Anaerobic digestion of SS-OFMSW: Impact of seed on methanogenic abundance and digester performance

S. Ghanimeh[1,2], M. El-Fadel[1*] and P. E. Saikaly[3]

[1] Department of Civil and Environmental Engineering, American University of Beirut, Lebanon
[*] Corresponding author: Email: *mfadel@aub.edu.lb*; Fax: 9611744462
[2] Currently at the Department of Civil and Environmental Engineering, Notre Dame University – Louaize, Lebanon (E-mail: *sghanimeh@ndu.edu.lb)*
[3] Water Desalination and Reuse Research Center, King Abdullah University of Science and Technology (KAUST), Thuwal 23955-6900, Saudi Arabia (E-mail: *Pascal.Saikaly@kaust.edu.sa*)

Abstract
This paper examines the impact of the seed source on methanogenic abundance and overall performance of thermophilic anaerobic digesters treating the source-sorted organic fraction of municipal solid waste (SS-OFMSW). Two digesters were started up with different seed mixes and operated for 250 days until steady-state was reached. Quantitative Polymerase Chain Reaction (Q-PCR) was used to determine the total number of *Bacteria* and the three main methanogenic orders: *Methanobacteriales*, *Methanomicrobiales* and *Methanosarcinales* (*Methanosarcinaceae and Methanosaetaceae*). Digester A was inoculated with a mix of digested waste and cattle manure, both of which are predominated by the acetotrophic methanogens *M.sarcinaceae* (95% and 85%, respectively), along with waste activated sludge. The latter presents equal abundance of hydrogenotrophic *M.microbiales* (50%) and acetotrophic *M.saetaceae* (46%) both of which are absent in the other 2 seeds. Digester B was seeded with the same mix as digester A with the addition of landfill leachate (94% *M.microbiales*) and municipal waste compost (41% *M.sarcinaceae*, 37% *M. saearaceae* and 22% *M.bacteriales*). The addition of compost and leachate induced an increase in the number of predominant orders (*M.sarcinaceae* and *M.bacteriales*) as well as minor ones (*M. sarcinaceae* and *M. microbiales*). With time, this impact was attenuated upon reaching steady-state with no significant differences in methane generation and biogas composition. Accordingly, the initial seed seems to have minor impact on the ultimate methanogenic composition and abundance, with environmental conditions (such as type of feed, temperature, mixing scheme, feeding mode and frequency) being the determinant factors. Ultimately, the digester with added compost and leachate exhibited lower VFAs (55%) and COD (27%) levels at steady-state which can be attributed to potential differences in composition (and function) of the bacterial flora.

Keywords
Thermophilic anaerobic digestion; seed source; methanogens

INTRODUCTION

To date, dissemination of thermophilic (55-60^{o}C) anaerobic digestion (AD) is hindered by operational difficulties and instability problems invariably associated with poor startup and lack of acclimated inocula. Thermophilic seeds are often not accessible, mainly in developing countries and at times in rural areas where the number of thermophilic digesters is limited, necessitating the use of mesophilic anaerobic inocula and resulting in long startup periods (Suwannoppadol et al., 2011; Fdéz-Güelfo et al., 2010). Thus, other readily available seeds have been suggested to aid in the startup of anaerobic digesters including, but not limited to, waste activated sludge (WAS), raw manure, communal compost and landfill leachate (El-Fadel et al., 2012). Such alternatives have been mostly considered for mesophilic applications with limited attempts to startup thermophilic digesters with seeds not originating from an AD system.

In this context, it has been shown that thermophilic methanogens (namely *Methanothermobacter* spp. and *Methanosarcina thermophila* spp.) are able to survive in communal biowaste compost (Thummes et al., 2007). Also, Neumann and Scherer (2011) reported that the addition of compost,

in mesophilic digesters treating fodder beet silage, can cause a shift in the methanogenic community towards hydrogenotrophic populations. Similarly, it has been shown that methanogens constitute 2% of the total microbial community in landfill leachate and that dominant clones in landfills are close relatives to thermophilic hydrogenotrophic methanogens (Huang et al., 2003; Chen et al., 2003).

Based on the above, this study investigates the impact of the seeding source on the ultimate methanogenic composition and overall digester performance using 2 inocula mixes. The mix in digester A consisted of digested waste, WAS and manure. The mix in digester B consisted of the same mix with the addition of leachate and compost.

MATERIALS AND METHODS

Operating conditions

The digesters were operated at 55^{o}C and were continuously mixed at 80rpm. The digesters were fed 3 times per week with SS-OFMSW collected from the university cafeteria and nearby food markets. During start-up, the organic loading rate (OLR) was increased stepwise to reach 2.5 gVS/l/d at a hydraulic retention time (HRT) of 30 days. The start-up was achieved in 134 days, after which the loading rate was maintained at 2.5 gVS/l/d for 116 days (~ 4HRT) to reach steady-state.

Analytical methods

The temperature and pH inside the digesters were monitored online via submerged probes. Biogas generation and composition were measured daily using a dual wavelength infrared cell with reference channels. The digesters liquor was tested for total, volatile and suspended solids content as well as COD, ammonia and alkalinity levels following the Standard Methods procedures. Major Volatile Fatty Acids (VFAs), namely acetate, propionate and butyrate, were measured using gas chromatography.

Quantitative Polymerase Chain Reaction (Q-PCR)

To quantify the abundance of various methanogenic orders (*Methanobacteriales*, *Methanomicrobiales* and *Methanosarcinales*) real-time PCR was performed in a 25-µL reaction volume containing 12.5 µL of 2 × iQ Supermix) (Bio-Rad Laboratories, Hercules, CA), 0.5 µM of each primer, 0.2 µM of each probe, 1 µl sample DNA, and RNase-free sterile water to a final volume of 25 µL. Amplification was performed using the CFX96 real-time PCR detection system (Bio-Rad Laboratories, Hercules, CA) with PCR conditions described by Yu et al. (2005). Primers, probes and plasmid standards used in this study are similar to those in Yu et al. (2005). Q-PCR assays with Ct values over 40 were considered negative. For each PCR run, a negative (no template) control was used to test for false positives or contamination.

RESULTS AND DISCUSSION

The results show that manure and digested waste were predominated with acetotrophic *M.sarcnia* (85% and 95%, respectively). In comparison, leachate was predominated by hydrogenotrophic *M.microbiales* (94%) which lacks in other seeds, except for a relatively low abundance in WAS (30 times lower number of DNA copies per unit volume). Also, compost exhibited a similar order of abundance of the remaining 3 methanogenic orders: *M.sarcinaceae* (41%), *M.saearaceae* (37%) and *M.bacteriales* (22%) (Table 1).

Table 1. Methanogenic composition of the different seeds.

	Hydrogenotrophs (%)		Acetotrophs (%)	
	M.microbiales	*M.bacteriales*	*M.sarcinaceae*	*M.saetaceae*
Digested waste	0%	5%	**95%**	0%
Manure	0%	15%	**85%**	0%
WAS	**50%**	3%	2%	**46%**
Compost	0%	22%	41%	37%
Leachate	**94%**	0%	6%	0%

At the end of the seeding process, the concentrations of predominant methanogens were higher in digester B: *M.sarcina* was higher by 5 orders of magnitude and *M.bacteriales* was 3 times higher than in digester A (Figure 1.a). The abundance of minor orders (*M.microbiales* and *M.saetaceae*) was also doubled (Figure 1.b).

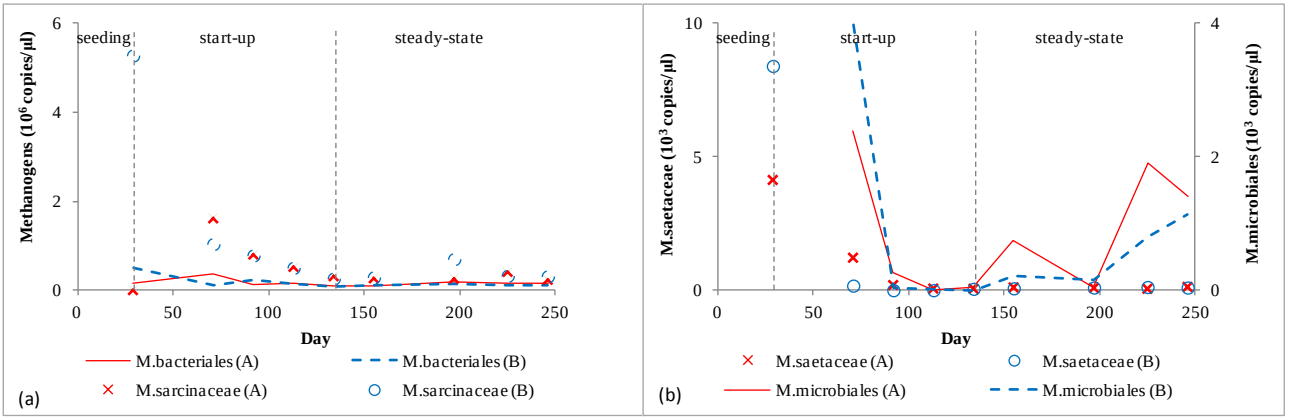

Figure 1. Abundance of methanogenic orders

Nevertheless, upon initiation of incremental loading, the abundance of methanogens (both hydrogenotrophs and acetotrophs) increased in A and decreased in B. By the end of the steady-state period, the absolute abundance of the various orders was comparable in A and B (Figure 1). Both digesters were dominated by the acetotrophic class *M.sarcinaceae* (66% in A, 76% in B) and the hydrogenotrophic order *M.bacteriales* (34% in A, 24% in B).

Thus, the impact of the initial seed on the final methanogenic composition was not discernible. Similarly, biogas generation and composition was identical in both digesters (Figure 2). Accordingly, it can be postulated that identical type of feed and operating conditions (including temperature, feeding mode and pattern, mixing scheme and sampling method) in the digesters resulted ultimately in closely similar methanogenic composition and abundance.

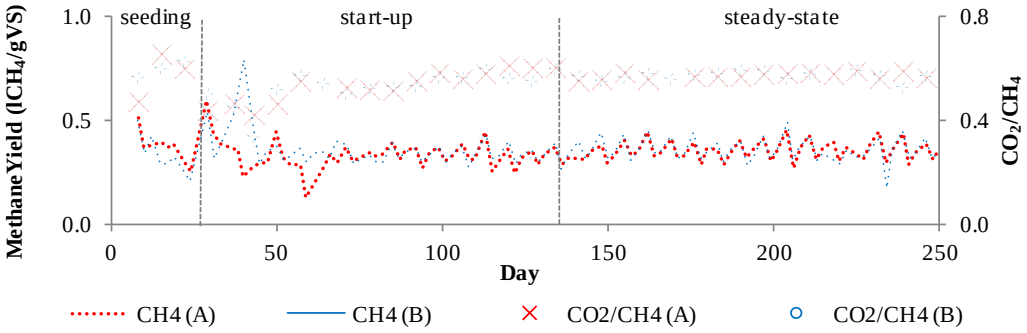

Figure 2. Methane generation and gas composition

Yet, the digesters exhibited a different behaviour for VFA degradation and COD removal, with digester B exhibiting lower VFA and COD levels (Figure 3). Even though the total number of bacteria at the end of the steady-state period was similar in both digesters (2 x 10^7 copies/µl), the difference in behaviour could be attributed to a difference in bacterial community composition. This is mostly expected when non-acclimated seeds are used. In fact, a lack of major propionate oxidizing bacteria in a manure inoculum sample was recorded (Ghanimeh, 2012). In such cases, propionate degradation can be reduced significantly, resulting in VFAs accumulation and subsequent instability problems. Pyrosequencing analysis of the domain *Bacteria* and *Archaea* has been initiated to ascertain these results.

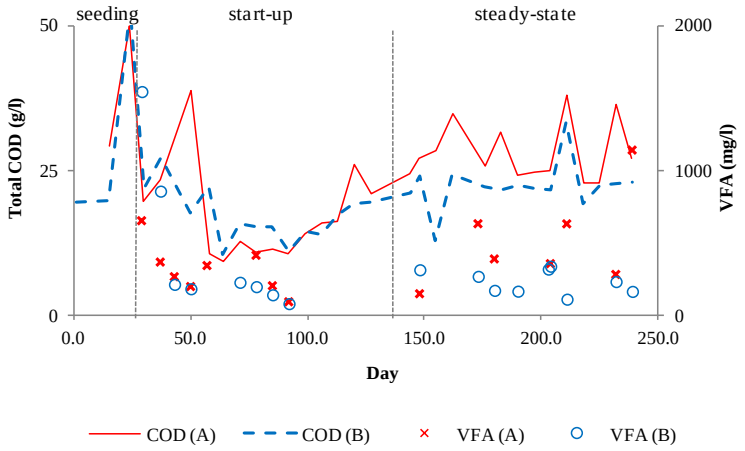

Figure 3. COD and VFA concentration

ACKNOWLEDGEMENTS

This work was supported by the National Council for Scientific Research, Lebanon, the Masri Institute of Energy and Natural Resources at the American University of Beirut, the US Agency for International Development. Special thanks are extended to the Bioscience Core Laboratory at KAUST.

REFERENCES

Chen, A.C., Imachi, H., Sekiguchi, Y., Ohashi, A., Harada, H. 2003. Archeal community compositions at different depths (up to 30m) of a municipal solid waste landfill in Taiwan as revealed by 16S rDNA cloning analyses. *Biotechnology Letters* 25, 719-724.

El-Fadel, M., Saikaly, P., Ghanimeh, S. 2012. Startup and stability of thermophilic anaerobic digestion of OFMSW. *Critical Reviews in Environmental Science and Technology* (in press).

Fdéz-Güelfo, L.A., Álvarez-Gallego, C., Sales Márquez, D., Romero García, L.I. 2010. Start-up of thermophilic-dry anaerobic digestion of OFMSW using adapted modified SEBAC inoculums. *Bioresource Technology* 101, 9031-9039.

Ghanimeh, S. 2012. Startup and stability of thermophilic anaerobic digesters treating organic waste in the absence of an acclimated seed. PhD Dissertation. American University of Beirut, Lebanon.

Huang, L.N., Zhu, S., Zhou, H., Qu, L.H. 2003. Characterization of methanogenic Archaea in the leachate of a closed municipal solid waste landfill. *FEMS Microbiology Letters* 46, 171-177.

Neumann, L., Scherer, P. 2011. Impact of bioaugmentation by compost on the performance and ecology of an anaerobic digester fed with energy crops. Bioresource Technology 102, 2931-2935.

Suwannoppadol, S., Ho, G., Cord-Ruwisch, R. 2011. Rapid start-up of thermophilic anaerobic digestion with the turf fraction of MSW as inoculums. *Bioresource Technology* 102, 7762-7767.

Thummes, K., Schafer, J., Kampfer, P., Jäckel, U. 2007. Thermophilic methanogenic Archea in compost material: Occurrence, persistence and possible mechanisms for their distribution to other environments. *Systematic and Applied Microbiology*. 30, 634-643.

Yu, Y., Lee, C., Kim, J., Hwang, S. 2005. Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction. Biotechnology & Bioengineering 89, 670-679.