

Addition of soluble bio-based substances select for Archaeal communities with higher performances in thermophilic Anaerobic digestion processes

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The biogas production through anaerobic digestion (AD) of the organic fraction of municipal solid waste treatment (OFMSW), has been broadly employed and investigated (Zahedi *et al* 2016). More recently researches were focused on beneficial supplementing of AD with different type of bio-based products like ashes (Shamurad *et al* 2020) or biochar (Pan *et al* 2019, Cimon *et al* 2020) in order to stabilize the process and enhance the biomethane yield. In a previous work compost derived-soluble bioorganic substances (CVD-SBO) were successfully used to decrease the ammonia level in the final digestate produced from OFMSW (Francavilla *et al* 2016) together with an increase of methane yield. In this work we focused our study on the evaluation of the *Bacteria* and *Archaea* microbial community structure and dynamics in a thermophilic anaerobic process treating OFMSW according to the addition of a CVD-SBO at different concentration. The microbial community structures were investigated using PCR-DGGE and 16SrRNA gene sequencing.

Materials and Methods

A lab-scale system running in parallel 6 bioreactors was used for the investigation. 6.5 L bioreactors were fed with fermentation slurry sampled from an industrial plant treating OFMSW. Digestate originating from the same industrial plant was used as inoculum. Duplicate runs were performed for the control slurry mix containing no added CVD-SBO, and for the same mix added at different amounts of CVD-SBO (0.05% and 0.20% respectively), starting the reactors at the same time by heating up to 55°C for 15 days. DNA was extracted from fermented liquors at the beginning and end of the batch experiment. PCR-DGGE was conducted for 16S rRNA genes of *Bacteria* and *Archaea*, respectively. Most prominent bands of both *Bacteria* and *Archaea* were cut and sequenced, and then analysed aided by Mega X software and compared to the GenBank database using BLASTn.

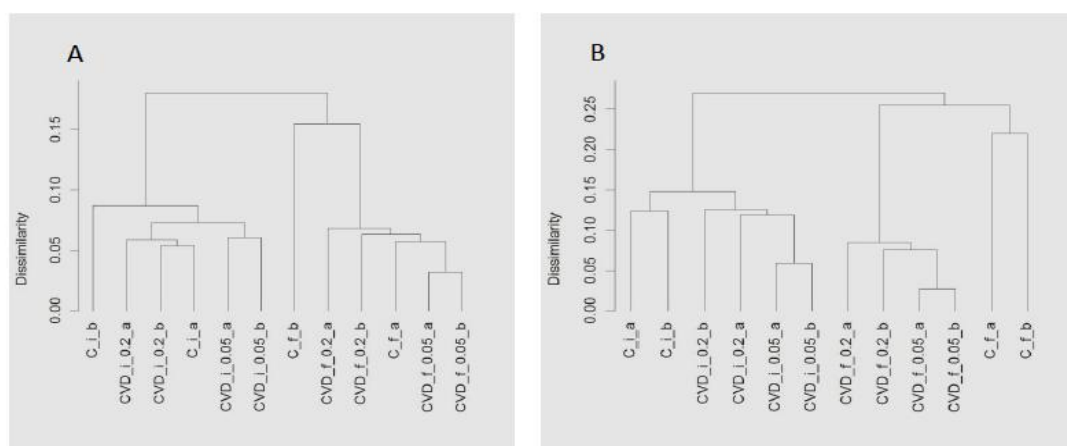


Figure 1. Cluster analysis of the bacterial (A) and archaeal (B) DGGE profiles based on the Bray-Curtis coefficient

Results and Discussion

A slight (+5%) increase in biogas productivity was observed when 0.2% CVD-SBO was added, while methane composition increased from 49 to 53% and CH₄/CO₂ ratio increased from 2.81 to 3.33. Both bacterial and archaeal community structure were highly similar in the batch reactors at the beginning of the reactor operation, but a clear shift occurred with time (Figure 1 A-B). Moreover, for *Bacteria* in both times no clear differentiations were observed between the control and the CVD reactors, while archaeal community clustered separately in the control and in the CVD supplemented reactors at the end of batch process. Generally, under the experimental conditions, archaeal

diversity is more affected, with a reduction of abundance and evenness, consistently with increased CVD concentration. Bacterial community was dominated by *Thermotogales*, thermophilic anaerobic rod shaped-bacteria, and in particular *Deffluviitoga tunisiensis*, originally isolated by mesophilic whey digester which produces acetate, H₂ and CO₂ by glucose degradation. Interestingly, the same affiliation of dominant has been reported by different authors in thermophilic AD (Roske *et al* 2014). The remaining sequenced bacterial DGGE bands shared high similarity with *Syntrophomonas wolfeii*, an acetogenic species able to degrade C4-C8 fatty acids syntrophically with hydrogenoclastic methanogens and frequently found in thermophilic anaerobic reactor (Pap *et al* 2015). The majority of archaeal sequences closely matched with *Methanosarcina mazei*, an acetoclastic methanogen with a dominance in thermophilic and mesophilic Ads (Merlino *et al* 2012) and *Methanoculleus thermophilus*, an hydrogenotrophic methanogen and a potential key species in different thermophilic AD lab scale and real scale plant (Wagner *et al* 2011). Worthy of interest is the increase in the 0.2% CVD-SBO reactors, of the sequences related to *Methanosarcina* spp. since it is considered the most versatile and robust genera of methanogenic archaea, because of the ability to grow under a wide temperature range, and to tolerate high ammonia contents and sudden pH changes (De Vrieze *et al* 2012).

Conclusion

Overall, Archaeal community was restricted to few key species, and diversity decreased following the process completion. Moreover, the effect of CVD on the reduction of archaeal diversity might be related to the higher biogas productivity of the reactor with 0.2% CVD added. The reported data confirm that the structure and dynamic of both bacterial and archaeal communities is mostly correlated with the AD process, while the addition of the CVD at two different concentration shows a measurable effect only for *Archaea*. This can be explained as a positive effect of CVD on the selection of the most active methanogenic taxa, triggering the production of methane. Further studies would allow an evaluation on the impact of N-cycle related microorganisms during the AD process.

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