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Published in:

Proceedings of the 2nd International Conference on Biogas Microbiology

Publication date:

2014

[Link back to DTU Orbit](#)

Citation (APA):

Kougias, P., De Francisci, D., Treu, L., Campanaro, S., & Angelidaki, I. (2014). Comparative microbial analysis before and after foaming incidents in biogas reactors. In Proceedings of the 2nd International Conference on Biogas Microbiology

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Comparative microbial analysis before and after foaming incidents in biogas reactors

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Foaming is one of the major problems that occasionally occurring in biogas plants affecting negatively the overall anaerobic digestion (AD) process. According to a recent survey, 15 out of 16 full-scale biogas plants, which were surveyed in Denmark, faced foaming incidents in the main reactor and/or in the pre-storage feeding tank, resulting in 30-50% biogas production loss (Kougias et al., 2014). In activated sludge systems and in wastewater treatment plants the major causes of foaming are organic overload, the presence of surface active agents, operational parameters (e.g. digester's shape, mixing system etc) and filamentous microorganisms (e.g. *Gordonia species*, *Microthrix parvicella*) (Ganidi et al., 2009). However, the contribution of specific microorganisms on foam generation in biogas reactors fed with agro-industrial wastes has not been previously investigated. The aim of the present study was to elucidate the microbiology of biogas reactors fed with different substrates prior and after foaming incidents.

The experiment was carried out in three continuous stirred tank reactors (CSTR) denoted as R1, R2 and R3. The total and the working volume of each reactor was 2 and 1.5 L, respectively. Each reactor was continuously stirred using a magnetic stirrer. The operating temperature was maintained at 54 ± 1 °C using thermal jackets. Each reactor was fed with a different mixed substrate, which was found to have an influence on foam formation in our previous study (Kougias et al., 2013). The hydraulic retention time (HRT) of all reactors was kept constant at 15 days. The whole experiment was divided into two periods. During the first period, the reactors were fed only with cattle manure. Once steady state conditions were reached, liquid sample from all reactors was obtained for DNA extraction and metagenomic analysis. After sampling, the feedstock composition of each reactor was changed by the addition of gelatine or Na-Oleate or glucose (second experimental period). As a consequence, foam formation was observed in all reactors approximately after one HRT period. Once the daily volume of the formed foam was steady, samples were taken again for DNA extraction and metagenomic analysis.

Results from the present study revealed significant variations in the microbiology of the manure-based biogas reactors after foam initiation. A number of genera could be linked to foaming as they produce biosurfactants (*Lactobacillus*, *Bacillus*, *Pseudomonas*, *Thermotoga*), others contain mycolic acid in their cell wall (*Thermoactinomyces*, *Pseudonocardia*) or decrease the surface tension of the media (*Micrococcus*, *Streptococcus*). *Frankia*, *Dialister* and *Paenibacillus* are known to be correlated to this phenomenon but their mechanism is still unclear. Finally, microorganisms that have a widely known association with foaming were identified when the identification threshold for the microorganisms was decreased to similar levels reported in the cited literature; however, the latter, due to its high importance, needs to be further investigated

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