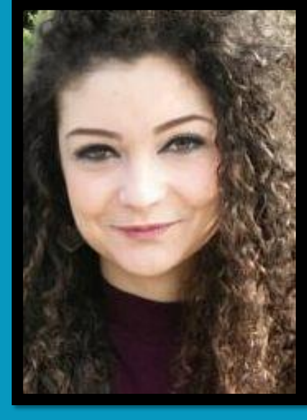

Addition of soluble bio-based substances selected for Archaeal communities with higher performances in thermophilic Anaerobic digestion processes.

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Introduction

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 evaluated 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.

Materials and Methods

A lab-scale system running in parallel six 6.5L batch bioreactors was used for the investigation. Bioreactors were fed with fermentation slurry sampled from an industrial plant treating OFMSW. Duplicate runs were performed for the control slurry mix containing no added CVD-SBO, and for the same mix added at 0.05% and 0.20% of CVD-SBO, starting the reactors at the same time by heating up to 55°C for 15 days. Microbial analyses were conducted by PCR-DGGE targeting 16SrRNA of Bacterial and Archaeal communities and by sequencing most representative DGGE bands.

Results and Discussion

Biogas production increased by 5% when 0.2% CVD was added. Moreover, the methane/CO_2 ratio increased from 2.8 to 3.3. (figures 1-2, table 1). The increase was related to the maximum specific methane production potential (Table 2).

By cluster analysis of PCR-DGGE fingerprinting we found that bacterial communities were not affected, while Archaeal communities clearly clustered according to the addition of CVD to the bioreactor (Figure 3). Generally, under the experimental conditions, archaeal diversity is more affected, with a reduction of abundance and evenness, consistently with increased CVD concentration (table 3).

By analyzing the DNA sequence of the most abundant bacterial and archaeal DGGE bands, we found a general selection of thermophilic bacteria typical of thermophilic AD and a selective effect of CVD towards the increase of acetoclastic *Methanosarcina* and decrease of hydrogenothrophic *Methanoculleus*. (Figure 4)

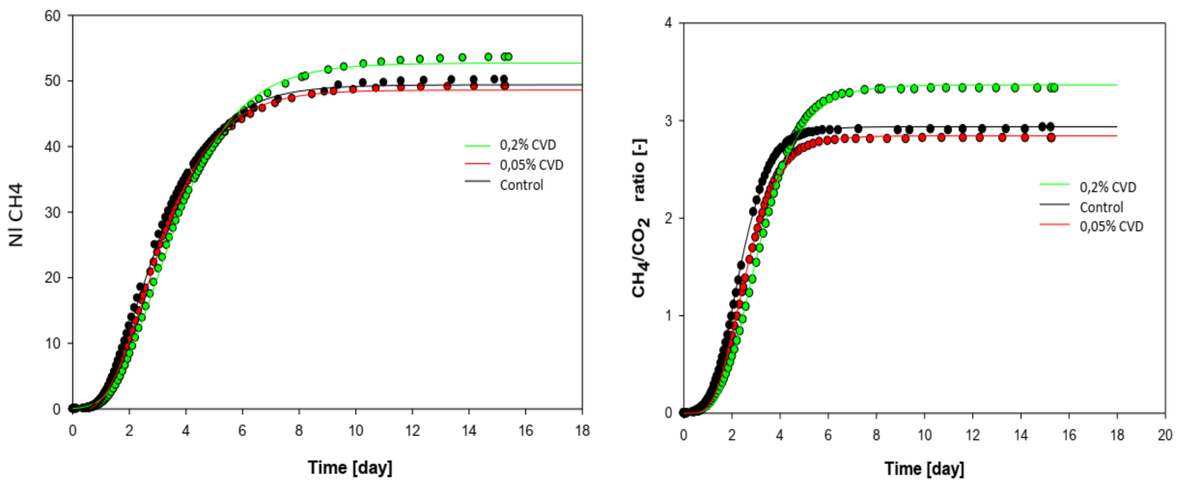

Figure 1. Methane production versus fermentation time.

Figure 2. CH_4/CO_2 evolution during the batch AD experiment.

Treatment	Parameters		
	Biogas (Nl)	CH_4 (Nl)	CH_4/CO_2 ratio
CVD 0.2	73.20±0.50[a]	53.60±0.51[a]	3.33±0,086[a]
CVD 0.05	70.50±0.45[b]	50.20±0.67[b]	2.90±0.075[b]
Control	70.25±0.30[b]	49.17±0.70[b]	2.81±0.072[b]
Significance	*	**	**

Table 1: Analysis of variance (ANOVA) of the main biogas production parameters. Means followed by the same letters in each colon are not significantly different at P ≤0.05 according to Tukey's test. *, F test significant at P<0.05; **, F test significant at P<0.01.

Treatment	Coefficients of the Gompertz equation		
	H	R	L
CVD 0.2	52.72±2.70[a]	1.43±0.03	2.94±0.60
CVD 0.05	48.45±2.45[b]	1.32±0.21	2.59±0.15
Control	49.10±1.10[b]	1.35±0.24	2.47±0.25
Significance	*	ns	ns

Table 2 - ANOVA results related to coefficients of Gompertz equation for CH_4/CO_2 ratio.

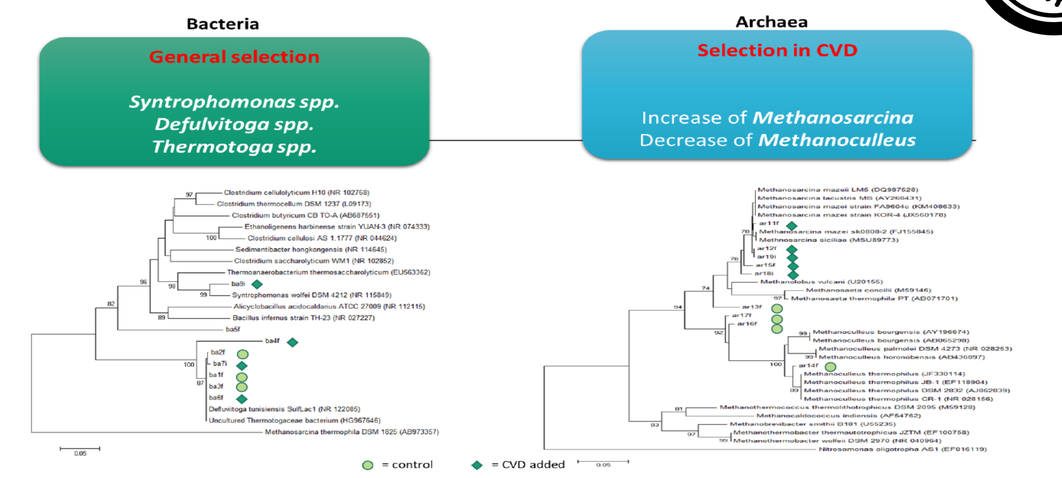

Figure 3. Phylogenetic tree showing the genetic relationships among the bacterial and archaeal sequenced DGGE bands and closely related database sequences. The tree is based on the minimum evolution method, and the bootstrap consensus tree is inferred from 999 replicates.

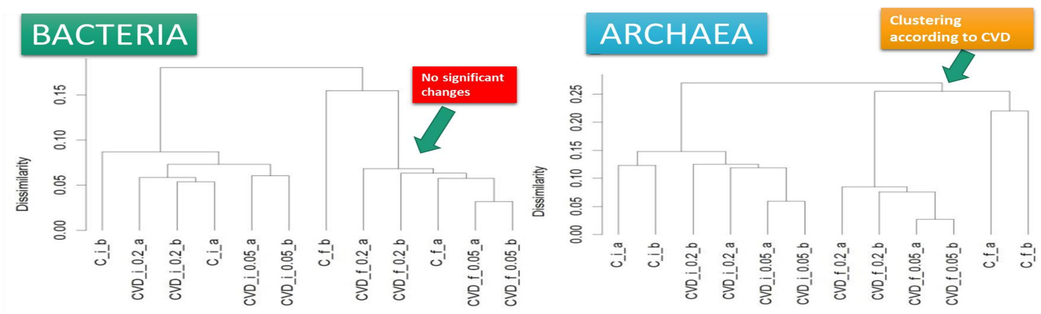

Figure 4. Cluster analysis of PCR-DGGE fingerprint of the bacterial and archaeal communities, based on the Bray-Curtis dissimilarity.

DIVERSITY INDICES		Initial (t_0)			Final (t_f)		
		Richness	Shannon	Pielou's	Richness	Shannon	Pielou's
Bacteria	Control	18 (0)[a]	2.73 (0.006)	0.95 (0.002)	13 (0)	2.29 (0.004)	0.89 (0.002)
	CVD 0.05	19 (0)	2.72 (0.048)	0.92 (0.017)	13 (0)	2.30 (0.004)	0.90 (0.001)
	CVD 0.2	17 (0)	2.68 (0.048)	0.95 (0.017)	13 (0)	2.35 (0.053)	0.92 (0.021)
Archaea	Control	28.5 (0.7)	3.07 (0.047)	0.92 (0.007)	17 (0)	2.59 (0.003)	0.92 (0.001)
	CVD 0.05	20 (0)	2.59 (0.016)	0.87 (0.005)	15 (0)	2.54 (0)	0.94 (0)
	CVD 0.2	20.5 (0.7)	2.63 (0.043)	0.87 (0.024)	14 (0)	2.40 (0.023)	0.91 (0.008)

Table 3. Diversity indices estimated by the bacterial and archaeal DGGE profiles. [a]Average of diversity indexes (standard deviation).

Conclusion

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.
Possible 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 DA process.