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## Real time monitoring of anaerobic digestion microbial communities as a foaming risk prediction method

"This EBNet PoC showed us the power of collaboration with the university and the potential of using multi-omics for understanding the pervasive foaming phenomena in the digester we are operating. While there is still a long way until the industry will be able to make routinely such analyses, we are proud to be one of the first AD companies in the UK who embarked on this path."

Stefan Weitz & Darren Smith, BioConstruct NewEnergy Ltd.

## Aim

The decomposition of the organic wastes generated by humans (estimated to be 105 billion tonnes/year) releases harmful methane and other greenhouse gas (GHG) emissions directly into the atmosphere. Methane is a much more potent GHG than  $CO_2$  and by simply managing the organic wastes more effectively we can cut global GHG emissions.

Anaerobic digestion (AD) is a "ready-to-use" technology which can prevent the methane emissions and generate biogas, bio-fertilisers, and other valuable bio-products. One of the major concerns for the AD companies is foaming, which has a significant impact on process efficiency and operational costs. Foaming is a manifestation of the instability of the AD process, and microorganisms are the main functional body ensuring AD performance. Therefore, foaming mechanisms need to be explored at the microbial community level. Industry is very keen to have a way to predict foaming as it affects productivity, can damage the infrastructure and is dangerous for the operators.

We propose to use microbial community fingerprinting through low-cost metagenomics (to answer, "who is there?") and metaproteomics (to answer, "what are they doing?") to provide data in real time for the risk prediction and control of foaming in AD. For the first time, we are going to apply this method for monitoring a full scale AD treating mixed waste.

## Results

Our samples were collected at the end of October 2021, from two similar reactors which received the same type of feed; one going through a foaming event and one without foaming.

Due to the complexity of the samples DNA extraction proved challenging. Firstly, we used the thirdgeneration sequencing technology provided by MinION (Oxford Nanopore Technologies). This technology generates longer reads and should improve the metagenome assembly. However, due to the challenging DNA extraction, we also sequenced the samples through Illumina, a second-generation sequencing technology which generate shorted reads.

Metagenomic data analysis showed that the microbial communities in the two reactors differ significantly. Some common species between the two plants had significantly higher relative abundance in the foaming reactor. Furthermore, metaproteomics also confirmed the presence of these putative foam-forming bacteria and has also identified numerous flagellin proteins that are markers for filamentous bacteria which are known to cause foam.

Our results are a first step towards using a combined omics approach in studying foaming in industrial reactors treating complex mixed waste.



## FUTURE ACTIVITY:

Work is underway to analyze a second set of samples collected in February 2022. Once completed, the combined results will be formalized in a manuscript (target journal: Journal of Biotechnology). This work is ongoing and soon we will be in a better position to have a full picture of the possible microbial markers for foaming in anaerobic digesters.