

Genome-centric resolution of anaerobic digestion microbiome in biogas reactors fed with Long Chain Fatty Acids

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One of the main inhibitions in biogas digesters is attributed to the accumulation of Long Chain Fatty Acids (LCFA). LCFA are amphiphilic compounds that are produced during the hydrolysis of lipid rich substrates. Several studies showed that LCFA are oxidized via β -oxidation process, which is executed by syntrophic interactions between specific bacteria and hydrogenotrophic archaea (Sousa et al., 2007). The present study elucidated by means of genome-centric metagenomics the microbiome of biogas reactors that is subjected to LCFA exposure. Samples from replicate reactors were obtained prior and after the addition of unsaturated LCFA to the manure-based feedstock. The analysis of the metagenomes provided a deeper insight into the diversity of the microbes that can tolerate LCFA and expanded our understanding on the fatty acid degradation.

The research work was conducted in triplicate thermophilic 1.5 L continuous stirred tank reactors operating at a hydraulic retention time of 15 days. The experiment was divided in two periods. In Period I, the reactors were fed exclusively with cattle manure. Once the reactors were running under steady state conditions, the influent feedstock was supplemented with Na-oleate at concentration 12 g/L-feed (Period II). Total Solids (TS), Volatile Solids (VS) and pH were measured according to standard methods (American Public Health Association (APHA) et al., 2005). Biogas composition and VFA concentrations were determined using gas-chromatography. Genomic DNA was extracted from each reactor at the end of Periods I and II. Genome centric metagenomics and bioinformatic analyses were performed using a previously developed strategy (Campanaro et al., 2016).

At the first operational period (Period I) during which all the reactors were fed exclusively with cattle manure, the average methane production rate reached 279 ± 8 mL/L-reactor/day. The total VFAs were maintained at low levels (below 1 g/L) and the pH was maintained between 8 and 8.4 indicating that the process was well functioning. At the second period (Period II), the addition of Na-Oleate significantly enhanced the methane production rate (892 ± 64 mL/L-reactor.day). Shortly after the addition of LCFA, the concentration of VFA was remarkably increased (approximately from 1 g/L to 4 g/L) and a concomitant slight decrease in pH was measured. This deterioration of the process was temporary as both VFA and pH returned to the initial levels during the steady state conditions.

The metagenomic analysis focuses on the most abundant metagenome assembled genomes (MAGs) and on those whose population remarkably changed in respect to LCFA addition. Therefore, emphasis is given to 19 MAGs out of the total 106 that were reconstructed in the study. The most abundant MAG (Fi01) belonged to *Clostridiales* family. Fi01 was inhibited by the presence of LCFA as its population decreased from 26% to approximately 17%. Nevertheless, it remained the predominant microbe in the community. Based on the KEGG database, Fi01 harbours numerous genes related to “carbon metabolism”, “two-component system” and “ABC transporters”. Based on a previous study, this MAG was assigned to be involved in the oligosaccharide utilisation (Campanaro et al., 2016). Moreover, the absence of genes related to fatty acid degradation indicates that the addition of LCFA did not favour its growth. The most abundant methanogen independently of the substrate composition belonged to *Methanoculleus* sp. (Eu01), indicating that the dominant methanogenic pathway was hydrogenotrophic. A recent study deeply investigating this particular MAG concluded that is a novel methanogen and provisionally named it as *Candidatus Methanoculleus thermohydrogenotrophicum* (Kougias et al., 2017). Moreover, it was found that specific syntrophic bacteria proliferated to accomplish the fatty acid degradation via β -oxidation pathway. Specifically, 3 MAGs affiliated to *Syntrophomonas* genus (Fi08, Fi09 and Fi10) increased in abundance by approximately 5.5 and 400 folds. Analysis of the KEGG pathways showed that these MAGs contain high number of genes encoding enzymes involved in fatty acid degradation and metabolism. Concordantly, a parallel investigation focusing on the metatranscriptome of these biogas reactors verified that Fi09 increased its transcriptional activity after the exposure to the LCFA (Treu et al., 2016).

To sum up, the addition of LCFA in biogas reactors deeply influences the microbiome stimulating the proliferation of specific syntrophic bacteria containing multiple genes related to fatty acid degradation. More specifically, members of *Syntrophomonas* genus increased significantly in abundance, possibly due to different mechanism of interspecies electron transfer. Regarding the archaeal community, it was concluded that the

predominant methanogenic pathway was hydrogenotrophic as supported by the high abundance of *Candidatus Methanoculleus thermohydrogenotrophicus*.

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