Influence of initial methanogenic diversities on the process performance of anaerobic methanation of acetate

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Anaerobic digestion (AD) is an important biomass-energy alteration process for the sustainable treatment of organic wastes and competitive bio-methane production with relatively low energy consumption. Among four, the first three biochemical steps of AD stands for the conversion of complex organic matters into methanogenic substrates, such as acetate, H2, and CO2 by the fermentative microorganisms, to produce methane as the final product by methanogens. Methanogens are slow growers and more susceptible to environmental parameters than fermenting groups; therefore, understanding methanogenic community dynamics has been considered as a crucial factor to aid in the effective monitoring and prediction of AD operations. The digester environment, mainly the volatile fatty acids (VFAs) concentrations, such as acetate navigates the composition and organization of the methanogenic community. The involvement of most of the AD steps, such as acidogenesis, acetogenesis, and methanogenesis steps in the production and degradation of acetate reinforced its detailed investigation in the AD system.

This study aims to evaluate the methanogenic dynamics when different initial communities are fed with the same concentration of acetate and to evaluate the absolute quantification of dominant archaeal populations to describe process performances reliably. To this end, eight different seeds from different sources treating various types of substrates (i.e., food waste, sludge, and co-digestion of both) were used for batch tests with acetate as the sole carbon source. The key acetate degraders were identified by the Ion Torrent PGM system through 16S rRNA gene sequencing and absolute cell densities were estimated from Quantitative polymerase chain reaction (QPCR) analysis by using order-specific primer and probe sets.

Methanomicrobiales (MMB), *Methanosarcinales* (MSL), and *Methanobacteriales* (MBT) were dominant archaeal orders among all the seeds at the end of the experiment. Seeds with a decrease in the relative abundance of MBT experienced the longest lag phase during batch operation. The maximum acetate degradation rate and methane production rate achieved seed used both acetoclastic and syntrophic acetate oxidation pathways in acetate degradation. *Methanosaeta*, *Methanoculleus*, and *Methanolinea* were the dominant methanogenic genus among all the seeds in this experiment.

According to the next generation sequencing (NGS) results, the best performing seed showed no change in dominant order MSL in terms of relative abundance, which cannot stand to explain process performance. Where the QPCR result showed a higher archaeal copy number for that seed. Hence, it was evident from this study that, the change in relative abundance does not necessarily relate to the process performance, where absolute quantification from QPCR is necessary to explain methanogenic dynamics. These results indicate that both relative and absolute abundances should be considered for a comprehensive biological interpretation of the AD system.

Table 1. Kinetic parameters of different seeds in batch reactions

Parameters (unit)		Seed sources[a]							
		Fa	Fb	Fc	Ca	Cb	Cc	Wa	Wb
Degradation rate (d -1)	k	0.19	0.35	0.17	0.47	0.20	0.66	0.33	0.49
	sd	0.01	0.02	0.03	0.02	0.01	0.04	0.01	0.00
Methane prod. Rate (mL/d)	Pmax	0.06	0.07	0.07	0.15	0.06	0.17	0.07	0.12
	sd	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.00
Lag phase (d)	λ	9.50	2.80	12.80	6.50	2.90	4.70	10.20	4.60
	sd	0.20	0.07	0.11	0.10	0.05	0.10	0.00	0.10

[a]Fa – Seed 1; Fb – Seed 2; Fc – Seed 3; Ca – Seed 4; Cb – Seed 5; Cc – Seed 6; Wa – Seed 7; Wb - Seed 8

Figure 1. Archaeal community structures at the order level. (a) Initial seeds; (b) Endpoints of the reactions. [Fa – Seed 1; Fb – Seed 2; Fc – Seed 3; Ca – Seed 4; Cb – Seed 5; Cc – Seed 6; Wa – Seed 7; Wb - Seed 8]

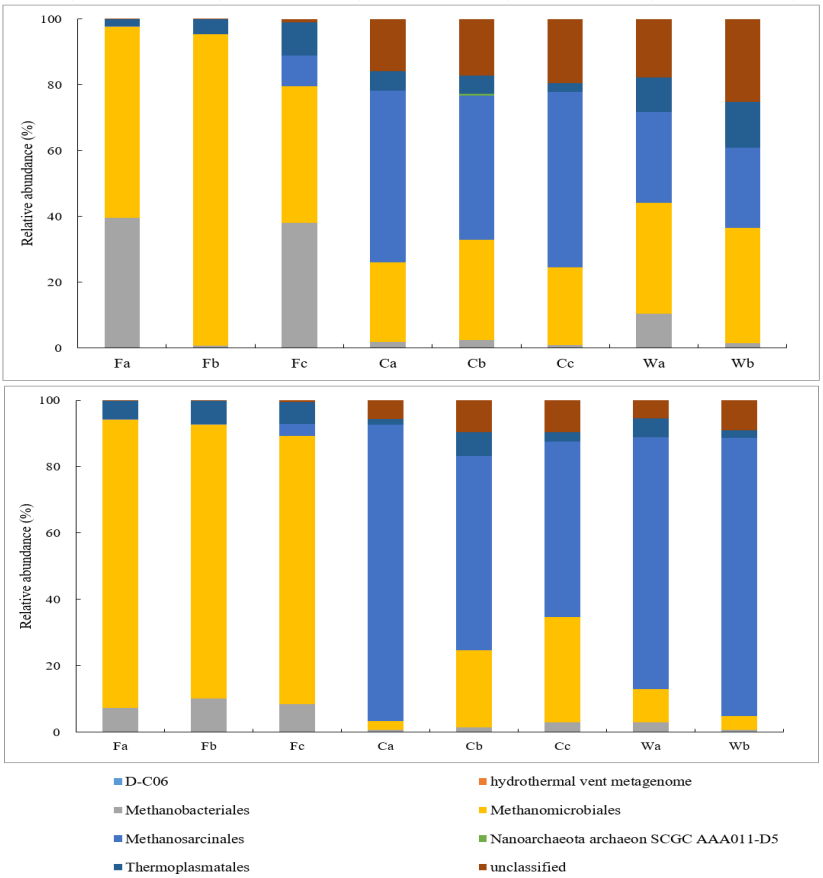

Figure 2. Heatmap of the absolute abundance (copies/mL) of total archaea (ARC) and three dominant orders (MBT, MMB, and MSL) in different seeds. [Initial – start points of the batch; Mid – midpoints of the batch; End – endpoints of the batch; Fa – Seed 1; Fb – Seed 2; Fc – Seed 3; Ca – Seed 4; Cb – Seed 5; Cc – Seed 6; Wa – Seed 7; Wb - Seed

Sites	qPCR results - Total ARC		
	Initial	Mid	End
Fa	2.16E+06	5.15E+06	7.34E+06
Fb	1.24E+06	5.61E+06	2.09E+06
Fc	4.86E+05	2.01E+06	1.52E+07
Ca	7.47E+05	4.40E+06	6.04E+06
Cb	3.41E+06	5.23E+06	6.07E+06
Cc	4.47E+06	7.94E+06	1.36E+07
Wa	9.28E+05	5.08E+06	4.55E+06
Wb	1.51E+06	5.46E+06	6.02E+06

Order	qPCR results - Order specific		
	Initial	Mid	End
MBT	2.18E+04	1.49E+04	3.66E+04
MMB	8.81E+05	2.76E+06	1.62E+06
MSL	1.45E+04	1.41E+04	3.95E+06
MBT	1.24E+03	2.84E+03	2.95E+03
MMB	8.00E+05	4.20E+06	1.97E+06
MSL	0.00E+00	0.00E+00	0.00E+00
MBT	8.23E+03	1.06E+04	1.72E+05
MMB	2.12E+05	1.64E+06	1.24E+07
MSL	1.81E+05	6.25E+04	8.92E+05
MBT	4.67E+03	4.70E+03	5.25E+03
MMB	4.21E+04	1.54E+05	1.62E+05
MSL	6.85E+05	2.52E+06	4.66E+06
MBT	1.17E+04	6.26E+03	5.63E+03
MMB	9.99E+05	1.18E+06	2.37E+06
MSL	2.12E+06	2.34E+06	2.89E+06
MBT	1.09E+04	6.12E+03	7.02E+03
MMB	1.33E+06	2.70E+06	4.93E+06
MSL	5.55E+05	9.59E+05	1.58E+06
MBT	1.00E+04	7.51E+03	6.81E+03
MMB	1.15E+05	3.80E+05	3.89E+05
MSL	4.40E+05	3.37E+06	3.56E+06
MBT	2.51E+03	5.03E+03	8.81E+03
MMB	1.39E+05	2.36E+05	4.51E+05
MSL	1.01E+06	3.14E+06	4.26E+06