The Study of Landfill Microbial Communities Using Landfill Gas and Landfill Gas Condensate

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Dedications

To my parents

for their love and support

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Abstract The Study of Landfill Microbial Communities Using Landfill Gas and Landfill Gas Condensate Mijin Kim R. Christopher Barry, Ph.D.

Landfills are today the most widely used form of municipal solid waste (MSW) disposal. A better understanding of the waste decomposition process is important to improving waste disposal systems. Landfills are extremely heterogeneous, and as a result, conventional grab sampling of waste can only provide a limited understanding of this microbial community. In this study, landfill gas and gas condensate were used, which allowed collecting samples without disrupting the waste. Samples were collected from the Burlington County Resource Recovery Complex, NJ. Cells from landfill gas were collected on membranes by vacuum from existing gas extraction wells. Microbial communities were studied by using PCR amplification and slot-blot hybridization with 16S rRNA-targeted oligonucleotide probes. This study found that in all three media (landfill gas, gas condensate, and waste), *Bacteria* dominated in the landfill populations. The relative abundance of *Bacteria* found by hybridization was approximately 86-87%. Also, bacterial 16S rRNA was detected throughout all landfill samples, except in one of the gas samples analyzed by PCR amplification. Quantitatively, Archaea seemed to be a minor component of the microbial community at the landfill despite their significant functional role. The relative abundances of Archaea were 7.2 % and 2.1 % in gas condensate and in waste, respectively, while they were not detected in the gas. However, limited sample volume may be responsible for non-detection in the gas. By PCR amplification analysis, archaeal rRNA was detected in the gas condensate and wastes, but not in the gas. Methanogens were identified only in the landfill gas condensate and in one waste sample. Eucaryotic rRNA was only detected in landfill wastes, where their relative abundance was 13%. A literature review suggested that no prior study has investigated the landfill microbial communities by using landfill gas or gas condensates. The present study suggests that gas and gas condensates could provide representative samples from the landfill environment. The results from this study should provide a foundation for study of the microbial ecology in landfills.

1. Introduction

Landfills are the most commonly used method for municipal solid waste (MSW) disposal, and so a better understanding of this system is important to improving municipal solid waste management. However, little study has been done on landfill microbiology, in large part because of the sampling difficulties involved. First, landfills are extremely heterogeneous anaerobic environments, and it is thus difficult to evaluate whole processes from individual samples unless very large amounts of waste are collected. Second, because some of the solid wastes in landfills are potentially hazardous, special precautions and training are required for excavation and drilling. Additionally, regulatory obstacles to disruption of landfills are also significant.

In landfills, the organic portion of waste is broken down by anaerobic microorganisms generating significant amounts of gases, mostly carbon dioxide (CO_2) and methane (CH_4 ; USEPA, 1995; Kortegast and Ampurch, 1997; Tchobanoglous, et al., 1993; WSDE, 1987). At most large landfills, gas is actively pumped from gas wells at meter-per-second velocities. A previous study (Barry and Kim, 2000) showed that particles of solid waste biofilms and other microbial aggregations come to the surface with the gas. This result suggested that the microbial communities in the solid waste environment might be reflected in the gas stream.

The diversity of microbial communities in the natural environment is difficult to understand with culture-based techniques alone. However, the recent development of molecular techniques, such as PCR amplification and hybridization with phylogenetic probes, can overcome the limitation of culture dependent methods. In this study,

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microorganisms were collected from landfill gas (LFG) and landfill gas condensate (LFGC), which is the liquid formed from water vapor in landfill gas when it cools. Landfill wastes were also collected at the same sampling sites for comparison. Then microbial populations from landfill samples were identified and quantified by using PCR amplification and slot-blot hybridization.

A literature review suggested that no prior study has investigated the landfill microbial communities by using landfill gas or gas condensates. This study describes a way of studying landfill microbial communities using landfill gas and landfill gas condensate, which allows collecting many numbers of samples without disrupting the waste. Comparisons between landfill waste and landfill gas microbial communities were made by using culture-independent methods. This study provides a foundation of the microbial ecology involved in landfill waste degradation.

2. Background

2.1 Sanitary Landfills 2.1.1 Overview of Sanitary Landfills

The term "sanitary landfill" refers to an engineered facility for the disposal of municipal solid waste designed and operated to minimize public health and environmental impacts (Tchobanoglous, et al., 1993). In the United States during the year 2000, approximately 128.3 million tons (55% by weight) of MSW were landfilled, while 69.9 millions tons (30% by weight) were recovered and 33.7 million tons (15% by weight) were combusted. The recovery has been substantially increased over last few decades: recovery was 6.4% in 1960 (U.S. Environmental Protection Agency, 2002). However, the types of wastes that can be recycled or composted are limited. Combustion is generally more expensive than landfilling, and air emissions from combustion, such as nitrogen oxides (NOx), sulfur dioxide (SO₂), carbon monoxide (CO), particulate matter (PM), dioxins and furans pose a significant environmental concern. In addition, ash residues from solid waste combustors require proper management, and these ashes are in fact often landfilled (Hickman, 1999). The number of landfills in the United States is steadily decreasing-from 8,000 in 1988 to 1,967 in 2000. The overall capacity, however, has remained relatively constant, because new landfills are usually much larger than in the past (U.S. Environmental Protection Agency, 2002). In general, economics and current trends suggest that disposal of solid waste in landfills will remain an important management strategy in the future.

Construction and operation of landfills is generally straightforward, despite the complexity of the natural processes involved in waste decomposition. Before wastes are

deposited in the ground, a site is often excavated to allow more waste to be deposited on a given plot of land. The lowest component of a landfill is the liner system, which includes drains and impermeable barriers designed to minimize the migration of leachate to groundwater. As solid waste is deposited in the landfill, waste is compacted and covered daily. The total amount of waste a site can receive is determined by the area of the plot and by the maximum slope of the sides of the landfill that still ensure slope stability. Once waste has reached the final design level of the landfill, a final cover is applied. The goals of the final cover are to minimize rainfall and snowfall infiltration, to limit the uncontrolled release of landfill gases, to suppress the proliferation of disease vectors, to limit the risk of fire, and to provide suitable conditions for use of the site after landfill closure (Fig. 1, Tchobanoglous, et al., 1993).

As waste degrades, it compacts as mass is converted to methane and escapes. The capacity of a landfill is defined by the available volume (a function of plot area and maximum slope). Faster decomposition provides faster settling, faster settling in turn yields more waste capacity in a given landfill resulting in less need for more landfills. Understanding the decomposition processes (and the gas production process) is therefore important to reducing the need for more landfills.



Figure 1. Section view through a typical solid waste sanitary landfill. Bench is used to maintain the slope stability of the landfill, for the placement of surface water drainage channels, and for the location of landfill gas recovery piping. (After Tchobanoglous, et al., 1993)

2.1.2 Landfill Gas Management

A solid waste landfill can be thought of as a biochemical reactor. Solid waste and water are the major inputs, and landfill gas and leachate are the principal outputs. Landfill gas is a product of the anaerobic biological decomposition of the organic fraction of waste. Carbon dioxide (CO₂) and methane (CH₄) are the principal gases and represent over 95% of landfill gas. Trace gases may include nitrogen, oxygen, hydrogen sulfide, disulfides, mercaptans, and various volatile organic compounds (USEPA, 1995; Kortegast and Ampurch, 1997; Tchobanoglous, et al., 1993; WSDE, 1987). Those trace gases sometimes are described as non-methane organic compound (NMOCs).

Landfill gas must be controlled because of its potential explosiveness, and because it induces vegetative stress that leads to the destruction of plants. Also, methane and carbon dioxide are recognized as greenhouse gases that contribute to global warming. Some trace gases can be toxic and therefore pose risks to public health. In the 1990s, attention turned to landfill gas, primarily due to the impact of landfill gas on air quality and global warming. This attention led the United States to regulate landfill gas such that municipal solid waste landfills are now required to monitor and manage both landfill gas migration and landfill gas surface emissions (USEPA, 1991; 1996). These regulations require the implementation of planned and regularly scheduled landfill gas monitoring at most municipal solid waste landfills. The landfill gas surface emission regulations address all new municipal solid waste landfills through New Source Performance Standards (NSPS), and regulate all existing municipal solid waste landfills with Emission Guidelines (EGs). These regulations establish the maximum allowable emission level of non-methane organic compounds (NMOCs). Also, these air emission regulations specify the best demonstrated technology (BDT) that must be used to control releases. Finally, these regulations specify quarterly air emission monitoring.

Two basic types of systems are used to control the migration and emission of landfill gas: passive and active systems. In the passive gas control systems the pressure of the gas that is generated in the landfill provides the driving force to move the gas through the collection system (Fig. 2). Such passive systems are usually limited to smaller sites or those producing little gas. In the active systems (Fig. 3), such as those at most large landfills, a vacuum is applied to control the flow of the gas. The active systems typically include a collection system and a processing system. The collection system provides a means to collect the gas and transport it to the processing station. Processing stations, broadly defined, are facilities which contain the equipment and technologies to manage and dispose of the gas after it is collected (Hickman, 1999).

Recovered landfill gases are either flared, used for the recovery of energy, or both. Flaring, or thermal destruction, is a common method of treatment for landfill gases. In flaring, landfill gases are combusted in the presence of oxygen to create carbon dioxide, sulfur dioxide, oxides of nitrogen, and other related gases. Modern flaring facilities are designed to meet rigorous operation specifications to ensure effective destruction of volatile organic compounds (VOCs) and other similar compounds that may be present in the landfill gas (Tchobanoglous, 1993). On the other hand, because landfill gas has a heating value in the range of 400 to 550 BTU/scf (14,904 – 20,492 kj/m³, USEPA, 1995; Kortegast and Ampurch, 1997; Tchobanoglous, et al., 1993; and WSDE, 1987), it provides a potentially recoverable energy source (landfill gas-to-energy). In the United States, landfill gas was used as a commercial energy source for the first time in 1975. To encourage and facilitate the development of environmentally and economically sound landfill gas projects, the USEPA created the Landfill Methane Outreach Program (LMOP) in 1999. LMOP is a part of the United States' commitment to reduce greenhouse gas emissions under the United Nations Framework Convention on Climate Change. LMOP is a voluntary assistance and partnership program, which promotes landfill gas as an important local energy resource. As a result, LMOP has more than 320 partners that have signed voluntary agreements to work with EPA to develop cost-effective LFG projects as of November 2002. LMOP estimates that more than 340 operational Landfill Gas-to-Energy projects have been initiated in the United States. In addition, about 200 projects are currently under construction or under consideration. The LMOP also estimates that the environmental benefits for these projects are equivalent to removing the emissions of 12.7 million cars per year or powering 630,000 homes and heating 1.3 million homes per year (Bolton, 2003; USEPA, 2003).



Figure 2. Typical gas vents used in the surface a landfill for the passive control of landfill gas. (Organic Waste Technologies/LFG Specialties, Inc., Hickman, 1999)



Figure 3. Typical vertical gas extraction well and wellhead assembly. (Source: Organic Wastes Technologies/LFC Specialties, Inc., Hickman, 1999)

2.1.3 Landfill Gas Condensate Management

In landfills, gas temperature is typically $38 - 49^{\circ}$ C as generated and $16 - 49^{\circ}$ C at the wellhead (USEPA, 1995; Kortegast and Ampurch, 1997; Tchobanoglous, et al., 1993; USEPA, 1996; WSDE, 1987), and saturated with moisture. As the landfill gas reaches the surface and flows through the collection system, it drops in temperature until it reaches a dew point resulting in formation of condensate. Gas condensate has high organic acid levels and low pH. For example, the chemical oxygen demand (COD) cited by Barry and Kim (2000) ranged from 14,400 mg/l to 18,800 mg/l and pH was 4.3. Condensate must be drained from the collection system to prevent it from collecting in the piping system and interfering with, or even stopping, the gas flow. Most landfills install in-line chambers, known as condensate traps or knockouts, in the collection lines and headers to drain the condensate. One common method of condensate management is returning it to the landfill if the landfill has a composite liner. This helps increase landfill moisture content and thereby accelerates waste decomposition. Another option is to combine the condensate with leachate for processing at a wastewater treatment plant. Such treatment may be either on-site or off-site (Hickman, 1999).

2.1.4 Landfill Leachate Management

Leachate forms as water moves through decomposing solid waste, mainly due to the infiltration of rainwater or snowmelt (Hickman, 1999). Leachate contains organic and inorganic compounds and decomposition products, some of them arising from incomplete decomposition. The chemical components of leachate will vary, largely depending on the age of the landfill. (This will be discussed in more detail later.). If leachate migrates away from a landfill, surface and groundwater contamination may result. Consequently, control of leachate is needed to eliminate this possibility. Landfill liners are now commonly used to prevent the movement of leachate and landfill gases from landfill sites (Tchobanoglous, et al., 1993). Further, the USEPA has established criteria that require the installation of a single composite liner, having both a flexible membrane component and a soil barrier layer (Fig. 4, USEPA, 1993). Also, the USEPA criteria require the installation of a leachate collection and removal system under municipal solid waste landfills designed to limit the depth of leachate over the top of the liner to one foot or less (USEPA, 1991). The same wastewater treatment technologies used on domestic and industrial wastewater can be applied to treat leachate from landfills, although consideration must be given to the characteristics of the leachate.



Figure 4. Composite liner system (Source: USEPA, 1993)

Besides the above technical considerations, political issues play a large role in solid waste management. For example, the Fresh Kills Landfill in Staten Island, NY, one of the longest operating in the US and maybe the largest in the world, was closed in January 2002 amid great controversy. Since the closing of Fresh Kills, all wastes from New York City are exported to out-of-state landfills at a cost of more than \$313 million a year. Disposal costs for solid waste have grown from \$44 per ton in 1997 to over \$88 per ton in 2001. To overcome this challenge, the city plans to utilize and upgrade the city's existing marine transfer station system. The waste will be containerized and compacted at the transfer stations. Then the sealed containers can be barged to container ships, rail facilities or trucks. The city expects that this plan will increase the flexibility and options for disposal dramatically. In addition, the city has created a task force to re-evaluate its recycling program and to ensure appropriate long-term strategies, which is to be fully integrated into the solid waste management plan (The City of New York, Office of the Mayor, Press Release, 2002).

Another political issue across the United States has been the conflict between local governments and Federal government, and in particular the dispute over "Flow Control Regulations". In this case, local governments prevented out-of-state solid waste processors from having access to the local solid waste market, as a way to assure that the local government would have revenue to fund its solid waste management program. By forcing waste haulers to use local facilities, revenue for operating the local facilities could be assured. This guarantee of future revenue streams was then used to secure bond funding to develop transfer stations, landfills, incinerators and other needed infrastructure. However, the US Supreme Court overturned these regulations, because the flow control ordinances were found to violate the Interstate Commerce Clause of the United States Constitution. These decisions included the cases of C&A Carbone, Inc. v. Town of Clarkstown, New York [11 US 383 (1994)] and Atlantic Coast Demolition & Recycling, Inc. v. Board of Chosen Freeholders of Atlantic County, New Jersey [48 F. 3rd 701 (1995)]. As a consequence of these decisions, local and regional solid waste management plans were left without funding. The stranded debt was estimated at \$ 1.5 million in the town of Clarkstown, New York, and \$ 1.65 billion in the State of New Jersey. In New Jersey, the state government has been forced to intervene to prevent widereaching financial default at the local level.

Also, the import and export of wastes to other states is another political issue. Pennsylvania is the largest importer of solid wastes in the United States and the governor suspended the development of new solid waste disposal capacity within the State. Overall, it can be seen that both technical and political aspects are a concern in solid waste management. Increased demand for landfill capacity fuels political debate. By accelerating waste decomposition rates, more wastes can be placed into existing facilities. This will help to relieve political stress. One outcome of the research presented here will be a better understanding of waste decomposition, which will in turn help to reduce demand for new landfill capacity and ease political tensions.

2.2 Biological and Chemical Changes in the Landfill 2.2.1 The Process of Decomposition

Municipal solid waste (MSW) is typically classified according to material content, such as paper, plastics, glass, etc. However, the overall organic component of MSW is a more useful measure for biodegradation studies (Barlaz, 1997). The organic composition of MSW contains 40 to 50% cellulose, 10 to 15% lignin, 12% hemicellulose, and 4% protein (Booker and Ham, 1982). Cellulose and hemicellulose are readily biodegradable, while lignin is recalcitrant under anaerobic conditions (Young and Frazer, 1987). In the decomposition of the organic fraction of municipal solid waste, several trophic groups of microorganisms work together to create a stable end product, methane (Fig. 5, Brock, et al., 1994). Overall, a variety of different processes occur, with reactions taking place both in series and in parallel.

The first class of reactions involves the hydrolysis of large-molecular-mass compounds (carbohydrates, proteins, and fats) into soluble sugars, amino acids, longchain carboxylic acids, and glycerol. Polymers are not readily transported across microbial cell membranes; thus, biological hydrolysis of polymers in waste is mediated by extracellular enzymes produced by microorganisms present in landfills (Palmisano, et al., 1993). Fermentative microorganisms then break down these hydrolysis products into short-chain carboxylic acids, ammonia, carbon dioxide, hydrogen, and acetic acid.

In the next step, obligate proton-reducing acetogens oxidize fermentation products, propionate and butyrate to acetate, carbon dioxide and hydrogen. This process is thermodynamically favorable only at very low hydrogen concentrations (10⁻⁵ to 10⁻⁴ atm; Zehnder, 1978). Therefore, the obligate proton-reducing acetogens work only in syntrophic association with hydrogen scavengers such as methanogens or sulfate reducers. Another reaction carried out by acetogens is the production of acetate from carbon dioxide and hydrogen. This process, however, has not yet been observed in the landfill environment (Barlaz, 1997).



Figure 5. Overall process of anaerobic decomposition of biological polymers (After Brock, et al., 1994)

The final step in the decomposition process involves the conversion of complex polymers into simpler end products, principally methane and carbon dioxide. The organisms responsible for producing methane are referred to as methanogens. These are a very diverse group of the Archaea (Woese, et al., 1990), and are oxygen-sensitive, fastidious anaerobes. Despite the enormous phylogenetic diversity, as a group methanogens can only use a small number of simple compounds, most of which contain one carbon (Zinder, 1993). Most methanogens can grow on molecular hydrogen and CO_2 as sole energy sources (Balch, et al., 1979; Jones, et al., 1987), except for a few obligate methylotrophic and acetotrophic species (Müller, et al., 1993). However, most of the methane produced in nature originates from acetate. Acetotrophs grow more slowly than CO₂-reducers, therefore, methane from acetate is not likely to predominate where the residence time for organic matter is short (Ferry, 1993). Other substrates include formate, methanol, methylated amines, and methylated sulfides (Barlaz, 1997). Because many methanogens use only one or two substrates, methanogens are dependent on other organisms for their substrates. Therefore, a food web of interacting groups of anaerobes is required to convert most organic matter to methane as described earlier, in contrast to aerobic ecosystems, where single organisms can usually effect the complete oxidation of complex organic molecules to carbon dioxide (Zinder, 1993). As a consequence, the relative amounts of methane produced in anaerobic systems can vary depending on the presence of other metabolic groups of anaerobes and the environment (Ferry, 1993).

2.2.2 Microbial Succession

In the landfill, there is a process of microbial succession that takes place during the first several years after placement of waste. The biological and chemical reactions in the landfill are considered to establish themselves during five more or less sequential phases, as illustrated in figure 6 (Farquhar and Rovers, 1973; Parker, 1978; Pohland, 1987; Pohland, 1991). Phase I is the initial adjustment phase, where biological activity occurs under aerobic conditions soon after waste is placed in the landfill. All of the microbial groups that will subsequently play a role in waste decomposition are generally considered to be present from the outset. The principal source of microorganisms is the soil material, which is used as daily and final cover. Digested wastewater treatment plant sludge, which is disposed of in many MSW landfills, and recycled leachate are other sources of organisms (Tchobanoglous, et al., 1993).

In phase II, called the transition phase, the oxygen is consumed and anaerobic conditions are established. As the landfill becomes anaerobic, nitrate and sulfate, which can serve as electron acceptors in biological reactions, are frequently reduced to nitrogen and hydrogen sulfide gases. In this phase, the pH of the leachate begins to decrease because of the accumulation of organic acids and CO_2 within the landfill as hydrolysis and fermentation proceed.

In phase III, the acid phase, the microbial activity initiated in phase II accelerates with increasing production of organic acids. The major gas produced in this phase is CO_2 , while smaller amounts of H_2 will also be released.



Figure 6. Generalized phases in the generation of landfill gases (I = initial adjustment, II = transition phases, III = acid phase, IV = methane fermentation, and V = maturation phase, Farquhar and Rovers, 1973; Parker, 1978; Pohland, 1987; Pohland, 1991)

As discussed previously, in anaerobic microbial ecosystems, H_2 produced by some species, e.g., fermentative bacteria, is used by other species, e.g., methanogens. Therefore, although large quantities of H_2 are produced in anaerobic environments, H_2 often does not accumulate (Wolin and Miller, 1982). The pH of the leachate can drop to 5 or even lower due to the accumulation of carboxylic acids as a result of an imbalance between fermentative activity and acetogenic and methanogenic activity (Barlaz, et al., 1989). On the other hand, the biochemical oxygen demand (BOD₅) and chemical oxygen demand (COD) of the leachate will increase significantly because of the high organic acid concentrations. Heavy metals can also increase in the leachate while pH is low as well.

In Phase IV, the methane fermentation phase, the activity of methanogens becomes predominant and methane production increases. Within the landfill, many previous trends reverse. Leachate pH will increase, achieving a neutral range from 6.8 to 8, because organic acids are transformed to methane and CO_2 . Leachate concentrations of BOD₅ and COD, and conductivity measurements, will likewise decrease. Increased pH contributes to lower heavy metal concentrations in the leachate because of lower solubility. The system should be largely at steady state, with relatively stable gas and leachate compositions. Presumably the microbial populations will remain largely stable as well.

Over time, however, the rate of decomposition should slow down as readilydegradable substrates are consumed. Eventually, this brings the beginning of phase V (the maturation phase). The gas production rate will significantly decrease in this phase. The leachate will often contain humic and fulvic acids, which are resistant to further biological degradation (Tchobanoglous, et al., 1993).

In considering this conceptual framework, it is important to recognize that landfills are created over periods of many years. Wastes deposited at one time are later covered with more waste, creating a layered structure that may extend over many hectares and to depths of several tens of meters. Thus, at any given time the different vertical and spatial zones within the landfill may be experiencing different decomposition phases. Also, many factors associated with the heterogeneity of the waste environment, such as the moisture content, waste density, and the availability of nutrients, will affect the duration and character of each phase. Microbial communities should likewise be expected to differ throughout the structure.

2.3 Molecular Approaches for the Study of Microbial Communities

Only a small percentage of the microorganisms in the natural environment are cultivable (Amann, et al., 1995), and this in turn has limited the ability of researchers to study the general composition of microbial communities. Therefore, significant bias can enter our understanding of microbial communities in the environment when using cultivation-dependent methods. For this reason, culture independent methods, such as molecular techniques, are important tools for examination of microorganisms in their environment. Such techniques are hybridization with gene probes and polymerase chain reaction (PCR) amplification which have made possible a very specific and sensitive evaluation of the microbial world. In the present study, slot blot hybridization and PCR amplification were used to describe the microbial community of the landfill. The details of each technique are described below.

2.3.1 Quantitative Slot-blot Hybridization

Gene probes are small pieces of DNA known as oligonucleotides that can bind (hybridize) to nucleotide sequences with homologous sequences in the target microorganisms. With the use of gene probes, specific microbial populations can be detected in environmental samples (Atlas and Bartha, 1997). In particular, the use of rRNA-targeted oligonucleotide probes for hybridization has become a powerful tool for describing the structure of microbial communities in many environments (Amann, et al., 1995; Stahl, 1995). 16S rRNA-targeted specific oligonucleotide probes are the most commonly used because rRNA is among the most conserved macromolecules in all living systems (Atlas and Bartha, 1997). 16S rRNA contains large enough molecules (1500 nucleotides) to compare sequences, but small enough to handle for analysis. The "S" stands for "Svedberg unit", a measurement of the rate at which proteins settle during centrifugation.

The basic protocols of hybridization were outlined by Atlas and Bartha (1998) and are summarized here in figure 7. First, single-stranded (denatured) target nucleic acids are attached to a membrane filter surface. Second, the filters are prehybridized to block nonspecific nucleic acid binding sites. Third, labeled probe is added to the membrane filters and the probe is allowed to hybridize. Probe is often labeled with radioactive chemical. Because of their high specific activity, ³²P-labeled nucleotides are routinely used to label nucleic acids in cell-free system (Lodish, et al., 1995). In addition, several nonradioactive alternatives are available. Such alternatives include probes labeled with digoxigenin (DIG), biotin, or fluorescein, which can be incorporated into the sequence by chemical synthesis (Marlowe, et al., 1999). After hybridization, excess unbound labeled probe is washed off and the hybrid (target-probe) sequences are detected by exposure Xray film, a process called autoradiography. During autoradiography a photon of light, beta particle, or gamma ray emitted by the filter activates silver bromide crystals on the film. When the film is developed, the silver bromide is reduced to silver metal and forms a visible grain or black spot on the film. This positive image indicates that the radioactively-labeled probe annealed to the target nucleic acid on the filter, and this in

turn implies that hybridization between the probe and the sample nucleic acid has occurred (Marlowe, et al., 1999).



Add radio labeled probe

Hybridize probe and wash away unbound labeled probe. Detect labeled hybrids by exposing X-ray film

Figure 7. Illustration of nucleic acid hybridization, which is the basis for gene probe detection (Adapted from Atlas and Bartha, 1997)

Nucleic acids can simply be placed directly on the membrane surface, although filtration manifolds have been designed to spot the samples in an evenly spaced manner with a large number of samples. This makes it is easy to scan film for quantification. Typically, deposits are made as thin lines or circles, referred to as "slot blots and "dot blots", respectively. Many types of manifold are commercially available, e.g., Minifold II by Schleicher and Schuell, and Bio-dot SF by Bio-Rad.

Using slot-blot hybridization, a certain 16S rRNA associated with a particular class of organism can be roughly quantified in comparison with the total amount of 16S rRNA in a sample. In a common approach, rRNAs from pure cultures at different concentrations are applied to the membranes, hybridized, and the signal intensity is measured. The least-squares method is then applied to construct reference RNA standard curves with RNA concentrations vs. signal intensity. The approximate amounts of each specific group of organism in the sample can now be calculated from these standard curves. Each amount is expressed as a percentage of the total amount of 16S rRNA that hybridizes to a universal probe, i.e. one that is complementary to a region of virtually all 16S-like rRNAs so far characterized (Pace, et al., 1986). Also, universal probes serve to normalize results obtained with probes targeting specific phylogenetic groups of microorganisms (Zheng, et al., 1996).

There are several advantages of using rRNA as a target to detect microorganisms. First, in active cells, rRNA molecules have a relatively high copy number per cell $(10^3 - 10^5)$, and so the rRNA can often be detected without amplification (Amann, et al., 1995). Second, huge sequence databases are currently available (Benson, et al., 1993; Rice, et al., 1993; Maidak, et al., 1994). With this sequence information, it possible to design
probes for species-specific or more general identification (at genus or family level). Furthermore, probes can be designed for non-culturable bacteria (Amann, et al., 1995). Another advantage is that it becomes possible to monitor microbial activity change by 16S rRNA hybridization techniques. For examples, it has been demonstrated that a correlation exists between RNA content and the growth rate of *E. coli* (Gausing, 1977), *Salmonella typhimurium* (Kjelgaard and Kurland, 1963), *Pseudomonas stutzeri* (Kerkhof and Ward, 1993), several marine isolates (Kemp, et al., 1993) and the sulfate reducing strain PT2 (Poulsen, et al., 1993). This correlation between RNA content and cell activity suggests that microbial activity can be monitored using hybridization with 16S rRNA targeted probes, rather than cell numbers.

However, there are several limitations to the use of rRNA targeted probe hybridization for quantification. First, the recovery of nucleic acids from the environment is complicated due to the variety of different environments and organism types (Stahl, 1995). This can be a general problem with application of molecular techniques to environmental samples. For example, the recovery of nucleic acids might be reduced by degradation or adsorption to matrix material, such as clays. Also, the recovered nucleic acids from an environmental sample could come from detritus or other sources, such as humic acids, instead of intact bacteria. Therefore, it is often necessary to use extensive purification steps for nucleic acid analyses for environmental samples. Even selection of purification is the degree to which recovered rRNA is representative of the total rRNA pool. Microorganisms have different cell wall structures, and therefore a cell that has an easy-to-break wall would be overestimated, while a cell that resists breakage would be underestimated. Environmental samples are even more complicated. The mechanisms of nucleic acid recovery from environmental samples are often poorly understood, so it is difficult to predict whether all organisms are lysed with equal efficiency or what fraction of the total nucleic acid is recovered. For example, Leff, et al. (1995) compared three different published DNA extraction methods and then used them on stream sediments in Aiken, SC. DNA yield varied with the different extraction methods, and one method was recommended by the authors for PCR amplification while another was preferred for Southern blots. Likewise, Niemi, et al. (2001) found that different lysis and purification methods affect PCR-DGGE analysis results when applied to soil microbial communities. Therefore, selection of appropriate lysis and purification methods must be based on experimental goals, and direct comparison of results from different methods may not be possible.

Another potential problem is the unclear relationship between the amount of rRNA and the actual numbers of cells present, since the content of rRNA per cell differs between species and with growth rate (Devereux, et al., 1996; Kemp, et al., 1993). Relative rRNA abundance cannot directly predict cell number. Therefore, it is possible that actual cell numbers of certain organisms are overestimated, while others are underestimated. To estimate biomass, an independent method would be required. On the other hand, the relative rRNA abundance should provide a reasonable measurement of the relative physiological activity of a respective population because it is the product of the number of detected cells and the average rRNA content (Wagner and Amann, 1997).

Another drawback is that the available database of sequences is still limited. Probes designed based on available databases (known sequences), and their specificity can only be evaluated against culturable reference organisms (Devereux, et al., 1992; Manz, et al., 1992). Therefore, with existing probes, the diversity of populations from any given environment would be underestimated. In addition, previously unknown populations may not be detected (see, for example, Daims, et al., 1999). In the case of unstudied environments, such as landfills, this limitation may be even more severe. However, this limitation can be addressed with greater adequacy as existing sequence databases grow more comprehensive, since this will provide refined information on the true specificity of old probes and allow better design of new probes (Wagner and Amann, 1997).

2.3.2 Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR), introduced by Saiki and colleagues in 1985, is an enzymatic reaction that allows amplification of specific DNA regions through a repetitive process. This process involves template denaturation, primer annealing, and the extension of the annealed primers by DNA polymerase resulting in a duplication of the starting target material. This process is repeated many times (usually 20 –30 cycles) resulting in an exponential increase in the amount of target DNA. The amplification product is visualized by agarose gel electrophoresis.

Although the PCR technique was originally used for genetic and clinical purposes, this technique has been used to detect and monitor microorganisms in complex environmental samples for a number of years (Bej, et al., 1991b; Steffan and Atlas, 1991). By exponentially amplifying a target sequence, PCR significantly increases the probability of detecting rare sequences in mixtures of DNA. Numerous studies have reported the detection of specific microorganisms in water, soils and sediments by PCR amplification without the need for cell cultivation (Bej, et al., 1991a, 1991c; Pillai, et al., 1991; Tsai and Olson, 1992a). Brauns, et al. (1991) detected a viable but nonculturable organism, *Vibrio vulnificus*, from a microcosm.

The advantages of PCR include speed, simplicity, and an inherent sensitivity to small amounts of DNA (Mullis and Faloona, 1987). However, there are some limitations to the PCR method. Specifically, the size of the region that can be amplified is limited and it is required that sequence information be known about the target. As a result, this means PCR amplification is most useful for DNA, for which there is already partial knowledge. Also, contamination results in false positives, therefore it is important to run controls (without DNA template) along with samples. It is often much more difficult to apply the PCR technique to environmental samples for these same reasons. Therefore, the following limitations should be addressed. First, the amount of samples that can be processed is limited because the PCR template is limited to only a few microliters. However, this limitation can be overcome to some extent by innovative sample processing. Second, the presence of PCR-inhibitory substances hinders the amplification process, and therefore extensive purification steps are often required from environmental samples. Also, samples must be subjected to PCR amplification by use of appropriately designed primers. Even with these disadvantages, the PCR technique has allowed environmental microbiologists to study important questions that were previously impossible to address with culture-dependent methods.

In addition, DNA fingerprint methods are commonly used for community structure study, such as denaturing gradient gel electrophoresis, DGGE (Gurtner et al.,

2000; Kozdrój and Elsas, 2001; Yang, et al., 2001) and restriction fragment length polymorphism, RFLP (Sandaa, et al., 2001; Dunbar, et al., 1999; Sessitsch, et al., 2001). Mostly, these methods are used in conjunction with PCR resulting in production of certain banding patterns. In the DGGE method, the universal primers derived from conserved 16S rDNA sequences are used to generate PCR amplification products of nearly identical lengths, but with variable sequence composition. Then these PCR amplification products are separated based on changes in the electrophoretic mobility of different DNA fragments migrating in a gel containing a linearly increasing gradient of DNA denaturants (urea/formaldehyde or temperature). Changes in fragment mobility are results from partial melting of double-stranded DNA in discrete regions, the so-called melting domain. Sequence variation within such domains changes their melting behaviors, and different PCR amplification products stop migrating at different positions in the denaturing gradient (Lerman, et al., 1984). DGGE analysis of PCR-amplified 16S rDNA fragments provides a rapid method to characterize community population structure. However, this method doesn't provide direct information on identity of specific microbial populations. More specific information of population composition can be obtained by secondary analysis of the DGGE banding pattern via sequencing or hybridization (Stahl, 1997).

In RFLP analysis, the amplified DNA using universal primers contains unique sequences that can be cut into smaller fragments by restriction enzymes and the fragments of DNA are usually separated by gel electrophoresis. The pattern of these fragments produces a fingerprint of the bacterial communities. RFLP analysis is most often used to identify specific bacterial isolates because the banding patterns produced by RFLP couples with gene probe analysis are unambiguous. In contrast, PCR-generated fingerprints can be difficult to reproduce and may contain "faint" or "ghost" bands, making interpretation difficult (Burr and Pepper, 1997). However, the choice of restriction enzymes used is usually empirical, and normally multiple enzymes must be used (Marlowe, et al., 1999).

Even with limitations such as those described previously, molecular techniques provide powerful tools over conventional culture dependent methods. Each method has advantages and disadvantages, and therefore methods should be chosen dependent on research interests. Combination of more than one method, even with culture dependent methods, will often give more accurate information. In the present study, the PCR method was used to analyze the diversity and distribution of microorganisms, and the results from slot blot hybridization provided quantitative information about the microbial community of the landfill.

2.4 Growth of Pure Cultures of Anaerobic Organisms

While this study sought to minimize the need to culture landfill organisms, it was nonetheless necessary to verify the accuracy and performance of molecular techniques with reference strains. As a result, it was necessary to apply anaerobic culture techniques since they were used to grow the reference strains for this study. A brief review of general methodology is presented here.

The most important requirement for growth of anaerobes is the maintenance of a highly reduced anaerobic environment. To obtain such an anaerobic environment, the primary tools are oxygen-free gases and materials that minimize the penetration of air from outside the anaerobic container. Modern approaches to the laboratory culture of obligately anaerobic microorganisms can be traced back to the work of Hungate (1950). In Hungate's methodology, medium is boiled to remove oxygen and an oxygen-free gas mixture flows from a gassing cannula into the medium to exclude further introduction of oxygen. However, these are not sufficient to remove all dissolved oxygen in the growth medium. Therefore, a reducing agent is added to lower the redox potential (typically, a mixture of cystein hydrochloride and sodium sulfide) and a redox-sensitive dye (e. g., resazurin) is supplied to indicate the oxidative state of the medium (Sowers and Noll, 1995).

Moore (1966) improved on the original gassing cannula technique by introducing a new anaerobic tube that had a reinforced tapered neck to minimize breaking when sealing with a stopper (Fig. 8a). The tubes clamp with a tube press to secure the stopper during autoclaving. Later, Miller and Wolin (1974) introduced further improvements by developing an anaerobic tube that featured a neck like that found on serum bottles (Fig. 8b). The tube can be sealed with an exceptionally thick butyl rubber stopper, which minimizes oxygen diffusion into the medium. The stoppers are then secured using aluminum crimp seals, which prevent the stopper from popping out as a result of pressure increases during methanogenesis or autoclaving. Since the thick rubber stopper can endure multiple needle punctures, the anaerobic gas mixture can easily be exchanged in the tube, and anaerobic cultures are simply inoculated and transferred by using a syringe to minimize exposure to oxygen. During this same period, the Aranki-Freter anaerobic glove box became commercially available, which provided a means of plating anaerobes on standard petri dishes without the use of gassing cannula (Edwards and McBride, 1975; Balch and Wolfe, 1976). Generally, any conventional techniques that would usually be performed at the benchtop can be performed with anaerobes in the anaerobic chamber (Sowers and Noll, 1995).



Figure 8. Culture tubes for anaerobic growth medium. The anaerobe tube (A) with the reinforced tapered neck is sealed with a conical butyl rubber stopper, and must be placed into a special press to remain tightly sealed. This system has largely been replaced with the crimp style tube (B), which is sealed with a butyl rubber septum secured in place with an aluminum crimp seal (after Sowers and Noll, 1995).

2.5 Cell Collection from Landfill Gas

While no previous literature was found that describes the sampling of

microorganisms from landfill gas, numerous studies have considered the collection of

airborne organisms from the ambient environment. The three major sampling methods

for airborne microorganisms are impaction, impingement, and filtration (Buttner, et al.,

1997). Impaction is the most commonly used method, where bioaerosol is divided from

the air-stream by utilizing the inertia of the particles to impact onto a soft agar or solid glass surface (Fig. 9a). The principle advantage of this method is that it allows direct collection of a bioaerosol onto culture media, therefore avoiding the need for further dilution or plating. However, it is biased towards viable microorganisms, and the collection surface is easily overloaded with high levels of viable microorganisms (Thorne, et al., 1992).

In the impingement method, bioaerosols impact into liquid, usually a dilute buffer solution, rather than onto an agar surface or a glass slide (Fig. 9b). The liquid impingement method overcomes the overloading problem when high bioaerosol concentrations are present, because microorganisms can be diluted in the liquid. Further, the liquid sample can easily be concentrated by filtration when low concentrations of microorganisms are present. The liquid samples may also be used for additional analysis, such as biochemical, immunological, and molecular biological assays. There are several limitations with impingement methods, such as the loss by re-entrainment in the exhaust flow caused by hydrophobicity, agitation, and misting within the impinger (Muilenberg, 1989). In addition, some organisms may suffer from the effects of sudden hydration upon impingement or osmotic shock (Cox, 1987).

In contrast, filtration methods collect bioaerosols onto filter membranes as the air passes through the filters. Inertial forces and other mechanisms such as interception, diffusion, and electrostatic attraction result in the collection of particles on the surface of the filter (Buttner, et al., 1997; Fig. 9c). In ambient air sampling, the filters are typically contained in disposable plastic cassettes, and a vacuum pump is required to draw sample through the filter. Filtration methods are simple, low cost, and different filter materials are available depending on the nature of the cells and the sample analysis method. Filtration methods can collect particles with almost 100% efficiency, down to particle sizes of about 0.1µm (Chatigny, et al., 1989). However, some cells can be dehydrated during sampling, resulting in loss of viability, damaging their biological activity and making detection difficult (DeCosemo, et al., 1992; Nevalainen, et al., 1993). Another potential limitation is the poor cell recovery from the filter surface. Despite these limitations, filtration methods continue to be used because of their simplicity and their ability to provide information on both viable and nonviable organisms (Palmgren, et al., 1986).

There is no single sampling method suitable for all types of bioaerosols, nor are standardized methods currently available for many situations. Therefore, it is often difficult to make comparisons between different sampling methods. In addition, ambient techniques cannot be directly applied to landfill gas sampling because of the high (condensing) moisture concentrations, and the enclosed nature of landfill gas collection systems. However, a study by Barry and Kim (2000) found that microorganisms from landfill gas and gas condensate could be collected by using a filtration method. This study is summarized in appendix A. In addition, numerous recent studies (Bartlett, et al., 1997; Olsson, et al., 1998; Stärk, et al., 1998; Maher, et al., 2001) reported the application of molecular techniques to the analysis of airborne microorganisms which were collected through filtration methods. Nucleic acids were directly extracted from filters for molecular analysis to avoid culturing. Consequently, for the present study, a filtration method was chosen to collect cells from landfill gas, and nucleic acids were then directly extracted from the filters prior to application of molecular techniques.



Figure 9. Mechanisms of collection utilized in bioaerosol sampling. A: solid plate impaction; B: liquid impingement; C: filtration; F: inertial force. (Adapted from Nevalainen, et al., 1992)

3. Materials and Methods

3.1 Sampling Site and Collection Methods

Samples for this study were collected from Burlington County Resource Recovery Complex, Burlington County, NJ. It is an operating municipal sanitary landfill approved by the New Jersey Department of Environmental Protection (DEP). Currently, fourteen commercial sanitary landfills are operating in New Jersey (Fig. 10). Burlington County landfill is located on the west side of the State near the town of Columbus. According to state records, the types of wastes permitted in Burlington County landfill are municipal waste (household, commercial and institutional), bulky wastes, construction and demolition debris, vegetative waste, animal and food processing waste, dry industrial waste, and waste material consisting of incinerator ash or ash containing waste.

3.1.1 Collection of Waste Samples

Landfill wastes were excavated to install gas wells as a part of ongoing environmental activities at the site. A 36-inch (91.4 cm) diameter bucket auger was used to drill the well holes. For this study, wastes were collected from four well sites between August 21 and August 28, 2000. Two wells were chosen in the older part of the landfill, where waste age was approximately 10 years (Wells No. 45 and 8A). Two other wells (Wells No. 41 and 38) were sampled where waste age was approximately 2 - 3 years. The depths of Wells No. 41 and 38 were both 90ft (27.4 m). Wells 45 and 8A had 60ft (18. 3 m) and 46ft (14.0 m) depths, respectively.



Figure 10. Map of commercial sanitary landfills which are currently operating in New Jersey State. Burlington County is located in the southwest of the State (NJ Department of Environmental Protection)

During the present study, excavated wastes were collected every 20 ft (6.1 m) and placed in air tight glass mason jars with metal lids (Ball. Broomfield, CO), purged with nitrogen gas and transported to the laboratory on ice. Immediately after arrival at the laboratory, the waste samples were transferred to storage at -20°C until processed.

3.1.2 Cell Collection from Landfill Gas

Landfill gases were collected around 1 - 1.5 year after the gas extraction wells were installed. Gas from areas containing older waste was collected at Wells 45 and 8A, where wastes were collected previously. Landfill gas from relatively new sites (Wells 38 and 41), where wastes were collected previously, could not be sampled due to final cover construction during the sampling period. Instead, gas from young waste was collected at Wells 28 and 33. These wells were the nearest accessible wells from Wells 38 and 41 during the sampling period. Gas sampling was done approximately once a week between the summer of 2001 and the spring of 2002.

Landfill gas was withdrawn from gas extraction wells by applying a vacuum (dry rotary vane vacuum pump, EW-07055-60, having a nominal free-air capacity of 4.5 cfm (133 L/min), Cole-Parmer. Chicago, IL) to well heads. However, actual gas flow rates through the filters were less than the nominal free-air flow rate of the pump due to resistance in the filter, and so it is not known exactly what sample volume was passed through each filter. The filter surfaces were overloaded with particles after approximately three hours sampling period in many cases. Thus, in this study, sampling generally took around three hours per filter.

Landtec brand well heads were installed on each gas extraction well, and the sampling collection apparatus was attached to ports on the well heads. Cells were collected on Millipore Express polyethersulfone (PES) membranes (25 mm diameter) with pore size of 0.22 μ m, which were mounted in sterile filter holders (Millipore Swinnex). The PES membrane is hydrophilic and offers fast flow, high filter capacity and low protein binding while remaining bacterially retentive. Filter holders were attached to well heads through sterile tubing and syringe barrels (Fig. 11). Gas temperatures typically ranged between 44 °C and 47 °C at the surface.

Because the gas is saturated with water vapor while in the waste, condensate forms when the gas cools upon reaching the surface. If this condensate collects on the filter membrane, gas flow will stop and no sample will be collected. In order to prevent formation of condensate on the membranes, the gas wells and filter holders were wrapped in heating tapes (Omega. Stamford, CT) and then insulated with commercially available closed-cell foam pipe insulation. The temperature of the heating tape was set as close to the gas temperature as possible with temperature controllers (Omega. Stamford, CT) in order to keep vapor from condensing prior to filtration. Two complete collection systems were simultaneously attached to the well head of one extraction gas well, so samples were collected onto two separate filters at the same time. Figure 12 shows the gas sampling process in the landfill. Immediately after collection, filters placed on ice in a cooler. After transport to the laboratory, filters were kept at -20°C until further analysis.



Figure 11. Filtration apparatus to collect cells from landfill gas well head, before attachment of filters to pumps or application of heat tape and insulation. When not being used for sampling, the well head was covered with the rubber cup shown hanging in the foreground in order to keep the sample ports relatively clean.



Pipe insulation (heating tape was inside)

Figure 12. Gas collection process to collect cells from landfill gas well, showing pumps, insulation and heating controller. The valve and pipe leading to left at top of picture are permanent parts of the landfill's gas collection system. The portable electric generator was kept at a considerable distance to prevent possible explosion hazards in the vicinity of the highly flammable landfill gas.

3.1.3 Cell Collection from Landfill Gas Condensate

Landfill gas condensate (LFGC) was collected from the landfill's condensate collection system at a point where the condensate was draining into a condensate sump. The condensate in the sump arose from numerous wells and had traveled over 100 m from the well heads. Gas condensate was collected into a clean bucket and transferred into 15 L polycarbonate Nalgene carboys. Carboys were transferred to the laboratory on ice. Immediately upon arrival at the laboratory, the landfill gas condensate was passed through Sterivex filter units (Millipore Corp. Bedford, MA) to concentrate microbial biomass, as suggested by Somerville, et al. (1989). Sterivex filter units have filters inside a cylindrical polyvinyl chloride housing and are designed for pressure-driven concentration of biomass. In this study, Sterivex GP filter units were used, which have Millipore Express polyethersulfone (PES) membranes with pore size 0.22 µm. Gas condensates were aseptically pumped through filters via a Masterflex pump (Cole-Parmer. Chicago, IL) using the layout shown in figure 13. Filtration continued until flow rate slowed, where gas condensate volume was usually around 1.5 - 2.0 L. After filtration, filters were rinsed with SET buffer (20% sucrose, 50 mM EDTA, 50 mM Tris hydrochloride [pH 7.6]; Rodriquez, et al., 1983). Then the inlet port was capped with the luer-lock end of a 3 or 5 ml disposable syringe while the outlet port was capped with a syringe tip cover, which was cut about in half. The filters were stored at -20°C until analyzed.



Figure 13. The scheme of filtration process to concentrate cells from landfill gas condensate.

3.2 Growth of Pure Cultures

To verify the performance of molecular techniques, the following strains were used as references: *Pseudomonas aeruginosa* (ATCC 10145) was used as a *Bacteria* representative, while *Saccharomyces cerevisiae* (ATCC 4108) was used as a *Eucarya* reference strain. *Methanococcus thermolithotrophicus* (ATCC 35097), *Methanosarcina acetivorans* (ATCC 35395) and *Methanosaeta concilii* (DSM 3013) were used as *Archaea* reference strains. Each strain was purchased from the American Type Culture Collection (ATCC. Manassas, VA) or Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSM. Braunschweig, Germany). Preparation of culture media and growth conditions were as described by ATCC and DSM protocols. A brief description of growth conditions is presented here.

P. aeruginosa (ATCC 10145) was grown under aerobic conditions at 37°C in nutrient broth (Difco 0003. Detroit, MI). *S. cerevisiae* (ATCC 4108) was grown at 30°C in YM broth (Difco 0711. Detroit, MI).

For anaerobic cultures, preparation medium and inoculation were performed as described earlier. The anaerobic tubes with extra thick rubber stoppers and aluminum seals were purchased from Bellco Glass (Vineland, NJ). For larger volumes, 125 mL serum bottles were used (Wheaton, Fisher Scientific). For anaerobic media preparation, a low-cost gassing manifold was used as described by Sowers and Moll (1995, Fig. 14). *M. thermolithotrophicus* (ATCC 35097) was grown at 60 °C in ATCC medium 1439 under a gas mixture of 80% H₂ + 20% CO₂. *M. acetivorans* (ATCC 35395) was grown at 37 °C in ATCC medium 1355 under a gas mixture of 80% N₂ + 20% CO₂. *M. concilii* (DSM 3013) was grown at 35°C in DSM media 334 under a gas mixture of 80% N_2 + 20% CO₂. Detailed media components and preparation are described in appendix B.

The cells were harvested by centrifugation at $4,303 \times g$ (in a Sorvall SS34 rotor at 6,000 rpm) for 15 min at 4°C. The supernatant was discarded, and the cells were resuspended in fresh growth medium. Then cell pellets were collected after centrifugation at $14,462 \times g$ (in a Sorvall SS34 rotor at 11,000 rpm) for 30 min at 4°C. The cells for nucleic acid extraction were stored at -80°C.



Figure 14. Apparatus for dispensing anaerobic media with gassing cannula (Sowers and Noll, 1995). The diagram shows a flask containing de-oxygenated medium and pipette that will be used to transfer the medium to the culture tubes. The flask, the pipette and the tubes are all flushed with oxygen free gas during the procedure.

3.3 Nucleic Acid Extraction

In order to avoid culturing, nucleic acids were directly extracted from landfill gas, gas condensates and wastes. In the case of RNA extraction, RNA is easily degraded by RNAases. Therefore, special precautions as described by Maniatis, et al. (1982) were used throughout the RNA extraction.

3.3.1 Nucleic Acid Extraction from Pure Cultures

RNA was extracted from *P. aeruginosa* (ATCC 10145), *S. cerevisiae* (ATCC 4108), and *M. acetivorans* (ATCC 35395). 3 mL of Na-Ac buffer (50 mM Na-Ac and 10 mM EDTA, Thomm and Gohl, 1995) and 300 µL 10% SDS were used as lysis buffer. Cells were disrupted by mechanical agitation for 3 min with a Beadbeater (BioSpec Products, Inc. Bartlesville, OK) in the presence of 0.1 mm ice-cold and acid-washed glassbeads (0.5 mm glassbeads for *S. cerevisiae*). After disruption, lysates were transferred into microcentrifuge tubes. Then, nucleic acids were extracted and purified 3 times with phenol-isoamyl-chloroform (125:24:1, pH 4.3. Fisher Scientific. Fair Lawn, NJ). Finally, nucleic acids were extracted with chloroform and precipitated with ethanol overnight at -20°C. After 30 min centrifugation at 14,000 rpm and 4°C, RNA pellets were rinsed with cold 70% ethanol. To remove residual DNA, DNase (DNA-free kit. Ambion. Austin, TX) was used according to the manufacturer's instructions. The amount of RNA was determined by spectrophotometer at 260 nm and the integrity of RNA preparation was determined by agarose gel electrophoresis.

DNA was extracted from *P. aeruginosa* (ATCC 10145), *M. concilii* (DSM 3013), and *M. thermolithotrophicus* (ATCC 35097). Except for *M. thermolithotrophicus* (ATCC 35097), 3 mL of TE buffer (10 mM Tris- Cl [pH 7.6] and 1 mM EDTA), 300 μL of 10% SDS were used as lysis buffer. Cell disruption was carried out with mechanical agitation as described previously. Cell disruption of *M. thermolithotrophicus* (ATCC 35097) was carried out by incubating in a lysis solution overnight at 4°C as described by Hoaki, et al. (1994). Then, DNA was extracted and purified with phenol-isoamyl-chloroform (25:24:1, pH 8.0, buffered with Tris, Fisher Scientific. Fare Lawn, NJ). Precipitation and collection of the DNA pellet was done as described previously.

3.3.2 Nucleic Acid Extraction from Landfill Gas Condensates

Cell lysis for condensate samples was performed based on the method described by Somerville, et al. (1989) in which cell lysis and proteolysis were carried out within the filter housing. After the crude lysates were drawn off from the filter unit, nucleic acid extraction, purification and precipitation were done as described previously.

3.3.3 Nucleic Acid Extraction from Landfill Gas

For landfill gas samples, the RNA was extracted directly from the filters by mechanical disruption. Each filter was cut into quarters with an industrial razor blade (VWR Scientific. Media, PA), which was cleaned with ethyl alcohol. The lysis buffer contained 3 mL SET buffer (20% sucrose, 50 mM EDTA, 50 mM Tris hydrochloride [pH 7.6]; Rodriquez, et al., 1983) and 300 μL of 10% SDS. Cell disruption, nucleic acid extraction, purification, precipitation and removal of DNA were achieved as described previously.

For DNA extraction, cell lysis was carried out directly on the filter by incubating the filter in a lysis solution described by Maher, et al. (2001). After incubation, lysates were transferred to microcentrifuge tubes and then DNA extraction, purification and precipitation were performed as described previously.

3.3.4 Nucleic Acid Extraction from Landfill Wastes

Five grams (wet basis) of landfill waste was added to a homogenizing chamber containing 15 ml – 20mL extraction buffer, 1.5 – 2 mL of 10 % SDS, 5g of 0.5 mm glass beads and 5g of 0.1 mm glass beads. For RNA extraction, the extraction buffer contained 20% sucrose, 50 mM EDTA, 50 mM Tris hydrochloride (pH 7.6), 1.7% polyvinylpyrrolidone (PVP) and skim milk (0.1g/25mL, Becton Dickinson. Sparks, MD). For DNA extraction, the extraction buffer contained TENP buffer (50 mM Tris, 20 mM EDTA, 100 mM NaCl, 1 % [wt/vol] polyvinylpolypyrrolidone, PVPP; Picard, et al., 1992). PVP (Felske, et al., 1996), skim milk (Volossiouk, et al., 1995) and PVPP were used to remove contaminants, which are mostly humic substances. Tris and EDTA (Ethylenediamine-tetraacetic acid) are known to protect the DNA from nuclease activity (Picard, et al., 1992). Cell lysis was carried out by mechanical disruption as described previously. After disruption, glass beads, particles, and cell debris were removed by centrifugation at $4,303 \times g$ (in a Sorvall SS34 rotor at 6,000 rpm) for 10 min at 4°C. The supernatant was collected into a new tube. The pellet was washed twice with fresh extraction buffer. After washing, the supernatant was collected and combined with previous supernatant, and the pellet was discarded. The supernatant was then centrifuged at $14,462 \times g$ (in a Sorvall SS34 rotor at 11,000 rpm) for 30 min at 4 °C. The resulting

supernatant was discarded and the pellet was resuspended with buffer. For RNA extraction, AE buffer (20 mM Na-Ac [pH 5.5] and 1 mM EDTA), and for DNA extraction, TE buffer (10 mM Tris-HCl [pH 7.6] and 1 mM EDTA) were used. Then nucleic acids were extracted, purified and precipitated as described previously.

The crude RNA extracts ranged from colorless to yellow to dark brown. Yellow and dark brown RNA extracts were purified with an Rneasy mini kit (Qiagen. Valencia, CA). First, guanidinium isothiocyanate (GITC)-containing buffer and ethanol were added to the sample to provide conditions which promote selective binding of RNA to the Rneasy membrane. The sample was then applied to the Rneasy mini spin column. RNA bind to the membrane, contaminants were efficiently washed away, and high-quality RNA was eluted in water. After purification with the kit, most samples appeared colorless, however, some of the extracts still appeared slightly yellow.

The crude DNA extracts ranged from yellow to dark brown, and were purified with a Wizard DNA Clean-up System (Promega. Madison, WI). This system is a simple batch binding and column washing method for purifying DNA from contaminants such as enzymes, nucleotides and salts. The purification treatments were carried out according to manufacturer's instructions. After purification, all DNA extracts appeared colorless.

3.4 rRNA Quantification by Slot-blot Hybridization

Group-specific hybridization probes targeting the 16S rRNA of *Bacteria* (EUB338, positions 338 to 355; Amann, et al., 1990), *Archaea* (ARC914, positions 943-915; Raskin, et al., 1994a) and *Eucarya* (EUK516, positions 502 to 516; Amann, et al., 1990) were used to measure the abundance of the corresponding populations in the

landfill microbial community. The abundances of each domain were expressed as percentages of the total 16S rRNA. The total 16S rRNA in each sample was determined by hybridization with universal probe (UNIV1390, Zheng, et al., 1994). The universal probe targets practically all organisms known at present. The sequences for each probe are given in table 1. Oligonucleotide probes were purchased from Integrated DNA Technologies Inc. (Coralville, IA).

Probe/ Primer	Target	Sequence (5'-3')	Tm (°C)
Probe			
UNIV1390	All know organisms,	GACGGGCGGTGTGTACAA	44
	16S, 18S rRNA		
EUB338	Bacteria 16S rRNA	GCTGCCTCCCGTAGGAGT	54
ARC915	Archaea 16S rRNA	GTGCTCCCCGCCAATTCCT	56
EUK516	Eucarya 18S rRNA	ACCAGACTTGCCCTCC	52
Primer			
f27 (f)	Bacteria 16S rRNA	AGAGTTTGATCMTGGCTCAG	60
r1492 (r)	Bacteria 16S rRNA	TACGGYTACCTTGTTACGACTT	60
1Af (f)	Archaea 16S rRNA	TCYGKTTGATCCYGSCRGAG	55
1100Ar (r)	Archaea 16S rRNA	TGGGTCTCGCTCGTTG	55
ME1 (f)	MCR α subunit	GCMATGCARATHGGWATGTC	50
ME2 (r)	MCR α subunit	TCATKGCRTAGTTDGGRTAGT	50

Table 1. Oligo Probes and PCR Primers Used in This Study

f: forward primer; r: reverse primer

Tm: final washing temperature for probe and annealing temperature for primers D: A, G or T; H: A, C or T; M:A or C; K:G or T; S:C or G; R:A or G; Y:C or T; W: A or T

Slot-blot hybridization was performed as described by Stahl, et al. (1988) and

Raskin, et al. (1994a), but the protocol was slightly modified. RNAs, extracted from pure

cultures and landfill samples, were denatured by the addition of 3 volumes of 2%

glutaraldehyde (Sigma) in 50 mM sodium phosphate (pH 7.0). After 10 min incubation at

room temperature, dilution water was added to obtain a final volume of 50 mL. Dilution water made up with RNAse-free water containing 1 µg of poly (A) (Amersham Biosciences. Piscataway, NJ) per ml and 0.02 µl of 2% bromophenol blue per ml. Then RNAs were loaded onto nylon membranes (Hybond-N. Amersham Life Science. Buckinghamshire, England) using a slot-blotting apparatus (Bio-Dot SF. Bio-Rad. Hercules, CA). The membrane was air dried and baked at 80°C for 2 hours to immobilize RNA.

Probes were prepared as follow. First, each oligo probe was mixed with 3μ l of 10 × kinase buffer (Roche Diagnostics Corp. Indianapolis, IN), 1.5 µl of 1% Nonidet P40 (IGEPAL CA-630. Sigma. St. Louis, MO), 1 µl polynucleotide kinase (3' phosphatase free; Roche Diagnostics Corp. Indianapolis, IN), an equimolar amount of [γ - ³²P]ATP at specific activity of > 7,000 Ci/mM and a concentration of >160 mCi/ml (initial activity level; ICN Inc. Irvine, CA), and RNase-free water to obtain a total volumes of 30 µl. Then this mixture was incubated for 30 min at 37°C. The unincorporated ³²P was removed by spun-column chromatography as described by Sambrook, et al. (1989).

Baked membrane was placed in hybridization tubes (Techne, Princeton, NJ) and hybridization buffer (0.9 M NaCl, 50 mM sodium phosphate [pH 7.0], 5 mM EDTA, $10 \times$ Denhardt solution (Sambrook, et al., 1989), 0.5 % sodium dodecyl sulfate (SDS), 0.5 mg of poly (A) per ml) was added. Membranes were prehybridized for 2 hr at 40 °C in a rotating incubator (Hybridizer. Techne. Princeton, NJ). Then labeled probe was added and incubation was continued for 14 to 18 hr at 40 °C. After incubation, the membrane was washed with buffer (1% SDS-1×SSC: 0.15 M NaCl and 0.015 M sodium citrate, pH 7.0) for 2 hr at 40°C. Washing buffer was changed every 15 min during the first 1 hr period. Then final washing was carried out for another 30 min in 1% SDS-1×SSC at the appropriated temperatures as indicated in table 1.

The membranes were air dried, and placed in the X-ray exposure holder (Kodak. Rochester, NY) with X-ray film (Kodak Scientific Imaging Film. Rochester, NY). Typically, hybridization with universal probe and *Bacteria*-specific probe needed less than 24 h of exposure for quantification. However, hybridization with *Archaea*-specific and *Eucarya*-specific probes required more than 2 days of exposure for adequate quantification, further, gas samples required more than 3 days of exposure. After exposure, films were developed in the film processor (Konica Corp. Scarborough, ME). Hybridization signal net intensity was measured by using KODAK 1D image analysis software (Eastman Kodak Company. Rochester, NY, 2000). Net intensity is the sum of the background-subtracted pixel values in the band rectangle.

For universal and specific probe quantifications, RNA standard curves were constructed. RNA standard curves were obtained from hybridization signals of the reference series of RNAs extracted from pure cultures at different concentrations. The least-squares method was used to calculate slopes and intercepts of the reference RNA standard curves. The concentrations of RNA in the landfill samples were then calculated from these standard curves. The abundances of each domain were expressed as percentages of the total 16S rRNA in a given sample.

3.5 Polymerase Chain Reaction (PCR) Amplifications

DNA extracts from landfill samples were used as templates for PCR amplification. Bacterial 16S rRNA genes were amplified using the *Bacteria*-specific primers f27 and r1492 (Giovannoni, 1991). The reactions were carried out for 30 cycles at 1 min at 92°C, 1 min at 60 °C, 1 min at 72°C, with a final extension at 72°C for 5 min (McDonald, et al., 1999). Amplification was also performed with *Archaea*-specific 16S rRNA primers 1Af and 1100Ar (Embley, et al., 1992). The reaction condition was 30 cycles with 95°C for 40 s, 55°C for 1 min, 72°C for 2 min, and a final extension step at 72°C for 3.5 min (Hales, et al., 1996).

As discussed previously, the methanogens are responsible for the terminal steps of anaerobic degradation of organic materials. In order to detect the presence of methanogens, the methanogen-specific primers ME1 and ME2 were used (Hales, et al., 1996). These primers target for the methanogen-specific *mcr*A gene, which encodes the α subunit of methyl coenzyme M reductase (MCR). MCR catalyzes the final step of methanogenesis and appears to be conserved in all methanogens (Hales, et al., 1996). The reaction conditions were 30 cycles of 94°C for 40 s, 50°C for 1.5min, 72°C for 3 min, and a final extension step at 72 °C for 10 min.

Dilution of DNA template provides a simple method that can facilitate PCR amplification, since it retains the target DNA sensitivity while diluting out inhibitors (Alvarez, et al., 1994; Tsai and Olson, 1992a; Tsai and Olson, 1992b; Xia, et al., 1995). In the present study, DNA from each landfill sample was diluted at different ratios, undiluted, 10^{-1} , and 10^{-2} dilution, and then PCR amplification was performed. Primers

were purchased from Integrated DNA Technologies Inc. (Coralville, IA). Primer sequences and the annealing temperatures are shown in table 1.

4. Results

4.1 Isolation of Nucleic Acids from Landfill Gas, Gas Condensate, and Waste

Nucleic acids from landfill gas and landfill gas condensate were successfully extracted with the common phenol purification method without any additional purification steps. In landfill gas samples, the amount of extractable DNA was very low, below the detection limit of gel electrophoresis. In contrast, recovered RNA from landfill gas showed two distinct bands at greater than 1.1 kb and these are assumed to be 23S rRNA and 16S rRNA (Fig. 15a). RNA from landfill gas condensate showed two bands at greater than 0.98 kb it is assumed that these are from 23S rRNA and 16S rRNA as well. A diffuse band of low-molecular weight RNA (less than 123 bp) was observed in gas condensate. Treatment of the samples with DNase clearly differentiated RNA and DNA in the gas condensate (Fig. 15b).

During excavation of the landfill, the correlation between the decomposition process of landfill materials and depths (or waste age) could easily be observed. Close to the surface, wastes contained identifiable materials, such as newspapers, glass and plastic, and recognizable decomposing materials. However, at greater depths (at or below 60 ft (18. 3 m)), samples largely consisted of homogenous dark materials without recognizable organic source materials. Ambient temperatures of the excavated wastes ranged from 36°C to 58°C. Generally temperature increased with increasing depth below the surface of the landfill (Fig. 16).



(b) Gas Condensate



Figure 15. Ethidium bromide-stained 1.5% agarose gels of nucleic acid extracts from landfill gas and gas condensate. Lane 1: 123 bp DNA ladder (1 μ g, Gibco BRL) (a) Lane 2: Well No. 33 gas (0.3 μ g ; lane 3 &4: Well No. 45 gas (0.3 μ g & 0.8 μ g, replicate, two independent extractions) (b) lane 2&3: nucleic acid from landfill gas condensates (~1 μ g/lane, replicate: two independent extractions).



Figure 16. Profile of *in situ* excavated waste temperature versus depth at four well sties. Temperatures were determined by measuring samples after they were brought to the surface during well drilling (W indicates the well number. Wells No. 41 & 38: new waste sites; Wells No. 45 & 8A: old waste sites)

For nucleic acid extraction from landfill wastes, extensive purification steps were required as discussed previously. Two major bands were observed in the RNA extracted from waste samples and were assumed to be 23S and 16S rRNA (Fig. 17a). Recovered DNA from wastes was fragmented (Fig. 17b), it seems because of shearing by the bead beater (Leff, et al., 1995). Leff, et al. found the bead beater method yielded a higher amount of DNA than other methods, such as freezing and thawing or cell lysis with a cation-exchange resin. In the present study, PCR amplification with DNA extracted from wastes was successfully achieved, and so this shearing did not appear to affect the quality of DNA. Recovered nucleic acids from landfill gas, gas condensate, and waste in the present study were found to be suitable for hybridization and PCR amplification.

(a) RNA



(b) DNA



Figure 17. Ethidium bromide-stained 1.5% agarose gels of nucleic acid extracts from landfill wastes. Lane 1: 123 bp DNA ladder (1 μ g) (a) lane 2&3: W41 (20ft, two independent extractions); lane 4: W41 (40ft); lane 5: W41 (60ft): lane 6&7: W41 (80 ft, two independent extractions); lane 8: W38 (20ft); lane 9: W45 (40ft). RNA amount of each lane was from 0.2 μ g to 0.6 μ g. (b) Lane 2: W41 (20ft); lane 3: W41 (40ft); lane 4: W41 (60ft); lane 5: W41 (80ft); lane 6: W38 (20ft); lane 7: W38 (40ft); lane 8: W38 (60ft); lane 9: W38 (80ft); lane 10:W45 (20ft); lane 11: W45 (40ft); lane 12: W45 (60ft); lane 13: W8A (20ft); lane 14: W8A (40ft). DNA amount of each lane was around 1.0 μ g. (W indicates the well number. The numbers in parentheses indicate the depth below landfill surface.)

4.2 Results of PCR Amplification

In order to analyze the diversity and distribution of microorganisms across the landfill environment, PCR amplifications of DNA from landfill gas, gas condensate and wastes were performed with *Bacteria*-specific primers (f27 and r1492), *Archaea*-specific primers (1Af and 1100Ar), and *Methanogen*-specific primers (ME1 and ME2). Table 2 summarizes the PCR amplification results from landfill samples with each primer.

		Bacteria-	Archaea-	Methanogen-
		Specific Primer	Specific Primer	Specific Primer
	W28	_	-	-
	W33	+	_	_
Gas				
	W45	+	_	-
	W8A	+	—	_
Gas Condensate		+	+	+
	W41	+	+	+
	W38	+	+	—
Waste				
	W45	+	+	—
	W8A	+	+	—

Table 2. PCR Amplification Results of DNA Extracted from Landfill Samples

+: amplification product; – : no amplification product W indicates well number.
Only bacterial 16S rRNA genes were detected in the landfill gas (Fig. 18),

except at Well no. 28, where response was negative. No amplification products were obtained from landfill gas samples with *Archaea* and *Methanogen*-specific primers.



Figure 18. Ethidium bromide-stained 1.5% agarose gel of PCR amplification of DNA from landfill gas with *Bacteria*-specific primers. Lane 1: 123 bp DNA ladder (Gibco); Lane 2: negative control (no DNA template); Lane 3: positive control (*P. aeruginosa*, ATCC10145); Lane 4: W 33; Lane 5: W 45; Lane 6: W 8A (W indicates well number.)

Amplification of 1.5 kb of the bacterial 16S rRNA gene and 1.1 kb of the archaeal 16S rRNA gene from landfill gas condensates were successfully achieved. To detect methanogens, which are members of the domain *Archaea*, PCR amplification was performed with primers, ME1 and ME2, which amplify the α -subunit gene for methyl coenzyme M reductase (MCR). PCR amplification was carried out with undiluted, and 10^{-1} and 10^{-2} dilutions of DNA templates. Undiluted DNA extracts caused inhibition, and 10^{-1} dilution still caused inhibition in some cases, while 10^{-2} dilution was generally found to be the most suitable ratio for PCR amplification for theses samples. Figure 19 shows

PCR amplification results for landfill gas condensate, as revealed by 1.5 % agarose gel electrophoresis.



Figure 19. Ethidium bromide-stained 1.5% agarose gels of PCR amplification of DNA from landfill gas condensate with primers. Lane 1: 123 bp DNA ladder (Gibco); Lane 2: negative control (no DNA template); Lane 3: positive control (for *Bacteria*, *P. aeruginosa* (ATCC 10145), for *Archaea*, *M. concilii* (DSM 3013), for *Methanogen*, *M. thermolithotrophicus* (ATCC 35395); Lane 4: undiluted; Lane 5: 10⁻¹ dilution; Lane 6: 10⁻² dilution

DNA was extracted from wastes collected at 20 ft (6.1 m) depth increments. These DNA samples were then used as templates in PCR amplification. Bacterial 16S rRNA genes were detected throughout all sampling sites and depths. Archaeal 16S rRNA genes were detected at all four sampling sites; however, the presence of *Archaea* varied. There was no distinct pattern of *Archaea* presence versus depth (Table 3). PCR amplification with methanogen-specific primers revealed the presence of methanogens only in one of the waste samples (Well No. 41 at 20 ft (6.1 m) below landfill surface). Each PCR amplification was performed with undiluted, 10^{-1} and 10^{-2} dilution DNA. In most cases, the 10^{-2} dilution was found to be the most suitable ratio for PCR amplification (Table 3). Figures 20, 21 & 22 show these PCR amplification results for wastes from four sampling sites, as revealed by 1.5% gel agarose electrophoresis.

			Bacteria-	Archaea-	Methanogen-
Well no.	depth	dilution	Specific	Specific	Specific
W41	20ft	10^{0}	_	+	+
		10-1	+	+	-
		10-2	_	-	+
	40ft	10^{0}	—	+	-
		10-1	_	-	_
		10 ⁻²	+	+	-
	60ft	10^{0}	—	—	—
		10-1	—	—	—
		10 ⁻²	—	+	-
	80ft	10^{0}	-	-	-
		10-1	-	-	-
		10 ⁻²	+	-	—
	20ft	10 ⁰	_	_	-
		10-1	_	_	_
		10-2	+	_	_
	40ft	10^{0}	_	_	_
		10-1	_	_	_
W/28		10 ⁻²	+	+	_
W 38	60ft	10^{0}	_	_	_
		10-1	_	_	_
		10-2	+	+	-
	80ft	10^{0}	_	_	_
		10-1	_	-	-
		10-2	+	_	_
W45	20ft	10^{0}	_	_	—
		10-1	_	_	_
		10-2	+	+	_
	40ft	10^{0}	_	_	_
		10-1	_	_	_
		10-2	_	_	_
	60ft	10^{0}	_	_	_
		10-1	_	+	_
		10-2	+	+	_
8A	20ft	10^{0}	—	-	—
		10-1	-	-	-
		10 ⁻²	+	+	—
	40ft	10^{0}	—	_	—
		10-1	+	_	_
		10-2	+	_	_

Table 3. PCR Amplification Results of Landfill Waste Samples at Each Depth

+: amplification product; - : no amplification product W indicates the well number.



W38

W8A



Figure 20. Agarose gels showing PCR amplification of DNA from landfill waste samples with *Bacteria*-specific primers. Lane 1: 123 bp DNA ladder; Lane 2: control without DNA; Lane 3: P. aeruginosa; Lane 4: 20 ft, undiluted; Lane 5: 20 ft, 10^{-1} dilution; Lane 6: 20ft, 10^{-2} dilution; Lane 7: 40 ft, undiluted; Lane 8: 40 ft, 10^{-1} dilution; Lane 9: 40ft, 10^{-2} dilution; Lane 10: 60 ft, undiluted; Lane 11: 60 ft, 10^{-1} dilution; Lane 12: 60ft, 10^{-2} dilution; Lane 13: 80 ft, undiluted; Lane 14: 80 ft, 10^{-1} dilution; Lane 15: 80ft, 10^{-2} dilution (W indicates the well number.)



W38

W8A



Figure 21. Agarose gels showing PCR amplification of DNA from landfill waste samples with *Archaea*-specific primers. Lane 1: 123 bp DNA ladder; Lane 2: (-) control (M. concilii) ; Lane 3: (+) control; Lane 4: 20 ft, undiluted; Lane 5: 20 ft, 10^{-1} dilution; Lane 6: 20ft, 10^{-2} dilution; Lane 7: 40 ft, undiluted; Lane 8: 40 ft, 10^{-1} dilution; Lane 9: 40ft, 10^{-2} dilution; Lane 10: 60 ft, undiluted; Lane 11: 60 ft, 10^{-1} dilution; Lane 12: 60ft, 10^{-2} dilution; Lane 13: 80 ft, undiluted; Lane 14: 80 ft, 10^{-1} dilution; Lane 15: 80ft, 10^{-2} dilution (W indicates the well number.)





Figure 22. Agarose gel showing PCR amplification of DNA from landfill waste samples with *Methanogen*-specific primers. Lane 1: 123 bp DNA ladder; Lane 2: (-) control; Lane 3: (+) control; Lane 4: 20 ft, undiluted; Lane 5: 20 ft, 10^{-1} dilution; Lane 6: 20ft, 10^{-2} dilution; Lane 7: 40 ft, undiluted; Lane 8: 40 ft, 10^{-1} dilution; Lane 9: 40ft, 10^{-2} dilution; Lane 10: 60 ft, undiluted; Lane 11: 60 ft, 10^{-1} dilution; Lane 12: 60ft, 10^{-2} dilution; Lane 13: 80 ft, undiluted; Lane 14: 80 ft, 10^{-1} dilution; Lane 15: 80ft, 10^{-2} dilution (W indicates the well number.)

4.3 Quantification of Domains by Slot-blot Hybridization

Landfill microbial populations were quantified with domain-specific oligonucleotide probes targeting the 16S rRNA of *Bacteria, Archaea* and *Eucarya* by slot-blot hybridization (Table 1). The abundance of each domain was expressed as a percentage of the total 16S rRNA. The total 16S rRNA in each sample was determined by hybridization with universal probe (UNIV1390). The three domain probes used in this study, EUB338, ARC915 and EUK 516, were highly specific against each representative pure culture, and the intensity of the hybridization signal increased with increasing RNA concentrations (See Fig. 23a, in which RNA concentration increases from left to right). Figure 23b showed the slot-blot hybridization response with ³²P-labeled oligonucleotide probes to rRNA from landfill gas, gas condensate and waste. The reference RNA standard curves were obtained from the intensity of the hybridization signal at each different RNA concentration from individual pure cultures (See for example, Fig. 24, 25 & 26).

Hybridization results are summarized in table 4 and the presented results are average from 3 –5 replicate analyses. The results showed that in the landfill gas, condensate, and waste, Bacteria were dominant and the relative contribution of Bacteria remained quite similar among landfill samples (Gas: $85.9\% \pm 2.4\%$; Gas condensate: $86.8\% \pm 7.9\%$; and Waste: $86.5\% \pm 4.3\%$, where \pm indicates the standard deviation, as shown Table 4 & Fig. 27). Archaea comprised a small but substantial component in the gas condensate $(7.2\% \pm 1.4\%)$. In the raw waste materials, response levels for Archaea were lower $(2.1\% \pm 0.61\%)$, while they were not detected in the gas. *Eucarya* were only detected in waste materials $(13.1\% \pm 3.1\%)$. Overall, the relative abundances of three domain populations in the landfill did not show any distinct pattern between relatively young wastes (approximately 3 year-old: Wells No. 28, 33, 38, &41) and old wastes (approximately 10 year-old: Wells No. 8A & 45, see Fig. 28). The three domain probes counted for most of the rRNA quantified with the universal probe. The sum of the three domain probes for landfill gas, gas condensate, and wastes were 84.6%, 94% and 101.9 %, respectively. These quantitative hybridization results for the landfill environment will be compared with those found by other researchers for natural anaerobic environments and anaerobic digesters in the discussion section.



(a) Specificity of 16S rRNA-targeted probes against pure cultures

(b) Landfill samples







Figure 24. Examples of the standard curves used to estimate the relative abundance of *Bacteria* in a gas sample (Well No. 33)





Figure 25. Examples of standard curves used to estimate the relative abundance of *Archaea* in a gas condensate sample







Figure 26. Examples of standard curves used to estimate the relative abundance of *Eucarya* in a waste sample (Well No. 45)

Target group (Probe name)		Bacteria (EUB338)	Archaea (ARC915)	Eucarya (EUK516)	Sum
Gas	W28	84.6 (± 15.4)	_	_	84.6
	W33	85.2 (± 11.1)	_	-	85.2
	W45	84.2 (± 11.3)	_	-	84.2
	W8A	89.4 (± 4.0)	_	_	89.4
Average		85.9 (± 2.4)		_	85.9
Gas condensate		86.8 (± 7.9)	7.2 (± 1.4)	_	94 .0
Waste	W41	92.4 (± 5.5)	2.2 (± 0.5)	12.7 (± 3.7)	107.3
	W38	85.0 (± 10.2)	2.9 (± 2.2)	14.7 (± 1.0)	102.5
	W45	86.6 (± 5.5)	1.7 (± 0.3)	16.0 (± 1.4)	104.3
	W8A	82.1 (± 5.3)	1.6 (± 0.2)	8.9 (± 1.8)	93.3
Average		86.5 (± 4.3)	2.1 (± 0.6)	13.1 (± 3.1)	101.9

Table 4. Relative Abundance of Target Groups in the Landfill Samples (% 16S rRNA)

The numbers in parentheses indicate the standard deviation.

-: no detection, W indicates the well number.



Figure 27. Three domains (*Bacteria*: EUB338; *Archaea*: ARC915; *Eucarya*: EUK516) composition in the landfill gas, gas condensate, and wastes; results are expressed as percentages of 16S rRNA of each phylogenetic target group. The error bars indicate the value of the standard deviation (see Table 4).

(a) Gas and gas condensate





Figure 28. Three domains (*Bacteria*: EUB338; *Archaea*: ARC915; *Eucarya*: EUK516) composition in the landfill gas, gas condensate, and wastes at different sampling sites. (W indicates the well number.) The results are expressed as percentages of 16S rRNA of each phylogenetic target group. The error bars indicate the value of the standard deviation (see Table 4).

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5. Discussion

Detection, distribution and relative abundance of Bacteria, Archaea and Eucarya in the landfill environment: The present study found that Bacteria dominated in the landfill populations at least in terms of rRNA representation. The relative abundance of Bacteria found by hybridization was approximately 86-87%. Also, bacterial 16S rRNA was detected throughout all landfill samples, except in one of the gas samples by PCR amplification (Well No. 28). In a previous study (Barry and Kim, 2000), the cell numbers were only 4.3 to 196/mL in the landfill gas phase, while 3×10^6 /mL to 9×10^6 /mL in the landfill gas condensate (See Appendix B for detailed information on this study). It is suspected that, due to the low cell numbers in the gas stream, only Bacteria were detected in the present study, i.e. the cell numbers of other groups may have been below the limit of detection. Other sampling protocols could later be found to yield detectable levels of archaeal biomass.

Archaea seemed to be a minor component of the microbial community at the landfill site in the present study (7.2% in the gas condensate, 2.1% in the waste). Archaeal 16S rRNA was detected in the gas condensate and wastes, but not in the gas stream by PCR amplification. No prior study of this kind has been done in the landfill environment, thus it is difficult to make direct comparisons, but the present study is comparable with other studies from natural anaerobic ecosystems. Specifically, studies in deep-sea sediments (1500 m deep, Vetriani, et al., 1999), two deep, anaerobic and alkaline aquifers (316m, and 1,270m below surface, Fry, et al., 1997), marine arctic sediments (Ravenschlag, et al., 2001), and Lake Michigan sediments (MacGregor, et al., 1997), all found that *Bacteria* were dominant and *Archaea* were present in only limited amounts (1.8–10%) based on relative rRNA abundances.

Along with anaerobic niches, *Archaea* have been most frequently isolated from extreme environments such as at high temperature, high salinity, and extremes of pH. Numerous studies found an unexpected diversity of *Archaea* in terrestrial and marine hydrothermal systems as well. For example, Barns, et al. (1994, 1996) found a great phylogenetic diversity in archaeal rDNA clones recovered from a Yellowstone National Park hot spring. The sediment was \approx 74°C at the site of sampling. Also, Takai and Sako (1999) found a great phylogenetic diversity in archaeal rDNA clones recovered from a shallow marine hydrothermal vent and an acidic hot spring in Japan. The effluent vent water temperature was 128°C and the temperature of the sediments was 25-75°C. The acidic hot spring environment in this study could be one of the most extreme habitats for life due to its high temperature and strong acidity (pH 2.8). However, it seems the *Bacteria* clearly dominated in most of these environments (Reysenbach, et al., 1998; Harmsen, et al., 1997; Hugenholtz, et al., 1998; Takai & Sako, 1999).

In the present study, PCR amplification results showed no distinct pattern with respect to the presence of *Archaea* as related to depth within the landfill. Consistent with these results, the depth-related profiles of 16S archaeal rRNA in natural ecosystems seems to vary in different studies as well. McDonald, et al. (1999) performed PCR amplification with *Archaea*-specific primers (using the same primers as the present study, i.e., 1Af and 1100Ar) in British bog peat, and found the presence of *Archaea* only below 8cm depth. Likewise, *Archaea* abundance generally increased in the deeper, anoxic regions in permanently cold marine sediments from the Arctic Ocean (Sahm, et al.,

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1998). However, MacGregor, et al. (1997) found *Archaea* abundance decreased with depth in Lake Michigan sediments. In addition, *Archaea* abundances in deep-sea sediment in the northwestern Atlantic Ocean (Vetriani, et al., 1999) and coastal marine sediment from Aarhus Bay, Denmark (Sahm, et al., 1999) varied with depth. The present study did not measure the relative abundances of *Archaea* with depth, but PCR amplification results showed variability in the detection of *Archaea* as a function of depth, and this variability in detection may be related to abundance.

In the landfill, the organic portion of waste is anaerobically broken down, producing gases, mainly methane and carbon dioxide. To identify methanogens, PCR amplification was performed with methanogen-specific primers. Methanogens were identified only in the landfill gas condensate and in one of the waste samples (Well No. 41 at 20 ft (6. 1 m) below the landfill surface). This suggests that methanogens may not be the major member of the *Archaea* in the landfill waste, an observation that is supported by previous research. Qian and Barlaz (1996) measured hydrolytic, acetogenic and methanogenic bacteria along with total anaerobes from different kinds of landfill wastes (grass, leaves, food wastes) by the most probable number (MPN) technique. Their results showed that methanogens were only a small portion (less than 0.2%) of total anaerobes. Hemicellulolytic organisms accounted for the major portion of total anaerobes. Also, in the natural anaerobic ecosystems studied by others, methanogens were detected in only limited amounts (Hales, et al., 1996; McDonald, et al., 1999).

As described earlier, methanogens use a severely limited number of substrates and have low growth rates. Also, it seems that the members of the *Bacteria* domain carry out most of the functions in the food web in the anaerobic environments (Huang, et al.,

2002). This may explain the low abundance of *Archaea* (including methanogens) in the landfill environment. Another possible explanation for the non-detection of *Archaea* (including methanogens) in some landfill samples is that the probes used in the present study may have been only limited applicability. As discussed earlier, probes are designed based on known sequences, thus the probes used in the present study may not detect certain novel microorganisms in the landfill samples. Further study is required to understand the diversity and role of *Archaea*, especially methanogens, despite their apparently low numbers in the present study.

Eucaryotic rRNA was only detected in landfill wastes, where they contributed approximately 13% of the signal of the universal probe. The origin of *Eucarya* in the wastes is unknown in the present study. However, several possible explanations exist. For example, numerous studies have reported the presence of anaerobic protozoa in landfills. Finlay and Fenchel (1991) isolated anaerobic protozoa, with symbiotic methanogens (probably *Methanobacterium formicicum*) in municipal landfill material. Röling, et al. (2001) found archaeal profiles that were clearly related to a methanogenic endosymbiont of an anaerobic protozoan in a landfill leachate-polluted aquifer. Also, protozoa have been detected in deep subsurface sediments (Sinclair and Ghiorse, 1989) and in relatively shallow subsurface sediments in many different regions (Ghiorse and Wilson, 1988). Further, protozoa possibly exist in an anaerobic groundwater from deep aquifers (Fry, et al., 1997).

Eucaryotic RNA may also be associated with fungi. Anaerobic fungi have been found in the rumen (Akin, et al., 1983; Bauchop, 1979; Orpin, 1975) and have been reported to attack and degrade lignified plant tissues resistant to ruminal bacteria (Akin,

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et al., 1983). Fungi also were detected in deep subsurface sediments by Sinclair and Ghiorse (1989), and in relatively shallow subsurface sediments by Ghiorse and Wilson (1988). Anaerobic fungi could play an important role in the landfill ecosystem, because anaerobic fungi can degrade recalcitrant lingnocelluolosic substrates. Barlaz, et al. (1989) enumerated cellulolytic and hemicellulolytic bacteria in refuse by an MPN (most probable number) technique, but, they found no evidence of anaerobic cellulolytic fungi in a refuse sample.

Based on rRNA sequence comparison, mold, animals and plants belong to the *Eucarya* domain (Woese and Fox, 1977). Therefore, it is possible that the *Eucarya* signal in wastes of the present study comes at least in part from mold, animal or plant sources in waste materials rather than organisms involved in the degradation of wastes. Overall, there is little information about the nature of eucaryotes in landfill wastes, but their presence in landfills and other anaerobic natural ecosystems suggests that further studies are needed to determine the role(s) that these microorganisms may play.

Technical considerations: Since little prior study focused on landfill microbiology, the techniques used in the present study, such as the cell collection method for landfill gas and the nucleic acid extraction protocols for landfill gas and wastes, were chosen mainly for their simplicity based on references. Therefore, it will be worthwhile to compare different methods in future studies. For example, a filtration method was used to collect cells from landfill gas in this study. However, it seems likely that limited sample size contributed to the lack of positive response to certain subsets of the anaerobic community (e.g. *Archaea*). Therefore, it is suggested that application of a different

sampling method, such as a liquid impingement method, may improve collection efficiency.

The extraction of nucleic acids from the environment is an essential step in all studies of microbial community analysis by molecular methods. A particular challenge is that RNA is easily degraded by RNases during the extraction process, and this makes it more difficult to study environmental samples. The present study successfully extracted nucleic acids from landfill samples. However, compared with landfill gas and gas condensate samples, nucleic acids from waste needed extensive purification steps. In addition, heterogeneity of wastes makes it more difficult to find a suitable purification method. As described earlier, different lysis and purification methods affect the results of any analysis of the microbial community structure. In the landfill environment, because of a lack of comparative studies, it is unclear what effect the different lysis and purification methods have on the study results. In particular, some methanogens, e.g. *Methanosarcina spp.*, have unusual outer cell layers (polysaccharide sacculus), which might lead to difficulties in nucleic acid extraction (Balch, et al., 1979). This may have contributed to the infrequency with which methanogens were detected in the present study. However, the intent of the present study was not focused on comparison of different extraction methods, and so this will be considered in the future.

Alm, et al. (2000) examined the effects on hybridization that resulted from the presence of humic substances and DNA in the RNA extracts. They demonstrated that the response in rRNA-targeted oligonucleotide probe hybridizations decreased as the concentrations of DNA and humic substances in RNA extracts increased. However, they observed that normalizations with a universal probe should help overcome this limitation,

because the decrease of hybridization signal by humic substances and DNA showed fairly uniform behavior for different target sites. Some of the sum percentages of the three domains in the present study were greater than 100%, an observation that has been reported in other studies (Delong, et al., 1992; Ogram, et al., 1995; Shi, et al., 1999). This may be due to partial degradation of sites for universal-probe annealing in some samples (Ogram, et al., 1995). Further study would be needed to explore this concern.

Comparison of microbial community structures of landfill gas, gas condensate, and waste: The sampling of landfill wastes is technically challenging as described previously. In the present study, landfill gas and landfill gas condensate were used to study the landfill microbial community. The results suggested that gas and gas condensates could provide representative samples for analysis. It is believed that this is the first report on investigation of the landfill microbial community using gas or gas condensate coupled with molecular techniques. Recently, however, several studies evaluated the landfill environment by using molecular techniques, but these studies examined the landfill leachate (Daly, et al., 2000; Röling, et al., 2001; Huang, et al., 2002; Van Dyke and McCarthy, 2002), or were limited to cellulolytic bacteria in a lowlevel radioactive waste disposal site (Lockhart, et al., 2002). In the studies of Daly, et al. (2000) and Van Dyke and McCarthy (2002), the authors designed the PCR primers for phylogenetic subgroups of sulfate-reducing bacteria, and cellulose-degrading bacteria. In particular, Daly, et al. (2000) mentioned that, due to the difficulty of sampling of landfill wastes, leachate was used as the source for samples in their study. The study by Röling, et al. (2000) focused on leachate contamination rather than waste degradation, while the

study by Huang, et al. (2002) examined the impact of leachate recirculation on microbial communities by studying leachate.

However, the microbial community of leachate is likely different from that of the landfill environment, because, in addition to inorganic compounds and heavy metals, leachate contains high levels of organic constituents. These factors are important, for example, metals are inhibitory to methanogens (Muller and Steiner, 1988). In addition, unlike the present study, Huang, et al. (2002) found that methanogen-like rDNAs were dominant in the archaeal library of the leachate from the recirculating landfill they studied.

The extremely high and varied organic carbon load together with long retention times in leachate may lead high diversity of certain microorganism groups. For example, the study by Daly, et al. (2000) found an unexpected high level of diversity among sulfate-reducing bacteria (SBR) in landfill leachate. SBR can compete with methanogens for electron donors such as acetate and H₂, and have the potential to inhibit methanogenesis (Gurijala and Suflita, 1993; Harvey, et al., 1997). Daly, et al. believed that leachate conditions favor to fermentative microorganisms for producing various volatile fatty acids that serve as substrates for SBR. Then they suggested that the scale of landfill sites and their extreme heterogeneity would promote microbial diversity. In addition, because leachate results from the percolation of water through the site, this may explain the high diversity of SBR in leachate. The inhibition of methanogenesis by sulfate has been found in landfill waste (Gurijala and Suflita, 1993), however, no comprehensive study has been done of SBR in landfills, therefore it will be considered in future. Landfills are often compared with anaerobic digesters. However, they are quite different from the waste environment. Anaerobic digesters are simple concrete tanks in which sludges are placed and allowed to decompose anaerobically. The hydraulic retention times in a digester are typically 30-60 days. Applications of anaerobic digesters are the treatment of liquid or sludge wastes from municipal and industrial sources, and for the stabilization of wastewater sludge (Tchobanoglous, 1979). However, unlike an engineered anaerobic digester (often operated under optimum conditions), the landfill environment is extremely heterogeneous, contains high solids with relatively little moisture, and has a retention time orders of magnitude greater than a digester. Clearly, this can be expected to lead to a different microbial population. Nonetheless, a comparison of results between there two environments can provide valuable insight into the factors affecting community dynamics.

A few studies (Raskin, et. al., 1994b; Raskin, et al., 1995; Griffin, et al., 1998) have evaluated the microbial community structure in anaerobic digesters by hybridization with 16S rRNA targeted probes. As in the present research, these studies found that *Bacteria* constituted the majority of the microorganisms in the anaerobic digester, while *Archaea* were present in limited amounts. However, since conditions in anaerobic digesters did not favor the growth of non-methanogenic *Archaea* (e.g. extreme halophiles, thermoacidophiles, the *Archaeoglobales*, the *Thermococcales*, and the thermophiles placed in the Crenarchaeota kingdom; Woese, et al., 1990), these authors suggested that no *Archaea* other than methanogens were expected to be present in the anaerobic digester. Also, they confirmed that the sum of the methanogenic signals was relatively close in magnitude to the signal of the *Archaea*-specific probe. However, in the present study, methanogens were only detected in landfill gas condensate and one waste sample. Further analysis is needed to compare the microbial community of the landfill with that of the anaerobic digester.

The present study shows that microbial populations from landfill gas are easily collected and analyzed, and that it is possible to apply molecular techniques to landfill gas samples. More information on microbial community structure could be obtained by using additional molecular techniques, such as hybridization with group-specific probes, or sequencing. For example, Raskin, et al. (1994a) designed eight probes which targeted the phylogenetically defined groups of methanogens. Also, Devereux, et al. (1992) designed a set of hybridization probes for quantifying different groups of the gramnegative mesophilic sulfate reducing bacteria (SRB), further Van Dyke and McCarthy (2002) designed the probes for the six major phylogenetic groups of SRB. By using these group-specific probes, detailed community information could be obtained.

Also, 16S rDNA sequencing analysis could provide more information on the diversity of microorganisms in landfills. New probes or primers could be designed based on this new sequence information. Detailed procedures for retrieval of rRNA sequence information and probe design for environmental samples is well addressed in the literature (Amann, et al., 1995). As described earlier, probes can only be designed based on known sequences, and therefore the probes used in the present study may not have detected previously uncharacterized microorganisms in landfill samples. It will be worthwhile in the future to design new probes based on more extensive knowledge of landfill samples.

Another possible application of molecular techniques is florescence in situ hybridization (FISH). Combined with microscopy, such as scanning confocal laser microscopy (SCLM), FISH allows the determination of cell morphology and the analysis of spatial distributions in situ (Amann, et al., 1995). As discussed earlier, in anaerobic systems, microorganisms depend highly on other microorganisms. Therefore, understanding spatial relationships within landfill samples will help to understand associations between microorganisms.

The present study focused on culture-independent molecular techniques. However, it is often impossible to infer the physiological role of organisms based only on their phylogenetic positions (Etchebehere, et al., 2002). To overcome this limitation, it may be necessary to consider the inclusion of conventional cultivation methods in future studies to overcome this limitation. Finally, the protocols developed in the present study could be used to monitor in situ microbial activity using landfill gas in order to provide information for site management, for example to optimize "bioreactor landfill" operation. A bioreactor landfill is a sanitary landfill that uses enhanced microbiological process to transform and stabilize the readily and moderately decomposable organic waste constituents within five to ten years of bioreactor process implementation. The underlying philosophy for a bioreactor landfill is to keep the waste sufficiently wet, such as through leachate recirculation, in order to maximize the biodegradation process (O'Brien, Elements 2004). By using protocol developed in the present study, landfill gas and gas condensate could be collected on regular basis and results from microbial community analysis can help to operate "bioreactor landfill".

A better understanding of microbial populations in landfills is important to improve municipal solid waste management. This will help to reduce the time required for stabilization of MSW in the landfill, and will result in smaller and reusable landfills. The present study should provide the foundation for a comprehensive investigation of the microbial ecology involved in landfill waste degradation. Information from slot-blot hybridization and PCR amplification should be used as references for future studies of the landfill environment.

6. Conclusions

The following general conclusions can be drawn from this study.

1. This study demonstrated that microorganisms from landfill gas and gas condensates can be successfully collected, and nucleic acids can be extracted for application of molecular techniques, such as PCR amplification and rRNA slot-blot hybridization.

2. This study found that *Bacteria* dominated in the landfill populations. The relative abundance of *Bacteria* found by hybridization was approximately 86-87%. Also, Bacterial 16S rRNA was detected throughout all landfill samples, except in one of the gas samples by PCR amplification.

3. *Archaea* were a minor component in the landfill environment. 16S rRNA was detected in the gas condensate and wastes, but not in the gas stream by PCR amplification. Also, hybridization results showed *Archaea* comprised 7. 2 % in the gas condensate and 2.1 % in the raw waste materials, while they were not detected in the gas. Lack of detection in the gas is believed to be a result of low sampling volumes rather than an indication of complete absence, but further testing is required before a definitive conclusion can be reached.

4. Methanogens were identified only in the landfill gas condensate and in one waste sample using PCR amplification. This suggests that methanogens may be the minor component of the *Archaea* domain in the landfill waste, and possibly in landfill gas. This result is different from what researchers have typically encountered in other engineered systems like anaerobic digesters.

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5. Eucaryotic rRNA was only detected in landfill wastes, where their relative abundance was approximately 13% based on relative rRNA contents.

6. The present study suggests that gas and gas condensates could provide representative samples for studying the landfill environment.

7. The present study should provide useful tools for description of the landfill microbial community, and add to our understanding of microbial community structure in landfills.

7. Future Work

On the basis of the present study, the following lines of research show promise for the future.

1. Application of different gas collection methods, and different lysis and purification methods for nucleic acid extraction, to optimize the analysis of microbial community structure and function.

2. Application of group-specific 16S rRNA hybridization probes, e.g., methanogenic groups and sulfate reducing bacteria groups, to describe the detailed microbial community of the landfill.

3. Sequencing analysis, which will help to identify new microorganisms and to design new probes that will allow development of detailed diversity information. In particular, new probes may help to detect unknown microorganisms in landfill environment.

4. Application of the florescence in situ hybridization (FISH), which allows analysis of the spatial relationships in the landfill microbial community.

5. A combination of cultivation methods and molecular methods should be used to better understand the ecological roles of organisms in landfills.

6. Application of the protocols developed in the present study to develop tools for site management, for example to optimize "bioreactor landfill" operation.

List of References

Akin, D. E., G. L. R. Gordon, and J. P. Hogan. 1983. Rumen bacterial and fungal degradation of *digitaria pentzii* grown with and without sulfur. Appl. Environ. Microbiol. **46**:738-748.

Alm, E. W., D. Zheng, and L. Raskin. 2000. The presence of humic substances and DNA in RNA extracts affects hybridization results. Appl. Environ. Microbiol. **66**:4547-4554.

Alvarez, A. J., M. P. Buttner, G. A. Toranzos, E. A. Dvorsky, A. Toro, T. B. Heikes, L. E. Mertikas, and L. D. Stetzenbach. 1994. Use of solid-phase PCR for enhanced detection of airborne microorganisms. Appl. Environ. Microbiol. **60**:374-376.

Amann, R. I., B. J. Binder, R. J. Olson, S. W. Chisholm, R. Devereux, and D. A. Stahl. 1990. Combination of 16S rRNA-targeted oligonucleotide probes with flow cytomertry for analyzing mixed microbial populations. Appl. Environ. Microbiol. 56:1919-1925.

Amann, R. I., N. Springer, W. Ludwig, H.-D. Görtz, and K.-H. Schleifer. 1991. Identification and phylogeny of uncultured bacterial endosymbionts. Nature (London) **351**:161-164.

Amann, R. I., W. Ludwig, and K.-H. Schleifer. 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol. Rev. 59:143-169.

Atlas, R., and R. Bartha. 1998. Microbial ecology: Fundamentals and application, 4th ed. Benjamin/Cummings Publishing Company Inc., Menlo Park, CA.

Balch, W. E., and R. S. Wolfe. 1976. New approach to the cultivation of methanogenic bacteria: 2-Mercaptoethanesulfonic acid (HS-CoM)-dependent growth of *Methanobacterium ruminantium* in a pressurized atmosphere. Appl. Environ. Microbiol. 32:781-791.

Balch, W. E., G. E. Fox, L. J. Magrum, C. R. Woese, and R. S. Wolfe. 1979. Methanogens: Reevaluation of unique biological group. Microbiol. Rev. **43**:260-296.

Barlaz, M. A. 1997. Microbial studies of landfills and anaerobic refuse decomposition, p. 541-557. *In* C. J. Hurst, G. R. Knudsen, M. I. McInerney, L. D. Stetzenbach, and M. V. Walter (ed.), Manual of environmental microbiology. American Society for Microbiology Press, Washington, D.C.

Barlaz, M. A., Schaefer, D. M., and R. K. Ham. 1989. Bacterial population development and chemical characteristics of refuse decomposition in a simulated sanitary landfill. Appl. Environ. Microbiol. **55**:55-65.

Barns, S. M., C. F. Delwiche, J. D. Palmer, and N. R. Pace. 1996. Perspectives on archaeal diversity, thermophily and monophyly from environmental rRNA sequences. Proc. Natl. Acad. Sci. USA **93**:9188-9193.

Barns, S. M., R. E. Fundyga, M. W. Jeffries, and N. R. Pace. 1994. Remarkable archaeal diversity detected in a Yellowstone National Park hot spring environment. Proc. Natl. Acad. Sci. USA **91**:1609-1613.

Barry, R. C., and M. Kim. 2000. Using landfill gas to study solid waste microbial communities. *In* abstracts of the 100th General Meeting of the American Society for Microbiology 2000. American Society for Microbiology, Washington, D.C.

Bartlett, M. S., S. H. Vermund, R. Jacobs, P. J. Durant, M. M. Shaw, J. W. Smith, X. Tang, J.-J. Lu, B. Li, S. Jin, and C.-H. Lee. 1997. Detection of *Pneumocystis carinii* DNA in air samples: Likely environmental risk to susceptible persons. J. Clinic. Microbiol. **35**:2522-2513.

Bauchop, T. 1979. Rumen anaerobic fungi of cattle and sheep. Appl. Environ. Microbiol. **38**:148-158.

Bej, A. K., J. L. DiCesare, L. Haff, and R. M. Atlas. 1991a. Detection of *Escherichia coli* and *Shigella* spp. in water by using the polymerase chain reaction and gene probes for *uid*. Appl. Environ. Microbiol. **57**:1013-1017.

Bej, A. K., M. H. Mahbubani, and R. M. Atlas. 1991b. Amplification of nucleic acids by polymerase chain reaction (PCR) and other methods and their applications. Critic. Rev. Biochem. Mol. Biol. **26**:301-334.

Bej, A. K., M. H. Mahbubani, J. L. DiCesare, and R. M. Atlas. 1991c. Polymerase chain reaction-gene probe detection of microorganisms by using filter-concentrated samples. Appl. Environ. Microbiol. **57**:3529-3534.

Benson, D., D. J. Lipman, and J. Ostell. 1993. GenBank. Nucleic Acids Res. 21:2963-2965.

Bolton, N. 2003. Landfill gas-to-energy: Solving problems. MSW Manage. 13:38-44.

Booker, T. J., and R. K. Ham. 1982. Stabilization of solid waste in landfills. J. Environ. Eng. ASCE **108**:1089-1100.

Brauns, L. A., M. C. Hudson, and J. D. Oliver. 1991. Use of the polymerase chain reaction in detection of culturable and nonculturable *Vibrio vulnificus* cells. Appl. Environ. Microbiol. **57**:2651-2655.

Brock, T. D., M. T. Madigan, J. M. Martinko, and J. Parker. 1994. Biology of Microorganisms. 7th ed., Prentice-Hall. Englewood Cliffs, NJ.

Burr, M.D., and Pepper. I. L. 1997. Variability in presence-absence scoring of AP PCR fingerprints affects computer matching of bacteria isolates. J. Microbiol. Methods **29**:63-68.

Buttner, M. P., K. Willeke, and S. A. Grinshpun. 1997. Sampling and analysis of airborne microorganisms, p. 629-640. *In* C. J. Hurst, G. R. Knudsen, M. J. McInerney, L. D. Stetzenbach, M. V. Walter (ed.), Manual of environmental microbiology. American Society for Microbiology, Washington, D. C.

Chatigny, M. A., J. M. Macher, H. A. Burge, and W. R. Solomon. 1989. Sampling airborne microorganisms and aeroallergens, p. 199-220. *In* S. V. Hering (ed.), Air sampling instruments, 7th ed. American Conference of Governmental Industrial Hygienists, Cincinnati.

Cox, C. S. 1987. The aerobiological pathway of microorganisms. John Wiley & Sons, Ltd., Chichester, England.

Daims, H., A. Brühl, R. Amann, K.-H. Schleifer, and M. Wagner. 1999. The domainspecific probe EUB338 is insufficient for the detection of all Bacteria: Development and evaluation of a more comprehensive probe set. Syst. Appl. Microbiol. **22**:434-444.

Daly, K., R. J. Sharp, and A. J. McCarthy. 2000. Development of oligonucleotide probes and PCR primers for detecting phylogenetic subgroups of sulfate-reducing bacteria. Microbiol. **146**:1693-1705.

DeCosemo, G. A. L., I. W. Stewart, W. D. Griffiths, and J. S. Deans. 1992. The assessment of airborne microorganisms. J. Aerosol Sci. **23**(Suppl. 1):S683-S686.

DeLong, E. F. 1992. Archaea in coastal marine environments. Proc. Natl. Acad. Sci. USA **89**:5685-5689.

Devereux, R., M. E. Hines, and D. A. Stahl. 1996. S-cycling: Characterization of natural communities of sulfate-reducing bacteria by 16S rRNA sequence comparisons. Microbiol. Ecol. **32**:283-292.

Devereux, R., M. D. Kane, J. Winfrey, and D. A. Stahl. 1992. Genus- and group-specific hybridization probes for determination and environmental studies of sulfate-reducing bacteria. Syst. Appl. Microbiol. **15**:601-609.

Dunbar, J., S. Takala, S. M. Barns, J. A. Davis, and C. R. Kuske. 1999. Levels of bacterial community diversity in four arid soils compared by cultivation and 16S rRNA gene cloning. Appl. Environ. Microbiol. **65**:1662-1669.

Edwards, T., and B. C. McBride. 1975. New method for the isolation and identification of methanogenic bacteria. Appl. Microbiol. **29**:54-0545.

Embley, T. M., B. J. Finlay, R. H. Thomas, and P. L. Dyal. 1992. The use of rRNA sequences and fluorescent probes to investigate the phylogenetic positions of the anaerobic ciliate *Metopus palaeformis* and its archaeobacterial endosymbiont. J. Gen. Microbiol. **138**:1497-1487.

Etchebehere, C., M. I. Errazquin, P. Dabert, and L. Muxi. 2002. Community analysis of a denitrifying reactor treating landfill leachate. FEMS Microbiol. Ecol. **40**:97-106.

Farquhar, G. H., and F. A. Rovers. 1973. Gas production during refuse decomposition. Water Air Soil Pollut. 2:483-495.

Felske, A., E. Engelen, U. Nübel, and H. Backhaus. 1996. Direct ribosome isolation from soil to extract bacterial rRNA for community analysis. Appl. Environ. Microbiol. **62**:4162-4167.

Ferry, J. G. 1993. Fermentation of acetate, p. 305-334. *In* J. G. Ferry (ed.), Methanogenesis. Chapman & Hall, Inc., New York.

Finlay, B. J., and T. Fenchel. 1991. An anaerobic protozoon, with symbiotic methanogens, living in municipal landfill material. FEMS Microbiol. Ecol. **85**:169-180.

Fry, N. K., H. K. Fredrickson, S. Fishbain, M. Wagner, and D. A. Stahl. 1997. Population structure of microbial communities associated with two deep, anaerobic, alkaline aquifers. Appl. Environ. Microbiol. **63**:1498-1504.

Gausing, K. 1977. Regulation of ribosome production in *Escherichia coli:* Synthesis and stability of ribosomal RNA and of ribosomal protein messenger RNA at different growth rates. J. Mol. Biol. **115**:335-354.

Ghiorse, W. C., and J. T. Wilson. 1988. Microbial ecology of the terrestrial subsurface. Adv. Appl. Microbiol. **33**:107-172.

Giovannoni, S. J. 1991. The polymerase chain reaction, p. 177-203. *In* E. Stackebrandt and M. Goodfellow (ed.), Nucleic acid techniques in bacterial systematics. John Wiley & Sons, Inc., New York.

Griffin, M. E., K. D. McMahon, R. I. Mackie, and L. Raskin. 1998. Methanogenic population dynamics during start-up of anaerobic digesters treating municipal solid waste and biosolids. Biotechnol. Bioeng. **57**:342-354.

Gurijala, K. R., and J. M. Suflita. 1993. Environmental factors influencing methanogenesis from refuse in landfill samples. Environ. Sci. Technol. 27:1176-1181.

Gurtner, C., J. Heyrman, G. Piñar, W. Lubitz, J. Swings, and S. Rölleke. 2000. Comparative analysis of the bacterial diversity on two different biodeteriorated wall paintings by DGGE and 16S rDNA sequence analysis. Int. Biodeter. Biodegr. **46**:229-239.

Hales, B. A., C. Edwards, D. A. Ritchie, G. Hall, R. W. Pickup, and J. R. Saunders. 1996. Isolation and identification of methanogen-specific DNA from blanket bog peat by PCR amplification and sequences analysis. Appl. Environ. Microbiol. **62**:668-675.

Harmsen, H. J., D. Prieur, and C. Jeanthon. 1997. Distribution of microorganisms in deep-sea hydrothermal vent chimneys investigated by whole-cell hybridization and enrichment culture of thermophilic subpopulations. Appl. Environ. Microbiol. **63**:2876-2883.

Harvey, R. W., J. M. Suflita, and M. J. McInerney. 1997. Overview of issues in subsurface and landfill microbiology, p. 523-525. *In* C. J. Hurst, G. R. Knudsen, M. J. McInerney, L. D. Stetzenbach, and M. V. Walter (ed.), Manual of environmental microbiology. American Society for Microbiology, Washington, DC.

Hickman, H. L. Jr. 1999. Principal of integrated solid waste management. American Academy of Environmental Engineers Publication.

Hoaki, T. M., M. Nishijima, M. Kato, K. Adachi, S. Mizobuchi, N. Hanzawa, and T. Maruyama. 1994. Growth requirements of hyperthermophilic sulfur-dependent heterotrophic *Archaea* isolated from a shallow submarine geothermal system with reference to their essential amino acids. Appl. Environ. Microbiol. **60**:2898-2904.

Huang, L.-N., H. Zhou, Y.-Q. Chen, S. Luo, C.-Y. Lan, and L.-H. Qu. 2002. Diversity and structure of the archaeal community in the leachate of a full-scale recirculating landfill as examined by direct 16S rRNA gene sequence retrieval. FEMS Microbiol. Lett. **214**:235-240.

Hugenholtz, P., C. Pitulle, K. L. Hershberger, and N. R. Pace. 1998. Novel division level bacteria diversity in Yellowstone hot spring. J. Bacteriol. **180**:366-376.
Hungate, R. E. 1950. The anaerobic mesophilic cellulolytic bacteria. Bacteriol. Rev. **14**:1-49.

Jones, W. J., D. P. Nagle Jr., and W. B. Whitman. 1987. Methanogens and the diversity of archaebacteria. Microbiol. Rev. **51**:135-177.

Kemp, P. F., S. Lee, and J. LaRoche. 1993. Estimating the growth rate of slowly growing marine bacteria from RNA content. Appl. Environ. Microbiol. **59**:2594-2601.

Kerkhof, L., and B. B. Ward. 1993. Comparison of nucleic acid hybridization and fluorometry for measurement of the relationship between RNA/DNA ratio and growth rate in a marine bacterium. Appl. Environ. Microbiol. **59**:1303-1309.

Kjelgaard, N. O., and C. G. Kurland. 1963. The distribution of soluble and ribosomal RNA as a function of growth rate. J. Mol. Biol. **6**:341-348.

Kortegast A. P., and A. R. Ampurch. 1997. Landfill site selection: A new approach to an old planning problem. Proceedings, ISEWA'97 World Conference. Wellington, New Zealand, International Solids Wastes Association. Copenhagen, Denmark.

Kozdrój, J., and J. D. van Elsas. 2001. Structural diversity of microbial communities in arable soils of a heavily industrialized area determined by PCR-DGGE fingerprinting and FAME profiling. Appl. Soil Ecol. **17**:31-42.

Leff, L.G., J. R. Dana, J. V. McArthur, and L. J. Shimkets. 1995. Comparison of methods of DNA extraction from stream sediments. Appl. Environ. Microbiol. 61:1141-1143.

Lerman, L. S., S. G. Fischer, I. Hurley, K. Silverstein, and N. Lumelsky. 1984. Sequence determined DNA separations. Annu. Rev. Biophys. Bioeng. 13:399-423.

Lockhart, R., P. Humphreys, I. Beadle, and A. J. McCarthy. 2002. Molecular biological analysis of a low level radioactive waste disposal site. *In* abstracts of the 102th General Meeting of the American Society for Microbiology 2002. American Society for Microbiology, Washington, D.C.

Lodish, H., D. Baltimore, A. Berk, S. L. Zipursky, P. Matsudaira, J. Darnell. 1995. Molecular cell biology. 3rd ed. Science American Books Inc., New York.

MacGregor, B. J., D. P. Moser, E. W. Alm, K. H. Nealson, and D. A. Stahl. 1997. Crenarchaeota in Lake Michigan sediment. Appl. Environ. Microbiol. **63**:1178-1181.

Maher, N., H. K. Dillon, S. H. Vermund, and T. R. Unnasch. 2001. Magnetic bead capture eliminates PCR inhibitors in samples collected from the airborne environment, permitting detection of *Pneumocystis carinii* DNA. Appl. Environ. Microbiol. **67**:449-452.

Maidak, B. L., N. Larsen, M. J. McCaughey, R. Overbeek, G. J. Olsen, and K. Fogel. 1994. The ribosomal database project. Nucleic Acids Res. 22:3485-3487.

Maniatis, T., E. Fritsch, and J. Sambrook. 1982. Molecular Cloning: A laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Manz, W., R. Amann, W. Ludwig, M. Wagner, and K. H. Schleifer. 1992. Phylogenetic oligodeoxynucleotide probes for the major subclasses of proteobacteria; Problems and solution. Syst. Appl. Microbiol. **15**:593-600.

Marlowe, E. M., K. Josephson, and I. L. Pepper. 1999. Nucleic acid-based methods of analysis, p. 287-318. *In* R. M. Maier, I. L. Pepper, and C. P. Gerba (ed.), Environmental microbiology. Academic Press, San Diego, CA.

McDonald, J. R., M. Upton, G. Hall, R. W. Pickup, E. Edwards, J. R. Saunders, D. A. Ritchie, and J. C. Murrell. 1999. Molecular ecological analysis of methanogens and methanotrophs in blanket bog peat. Microbiol. Ecol. **38**:225-233.

Miller, T. L., and M. J. Wolin. 1974. A serum bottle modification of the Hungate techniques for cultivating obligate anaerobes. Appl. Environ. Microbiol. 27:985-987.

Moore, W. E. C. 1966. Techniques for routine culture of fastidious anaerobes. Int. J. Syst. Bacteriol. **16**:173.

Muilenberg, M. L. 1989. Aeroallergen assessment by microscopy and culture. Immunol. Allergy Clinic. North Am. **9**:245-268.

Muller, R., and A. Steiner. 1988. Influence of nickel and copper on anaerobic sludge digestion, p. 41-144. Poster Papers. 5th International Symposium on Anaerobic Digestion, Bologna, Italy.

Müller, V., M. Blaut, and G. Gottschalk. 1993. Bioenergetics of methanogenesis, p. 361-406. *In* J. G. Ferry (ed.), Methanogenesis. Chapman & Hall, Inc., New York.

Mullis, K. B., and F. A. Faloona. 1987. Specific synthesis of DNA *in vitro* via a polymerase-catalyzed chain reaction. Methods Enzymol. **155**:335-351.

Nevalainen, A., K. Willeke, F. Liebhaber, J. Pastuszka, H. Burge, and E. Henningson. 1993. Bioaerosol sampling, p. 417-492. *In* K. Willeke and P. A. Baron (ed.), Aerosol measurement: Principals, techniques, and applications. Van Nostrand Reinhold, New York.

Nevalainen, A., J. Pastuszka, F. Liebhaber, and K. Willeke. 1992. Performance of bioaerosol samplers: Collection characteristics and sampler design consideration. Atmos. Environ. **26A**:531-540.

New Jersey Department of Environmental Protection. 2003. Division of Solid and Hazardous Wastes. [Online] <u>www.state.nj.us/dep/dshw</u>

New York City Office of the Mayor. 2002. Press Release, July 31, 2002.

Niemi, R. M., I. Heiskanen, K. Wallenius, and K. Lindström. 2001. Extraction and purification of DNA in rhizosphere soil samples for PCR-DGGE analysis of bacterial consortia. J. Microbiol. Methods **45**:155-165.

O'Brien, J. K. Elements 2004. The solid waste manager's guide to the bioreactor landfill. MSW Manage. **13**:12-16.

Ogram, A., W. Sun, F. J. Brockman, and J. K. Fredrickson. 1995. Isolation and characterization of RNA from low-biomass deep-subsurface sediments. Appl. Environ. Microbiol. **61**:763-768.

Olsson, M. C., H. Lidman, S. Latouche, A. Björkman, R. Roux, E. Linder, and M. Wahlgren. 1998. Identification of *Pneumocystis carinii f. sp. hominis* gene sequences in filtered air in hospital environments. J. Clinic. Microbiol. **36**:1737-1740.

Orpin, C. G. 1975. Studies another rumen flagellate *Neocallimastix frontalis*. J. Gen. Microbiol. **91**:249-262.

Pace, N. R., D. A. Stahl, D. J. Lane, and G. J. Olsen. 1986. The analysis of natural microbial populations by ribosomal RNA sequences. Adv. Microb. Ecol. 9:1-55.

Parker, A. 1983. Behavior of wastes in landfill-leachate. *In* R. Holmes (ed.), Practical waste management. John Wiley & Sons Ltd., Chichester, England.

Palmgren, U., G. Ström, P. Malmberg, and G. Blomquist. 1986. The Nuclepore filter method: A technique for enumeration of viable and nonviable airborne microorganisms. Am. J. Ind. Med. **10**:325-327.

Palmisano, A. C., B. S. Schwab, D. A. Maruscik. 1993. Hydrolytic enzyme activity in landfilled refuse. Appl. Microbiol. Biotechnol. 38:823-832.

Picard, C., X. Ponsonnet, E. Paget, X. Nesme, and P. Simonet. 1992. Detection and enumeration of bacterial in soil by direct DNA extraction and polymerase chain reaction. Appl. Environ. Microbiol. **58**:2717-2722.

Pillai, S. D., K. L. Josephson, R. L. Bailey, C. P. Gerba, and I. L. Pepper. 1991. Rapid method for processing soil samples for polymerase chain reaction amplification of specific gene sequences. Appl. Environ. Microbiol. **57**:2283-2286.

Pohland, F. G. 1987. Critical review and summary of leachate and gas production from landfills. EPA/600/S2-86/073, US EPA hazardous Waste Engineering Research Laboratory, Cincinnati, OH.

Pohland, F. G. 1991. Fundamental principles and management strategies for landfill codisposal practice. Proceedings Sardinia 91 Third International Landfill Symposium, Vol. II, p. 1445-1460. Grafiche Galeati, Imola, Italy.

Poulsen, K. G. Ballard, and D. A. Stahl. 1993. Use of rRNA fluorescence in situ hybridization for measuring the activity of single cells in young and established biofilms. Appl. Environ. Microbiol. **59**:1354-1360.

Qian, X., and M. A. Barlaz. 1996. Enumeration of anaerobic refuse-decomposing microorganisms on refuse constituents. Waste Manage. Res. 14:151-161.

Raskin, L. J., M. Stromley, B. E. Rittmann, and D. A. Stahl. 1994a. Group-specific 16S rRNA hybridization probes to describe natural communities of methanogens. Appl. Environ. Microbiol. **60**:1232-1240.

Raskin, L., K. Poulsen, D. R. Noguera, and B. E. Rittmann. 1994b. Quantification of Methanogenic groups in anaerobic biological reactors by oligonucleotide probe hybridization. Appl. Environ. Microbiol. **60**:1241-1248.

Raskin, L., D. Zheng, M. E. Griffin, P. G. Stroot, and P. Misra. 1995. Characterization of microbial communities in anaerobic bioreactors using molecular probes. Antonie Leeuwenhoek **68**:297-308.

Ravenschlag, K., K. Sahm, and R. Amann. 2001. Quantitative molecular analysis of the microbial community in marine arctic sediments (Svalbard). Appl. Environ. Microbiol. **67**:387-395.

Reysenbach, A.-L., G.S. Wickham, and N. R. Pace. 1994. Phylogenetic analysis of the hyperthermophilic pink filament community in Octopus spring, Yellowstone National Park. Appl. Environ. Microbiol. **60**:2113-2119.

Rice, C. M., R. Fuchs, D. G. Higgins, P. J. Stoehr, and G. N. Cameron. 1993. The EMBL data library. Nucleic Acids Res. 21:2967-2971.

Rodriquez, R. L., and R. C. Tait. 1983. Recombinant DNA techniques: An introduction. Benjamin/Cummings Publishing Company, Inc., Menlo Park, CA.

Röling, W. F. M., B. M. van Breukelen, M. Braster, B. Lin, and H. W. van Verseveld. 2001. Relationships between microbial community structure and hydrochemistry in a landfill leachate-polluted aquifer. Appl. Environ. Microbiol. **67**:4619-4629.

Sahm, K. and U.-G. Berninger. 1998. Abundance, vertical distribution, and community structure of benthic prokaryotes from permanently cold marine sediments (Svalbard, Arctic Ocean). Mar. Ecol. Prog. Ser. 165:71-80.

Sahm, K. B., J. MacGregor, B. B. Jorgensen, and D. A. Stahl. 1999. Sulphate reduction and vertical distribution of sulphate-reducing bacteria quantified by rRNA slotblot hybridization in a coastal marine sediment. Environ. Microbiol. 1:65-74.

Saiki, R. K., S. Scharf, F. Faloona, K. B. Mullis, G. T. Horn, H. A. Erlich, and N. A. Arnheim. 1985. Enzymatic amplification of β -globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science **230**:1350-1354.

Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning; A laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Sandaa, R.-A., and V. Torsvik, and Ø. Enger. 2001. Influence of long-term heavymetal contamination on microbial communities in soil. Soil Biol. Biochem. 33:287-295.

Sessitsch, A., A. Weilharter, M. H. Gerzabek, H. Kirchmann, and E. Kandeler. 2001. Microbial population structures in soil particle size fractions of a long-term fertilizer field experiment. Appl. Environ. Microbiol. **67**:4215-4224.

Shi, Y., M. D. Zwolinski, M. E. Schreiber, J. M. Bahr, G. W. Sewell, and W. J. Hickey. 1999. Molecular analysis of microbial community structures in pristine and contaminated aquifer; Field and laboratory microcosm experiments. Appl. Environ. Microbiol. **65**:2143-2150.

Sinclair, J. L., and W. C. Ghiorse. 1989. Distribution of aerobic bacteria, protozoa, algae, and fungi in deep subsurface sediments. Geomicrobiol. J. 7:15-31.

Somerville, C. C., I. T. Knight, W. L. Straube, and R. R. Colwell. 1989. Simple, rapid method for direct isolation of nucleic acids from aquatic environments Appl. Environ. Microbiol. **55**:548-554.

Sowers, K. R., and K. M. Moll. 1995. Techniques for anaerobic growth, p. 15-48. K. R. Sowers, and H. J. Schreier (ed.), *In* Archaea: A laboratory manual, methanogens. Cold Spring Harbor Laboratory Press, Plainview, NY.

Stahl, D A., B. Flesher, H. R. Mansfield, and L. Montgomery. 1988. Use of phylogenetically based hybridization probes for studies of ruminal microbial ecology. Appl. Environ. Microbiol. **54**:1079-1084.

Stahl, D. A. 1995. Application of phylogenetically based hybridization probes to microbial ecology. Mol. Ecol. **4**: 535-542.

Stahl, D. A. 1997. Molecular approaches for the measurement of density, diversity, and phylogeny, p. 102-114. *In* C. J. Hurst, G. R. Knudsen, M. J. McInerney, L. D. Stetzenbach, and M. V. Walter (ed.), Manual of environmental microbiology. American Society for Microbiology, Washington, DC.

Stärk, K. D. C., J. Nicolet, and J. Frey. 1998. Detection of *Mycoplasma hyopneumoniae* by air sampling with a nested PCR assay. Appl. Environ. Microbiol. **64**:543-548.

Steffan, R. J., and R. M. Atlas. 1991. Polymerase chain reaction: Application in environmental microbiology. Ann. Rev. Microbiol. **45**:137-161.

Takai, K., and Y. Sako. 1991. A molecular view of archaeal diversity in marine and terrestrial hot water environments. FEMS Microbiol. Ecol. **28**:177-188.

Tchobanoglous, G. 1979. Wastewater engineering: Treatment, disposal, reuse, 2nd ed. McGraw-Hill, New York.

Tchobanoglous, G., T. Hilary, and S. Vigil. 1993. Integrated solid waste management: Engineering principles and management issues. McGraw-Hill, New York.

Thomm, M., and H. P. Gohl. 1995. Isolation of RNA from methanogenic Archaea, p. 379-381. K. R. Sowers, and H. J. Schreier (ed.), *In* Archaea: A laboratory manual: Methanogens. Cold Spring Harbor Laboratory Press, Plainview, NY.

Thorne, P. S., M. S. Kiekhaefer, P. Whitten, and K. J. Donham. 1992. Comparison of bioaerosol sampling methods in barns housing swine. Appl. Environ. Microbiol. **58**:2543-2551.

Tsai, Y. L., and B. H. Olson. 1992a. Detection of low numbers of bacterial cells in soils and sediments by polymerase chain reaction. Appl. Environ. Microbiol. **58**:754-757.

Tsai, Y. L., and B. H. Olson. 1992b. Rapid method for direct extraction of DNA from soil and sediments. Appl. Environ. Microbiol. **57**:1070-1074.

U.S. Environmental Protection Agency. 1991. Solid waste disposal facility criteria 40 CFR Parts 257-258. U.S. Environmental Protection Agency. Washington, D.C.

U.S. Environmental Protection Agency. 1993. Solid waste disposal facility criteria, technical manual. EPA/530-R-93-017. U.S. Environmental Protection Agency. Washington, D.C.

U.S. Environmental Protection Agency. 1995. Decision-Maker's guide to solid waste management, 2nd ed., US Environmental Protection Agency. EPA/530-R-95-023. U.S. Environmental Protection Agency. Washington, D.C.

U.S. Environmental Protection Agency. 1996. New source performance and emission guidelines for municipal solid waste landfills, 40 CFR Part 60. U.S. Environmental Protection Agency. Washington, D.C.

U.S. Environmental Protection Agency. 2002. Municipal solid waste in the United States: 200 facts and figures. U.S. Environmental Protection Agency. Washington, D.C.

U.S. Environmental Protection Agency. 2003. Landfill methane outreach program. [Online.] <u>www.epa.gov/lmop</u>.

Van Dyke, M. I., and A. J. McCarthy. 2002. Molecular biological detection and characterization of *Clostridium* populations in municipal landfill sites. Appl. Environ. Microbiol. **68**:2049-2053.

Vetriani, C., H. W. Jannasch, B. J. MacGregor, D. A. Stahl, and A.-L. Reysenbach. 1999. Population structure and phylogenetic characterization of marine benthic *Archaea* in deep-sea sediments. Appl. Environ. Microbiol. **65**:4375-4384.

Volossiouk, T., E. J. Robb, and R. N. Nazar. 1995. Direct DNA extraction for PCRmediated assays of soil organisms. Appl. Environ. Microbiol. **61**:3972-3976.

Wagner, M., and R. Amann. 1997. Molecular techniques for determining microbial community structures in activated sludge, p. 61-72. *In* T. E. Cloete and N. Y. O. Muyima (ed.), Microbial community analysis: The key to the design of biological wastewater treatment systems. International Association of Water Quality. London, England.

Woese, C. R., and G. E. Fox. 1977. Phylogenetic structure of the prokaryotic domain: The primary kingdoms. Proc. Natl. Acad. Sci. USA **74**:5088-5090.

Woese, C. R., O. Kandler, and L. Wheelis. 1990. Towards a natural system of organisms: proposal for the domains *Archaea*, *Bacteria* and *Eucarya*. Proc. Natl. Acad. Sci. USA **87**:4576-4579.

Wolin, M. J., and T. L. Miller. 1982. Interspecies hydrogen transfer: 15 Years later. ASM New. Am. Soc. Microbiol. **48**:561-565.

WSDE. 1987. Solid waste landfill design manual. Washington State Department of Ecology. Olympia, WA.

Xia, J. Q., C. V. Yason, and F. S. B. Kibenge. 1995. Comparison of dot blot hybridization, polymerase chain reaction, and virus isolation for detection of bovine herpesvirus-1 (BHV-1) in artificially infected bovine semen. Can. J. Vet. Res. **59**:102-109.

Yang, C.-H., D. E. Crowley, and J. A. Menge. 2001. 16S rDNA Fingerprinting of rhizosphere bacterial communities associated with healthy and phytophthora infected avocado roots. FEMS Microbiol. Ecol. **35**:129-136

Young, L. Y., and A. C. Frazer. 1987. The fate of lignin and lignin derived compounds in anaerobic environments. Geomicrobiol. J. 5:261-293.

Zehnder, A. J. B. 1978. Ecology of methane formation. *In* R. Mitchell (ed.), Water pollution microbiology. John Wiley & Sons Inc., New York.

Zheng, D. E., W. Alm, D. A. Stahl, and L. Raskin. 1996. Characterization of universal small-subunit rRNA hybridization probes for quantitative molecular microbial ecology studies. Appl. Environ. Microbiol. **62**:4504-4513.

Zinder, S. H. 1993. Physiological ecology of methanogens, p. 128-296. *In* J. G. Ferry (ed.), Methanogenesis. Chapman & Hall, Inc., New York.

Appendix A: Using Landfill Gas to Study Solid Waste Microbial Communities

This is a summary of a poster presentation presented by R.C. Barry and M. Kim at the 100th General Meeting of the American Society for Microbiology 2000. The results from this preliminary investigation provided an initial conformation that landfill organisms are indeed brought to the surface with gas flows.

Background

During the summer of 1998, samples of landfill gas and landfill gas condensates were collected from four landfills located in the Mid-Atlantic regions of the United States. Samples were evaluated for the presence and viability of microorganisms. The facilities participating in this study were the Burlington County Landfill in Florence, NJ; the Middlesex County (Edgeboro) Landfill in East Brunswick, NJ; the Waste Management Tullytown Landfill in Fairless Hills, PA; and the Central Solid Waste Management Center in Sandtown, DE. All of these facilities have active gas collection systems that apply vacuum to networks of gas extraction wells. Samples were evaluated by direct count and MPN procedures as well as microscopic examination.

Procedures

Direct counts of landfill gas samples

A gas sampling pump (Bendix type C115, 2 L/min nominal flow rate) was used to draw gas from well heads through a sterile membrane filter (Nucleopore, 2 μ m polycarbonate). Gas flow rates were measured using a rotameter calibrated against a laboratory standard at Drexel University. The filters were then stained with either DAPI or Acridine orange using an adaptation of the protocols described by Kepner and Pratt (1994). Counts were made under epifluorescent microscope, and results extrapolated to estimate concentrations in the original gas sample. In addition, at the Edgeboro landfill, samples were collected to compare microbial concentrations in wells with high gas flow rates to wells with low gas flow rates.

Direct counts of condensate

Samples of landfill gas condensate were collected from the Burlington County and Tullytown landfills. Condensate from Burlington County was collected from a drainage network and so originated at several wells. Condensate from Tullytown came from a single well. Samples were transported in sterile containers at 4 °C and were stained directly using DAPI or Acridine orange. Samples were then passed through sterile membrane filters (2 μ m polycarbonate) for viewing (Kepner and Pratt, 1994). A Petroff-Hauser cell counter was also used to estimate microbial concentrations in the landfill gas condensate.

Chemical oxygen demand (COD)

The COD of Burlington County landfill gas condensate was measured using the closed reflux colorimetric method (Standard Methods 522D, using Hach COD vials and a Spectronic Genesays 2 UV-Vis spectrophotometer). Where available, Landtec GEM-500 landfill gas analyzers and Landtec pitot-tube well heads owned by landfill operators were used to estimate landfill gas flow rates and composition.

Most probable number (MPN) counts

The 5-tube MPN method for total anaerobes described by Qian and Barlaz (1996) was used to estimate the number of viable bacteria in landfill gas and landfill gas condensate from the Burlington County landfill. Sterile syringes were used to withdraw 5 mL aliquots of landfill gas from sterile gas sampling lines, and the gas was then injected into serum bottles containing oxygen-free dilution buffer. Serial dilutions were prepared and used to inoculate vials of total anaerobe medium. The vials were incubated for 60 days; positive results were noted by visible changes in optical density.

Results

Findings are summarized in tables A and B. Significant concentrations of microorganisms were found in both landfill gas and landfill gas condensate. Direct count concentrations ranged from 4.3. to 196/mL in the gas phase, and from 3×10^{6} /mL to 9×10^{6} / mL in the liquid condensate. MPN results for Burlington County samples were between 7 and 11 times lower than direct count results.

Photomicrographs of DAPI stained particles from gas phase are shown in figure A, while microbes collected from the condensate are shown in figure B. Microbial assemblages, perhaps pieces of detached biofilms, were evident in samples collected from all sites, both in gas and in condensates.

The condensate from Burlington County had a surprisingly high COD value of 14,400 mg/l, a result late verified by Burlington County during independent testing. A second round of sampling on March 2, 1999, showed a condensate COD of 18,800 mg/L and a pH of 4.26 (Leachate COD at the site is typically between 7,000 and 8,000 mg/L). The high COD and low pH are almost certainly the result of short-chain volatile fatty acids and related fermentation products condensing out of the gas phase along with the water.

Reference

Kepner, R. L., Jr., and J. R. Pratt. 1994. Use of fluorochromes for direct enumeration of total bacteria in environmental samples: past and present. Microbiol. Rev. 58:603-615.

Qian, X., and M. A. Barlaz. 1996. Enumeration of anaerobic refuse-decomposing micro-organisms on refuse constituents. Was. Manage. Res. 14:151-161.

Facility	Well	Gas Flow	Count	Method
		(scfm)	(per mL)	
Edgeboro Landfill	EW-98	20	72	AO
East Brunswick, NJ	EW-75	> 100	78	AO
	GW-26	73	124	AO
Burlington County, NJ	W-18	40	77	DAPI
	W-1	N/A	4.3	DAPI
	W-1	N/A	0.4	MPN
Tullytown	W-20	N/A	27	DAPI
Fairless Hills, PAW-28		N/A	196	DAPI

Table A. Direct Count and MPN Results for Landfill Gas

Table B. MPN, Direct count, Chemical Oxygen Demand and pH Results for Landfill Gas Condensate

Facility	Analysis	Result
Burlington County, NJ	MPN	5×10^5 /ml
(Sample Date 7/27/98)	Petroff-Hause Count	3.3×10^{6} /ml
	COD	14,400 mg/l
Burlington County, NJ	COD	18,800 mg/l
(Sample Date 3/2/99)	pH	4.26
Tullytown Landfill	DAPI	8.8×10^{6} /ml
Fairless Hills, PA		
(Sample Date 7/28/98)	Petroff-Hauser Count	8.2×10^{6} /ml



Figure A. Microorganisms filter from landfill gas and stained with DAPI. The sample was collected from the Burlington County Landfill, New Jersey.



Figure B. A particle collected from landfill gas condensate and stained with DAPI. This sample was collected from the Burlington County Landfill, New Jersey.

Appendix B: Media for Methanogens

ATCC medium: 1439 Methanogenium medium

KCl	0.335 g
MgCl ₂ •7H ₂ O	2.75 g
MgSO ₄ ·7H ₂ O	3.45 g
NH ₄ Cl	0.25 g
CaCl ₂ •2H ₂ O	0.14 g
K ₂ HPO ₄	0.14 g
NaCl	18.0 g
Trace Elements Solution SL-6 (see below)	10.0 ml
Wolfe's Vitamin Solution (see below)	10.0 ml
$Fe(NH_4)_2(SO4)_2$ ·7H ₂ O	2.0 mg
NaHCO ₃	5.0 g
Sodium acetate	1.0 g
Yeast extract	2.0 g
Trypticase (BBL 11921)	2.0 g
Resazurin	1.0 mg
L-Cysteine•HCl•H ₂ O	0.5 g
Na ₂ S•9H ₂ O	0.5 g
Distilled water to	1.0 L

Adjust for final pH 6.8. Prepare anaerobically under 80% N_2 and 20% CO_2 . Autoclave at 121°C for 15 minutes.

Trace Elements Solution SL-6:

$ZnSO_4 \cdot 7H_2O$	0.10 g
MnCl ₂ •4H ₂ O	0.03 g
H ₃ BO ₃	0.3 g
CoCl ₂ •6H ₂ O	0.2 g
CuCl ₂ ·H ₂ O	0.01 g
NiCl ₂ •6H ₂ O	0.02 g
Na ₂ MoO ₄ •H ₂ O	0.03 g
Distilled water	1.0 L
Distilled water	1.0 L

Adjust final pH of Trace Elements Solution SL-6 to 3.4.

Wolfe's Vitamin Solution:

2.0 mg
2.0 mg
10.0 mg
5.0 mg
5.0 mg
5.0 mg
5.0 mg
0.1 mg
5.0 mg
5.0 mg
1.0 L

ATCC medium: 1355 Methanosarcina acetivorans medium

NaCl	23.4 g
MgSO ₄	6.3 g
Yeast extract	1.0 g
Na ₂ CO ₃	5.0 g
NH ₄ Cl	1.0 g
KCl	0.8 g
CaCl ₂ •2H ₂ O	0.14 g
Na ₂ HPO ₄	0.6 g
Resazurin	1.0 mg
L-Cysteine•HCl•H ₂ O	0.25 g
Na ₂ S·9H ₂ O	0.25 g
Trimethylamine HCl*	3.0 g
Wolfe's Mineral Solution (see below)	10.0 ml
Distilled water to	1.0 L

Adjust pH of the medium to 7.2 with 6 N HCl before autoclaving. Slants contain 1% purified agar.

*Methanol or methylamine HCl may be substituted for trimethylamine HCl at a concentration of 50 mM.

1. Melt agar in a round-bottom flask with all components except sodium sulfide. The best results are obtained by autoclaving under low pressure for 5 minutes.

2. Place medium in a water bath adjusted to 50° C with a gas mixture of 80% N₂ and 20% CO₂ flowing through the headspace.

3. If there is a large amount of precipitate, add HCl and mix thoroughly

by swirling. As the precipitate goes into solution the pH will decrease.

A small amount of precipitate may remain.

4. Add sodium sulfide.

5. Dispense into tubes under 80% N₂ and 20% CO₂; seal with butyl rubber stoppers and autoclave at 121° C for 15 minutes. A precipitate will form during autoclaving but will go back into solution as the medium cools. Gently inverting the tubes before the medium solidifies will facilitate dissolution.

6. Broth medium is prepared in the same fashion but a water bath is not required.

Wolfe's Mineral Solution:

1.5 g
3.0 g
0.5 g
1.0 g
0.1 g
0.1 g
0.1 g
0.1 g
0.01 g
0.01 g
0.01 g
0.01 g
1.0 L

Add nitrilotriacetic acid to approximately 500 ml of water and adjust to pH 6.5 with KOH to dissolve the compound. Bring volume to 1.0 L with remaining water and add remaining compounds one at a time.

DSM Medium 334: Methanotrhix Medium

Use medium 318 without yeast extract, trypticase, and methanol. Add 6.8 g/L sodium acetate and increase the amount of KHCO₃ to 4.0 g/L. Final pH should be 7.0. Use 20% inoculum. Pressurize culture bottles to 1 bar overpressure with 80% N_2 + 20% CO₂.

DSM Medium 318: Methanosarcina (Bcyt) Medium

KH ₂ PO ₄	0.3 g
NaCl	0.6 g
MgCl ₂ ·6H ₂ O	0.1 g
CaCl ₂ ·2H ₂ O	0.08 g
Trace element solution (see below)	10.0 ml
Vitamin solution (see medium 141)	10.0 ml
NH ₄ Cl	1.0 g
Yeast extract	0.5 g
Trypticase (BBL)	0.5 g
Methanol	5.0 ml
Resazurin	1.0 mg
KHCO ₃	2.0 g
Cysteine-HCl·H ₂ O	0.3 g
Na ₂ S·9H ₂ O	0.3 g
Distilled water	1.0 L

Adjust final pH to 6.8. Gas phase: $80\% N_2 + 20\% CO_2$. Sterilize the vitamins (by filtration), cysteine and sulfide separately.

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Nitrilotriacetic acid (NTA)	12.8 g
FeCl ₃ ·6H ₂ O	1.35 g
MnCl ₂ ·4H ₂ O	0.1 g
CoCl ₂ ·6H ₂ O	0.024 g
CaCl ₂ ·2H ₂ O	0.1 g
ZnCl ₂	0.1 g
CuCl ₂ ·2H ₂ O	0.025 g
H ₃ BO ₃	0.010 g
Na ₂ MoO ₄ ·2H ₂ O	0.024 g
NaCl	1.0 g
NiCl ₂ ·6H ₂ O	0.12 g
Na ₂ SeO ₃ ·5H ₂ O	0.026 g

First dissolve NTA in 200 ml of water and adjust the pH to 6.5 with KOH.

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Grant Award

Cooperation Activities Program Student Travel Grant. 2003. American Society for Microbiology, Washington, D.C.

Poster Presentation at Professional Meetings

Kim, M., and R. C. Barry. 2003. Characterization of landfill microbial communities using landfill gas and landfill gas condensate with rRNA slot-blot hybridization. *In* abstracts of the 103rd General Meeting of the American Society for Microbiology 2003. American Society for Microbiology, Washington, D.C.

Kim, M., and R. C. Barry. 2002. Sampling and extraction of total RNA from landfill gas for analysis of microbial community structure. *In* abstracts of the 102nd General Meeting of the American Society for Microbiology 2002. American Society for Microbiology, Washington, D.C.

Kim, M., and R. C. Barry. 2001. Comparison of microbial communities in landfills using fatty acid methyl ester (FAME) profiles. *In* abstracts of the 101st General Meeting of the American Society for Microbiology 2001. American Society for Microbiology, Washington, D.C.

Barry, R. C., and M. Kim. 2000. Using landfill gas to study solid waste microbial communities. *In* abstracts of the 100th General Meeting of the American Society for Microbiology 2000. American Society for Microbiology, Washington, D.C.

Vita