Characterization of Microbial Community during Anaerobic Digestion of the Organic Fraction of Municipal Solid Waste in Bioreactor Landfill Simulators

X. Fei*, D. Zekkos*, S. Tibbetts* and L. Raskin*

* Department of Civil and Environmental Engineering, University of Michigan, 1351 Beal Ave., Ann Arbor, Michigan, USA
(E-mail: *xcfei@umich.edu, zekkos@geoengineer.org, sktibb01@gmail.com, raskin@umich.edu*)

Abstract
Anaerobic digestion (AD) of the organic fraction of municipal solid waste (OFMSW) in bioreactor landfills was investigated with parallel large-scale (40-L) simulators operated for 250 days. Concentrations and generation rates of methane (CH_4) and carbon dioxide (CO_2) were measured over the period, as well as concentrations of volatile fatty acids (VFAs) and pH in leachate. Multiple solid waste and leachate samples were processed for DNA extraction, Polymerase Chain Reaction (PCR) and pyrosequencing of bacterial and arachaeal 16S rRNA genes for microbial community characterization. The biogas and leachate characteristics changed with time as expected based on typical AD studies with other waste streams. DNA concentrations in the leachate were used to follow changes in biomass levels and they correlated well with CH_4 generation rates, suggesting that biomass in the leachate can be used to monitor AD processes in bioreactor landfills, which are difficult to sample due to accessibility problems and large spatial heterogeneity. Microbial community characteristics (to be determined with pyrosequencing data) are anticipated to shift between different phases (transition, acid formation and methanogenesis), as delineated by distinct changes of biogas and leachate qualities.

Keywords
municipal solid waste; bioreactor landfill; microbial community; pyrosequencing

INTRODUCTION

The United States (US) and the European Union (EU) each generate over 200 million tons of municipal solid waste (MSW) each year (EPA 2011; EuroStat 2011). Final disposal methods vary widely between states in the US and countries in the EU due to different regulations, economic development levels and land usage restrictions. In the US, over 50% of MSW goes to landfills, while the corresponding number in the EU is around 40%. Anaerobic digestion (AD) of OFMSW reduces the waste volume before final disposal of residue and generates CH_4 as an energy source. Traditionally, AD of the organic fraction of MSW (OFMSW) has been carried out in anaerobic digesters similar to those operated for the AD of municipal sewage sludge and with a variety of different designs (Mata-Alvarez *et al.* 2000). Alternatively, AD of OFMSW can be realised in-situ in bioreactor landfills. In such systems, methane extraction and leachate recirculation are implemented with vertical wells and pipeline systems. Volume reduction is achieved, which increases landfilling capacity and the lifespan of a landfill (Reinhart & Townsend 1998; Kim & Pohland 2003). New landfills can be designed to operate as bioreactor landfills, which are gaining popularity in the US (Benson *et al.* 2007). In addition, existing landfills may be retrofitted to operate as bioreactor landfills, which are more often the case in the EU (Berge *et al.* 2009).

A significant amount of work has been done on the treatment of OFMSW in anaerobic digesters, including influent characterization (Zaher *et al.* 2009), steady state and dynamic performance evaluation (Stroot *et al.* 2001), and the characterization of microbial communities (McMahon *et al.* 2004). In contrast, only a few researchers have studied the overall performance (Kim & Pohland 2003; Barlaz *et al.* 2010) and the microbial communities (Staley *et al.* 2011) of bioreactor landfills, either in full- or laboratory-scale systems. Available data on full-scale bioreactor landfill are scarce and sometimes inconsistent due to waste heterogeneity, as well as difficult to measure parameters and uncontrollable factors in the field. The OFMSW specimens used in laboratory-scale studies are often shredded and/or suspended in water, so that any heterogeneity is removed. As a result, the results from such laboratory-scale studies may not be representative of full-scale conditions. The current study adopted large-scale (0.3 m-diameter, 0.6-m height, and 40-L volume) and heavily-instrumented laboratory-scale simulators capable of containing real-size waste particles from the field to simulate in-situ heterogeneity, and enabled evaluation of field-scale variables during the process. Biogas, leachate, and microbial communities were characterized during long-term operation of the simulators (Fei *et al.* 2013).

MATERIALS & METHODS

Municipal solid waste was excavated from a landfill in Austin, Texas and shipped to the laboratory in air-tight 55-gallon drums. Waste composition was characterized according to Zekkos *et al.* (2010). The organic fraction of MSW was the sum of paper, cardboard and food wastes. Two waste specimens were re-constituted using actual waste representing the overall by weight composition of the sampled waste: 15.0% paper, 5.5% soft plastic, 5.0% wood, and 74.5% soil-size particles (passing 20-mm sieve). Each specimen had a total weight of 30.5 kg and moisture content of 21% (kg-moisture/kg-dry MSW). The specimens were placed into two identical simulators (#2 and #3) (Fig. 1). The simulators were monitored for 250 days. On day 10 (10 days after the MSW specimen was prepared), drainage valves were closed and deionized water was added into the simulators. After the specimen was submerged and saturated with leachate for 30 minutes, leachate was drained. Leachate was stored in a leachate tank and recirculated three times a week. Both simulators were operated at a temperature of 40 ±3°C with heating blankets.

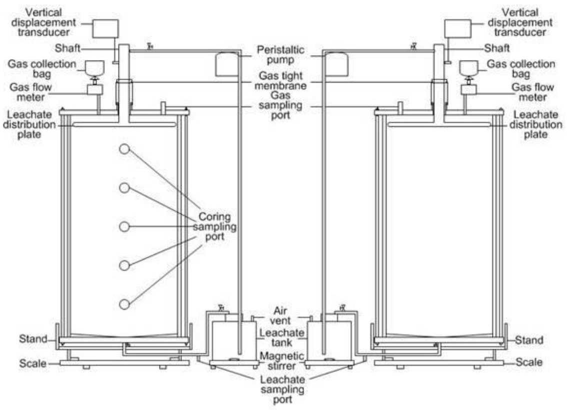

Figure 1. Schematic of simulators #2 (right) and #3 (left).

Biogas and leachate were sampled and analysed according to Fei *et al.* (2013). Gas generation rates were calculated by multiplying concentrations of CH_4 (r_{CH4}) and CO_2 (r_{CO2}) to the volume of the generated biogas, adjusted for a temperature of 20 °C.

DNA extraction method applied for both solid waste (day 47, 82, 111 and 179) and leachate samples (day 23, 34, 46, 83, 109 and 178) was modified from Zhou et al. (1996). Preparation for pyrosequencing, pyrosequencing, and data analyses were identical to Pinto et al. (2012) except that PCR forward primers used to target the 16S rRNA genes of *Bacteria* was Bact-563F and of *Archaea* was Arch-519F.

RESULTS & DISCUSSION

Biogas generation

Methane generation rates (r_{CH4}, L-CH_4/day) are shown in Fig. 2a. Good reproducibility was observed between the simulators that had the same waste composition. After day 10, when recirculation of leachate was initiated, the r_{CH4} increased rapidly. A maximum r_{CH4} of 10.5 L-CH_4/day was observed on day 33 for both simulators. The r_{CH4} dropped considerably between day 33 and day 100 and decreased more slowly afterwards. The r_{CH4} remained below 1 L-CH_4/day after day 150, indicating the completion of AD of most of the OFMSW in the simulators. A total of approximately 450 L CH_4 was generated in each simulator, which corresponds to 17.9 L-CH_4/kg-dry MSW, 97.8 L-CH_4/kg-paper, or 58.0 L-CH_4/kg-volatile solids (consisted of biodegradable paper and non-biodegradable soft plastics and wood). The last number fell within the reported range between 50 and 200 L-CH_4/kg-volatile solids (Eleazer *et al.* 1997).

Carbon dioxide generation rates were calculated and the ratios between the r_{CH4} and the r_{CO2} (r_{CH4}:r_{CO2}, L/day:L/day) are plotted in Fig. 2b. The ratio increased sharply after day 10 and reached a maximum value of approximately 1.5 on day 33. The ratio decreased slightly and fluctuated around 1.2 after day 49. The stable ratio suggested that methanogenesis was fully established in both simulators and that functionally stable microbial communities had been formed.

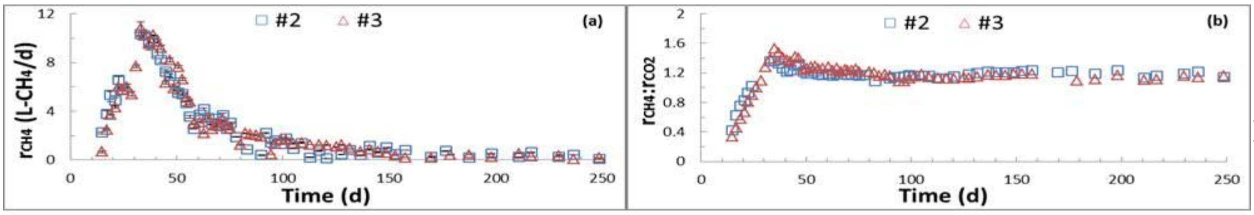

Figure 2. Methane generation rate (a) and ratio between r_{CH4} and r_{CO2} (b) for simulators #2 (blue) and #3 (red).

Leachate quality

Leachate drained after recirculation was analysed. Concentrations of VFAs in leachate samples are shown in Fig. 3a. Propionate concentrations increased starting on day 10 until day 25-30. Peak propionate concentrations were 2,032 and 2,795 mg/L for simulators #2 and #3, respectively. Propionate was completely consumed within the next 15 days. Acetate accumulated to a lesser extent and was also removed between days 20 and 46. The concentrations of formate, butyrate, and valerate were low compared to those of propionate and acetate (data not shown). The dynamics of VFA concentrations clearly delineated the transition phase, acid formation phase, and the methanogenesis phase (Barlaz *et al.* 1989; Kim & Pohland 2003; Fei & Zekkos 2012). Production and consumption of VFAs also correspond well with r_{CH4} trend. The leachate pH values for simulators #2 and #3 are shown in Fig. 3b. The leachates were slightly acidic in the transition and acid formation phases, but the pH environment was close to neutrality during stable methanogenesis. No pH adjustment was performed.

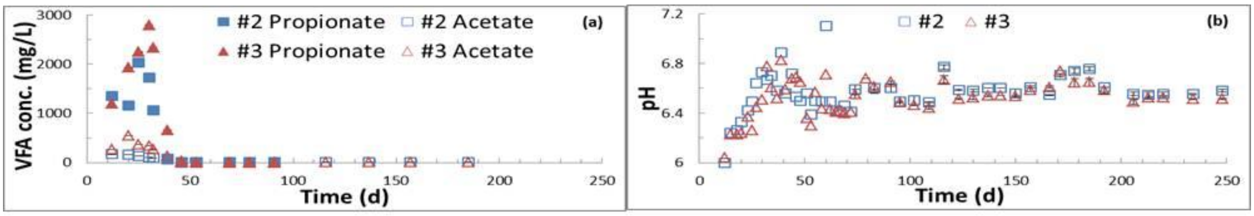

Figure 3. Concentrations of VFAs (a) and pH (b) of leachate collected from simulators #2 (blue) and #3 (red).

The data presented in Figures 2 and 3 indicate that AD of OFMSW in a bioreactor landfill resembles a typical AD process, which proceeds from disintegration and hydrolysis of organic particulates to acidogenesis and methanogenesis processes (Batstone *et al.* 2002). Both r_{CH4} and VFAs concentrations in the leachate were shown to be indicative of simulators' performances.

Analyses of microbial communities on solid waste and in leachate

Biomass concentrations in leachate represented by the amount of DNA extracted per volume of leachate (ng-DNA/ml-leachate) are plotted in Fig. 4a. The highest biomass concentration concurred with peak r_{CH4} and VFA concentrations. The biomass concentration subsequently decreased, and roughly 10% of the maximum biomass concentration remained on day 179. The biomass concentration in the leachate correlated well with the r_{CH4}, as depicted in Fig. 4b. The biomass concentration data collected so far suggest that sampling the leachate will be informative to characterize the AD process in bioreactor landfills. This is an important finding as it is very difficult to obtain representative MSW and biomass samples from full-scale landfills, whereas leachate samples are much easier to collect.

Biomass/DNA concentrations in MSW samples collected from the simulators and pyrosequencing data will be obtained in the near future (before June 2013). These results will further indicate if the leachate can be used to represent AD processes in bioreactor landfills. It will be determined if bacterial and archaeal population dynamics will agree with the patterns observed in semi-batch AD systems treating OFMSW (McMahon *et al.* 2004) and mathematical modelling results of OFMSW biodegradation in landfills (Vavilin & Angelidaki 2005).

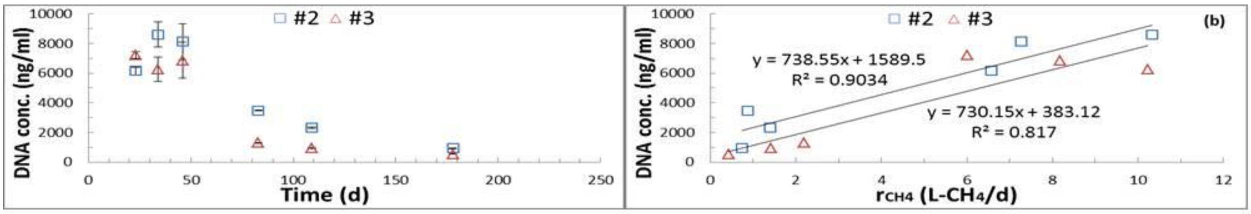

Figure 4. Biomass concentrations in leachate of simulators #2 (blue) and #3 (red) (a) and correlations with r_{CH4} (b).

ACKNOWLEDGEMENT

We would like to thank Adam Smith and Tara Clancy for their suggestions on analyses methods, and Andhika Sahadewa for help with characterizing MSW and preparing the specimens.

REFERENCES

Barlaz M. A., Bareither C. A., Hossain A., Saquing J., Mezzari I., Benson C. H., Tolaymat T. M. and Yazdani R. 2010 Performance of North American Bioreactor Landfills. II: Chemical and Biological Characteristics. *Journal of Environmental Engineering-Asce* **136**(8), 839-53.

Barlaz M. A., Schaefer D. M. and Ham R. K. 1989 Bacterial population development and chemical characteristics of refuse decomposition in a simulated sanitary landfill. *Applied and Environmental Microbiology* **55**(1), 55-65.

Batstone D. J., Keller J., Angelidaki I., Kalyuzhnyi S. V., Pavlostathis S. G., Rozzi A., Sanders W. T. M., Siegrist H. and Vavilin V. A. 2002 The IWA Anaerobic Digestion Model No 1 (ADM1). *Water Science and Technology* **45**(10), 65-73.

Benson C. H., Barlaz M. A., Lane D. T. and Rawe J. M. 2007 Practice review of five bioreactor/recirculation landfills. *Waste Management* **27**(1), 13-29.

Berge N. D., Reinhart D. R. and Batarseh E. S. 2009 An assessment of bioreactor landfill costs and benefits. *Waste Management* **29**(5), 1558-67.

Eleazer W. E., Odle W. S., Wang Y. S. and Barlaz M. A. 1997 Biodegradability of municipal solid waste components in laboratory-scale landfills. *Environmental Science & Technology* **31**(3), 911-7.

EPA 2011 Municipal Solid Waste Generation, Recycling, and Disposal in the United States: Facts and Figures for 2010. http://www.epa.gov/osw/nonhaz/municipal/msw99.htm.

EuroStat 2011 Municipal waste statistics. http://epp.eurostat.ec.europa.eu/statistics_explained/index.php/Municipal_waste_statistics.

Fei X. and Zekkos D. 2012 Factors Influencing Long-Term Settlement of Municipal Solid Waste in Laboratory Bioreactor Landfill Simulators. *Journal of Hazardous, Toxic, and Radioactive Waste* **published online ahead of printing**.

Fei X., Zekkos D. and Raskin L. 2013 Joint Microbial, Chemical, and Geotechnical Characterization of Biodegradation Processes of Solid Waste in Laboratory Simulators. In: *Coupled Phenomena in Environmental Geotechnics*, Torino Italy, in preparation.

Kim J. and Pohland F. G. 2003 Process enhancement in anaerobic bioreactor landfills. *Water Science and Technology* **48**(4), 29-36.

Mata-Alvarez J., Mace S. and Llabres P. 2000 Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives. *Bioresource Technology* **74**(1), 3-16.

McMahon K. D., Zheng D. D., Stams A. J. M., Mackie R. I. and Raskin L. 2004 Microbial population dynamics during start-up and overload conditions of anaerobic digesters treating municipal solid waste and sewage sludge. *Biotechnology and Bioengineering* **87**(7), 823-34.

Pinto A. J., Xi C. and Raskin L. 2012 Bacterial Community Structure in the Drinking Water Microbiome Is Governed by Filtration Processes. *Environmental Science & Technology* **46**(16), 8851-9.

Reinhart D. R. and Townsend T. G. 1998 *Landfill Bioreactor Design and Operation*. Lewis Publishers, Boca Raton, FL.

Staley B. F., de los Reyes F. L. and Barlaz M. A. 2011 Effect of Spatial Differences in Microbial Activity, pH, and Substrate Levels on Methanogenesis Initiation in Refuse. *Applied and Environmental Microbiology* **77**(7), 2381-91.

Stroot P. G., McMahon K. D., Mackie R. I. and Raskin L. 2001 Anaerobic codigestion of municipal solid waste and biosolids under various mixing conditions - I. Digester performance. *Water Research* **35**(7), 1804-16.

Vavilin V. A. and Angelidaki I. 2005 Anaerobic degradation of solid material: Importance of initiation centers for methanogenesis, mixing intensity, and 2D distributed model. *Biotechnology and Bioengineering* **89**(1), 113-22.

Zaher U., Buffiere P., Steyer J. P. and Chen S. 2009 A Procedure to Estimate Proximate Analysis of Mixed Organic Wastes. *Water Environment Research* **81**(4), 407-15.

Zekkos D., Kavazanjian E., Bray J. D., Matasovic N. and Riemer M. F. 2010 Physical Characterization of Municipal Solid Waste for Geotechnical Purposes. *Journal of Geotechnical and Geoenvironmental Engineering* **136**(9), 1231-41.

Zhou J. Z., Bruns M. A. and Tiedje J. M. 1996 DNA recovery from soils of diverse composition. *Applied and Environmental Microbiology* **62**(2), 316-22.