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De Francisci, Davide; Kougias, Panagiotis; Treu, Laura; Campanaro, Stefano; Angelidaki, Irini

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||||| **Changes in the microbial profile of biogas reactors due to variations in the feedstock composition**

First author: Postdoc Davide De Francisci, Technical University of Denmark, Environmental Engineering, Miljøvej, Building 113, 2800 Kgs. Lyngby, Denmark

Co-author(s): Panagiotis G., Kougias (Department of Environmental Engineering, Technical University of Denmark)

Laura, Treu (Department of Environmental Engineering, Technical University of Denmark)
Stefano, Campanaro (Department of Biology, University of Padua)

Irini, Angelidakis (Department of Environmental Engineering, Technical University of Denmark)

Anaerobic digestion (AD) is a widely applied methodology for treating different types of wastes, which can be extremely complex and contain several compounds that could inhibit the AD process. Aim of this study is to gain a deeper understanding on how the microbial ecology of biogas reactors responds to radical changes of substrate.

The experimental work was carried out in three continuous stirred tank reactors (CSTR) denoted as R1, R2 and R3, operating under thermophilic conditions ($54 \pm 10^\circ\text{C}$). The hydraulic retention time (HRT) of the reactors was kept to 15 days. During the start-up period all the reactors were inoculated with thermophilic inoculum obtained from Hashøj biogas plant, Denmark. The whole experiment was divided in two periods; during the first experimental period all the reactors were fed with cattle manure, while in the second period the substrate contained cattle manure supplemented with proteins (R1), lipids (R2) and carbohydrates (R3). Samples were taken from each reactor prior to the feedstock change and after a time period of one HRT during which the reactors were fed with the corresponding mixed substrate. DNA was extracted from each sample and the corresponding microbial composition was determined via analyses of 16S amplicons.

Before the substrate change, the microbial composition of the three reactors was found to be extremely similar. The most abundant within the identified genera (relative abundance >1%) were *Methanobrevibacter*, *Megamonas*, *Flectobacillus*, *Bacteroides*, *Clostridium*, *Myroides*, *Flavobacterium* and *Bacillus*. After the substrate modification the profile of the microbial communities substantially changed within the relatively short period of time corresponding to one HRT.

In the reactor supplemented with carbohydrates, the relative abundance of microorganisms belonging to the genera *Methanobrevibacter* and *Bacteroides* decrease 12 and 9 folds, respectively. *Myroides* and *Flavobacterium* also decreased their relative abundance. These decrements are compensated by the enormous increase (69 folds) of *Lactobacillus*. In the reactor supplemented with lipids we again observed an increment of *Methanobrevibacter* and *Bacteroides* (6 and 4 folds, respectively) together with *Myroides* (14 folds) *Flectobacillus* (2 folds) and *Bacillus* (decrease of 2 folds). *Megamonas* is the only genus that increased its relative abundance upon the addition of lipids (increase of 2 folds). In contrast with the aforementioned reactors, the one supplemented with proteins did not undergo substantial changes in its microbial composition. *Methanobrevibacter* is the only genus whose relative abundance varies significantly (decrease of 2 folds).



This simple analysis underlines the dynamicity of microbial communities populating biogas reactors showing that by changing the composition of the main substrate the profile of the microbial community undergoes a profound transformation and is significantly altered already after a relatively short period of time corresponding to one HRT.