

Origin of the sludge

The thickened waste activated sludge was taken from the buffer tank of the WWTP of Mechelen-Noord (Belgium), stored at 4°C and used the same day. The original inoculum used for the start-up of the digesters was taken from a full-scale digester of the WWTP of Antwerpen-Zuid (Belgium).

Semi-continuous anaerobic digestion

Three pilot scale semi-continuous digesters (50 L working volume each) were run in parallel at mesophilic conditions (37°C) for 67 days, with a hydraulic retention time of 20 days. One digester was fed with untreated thickened waste activated sludge (i.e. the blank reactor) as a reference, while the others were fed with ultrasound (US) and microwave (MW) pre-treated thickened waste activated sludge, respectively. The methane production was recorded daily via a drum-type gas meter (Ritter, Germany).

US and MW pre-treatment

For each treatment, 500g of sludge was subjected to ultrasound waves (100W for 8min) or introduced in the MW oven (MW irradiated at 800W for 1min). The ultrasonic waves were applied using a US horn with a maximum power output of 150W (Bandelin Sonopuls HD 3200); for the microwave treatment, sludge samples were placed in a microwave oven (Sharp R-212, 2,45 GHz) with a maximum power output of 800W. A total energy of 2100 kJ/kg sludge was applied to the sludge in both treatments.

Sludge analyses

The dry matter (DM) and organic dry matter (ODM) content were determined following APHA (2006). The COD was determined using Nanocolor® COD 1500 test tubes (Macherey-Nagel) and a digital photometer Nanocolor® 500D (Macherey-Nagel).

Microbial analyses

Samples of the effluent for microbial characterization were taken about every three weeks during the 3.5 month experiment (t_1 to t_7), as well as a sample of the original inoculum. Samples were stored at -18°C until further use. Total DNA was extracted from the sludge samples in duplicate using the MOBIO Powersoil kit (MOBIO Laboratories, USA). Amplicon samples were obtained by amplifying extracted DNA with universal primers 515F and 806R constructed for 454 pyrosequencing targeting the 16S rRNA gene of both Bacteria and Archaea (Bates et al., 2009). Both primers contained a Roche adapter sequence and the forward primer contained a barcode sequence. After gel electrophoresis of the amplicons, bands with the appropriate length were excised from the gel and purified using the Qiagen gel extraction kit. The obtained pooled sample was sequenced using the Roche FLX 454 pyrosequencer. In total, approximately 370,000 sequences were obtained, resulting in 6,400 sequences per sample on average. Mothur version 1.27 (Schloss et al., 2009) was used to process sequences, align quality-trimmed sequences to the Silva reference alignment, remove chimeras and contaminants, and cluster the aligned sequences based on a 3% sequence similarity threshold using furthest neighbor clustering. This resulted in 8,542 operational taxonomic units (OTUs) across all samples, of which 5,167 are only represented by 1 sequence. A data matrix was constructed starting with the 40 most dominant OTUs for each sample (representing at least 60% of all sequences of each sample) and amended with other OTU's found in the top 40 of the other samples (representing at least 73% of all sequences). In total, 142 OTUs were included in the data matrix. In order to graphically compare the microbial community among treatments and time two-dimensional nMDS ordination based on the Bray-Curtis (Sørensen) similarity measure (default parameters, PC-ORD v5) was performed using this data matrix (Ramette, 2007).

RESULTS & DISCUSSION

Sludge parameters & biogas production

Table 1 depicts the values of (O)DM and COD determined for the three reactors. As expected, the concentrations of the (O)DS and total COD do not differ much between the three reactors. The soluble COD concentration, i.e. the amount of organic matter released into the water phase of the sludge, is clearly affected by the treatments. It is seen that, although the same amount of energy is applied to the sludge, an ultrasound treatment is able to increase the soluble organics concentration much better than the microwave treatment. An explanation must be found in the manner in which the energy is applied to the sludge particles.

Table 1. Average values of several composition variables for untreated, ultrasound and microwave pre-treated sludge

	Blank		US pre-treated		MW pre-treated	
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
DM (g/kg)	43,6	7,3	42,1	6,9	41,3	7,1
ODM (g/kg)	30,8	5,2	29,0	5,1	29,4	5,3
COD (g O_2/L)	40981	10184	42306	6158	43931	10450
sCOD (g O_2/L)	104	24	1923	954	226	168

The biogas production was also affected by the treatments: an increase of 17 and 21% for the US and MW treatment, respectively.

Changes of the microbial community structure during the experiment

454 sequencing resulted in, on average, ca. 6000 sequences per sample. Overall, 2269 unique OTUs across all samples were identified. Of the total number of OTUs after elimination of singletons and doubletons, 1-3% was identified as Archaea and the remainder as Bacteria.

In Figure 1, the microbial community structure of all three digesters during 3.5 months feeding is given after 2D nMDS (i.e. non-metric MultiDimensional Scaling) ordination using Sorensen as a similarity measure. The further two treatments are separated, the more dissimilar they are. Axis 1 explained ca. 60% of the variation, whereas the second axis explained ca. 30%. Therefore, the dissimilarity is more pronounced along Axis 1. During 3.5 months of feeding the anaerobic digester, the microbial community changed in the digester fed with untreated sludge.

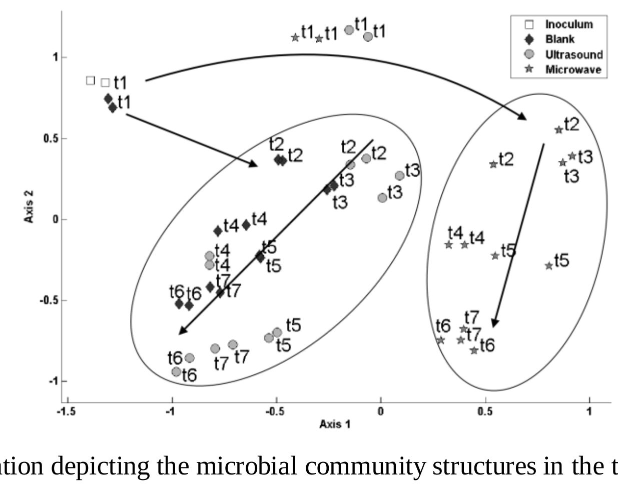

Figure 1. 2D nMDS ordination depicting the microbial community structures in the three reactors during 3.5 months feeding (t_1-t_7).

The arrows on Figure indicate the evolution of the bacterial community in the three digesters. From one month feeding onwards (t_2-t_7) a pattern in the community structures can be distinguished. In the digester receiving microwave pre-treated sludge, the bacterial community initially changed similarly as in the ultrasound pre-treated digester (cfr. t_1). Afterwards, the bacterial community diverges away from the two other digesters, showing that a different bacterial community was established due to the microwave pre-treatment. In the digester receiving ultrasound pre-treated sludge, the bacterial community first diverges away from the inoculum (t1), but subsequently, a similar bacterial community as in the blank digester is observed (t_2-t_7) showing that ultrasound pre-treatment did not alter the bacterial community established after 3.5 months feeding compared to the blank digester.

Contrary to what was expected, these observations cannot be linked to disintegration or solubilisation of the sludge due to the respective pre-treatments. Although ultrasound treatment caused a significant COD solubilisation, it did not lead to a shift in the bacterial community structure during digestion. On the other hand, the COD solubilisation caused by the microwave treatment was far less pronounced, however, a clear shift in the bacterial community was observed. The results (data not shown) revealed that the US treatment yielded the lowest biogas increment (23%) although a much higher sCOD concentration was achieved. In contrast, the MW treatment resulted in a 27% increase in biogas production, although the sCOD concentration did not increase much.

CONCLUSIONS

In this paper, the effects of an ultrasound and a microwave pre-treatment on sludge COD, biogas production and microbial community is discussed. It was seen that the US treatment resulted in the highest COD solubilisation (increase with factor 17.5) compared to the MW treatment (increase with 17%), even though the same amount of energy was applied to the sludge. An explanation must be found in the manner in which the energy is applied to the sludge, since this is different for both treatments. The 2D nMDS plot showed the change of the bacterial communities for all three digesters. From the start of the digestion experiment, the communities started to change as a result of acclimation to a new environment. However, it was seen that changes in the structure of the organic part of the sludge did not lead to a shift in the bacterial community during the digestion period. A shift in the bacterial community structure was observed for the MW treatment with low sCOD solubilisation and highest biogas increment (+27%). It is clear that more research is needed to unravel the complexity of the digestion and microbial community dynamics.

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