MICROBIAL POPULATION DYNAMICS AND DIVERSITY IN MUNICIPAL SOLID WASTE ANAEROBIC LABORATORY REACTORS

PROJECT REPORT

by

Christopher A. Bareither
Dept. of Geological Engineering
University of Wisconsin-Madison

Steven J. Fong
Dept. of Bacteriology
University of Wisconsin-Madison

Georgia L. Wolfe
Dept. of Bacteriology
University of Wisconsin-Madison

Katherine D. McMahon
Depts. of Civil and Environmental Engineering and Bacteriology

Submitted to
The University of Wisconsin System
Solid Waste Research Program

August 5, 2009
ABSTRACT

This study is directed towards developing relationships between physical and environmental characteristics of bioreactor landfills, microbial community composition, and methanogen populations. Anaerobic reactors degrading municipal solid waste are operated with temperature control and leachate recirculation to optimize biodegradation. Leachate samples are collected weekly and analyzed for pH, electrical conductivity, oxidation-reduction potential, and chemical oxygen demand. Biogas produced during biodegradation is measured volumetrically and composition is assessed for H₂, N₂, O₂, CO₂ and CH₄. Microbial community composition is assessed using automated ribosomal intergenic spacer analysis and methanogen populations are assessed using quantitative polymerase chain reaction. The reactors have been in operation for approximately 160 d and all exhibit typical leachate chemistry trends of anaerobic degradation. Coupled with the methane production, the reactors have progressed through the acid phase and accelerated methane production phase. A DNA extraction methodology was developed to optimize the concentration of DNA, which involves filtering leachate on a 0.2 µm filter and extraction with a Mo Bio Powersoil Kit.
# TABLE OF CONTENTS

Abstract.......................................................................................................................... ii

Table of Contents........................................................................................................... iii

List of Figures................................................................................................................ iv

Introduction .................................................................................................................. 1

Background.................................................................................................................... 2
  Municipal Solid Waste Biodegradation ................................................................. 2
  Environmental Factors Affecting Biodegradation .................................................. 3
  Microbial Composition and Dynamics in Anaerobic Biodegradation ................. 4

Materials and Methods................................................................................................ 6
  Municipal Solid Waste .......................................................................................... 6
  Laboratory Anaerobic Reactors .......................................................................... 7
  Leachate Volume and Chemistry ...................................................................... 8
  Gas Production and Chemistry ......................................................................... 8

Laboratory Anaerobic Reactor Operation Data...................................................... 9
  Temperature ....................................................................................................... 9
  Leachate Chemistry ........................................................................................... 9
  Biogas Composition and Methane Production ................................................... 10

Microbial Method Development.............................................................................. 11
  DNA Extraction .................................................................................................. 11
  Polymerase Chain Reactor Conditions ............................................................... 12
  Automated Intergenic Spacer Analysis................................................................. 14

Future Work .............................................................................................................. 15
  Reactor Operation .............................................................................................. 15
  Compression Cells and Scale Comparison .......................................................... 16
  Microbial Analyses ........................................................................................... 16

References.................................................................................................................. 18
LIST OF FIGURES

Fig. 1. Gas, leachate, solids, and microbial trends in laboratory-scale anaerobic reactors (Barlaz et al. 1989). .......................................................... 22

Fig. 2. Box-plots of the percent composition of material groups of municipal solid waste samples on a dry mass basis. .......................................................... 23

Fig. 3. Schematic of the laboratory anaerobic reactors .......................................................... 24

Fig. 4. Average waste temperature in the laboratory anaerobic reactors .......................... 25

Fig. 5. Temporal relationships of leachate chemical parameters in the laboratory anaerobic reactors: (a) pH, (b) oxidation reduction potential, (c) chemical oxygen demand, and (d) electrical conductivity .................................................. 26

Fig. 6. Temporal relationships of methane flow rate and cumulative methane production for (a) Reactor 1, (b) Reactor 2, and (3) Reactor 3 ......................... 27

Fig. 7. PCR amplification detection of Methanobacteriales and Methanomicrobiales .......................................................... 28

Fig. 8. Raw ARISA profiles of the bacterial community in the MSW leachate of Day 75 for (a) Reactor 1, (b) Reactor 2, and (3) Reactor 3. .......................... 29
INTRODUCTION

The landfill industry is currently in transition from the conventional landfill, where municipal solid waste (MSW) biodegradation is minimized due to limited moisture addition to the refuse, to the bioreactor landfill, where MSW biodegradation is a primary objective. Biodegradation is optimized through the increase in moisture content, increase in temperature, and/or nutrient/microbial seed addition to the refuse (Reinhart et al. 2002). The most widely used approach is to increase the moisture content through recirculation of leachate or addition of supplemental liquids (e.g., sewage or industrial wastewater). Although current bioreactor landfill operation is ad-hoc (Benson et al. 2007), the bioreactor landfill has potential to treat leachate in situ, accelerate waste stabilization, maximize gas generation, and increase waste settlement (Reinhart et al. 2002; Mehta et al. 2002). In effect, the waste mass serves as an anaerobic treatment system in which organic carbon in the leachate is converted to landfill gas (Pohland 1975; Reinhart et al. 2002).

In 2006 the United States discarded nearly 169 million tons of MSW to landfills and incinerators (USEPA 2006). The discarded fraction contained approximately 50% biodegradable materials (USEPA 2006). Biodegradation these materials is a microbial mediated process. Organic polymers are broken down through syntrophic relationships between hydrolytic, fermentative, acetogenic, and methanogenic microorganisms to ultimately yield carbon dioxide and methane (Barlaz et al. 1989; Levén et al. 2007). Molecular analyses have identified factors such as age, location, and operational conditions of landfills, which affect the microbial, and specifically the methanogenic archaeal, diversity and population (Huang et al. 2002; Huang et al. 2003; Chen et al. 2003a; Laloui-Carpentier et al. 2006). Methanogens are one of the key microbial groups of interest in landfill research, since they are the primary producers of methane. Limited studies have assessed the temporal influence on methanogenic diversity and community
structure throughout the MSW biodegradation process. Optimization of MSW biodegradation through the addition of microbial enhanced leachate is a realistic possibility through state-of-the-art molecular techniques; however, there is a need to first further our understanding of the microbial dynamics of MSW biodegradation.

This objective of this study is to develop relationships between chemical characteristics of MSW biodegradation, microbial community composition, and methanogen populations. Three laboratory anaerobic reactors are operated with temperature control and leachate recirculation to optimize biodegradation. Leachate samples are collected weekly for analysis of leachate quality and for DNA extraction. Biogas production and composition are also monitored. This report summarizes approximately 160 d of reactor operation, as well as molecular microbial methodologies developed for DNA extraction, amplification, and analysis.

BACKGROUND

Municipal Solid Waste Biodegradation

Cellulose, hemicellulose, and lignin are the primary organic polymers that constitute the biodegradable fraction of MSW. Cellulose and hemicellulose constitute approximately 45-60% of MSW by dry weight, and biodegrade anaerobically to yield methane and carbon dioxide (Barlaz et al. 1990; Mehta et al. 2002). Lignin, however, is recalcitrant under anaerobic conditions, and the fraction of MSW dry weight comprised of lignin (≈ 15% typically) remains stable throughout the life of a landfill (Colberg 1988). Lignin partially surrounds the cellulose and hemicellulose polymers, reducing complete biodegradation of cellulose and hemicellulose in MSW to approximately 60% (Barlaz et al. 1990).

Biodegradation of MSW occurs through syntrophic microbial interactions with hydrolytic, fermentative, acetogenic, and methanogenic microorganisms (Barlaz et al.
1989; Levén et al. 2007), and is typically explained in a series of sequential phases (Barlaz et al. 1989; Pohland and Kim 1999). Barlaz et al. (1989) used data from nine 2-L MSW reactors operated at 41 °C with leachate recirculation to generate a four-phase MSW biodegradation relationship shown in Fig. 1. The aerobic phase is characterized by depletion of oxygen and transition to anaerobic conditions, whereupon fermentative bacteria begin hydrolyzing cellulose and hemicellulose to soluble molecules. Hydrolytic bacteria then convert the soluble molecules to volatile fatty acids (VFAs) (e.g., acetate, propionate, and butyrate), CO₂, and H₂ during the anaerobic acid formation phase, resulting in the accumulation of carboxylic acids. This acid accumulation produces a decrease in pH and increase in chemical oxygen demand (COD) (Pohland and Kim 1999).

Methanogen population (MPN, Fig. 1) increases, as does the percent composition of methane, to mark the onset of methanogenesis. During the accelerated methane production phase, acetogens and methanogens increase the production of carbon dioxide and methane primarily by utilizing readily available carboxylic acids. Biodegradation of cellulose and hemicellulose occurs during methane production and there is an overall increase in pH and decrease in acid concentration and COD (Pohland and Kim 1999). A peak in the methane production rate marks the transition to the decelerated methane production phase (Fig. 1), and although gas composition remains nearly constant, overall gas production decreases. The rate of solids decomposition is at a maximum during the decelerated methane production phase, and is largely controlled by the rate of cellulose and hemicellulose hydrolysis.

Environmental Factors Affecting Biodegradation

The variability of biodegradation in full-scale landfills results in a range of cumulative methane generation from 0.34 to 68 L-CH₄/kg-MSW (Barlaz et al. 1990).
The primary environmental factors influencing biodegradation include the water content, moisture movement/recirculation, temperature, and nutrient availability. The water content of MSW at placement is approximately 20% (wet weight basis), which is below optimum for anaerobic microorganisms (Themelis and Ulloa 2007). Farquhar and Rovers (1973) report maximum biogas production (i.e., biodegradation) in MSW reactors with water contents ranging from 60-80%. Biogas production has also been shown to increase with leachate recirculation compared to conventional landfilling (Demir et al. 2003). Chugh et al. (1998) operated 200 L reactors at 38 °C with varying recirculation rate and reported increased daily gas production with more intense recirculation rate. However, the increase in gas production varied non-linearly with recirculation rate and a threshold recirculation rate exists that provides a balance of leachate residence time for microbial population development and leachate flux to remove inhibitory volatile fatty acids. Farquhar and Rovers (1973) identified an optimum temperature of 37 °C for biogas production for temperatures ranging from 0-55 °C. Barlaz et al. (1990) reported a range of optimum temperatures for mesophilic microorganisms to be between 38-42 °C. Biogas production has also been shown to increase with increasing concentration of organic solids (Rao and Singh 2004). However, Rao and Singh (2004) identified a threshold organic solids concentration of approximately 60 g-VS/L, whereupon biodegradation decreased due to inhibiting effects of increased carboxylic acid concentrations.

Microbial Composition and Dynamics in Anaerobic Biodegradation

Biodegradation of MSW is a process mediated by a complex community of microorganisms. For many years, the microbial ecology of MSW biodegradation was a “black box” that could not be dissected due to methodological limitations. Several more recent studies have used modern molecular tools to identify factors such as age,
location and operational conditions of landfills, which affect the microbial community structure and diversity.

The most abundant members of the bacterial community (as determined using molecular techniques) are usually members of the low-GC Gram-positive phylum that includes *Clostridia* and *Bacillus*, and the Bacteroidetes phylum that includes *Cytophaga* and *Bacteroides* (Huang et al. 2003; Huang et al. 2005; Levén et al. 2007). These organisms likely initiate polymer degradation, which is the initial step in the syntrophic biodegradation pathway of MSW. In thermophilic systems, members of the *Thermotogae* can be present in high numbers (Levén et al. 2007). Methanogenic Archaea are also critical for biogas production since they catalyze methane formation. Hydrogenotrophic *Methanomicrobiales* and acetoclastic *Methanosarcinales* have been frequently detected in bioactive landfills (Huang et al. 2003; Laloui-Carpentier et al. 2006).

Methanogens are one of the key microbial groups of interest in bioactive landfill research, since they are the primary producers of methane. Methanogen population dynamics has been studied in some detail in other anaerobic systems such as municipal sewage sludge digesters (McHugh et al. 2003; Conklin et al. 2006), granular sludges (Collins et al. 2003; Periera et al. 2002), and co-digestion of MSW and sewage (Griffin et al. 1998; McMahon et al. 2001). The ecology of acetoclastic methanogens (e.g. *Methanosarcina* and *Methanoseta*) is particularly interesting since these two genera seem to niche partition between low- and high-acetate concentrations (McMahon et al. 2001; Karakashev et al. 2005; Conklin et al. 2006). Thus, the concentration of acetate in leachate could have a significant impact on the route of carbon flow through acetate to CH$_4$ and CO$_2$. These two genera are also known to exhibit different uptake and growth kinetics (Conklin et al. 2006).
A significant limitation in the past investigations of microbial community ecology is the analysis of a single sample in time. Research has focused on variations of depth in landfills (Chen et al. 2003a,b), temperature (Levén et al. 2007), degree of waste stabilization (Calli and Girgin 2005), and leachate recirculation (Huang et al. 2002) versus conventional landfills (Huang et al. 2003). Although these studies have generated much new information about the distribution and preferences of anaerobic microbes in bioactive landfills, much remains to be learned about the temporal variation in community composition and how this relates to biogas production and waste stabilization.

MATERIALS AND METHODS

Municipal Solid Waste

Municipal solid waste (MSW) and leachate were collected from Deer Track Park Landfill, which is a Waste Management site located in Johnson Creek, Wisconsin. The MSW was approximately 3-4 months old at the time of sampling. A box-plot of the MSW material composition on a dry mass basis is shown in Fig. 2. The average material composition reported in Hull et al. (2005) for 1-3 year old MSW in a New Jersey landfill is also included for comparison. The MSW fine fraction (Fig. 2) is the material passing a 25-mm screen, which contains significant soil and other fine material difficult to visually identify. With the exception of a smaller fraction of paper/cardboard, likely due to decomposition, and a larger fraction of miscellaneous, due to increasing difficulty of visual identification with increasing age of waste, the relative material composition identified in this study is similar to that in Hull et al. (2005).

The MSW composition of the laboratory anaerobic reactors is composed of the average of each material group: 17.2% paper/cardboard, 5.3% flexible plastic, 4.8% rigid plastic, 2.8% textile, 7.3% wood, 7.7% gravel/ceramics/inerts, 0.1% yard waste, 0.2%
food waste, 5.1% metal, 0.5% glass, 2.3% miscellaneous, and 46.6% fine fraction. This composition is the average of a field-scale lysimeter experiment (Breitmeyer et al. 2008), which will be used to evaluate scale effects on microbial community composition and population. All MSW, minus the fine fraction, was shredded and passed through a 25-mm screen prior to placement in the reactors. The composite MSW was blended thoroughly, hydrated to a water content of approximately 28%, and compacted in five lifts to an average total unit weight of 7.7 kN/m³.

**Laboratory Anaerobic Reactors**

A schematic of the laboratory anaerobic reactors is shown in Fig. 2. The reactors consist of a 0.6-m diameter by 0.9-m tall stainless steel tank. Shredded MSW was placed in the reactors with a gravel layer at the base for leachate collection and a gravel layer at the top for leachate distribution. A fine-mesh aluminum screen was placed between the MSW specimen and drainage gravel to prevent clogging of the effluent port. Leachate is collected from the effluent port in an intravenous (IV) bag to minimize exposure to oxygen, and is recirculated via a perforated PVC pipe distribution network installed in the distribution gravel. Gas produced during MSW biodegradation is collected in flexfoil SKC gasbags.

The stainless steel tank is heated via two 300-mm x 600-mm flexible silicone rubber heaters. The heaters are regulated with an on/off relay switch connected to a Campbell Scientific, Inc. CR23X Micrologger. Omega Engineering, Inc. type-T thermocouples are used to monitor temperature in the drainage gravel, distribution gravel, and at four vertically staggered locations in the MSW. Real time temperature measurements are monitored by the Micrologger via an external thermocouple adjacent silicone rubber heaters to trigger the on/off relay switch and maintain refuse temperature near 40 °C for optimal biodegradation.
Leachate Volume and Chemistry

Leachate collected from Deer Track Park Landfill is stored in zero head-space containers at 4 °C to minimize biological activity. Leachate addition to the reactors began on Day 41 of reactor operation with an initial volume of 10 L. A second 10 L dose was applied on Day 43. Subsequent leachate addition/recirculation has been executed in 1 L volumes every 1-2 d. The initial larger dose volumes were applied to seed the reactors with an active anaerobic microbial culture to initiate biodegradation. Subsequent smaller volumes were chosen for ease of leachate management. Leachate collected in the IV bags is consistently recirculated in the reactors, with additional fresh leachate added if needed to achieve 1 L.

Chemical parameters of the leachate are used to provide a measure of the biodegradation activity. Effluent samples are collected weekly and analyzed for pH, electrical conductivity (EC), oxidation-reduction potential (ORP), and chemical oxygen demand (COD) in accordance with Standard Methods (1999).

Gas Production and Chemistry

Gas produced during biodegradation is measured via water displacement method in a calibrated carboy submerged in acidified water (pH ~ 3.0). Gas composition is measured in a Shimadzu Gas Chromatograph (GC-2014) equipped with a flame ionization and thermal conductivity detector. Relative compositions of hydrogen (H₂), nitrogen (N₂), oxygen (O₂), carbon dioxide (CO₂), and methane (CH₄) are calculated with respect to standard gases (Scott Specialty Gases and Linde Gas Corp).
LABORATORY ANAEROBIC REACTOR OPERATION DATA

Temperature

Daily average waste temperatures in the three reactors are shown in Fig. 4. Daily average waste temperatures were computed as arithmetic means of hourly measurements recorded at each of the four vertically staggered thermocouples within the waste specimens. Electrical problems with the relay switch on Reactor 1 caused the heaters to malfunction and burn out during the first day of operation. Elevated temperatures during this malfunctioning damaged all thermocouples in Reactor 1 except the one in the distribution gravel and one within the waste. Subsequent electronic interference between the damaged thermocouples in Reactor 1 and the datalogger caused elevated temperatures in reactors 2 and 3 during the first 20 d.

The heaters on Reactor 1 were replaced and electronic interference problems remedied such that by Day 50 of reactor operation an approximately stable temperature was achieved in each reactor. The average waste temperature between days 50-150 in Reactor 1 was 38±2 °C, in Reactor 2 was 34±4 °C, and in Reactor 3 was 34±2 °C.

Leachate Chemistry

Temporal relationships of pH, oxidation reduction potential (ORP), chemical oxygen demand (COD), and electrical conductivity (EC) for the three reactors are shown in Fig. 5. Leachate addition to the reactors began on Day 41 of operation, approximately corresponding to the time of temperature equilibrium. Similar temporal trends for all four leachate parameters are exhibited in the three reactors. As leachate is added to the reactors the pH decreases due to accumulation of carboxylic acids. This acid accumulation is characterized by an increase in COD from 2,400 mg/L in the leachate inoculum to between 37,000 and 42,000 mg/L by Day 62.
The EC relationships show a similar decreasing and subsequent stabilization trend compared to the COD. A similar phenomenon was reported by Ham and Bookter (1982), but at lower conductivities ranging from 5 to 20 mS/cm. The ORP is typically used as an indicator of methanogenesis in MSW research, where a negative ORP, typically less than 200 mV, is indicative of active methane production (Farquhar and Rovers 1973). The initial increase in ORP relative to the leachate inoculum (i.e., ORP = -370 mV) is representative of acid accumulation and inactive methanogenesis. As COD decreases, pH increases due to removal of the available carboxylic acids, and the ORP for all three reactors decreases, indicating active methanogenesis.

**Biogas Composition and Methane Production**

Methane flow rate and cumulative methane production for the three reactors are shown in Fig. 6. Although the three reactors are operated similarly and exhibit similar trends in leachate chemistry, methane production is variable. Gas production began approximately on Day 70 for reactors 1 and 2, whereas gas production began simultaneously with leachate addition in Reactor 3, approximately Day 41. Gas composition is similar in all three reactors (data not shown). The percent CH$_4$ increased during the first 20 d of gas production from approximately 20% to 60%. Between days 80 and 100 the relative CH$_4$ composition stabilized in all three reactors at 61-63%, with the balance being CO$_2$.

Methane flow rates and cumulative production in Fig. 5 are normalized with respect to MSW dry mass. The peak CH$_4$ flow rate in Reactor 3 was 0.69 L-CH$_4$/kg-MSW/d and occurred on Day 86. Peak CH$_4$ flows in reactors 1 and 2 were of similar magnitude, 0.43 L-CH$_4$/kg-MSW/d in Reactor 1 and 0.53 L-CH$_4$/kg-MSW/d in Reactor 2, but occurred later in operation due to the lag period of biogas initiation. The lag period for gas production in Reactor 1 agrees with the leachate chemistry (Fig. 5); however,
leachate chemistry in reactors 2 and 3 is very similar and cannot be used to determine the lag in gas production for Reactor 2.

Cumulative CH$_4$ production has been reported to range from 0.34 to 68 L-CH$_4$/kg-MSW for full-scale landfills (Barlaz et al. 1990). The broad range of CH$_4$ production is attributed primarily to variability in MSW composition, and secondarily to variability in environmental conditions. An increase in CH$_4$ production measured in the laboratory compared to full-scale operations is typical due to increased contact between microbes, substrates, and necessary growth factors from waste shredding and efficient leachate recirculation (Barlaz et al. 1990). Biochemical methane potential experiments are currently in progress to determine the maximum CH$_4$ yield for the MSW used in this study.

**MICROBIAL METHOD DEVELOPMENT**

**DNA Extraction**

Preliminary testing was directed towards developing a methodology to extract DNA from the leachate fraction of MSW. Techniques to separate the microbial community from the leachate were centrifugation and vacuum filtration. Centrifugation involved centrifuging the leachate at 10,000 g for 10 minutes to pelletize the solid matter. The supernatant was then decanted and the pellet was used for DNA extraction. Vacuum filtration was conducted on a 0.2 µm filter paper. After filtration the filter paper cut into 5-mm squares for DNA extraction. Equal 13.25 mL aliquots of fresh leachate from Deer Track Park landfill were used in this preliminary process.

Extraction of DNA from the centrifuged pellet and the filter paper was completed by two methods: (1) a traditional phenol-chloroform method with bead-beating and alcohol precipitation and (2) the Powersoil DNA Kit (MO BIO, Carlsbad, CA). The Powersoil DNA Kit was used with the manufacturer’s instructions with slight
modifications. The bead-beating step described in the instructions was optimized to a 3.5 setting for 3 minutes on a Biospec Bead Beater. Additionally, 2 µL of 50x ethylenediaminetetraacetic (EDTA) was added to the final DNA solution (100 µL volume) to prevent DNA degradation prior to storage in a -20 °C freezer.

The total DNA yield and purity was assessed using a Nanodrop spectrometer. This instrument measures the absorbance of light by a specimen at various wavelengths. The ratio of absorbance at wavelengths 260 to 280 nm ($A_{260}/A_{280}$) is used to assess the purity of the DNA extraction, where $A_{260}/A_{280} = 1.8$ indicates pure DNA. The total DNA yield is reported by the instrument as a concentration (mass of DNA per volume of solution). A DNA extraction methodology incorporating vacuum filtration on a 0.2-µm filter combined with the Powersoil DNA Kit yielded the highest concentration and purest DNA sample. This method was used subsequently for all weekly leachate samples collected from the reactors.

**Polymerase Chain Reactor Conditions**

Polymerase chain reaction (PCR) is a method to amplify DNA targets of interest for use in subsequent molecular analyses (e.g., sequencing, ARISA) (Madigan and Martinko 2006). PCR was performed on individual DNA samples extracted with the vacuum filtration/Powersoil Kit method described above. PCR was conducted with 24 µL of a master mix solution and 1 µL of sample DNA. The master mix solution contains 10.85 µL H₂O, 5.0 µL 5x GoTaq Buffer (Promega, Madison, WI), 5.0 µL betaine, 1.5 µL MgCl₂, 0.15 µL GoTaq polymerase (Promega, Madison, WI), 0.5 µL of 4x10 mM dNTP’s, and 0.5 µL of forward and reverse primers of interest. Thus, final concentrations of the forward and reverse primers were 0.4 µM in the master mix solution. Concentrations of MgCl₂ and betaine were optimized using concentration gradients run through the PCR amplification process. PCR products were then analyzed with gel electrophoresis on a
1% agarose gel and viewed with a FOTODYNE System UV Transilluminator (Harland, WI). For example, betaine was optimized by running a concentration gradient between 0.5 M and 2.0 M with 0.5 M increments. Betaine is used to prevent primer dimerization, which leads to non-specific amplification.

PCR was performed in an Eppendorf Mastercycler (Eppendorf AG, Hamburg, Germany) with the following steps: initial denaturing at 95°C for 5 minutes, 35 cycles of denaturation at 95°C for 1 minute, annealing at 58°C for 1 minute, extension at 72°C for 1.5 minutes, and final extension at 72°C for 10 minutes. Thermocycler settings were adapted from Staley (2009), but optimized and revised due to differing thermocyclers.

Primers targeting four methanogenic orders (Methanococcales, Methanobacteriales, Methanomicrobiales, and Methanosarcinales) and two families (Methanosarcinaceae and Methanosaetaceae) were selected from Yu et al. (2005) to quantify the methanogenic community in MSW leachate. These primers will be used in a quantitative PCR (qPCR) procedure currently being developed; however, preliminary qualitative screening of the primers was conducted to optimize PCR thermocycler settings.

An image of the gel electrophoresis for the Methanobacteriales and Methanomicrobiales primer sets is shown in Fig. 7. Bands in rows 1 and 10 correspond to a ladder, which is a mixture of fragments of known basepairs to compare with PCR products. Methanobacteriales primer sets were used to screen three leachate samples and a positive detection is indicated by the single bands shown in rows 3, 4, and 5. Methanomicrobiales primer sets were also used to screen three leachate samples and showed positive detection in all three (rows 7-9 in Fig. 7). Methanobacteriales was detected with primers MBT857f (5’–CGWAGGGAAGCTGTTA GT) and MBT1196R (5’-TACCGTCGTCCACTCCTT) and Methanomicrobiales was detected with primers MMB282F (5’–ATCGRTACGGGTTGTGGG) and MMB832R (5’–
CACCTAACGCRCAHTGTTTAC). Positive detection of Methanosarcinaceae was also achieved with primers Msc380F (5’ – GAACCGYGATAAGGGGA) and Msc828R (5’ – TAGCGARCATCGTTTACG) (Yu et al. 2005).

Automated Intergenic Spacer Analysis

Automated ribosomal intergenic spacer analysis (ARISA) fingerprints provide a unique snapshot of a bacterial community, with taxa inferred from the base pair length of their variable 16S-ITS-23S region (Fisher and Triplett 1999). The ITS region is amplified using a fluorescently tagged forward primer, specific to either bacteria or archaea, and capillary electrophoresis is used to separate fragments by length while recording the fluorescence intensity of each fragment. ARISA profiles show a range of peaks, of increasing length and varying height. The height of ARISA fluorescence peaks at a given base pair length is a proxy for the relative abundance of that taxon, or “Operational Taxonomic Unit” (OTU). ARISA profiles from bacterial or archaeal DNA can be compared across samples to explore changes in presence and abundance of particular OTUs. This process allows observations to be made regarding the dynamics of the bacterial or archaeal community. Changes in community profiles can be correlated to environmental parameters using multivariate statistics.

The intergenic spacer of the 16S-ITS-23S rRNA operon was PCR amplified and analyzed using ARISA essentially as described in Fisher and Triplett (1999), with minor modifications as described elsewhere (Shade et al 2007). 1 µl of extracted leachate DNA was used as a template for 30 cycles of PCR with GoTaq Flexi DNA polymerase (Promega Corporation, Madison, WI, USA), performed on an Eppendorf Mastercycler (Eppendorf AG, Hamburg, Germany). Bacterial-specific 1406 forward and universal 23S reverse primers were used. The 1406F primer was tagged with a fluorescent dye, 6-
FAM, on the 5’ end, enabling detection of the amplified product with capillary electrophoresis on an ABI PRISM 3730 DNA analyzer (Applied Biosystems, Foster City, CA, USA).

Raw ARISA profiles of the leachate bacterial community collected from each of the three reactors on Day 75 are shown in Fig. 8. Raw community profiles were size-calibrated with an internal standard and examined for quality control using GeneMarker (SoftGenetics, PA, USA). Fragment length increases positively along the x-axis, and fluorescence intensity increases positively along y-axis. The height of the fluorescence peak is an indirect measure of abundance, where abundance increases with peak height. Many OTUs are present in high relative abundance, as expected based on previous studies showing diverse bacterial communities in bioreactor leachate (Huang et al. 2005; Levén et al. 2007).

**FUTURE WORK**

**Reactor Operation**

The three laboratory anaerobic reactors will be disassembled intermittently during the next 6 months of operation. Reactor 2 will be disassembled at the end of June 2009, Reactor 1 will be disassembled in September 2009, and Reactor 3 will be disassembled in December 2009. Solid samples of the degraded refuse will be extracted from the reactors and processed for microbial analysis following a phosphate buffer protocol described in Staley (2009). An assessment of the microbial community based solely on the leachate fraction can be skewed towards planktonic members more adapted to a liquid environment, and a more complete characterization of the microbial community of MSW should account for both the leachate and solids fractions (Staley 2009).
Compression Cells and Scale Comparison

Municipal solid waste with the same composition, but varying particle size, as the laboratory anaerobic reactors is being tested in three varying sized compression cells as part of a larger project. The compression cells have diameters of 64, 100, and 300-mm and are all equipped with similar temperature control and gas and liquid management capabilities as the reactors. Additionally, the compression cells have stress control and are capable of generating stresses in the range of full-scale landfills (e.g., up to 400 kPa). Leachate will be recirculated in these compression cells are liquid addition rates typical of full-scale bioreactor landfills (Bareither et al. 2008). Leachate samples will be collected from these compression cells on a weekly basis for leachate chemistry and microbial analysis.

In accompaniment to the laboratory experiments, a field-scale lysimeter experiment is being conducted to assess hydraulic and mechanical at near full-scale bioreactor conditions (Breitmeyer et al. 2008). Leachate samples are being collected and processed for leachate chemistry and microbial community composition and methanogen population. Data from this field-scale project will provide an invaluable comparison to the applicability of simulating bioreactor operations at laboratory-scale.

Microbial Analyses

ARISA will also be performed on all samples using archaeal-specific primers and the same methods of analysis used on the bacterial ARISA to generate archaeal community data. Assignment of peaks in community profiles and standardization between runs will be performed using R v2.7 statistical software (http://cran.r-project.org/) and a script used in Kara and Shade (2009). Briefly, the algorithm calibrates profile data to an internal size standard, and then assigns peaks to OTU bins (window of base pair size in which a given ITS fragment may occur). These
bins will be manually determined in Genemarker, based on an overlay of all community profiles. Fluorescence will be used as a proxy for the relative abundance of an OTU in the community profile. Bray-Curtis similarity indices will be used to observe patterns through time in reactor bacterial and archaeal communities, and analysis of similarity (ANOSIM) will be used to rigorously test for differences between reactors and between reactor communities at various stages of the decomposition process. Correspondence Analysis (CA) will be used to search for patterns in multi-dimensional ordinations of the data and to link environmental variables, such as pH or CH$_4$ flow rate, to the observed variation in bacterial and archaeal communities. Clone libraries will be constructed to link ARISA OTUs to known taxa by comparing sequences to public databases (e.g. NCBI Genbank and the Ribosomal RNA Database Project). This will allow exploration of variations in functional microbial communities through time and in response to changing environmental parameters.
REFERENCES


anaerobic digesters treating synthetic industrial wastewaters, FEMS Microbiology Ecology, 46(2), 159-170.


Fig. 1. Gas, leachate, solids, and microbial trends in laboratory-scale anaerobic reactors (Barlaz et al. 1989).
Fig. 2. Box-plots of the percent composition of material groups of municipal solid waste samples on a dry mass basis.
Fig. 3. Schematic of the laboratory anaerobic reactors.
Fig. 4. Average waste temperature in the laboratory anaerobic reactors.
Fig. 5. Temporal relationships of leachate chemical parameters in the laboratory anaerobic reactors: (a) pH, (b) oxidation reduction potential, (c) chemical oxygen demand, and (d) electrical conductivity.
Fig. 6. Temporal relationships of methane flow rate and cumulative methane production for (a) Reactor 1, (b) Reactor 2, and (3) Reactor 3.
Fig. 7. PCR amplification detection of Methanobacterales and Methanomicrobiales.
Fig. 8. Raw ARISA profiles of the bacterial community in the MSW leachate of Day 75 for (a) Reactor 1, (b) Reactor 2, and (c) Reactor 3.