Anaerobic co-digestion of vegetable oil and thickened waste-activated sludge: effects on biogas production and microbial community structure


*Chemical and Biochemical Process Technology and Control Section, Department of Chemical Engineering, KU Leuven, Willem De Croylaan 46, B-3001 Heverlee, Belgium. raf.dewil@cit.kuleuven.be
**Laboratory for Process Microbial and Bioinspirational Management (PME & BIM), Department of Microbial and Molecular Systems (M2S), KU Leuven, Sint-Katelijne-Waver, Belgium. (stefan.ruyters@leuven.kuleuven.be)

Objectives and conclusions
In this research the effect of co-digesting fats, oils and greases (FOG) on the operational parameters (biogas production, volatile solids, etc.) was examined, using vegetable oils as a representative for FOG. For a period of 133 days, two CSTR-type digesters where run parallel and fed with identical amounts of the thickened waste-activated sludge (WAS). On the 54th day, one of the digesters was fed with additional oil. As expected, the biogas production in the oil-fed digester fed increased due to the addition of oil: about ~60 % for an oil load of 10-15 % of the volatile solids (VS) and ~200% for an oil load of 37-44 %, compared to the digester fed solely with WAS. No extraordinary values (e.g., pH, biogas production) were recorded that indicate a possible inhibition.

Additionally, the bacterial and archaeal community structure in both digesters was analyzed using 454 pyrosequencing and visualized at OTU level using non-metric multidimensional scaling. In addition, major groups were identified and relative abundances were calculated. Methanogens and methanotrophs are the dominant groups within the microbial community. Methanotrophs are the methane consuming bacteria, and Methanogens are the methane producing bacteria. These groups differ in the oil-fed digester differences from the WAS digester, but these differences are mainly the result of the stabilization phase, rather than due to oil addition. Indeed, changes of the relative abundance of the major Bacteria phyla are most pronounced during the stabilization phase. The relative abundance of the phylum Chloroflexi and the classes Deltaproteobacteria and Negativicutes changed in the oil-fed digester, indicating a possible connection. No change in the relative abundance of the class Chloroflexi, which are considered to be the main acetate producers in the digester, is observed during the course of the experiment. All major bacterial OTUs are associated with anaerobic and/or sludge environments. In addition, H2 producing bacteria were detected in level relative abundance.

**Experimental set-up**
- Two CSTR-type digesters (Fig. 1)
  - WAS
  - Kultivierter Ablauf
  - Oil: liquid olive, 20 L, overhead press
  - Anaerobic digester: C3-C
  - Feed is fed to buffer tank: 2-3 days RT
  - Biogas production (C2)
  - Content of both digesters is identical during phase 1 and 2

**Substrates**
- TOS: 4.4 ± 0.9 kg/L
- VS: 31.4 ± 3.1 g/L
- Oil: 4.25 ± 0.23
- Vegetable oil
- 100% refined fats
- 44% lromoinsaturated fats
- 41% polyunsaturated fats

**Microbial Analysis**
- ca. 3305 sequences per sample were recovered
- 3356 unique Operational Taxonomic Units (OTU) of which 97% are non-archaeal bacteria
- General community structure evolution at OTU level is visualized using two-dimensional non-metric multidimensional scaling (NMDS)

**Archaea**
- 13% of the obtained sequences for each sample were identified as Archaea
- 97% of the total number of OTUs is identified as Archaea
- Similar changes of the community structure are observed in the archaeal community of both digesters during the experiment. No major changes due to oil addition are observed.
- 97-100% of the Archaeal OTUs of the phylum Euryarchaeota are exclusively Methanobacterium and Methanococcales

**Bacteria**
- 97% of the total number of OTUs are identified as Bacteria
- 97% of the total number of OTUs are identified as Bacteria
- The bacterial community in 2 steps: a major shift during the stabilization phase (phase 1) in one digester, this is a very well parallel in both digesters.
- Diverse: majority phyla are Firmicutes, Proteobacteria, Verrucomicrobii, Bacteroidetes, Chloroflexi and Actinobacteria (45%).

**Fig. 1. Digester set-up**

**Fig. 2. NMDS plot of the archaea. 60% and 30% of the variation is explained along axis 1 and axis 2 respectively.**

**Fig. 3. Dynamics of the relative abundance of the class Chloroflexi of the Bacteria. A small shift occurs in the stabilization phase (phase 1). In general, the relative abundance does not change during the course of the experiment.**

**Fig. 4. NMDS plot of the Bacteria. 60% and 30% of the variation is explained along axis 1 and axis 2 respectively.**

**Fig. 5. Dynamics of the relative abundance of the class Chloroflexi of the Bacteria. A small shift occurs in the stabilization phase (phase 1). In general, the relative abundance does not change during the course of the experiment.**

**Fig. 6. Dynamics of the relative abundance of the phylum Chloroflexi of the Bacteria. During the stabilization phase (phase 1), the Bacteroidetes content increases and the Verrucomicrobii decreases, causing both communities to shift apart. After stabilization, the relative abundance of both communities does not change during the remaining course of the experiment.**

**Fig. 7. Dynamics of the relative abundance of the phylum Bacteroidetes and Verrucomicrobii of the Bacteria. During the stabilization phase (phase 1), the Bacteroidetes content increases and the Verrucomicrobii decreases, causing both communities to shift apart. After stabilization, the relative abundance of both communities does not change during the remaining course of the experiment.**

**Fig. 8. Dynamics of the relative abundance of the bacterial community in both digesters decreases equally for each digester during the course of the experiment.**

**Fig. 9. Dynamics of the relative abundance of the class Chloroflexi of the Bacteria. A small shift occurs in the stabilization phase (phase 1). In general, the relative abundance does not change during the course of the experiment.**

**Fig. 10. Dynamics of the relative abundance of the phylum Chloroflexi of the Bacteria. In phase 3 and 4, the Chloroflexi content of the oil digester increases, causing both communities to shift apart.**

**Fig. 11. Dynamics of the relative abundance of the phylum Chloroflexi of the Bacteria. In phase 3 and 4, the Chloroflexi content of the oil digester increases, causing both communities to shift apart.**

**Fig. 12. Dynamics of the relative abundance of the phylum Clostridiales of the Bacteria. In general, the relative abundance of both communities change parallel, with the Atribacteria abundance decreasing and the Bacteroidales abundance staying relatively constant. The abundance of the Clostridiales content of the oil digester increases during the oil feeding phases.**