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Archaeal community structure in leachate and solid waste is correlated to methane generation and volume reduction during biodegradation of municipal solid waste

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ABSTRACT

Duplicate carefully-characterized municipal solid waste (MSW) specimens were reconstituted with waste constituents obtained from a MSW landfill and biodegraded in large-scale landfill simulators for about a year. Repeatability and relationships between changes in physical, chemical, and microbial characteristics taking place during the biodegradation process were evaluated. Parameters such as rate of change of soluble chemical oxygen demand in the leachate (r_{sCOD}), rate of methane generation (r_{CH4}), rate of specimen volume reduction (r_{Vt}), DNA concentration in the leachate, and archaeal community structures in the leachate and solid waste were monitored during operation. The DNA concentration in the leachate was correlated to r_{CH4} and r_{Vt}. The r_{CH4} was related to r_{sCOD} and r_{Vt} when waste biodegradation was intensive. The structures of archaeal communities in the leachate and solid waste of both simulators were very similar and *Methanobacteriaceae* were the dominant archaeal family throughout the testing period. Monitoring the chemical and microbial characteristics of the leachate was informative of the biodegradation process and volume reduction in the simulators, suggesting that leachate monitoring could be informative of the extent of biodegradation in a full-scale landfill.

1. Introduction

Approximately 150 million tons of municipal solid waste (MSW) are annually disposed of in landfills in the United States. More than 50% of the landfilled waste consists of paper, food and yard waste (EPA, 2011), which are biodegradable under anaerobic conditions (Barlaz et al., 2010). MSW biodegradation and methane (CH_4) generation are greatly accelerated in bioreactor landfills compared to Subtitle D landfills, and generated CH_4 is collected efficiently via landfill gas pipelines deployed for active biogas extraction.

Significant challenges associated with the monitoring and operation of bioreactor landfills remain (Reinhart et al., 2002). Specifically, high variability in landfill monitoring data makes them hard to interpret (Wang et al., 2013) and guide decisions on landfill operations. The heterogeneity of landfilled waste often contributes to the variability in the parameters monitored during MSW biodegradation in bioreactor landfills (Staley et al., 2011). Because studies to evaluate the repeatability of the biodegradation process under the same conditions are rare (Fei et al., 2014b), the expected differences in degradation characteristics in bioreactor landfills are not well established, impacting the optimization of MSW biodegradation and CH_4 collection.

Physical, chemical, and microbial processes take place simultaneously during MSW biodegradation altering the chemical, physical and mechanical properties of the solid, liquid and gas phases of MSW (McDougall, 2007; Gawande et al., 2010; Fei et al., 2014a). While the evolution of biodegradation process parameters during MSW biodegradation has been studied, relationships among the many interdependent parameters have only been explored in a few studies (Reddy et al., 2011; Bareither et al., 2013), thus, remains unclear which parameters are most characteristic of each process and most appropriate for monitoring MSW biodegradation in bioreactor landfills.

Physical and chemical processes taking place during MSW biodegradation are mostly driven by microbial processes conducted by a complex consortium of microorganisms. These microorganisms are present as biofilms attached to the solid waste particles and suspended in the leachate that percolates through the waste (Barlaz et al., 2010). To the authors' knowledge, retrieval of solid waste and leachate samples from the same landfill simulator at different times during the biodegradation process, as presented herein, has not been attempted to date (see Fei et al., 2014a). Thus,

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direct comparison between the microbial communities in the solid waste and leachate during MSW biodegradation has not yet been established.

In the current study, MSW specimens of well-defined waste composition were prepared and degraded in duplicate laboratory landfill simulators using MSW excavated from a landfill with the intent to evaluate the repeatability of the MSW biodegradation process. Changes in the volume of waste, chemical properties of leachate, and microbial parameters in both leachate and solid waste were monitored for about a year to characterize their respective dynamics and investigate the relationships among them.

2. Material and methods

2.1. Specimen preparation and experimental setup

The MSW used in this study was excavated from a landfill in Austin, Texas, after two to three years of disposal and shipped in sealed drums to the University of Michigan. The waste composition was characterized according to the procedures described by Zekkos et al. (2010). The waste was first separated into a finer fraction of soil-like material that passed through a 20-mm sieve and a coarser fraction. The coarser fraction was subsequently segregated based on the type of waste constituents (i.e., paper, soft plastic, and wood). Two MSW specimens were reconstituted using these waste fractions based on the field waste composition on a wet weight basis. Each specimen weighed approximately 30 kg and consisted of 74.5% by weight soil-like material, 15.0% various types of paper, 5.5% soft plastic, and 5.0% wood. The gravimetric average moisture content of each specimen was 23% on dry basis.

Detailed descriptions of the simulators and operating procedures are presented by Fei et al. (2014a). In summary, each 42-L simulator (diameter = 0.3 m, height = 0.6 m) was filled manually with MSW on day 1. Initial volumes of the specimens were 37.1 L (simulator A) and 36.6 L (simulator B), and initial total unit weight was 7.9 and 7.8 kN/m^3, respectively. No moisture was added and there was no leachate recirculation in the first 11 days of the experiment. On day 12, the temperature of the simulators was raised from room temperature to 40 ± 3 °C using a heating blanket. On the same day, drainage valves at the bottom of the simulators were closed and deionized water was added to the simulators to completely submerge the specimens. The specimens remained submerged for 10 min before the valves were opened and the leachate drained by gravity. Thus, the specimens were maintained at field capacity (the maximum moisture content of the specimen under gravitational drainage condition) in between saturations. The leachate was collected in a leachate tank and recirculated three times a week, resulting in a leachate recirculation rate of 20 L, on average, per week. The simulators were operated for about a year.

2.2. Sampling and measurements of biogas, leachate and solid waste

The biogas was collected in a gas sampling bag (SKC Inc., Eighty Four, PA) and triplicate biogas samples were taken from the headspace of each simulator immediately before leachate recirculation. Leachate was mixed using a magnetic stir plate and a sample was collected 1 h after specimen drainage started. Biogas and leachate were sampled three times a week until day 100, and once per week thereafter.

The biogas composition was measured by a gas chromatograph equipped with a thermal conductivity detector (HP5890, Agilent, Santa Clara, CA). The biogas volume was measured by a gas mass flow meter (XFM series, Aalborg, Orangeburg, NY) and adjusted to standard temperature and pressure conditions. The rate of CH_4 generation (r_{CH4}, L/day) was calculated by multiplying the daily biogas generation volume by its corresponding CH_4 concentration. The cumulative volume of generated CH_4 (ΣV_{CH4}) was calculated over time.

Leachate samples were centrifuged at 10,000*g* for 15 min and the precipitates were stored at −80 °C for the extraction of biomass DNA. The supernatants were filtered through 0.45 μm nylon membrane filters and filtrates were analyzed for soluble chemical oxygen demand (sCOD) (APHA, 2005). The change rate of sCOD (r_{sCOD}, mg/L day) was calculated between each pair of measurements. The concentrations of volatile fatty acids (VFAs), i.e., acetic acid, propionic acid, butyric acid and valeric acid, in the filtrates were analyzed using an ion chromatograph system (Dionex, Sunnyvale, CA) and converted to equivalent COD values (Smith et al., 2013). The detection limits were 5.0, 3.5, 5.5, and 3.6 mg COD/L for the respective VFAs.

The height of the MSW specimen in the simulators was measured continuously using a cable extension transducer (PT1 series, Celesco, Chatsworth, CA). The volume reduction (ΣV_t) of the specimens was calculated, and the rate of specimen volume reduction (r_{Vt}, L/day) between two consecutive r_{CH4} values was averaged to facilitate pair-wise data analysis. Solid waste samples were collected using a core sampling technique from three sampling ports located along the height of simulator B (Fei et al., 2014a). Each sample was retrieved by augering a piece of sterilized thin-wall stainless steel tubing attached to a power drill into the waste (Fei et al., 2014a) and was stored at −80 °C for the extraction of biomass DNA. The total mass of each waste specimen was measured over time and the total unit weight was calculated. The total unit weight of the specimens in simulators A and B increased to 9.8 and 9.7 kN/m^3 by day 20 and changed to 10.8 and 10.6 kN/m^3 by day 350, respectively.

2.3. DNA extraction, PCR amplification and pyrosequencing

Total DNA was extracted from the pellets obtained by centrifuging the leachate samples of the duplicate simulators collected on days 23, 34, 46, 83, 109, and 178. A 2xTENS-C buffer was prepared with 100 mM Tris–HCl, 40 mM Ethylenediaminetetraacetic acid, 200 mM NaCl, and 2% sodium dodecyl sulfate mixed with 1% hexadecyltrimethyl ammonium bromide. The biomass of each leachate sample was re-suspended with 0.4 ml of 2xTENS-C buffer and 15 μl of 10 mg/ml Proteinase K (Promega Corporation, Madison, Wisconsin) was added. Solid waste samples collected on days 47, 82, 111, and 179 were thawed on ice for about 1 h and 0.4 g of each sample was weighed and transferred to a screw cap tube with a sterile spatula. 0.4 ml of 2xTENS-C buffer and 15 μl of 10 mg/ml Proteinase K was added. Duplicates were prepared for DNA extraction of each leachate and solid waste sample. Following this, a standard bead-beating and phenol-chloroform extraction protocol, as described in Urakawa et al. (2010), was performed.

DNA concentrations were measured using a spectrophotometer (NanoDrop 1000, Thermo Scientific, Wilmington, DE). The mass of DNA recovered from the leachate samples was normalized using the original volume of the collected leachate sample (ng DNA/ml leachate). The DNA extracted from the solid waste taken from the three sampling ports at each sampling time was pooled. Six leachate samples of each simulator (12 in total) and four solid waste samples of simulator B were processed. The leachate and solid waste samples were not retrieved on the same day, but were collected only one to two days apart.

PCR amplification of the 16S rRNA gene was performed using the protocol of Pinto and Raskin (2012) except that archaeal pyrosequencing primers Univ-519F/Arch-915R were used (Klindworth et al., 2013). Quantification, purification and pooling of the ampli-