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ORIGINAL ARTICLE

Microbial population dynamics in laboratory-scale solid waste bioreactors in the presence or absence of biosolids

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Abstract

Aims: Decomposition of solid waste is microbially mediated, yet little is known about the associated structure and temporal changes in prokaryotic communities. Bioreactors were used to simulate landfill conditions and archaeal and bacterial community development in leachate was examined over 8 months.
Methods and Results: Municipal solid waste (MSW) was deposited in laboratory bioreactors with or without biosolids and combustion residues (ash). The near-neutral pH fell about half a log by day 25, but recovered to ~7·0 by day 50. Cell concentrations in bioreactors containing only MSW were significantly higher than those from co-disposal bioreactors. Archaeal and bacterial community structure was analysed by denaturing gradient gel electrophoresis targeting 16S rRNA genes, showing temporal population shifts for both domains. *mcrA* sequences retrieved from a co-disposal bioreactor were predominantly affiliated with the orders *Methanosarcinales* and *Methanomicrobiales*.
Conclusion: Regardless of waste composition, microbial communities in bioreactor leachates exhibited high diversity and distinct temporal trends. The solid waste filled bioreactors allowed simulation of solid waste decomposition in landfills while also reducing the variables.
Significance and Impact of the Study: This study advances the basic understanding of changes in microbial community structure during solid waste decomposition, which may ultimately improve the efficiency of solid waste management.

Introduction

Disposal of municipal solid waste (MSW) in landfills supports the development of diverse microbial populations (Kjeldsen *et al.* 2002). The composition of microbial communities is influenced by many factors such as the types of wastes deposited, moisture availability, oxidation-reduction states and temperature (Barlaz *et al.* 1989a; Kjeldsen *et al.* 2002). Co-disposal of waste includes MSW and other types of wastes, including biosolids from wastewater treatment facilities, ash residues from waste-to-energy and other combustion processes, electronic wastes, construction and demolition wastes.

Understanding microbial population development in landfills over a period of time is challenging due to the complexity of waste materials deposited and the spatial heterogeneity of landfills. Previous studies have focused on particular aspects of microbial populations in waste degradation processes. Group-specific primers were employed to detect cellulolytic clostridia (Van Dyke and McCarthy 2002) and fungi (Lockhart *et al.* 2006) in landfill leachate. Quantitative real-time polymerase chain reaction (PCR) was used to study the development of type I methanotrophic communities during composting of organic matter (Halet *et al.* 2006) and to determine the abundance of cellulolytic *Fibrobacter* species in landfills (McDonald *et al.* 2008). Sequencing of cloned DNA (clone libraries) was used to study archaeal populations in the leachate of a full-scale recirculating landfill and bacterial populations in the leachate of a closed landfill

(Huang *et al.* 2002, 2005). Several studies have also investigated methanogenic *Archaea* populations in landfills (Huang *et al.* 2003; Uz *et al.* 2003), yet none of them attempted a temporal comparison.

Methanogenesis is a process that generates useful methane gas during waste degradation in landfills (Barlaz *et al.* 1989a; Senior *et al.* 1990). However, methane recovery rates are affected by waste composition, microbial degradation dynamics and economic feasibility. Methane is also a potent greenhouse gas and landfills account for 34% of all methane emissions (U.S. EPA 1999). Methanogens can be detected and analysed using molecular techniques such as fluorescence *in situ* hybridization (FISH) (Calli *et al.* 2005) and construction of 16S rDNA clone libraries (Huang *et al.* 2003; Mori *et al.* 2003) or biomarkers (Hales *et al.* 1996; Nercessian *et al.* 1999; Earl *et al.* 2003; Dhillon *et al.* 2005). Functional genes are used as biomarkers because their higher evolutionary rates can enhance the resolution of sequences at the species level compared to the 16S rRNA gene (Braker *et al.* 2000; Junca and Pieper 2004).

Conditions characteristic of solid waste degradation in a landfill were mimiced in bioreactors filled with solid waste and maintained in the laboratory. The chemical data associated with the study have been published (Cardoso *et al.* 2006). The microbial data that were collected simultaneously with the chemical data are presented in this work. We hypothesized that the microbial community development would vary in bioreactors with different waste composition. Denaturing gradient gel electrophoresis (DGGE) was used to evaluate the community development in terms of dominant members of the *Archaea* and *Bacteria*, providing a broad snapshot of changes in the microbial community over an 8-month period. Methanogen sequences from leachate samples were obtained using primers targeting the *mcrA* gene coding for the alpha subunit of the methyl coenzyme-M reductase (MCR) enzyme and compared over time.

Materials and methods

Bioreactor design

Bioreactors were designed to simulate landfill disposal practices in the US. They were constructed from 1·4 m tall, 30·5 cm diameter PVC pipes. The waste mixtures were hydrated to field capacity and leachate was recirculated daily to simulate rainfall of 8 cm d^{-1}. The leachate collection system was designed to simulate field conditions and consisted of a perforated 32 mm diameter PVC. The leachate collection pipes were surrounded by gravel (50·8 mm) with geotextiles above and below the gravel layers. The drainage system separating the waste from the leachate collection pipe consisted of 5 inches of granular material (25·4 mm gravel or Cholee sand). The bioreactors were operated in duplicate. A diagram of the bioreactor design is presented in our previously published paper (Cardoso *et al.* 2006).

Four bioreactors were filled with either MSW alone or MSW co-disposed with biosolids and combustion residues from waste-to-energy facilities. The waste materials were obtained from the North County Resource Recovery Facility in Palm Beach County, FL, USA. Duplicate bioreactors were packed with either 100% MSW or 60% MSW co-disposed with 30% combustion residues (6% fly ash + 24% bottom ash) and 10% biosolids (comprised of 50% material from drinking water treatment + 50% material from wastewater treatment).

Sample processing

Leachate samples were collected and 3 ml of each sample was filtered through 0·45 μm filters and the filters were stored at −20°C. Community DNA was extracted from the filters using the Ultraclean Soil DNA Kit (MoBio Laboratories, Inc., Carlsbad, USA) per manufacturer's instructions. The extracted DNA was stored at −20°C until further processing (1 week maximum).

Total microbial concentrations

Duplicate 1 ml samples of leachate from each bioreactor were individually centrifuged. The cells were washed in sterile phosphate buffered saline (PBS), stained with DAPI (4,6-diamidine-2-phenylindole) (1 mg ml^{-1} DAPI) and filtered through a 0·2 μm polycarbonate filter (Millipore, Billerica, USA). The stained cells were observed under a fluorescent microscope using a UV2B filter. Cells from five different fields of view were counted and the average was used to calculate the cell concentration ml^{-1} of sample. Measurements in duplicate samples varied from one another by less than 10%.

Polymerase chain reaction for community analysis

Direct amplification of the 16S rRNA genes of *Archaea* using the primer set 344f and 517r was not consistently successful; therefore, a nested approach was used. The first round of PCR was performed using the primer set 21f and 958r (Delong 1992; Pearson *et al.* 2004). Acetamide was added to a final concentration of 2% (v/v) to increase the specificity of the reaction. PCR conditions were as follows: initial denaturation at 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 45 s, extension at 72°C for 1 min, and a final extension at 72°C for 5 min. This procedure was followed by a second round of PCR using *Archaea*-specific forward primer 344f

and a universal reverse primer 517r (Bano *et al.* 2004; Pearson *et al.* 2004). A 40 bp GC-clamp was attached to the 5′-end of the forward primer. Acetamide was excluded from the reactions. Templates were amplified using a 'touchdown' PCR to increase primer specificity (Ferrari and Hollibaugh 1999). The archaeal primer sets used in this study amplify both euryarchaeotes and crenarchaeotes and do not amplify nonarchaeal templates (Delong 1992; Raskin *et al.* 1994). *Methanosarcina acetivorans* strain C2A (DSM 2834) was used as the positive control.

Bacterial 16S rRNA genes were amplified using the primer set 1070f and 1392r (Ferris *et al.* 1996). A 40 bp GC-clamp was attached to the 5′-end of the reverse primer. PCR conditions were the same as those used for the first round of archaeal amplification. *Escherichia coli* ATCC 9637 was used as the positive control.

Denaturing gradient gel electrophoresis

DGGE was carried out using the Bio-Rad (Richmond, USA) DCode Universal Mutation Detection System. A 1 mm thick 7% (w/v) polyacrylamide gel containing a 40–65% linear denaturing gradient of formamide and urea (100% denaturant = 7 mol l^{-1} urea and 40% (v/v) formamide) was prepared for archaeal community analysis whereas a 45–60% gradient was used for bacterial community analysis.

DGGE standards were created by loading GC-clamped PCR products (approx. 150–300 ng total DNA) of the small subunit rRNA gene from *Aiptasia pallida* (brown sea anemone) (18S rRNA), *Gallus domesticus* (chicken) (18S rRNA), *M. acetivorans* strain C2A (DSM 2834) (16S rRNA), *Clostridium perfringens* (Sigma D5139) (16S rRNA), *E. coli* ATCC 9637 (16S rRNA) and *Streptomyces fradiae* ATCC 10745 (16S rRNA) mixed with 10 μl of loading dye. Two standard lanes were loaded per gel. Approximately 650–800 ng (total) of PCR product amplified from leachate samples was loaded in individual lanes of the gel. Gels were electrophoresed at 47 V, 60°C for 16 h, stained with SYBR Green I and images were obtained using a Foto/Analyst Imaging System (Fotodyne Inc., Hartland, USA).

Cloning and sequence analysis of *mcrA* gene

Since co-disposal (MSW + ash + biosolids) is a widespread method of waste disposal, one of the two co-disposal bioreactors was selected for the study of methanogen populations. An early sample of leachate (day 50) and a late sample (day 218) were selected for methanogen population analysis. DNA extracted from the two samples was amplified using the ME1 and ME2 primers (Hales *et al.* 1996; Nercessian *et al.* 1999) that target the *mcrA* gene. PCR conditions were as described previously (Hales *et al.* 1996). *Methanosarcina acetivorans* strain C2A (DSM 2834) was used as the positive control.

The day 50 and day 218 amplicon bands (760 bp) were excised from the gel using a QIAQuick Gel Extraction Kit (Qiagen, Valencia, CA, USA), as per manufacturer's instructions. The TOPO TA Cloning Kit for Sequencing (Invitrogen, CA, USA) was used for both cloning and transformation (as per manufacturer's instructions). The vectors (plasmids) were subsequently transformed into One Shot TOP10 chemically competent *Escherichia coli* cells (Invitrogen, Carlsbad, CA, USA). Cells were gently plated onto Luria broth (LB) agar plates amended with 100 μg ml^{-1} ampicillin and individual colonies were re-streaked on new plates. Plasmids were extracted using the FastPlasmid Mini kit (Eppendorf, Hamburg, Germany) per manufacturer's instructions. Insert DNA from the extracted plasmids was amplified using the ME primer set and confirmed by agarose gel electrophoresis. PCRs were purified using QIAQuick PCR Purification Kit (Qiagen). The Genome Lab DTCS-Quick Start Kit (Beckman Coulter, Fullerton, CA, USA) was used for the final sequencing PCR. The amplified DNA was purified, concentrated by ethanol precipitation and sequenced using a Beckman CEQ™ 8000 Genetic Analysis System (Beckman Coulter, Fullerton, USA). Sequences were analysed using Blast (http://www.ncbi.nlm.nih.gov/BLAST/) to confirm their identities as methanogens. Possible methanogen sequences were designated JME for clones from the day 50 sample, and DME for clones from the day 218 sample.

Statistical analysis

Gel images were imported into Bionumerics (Version 3.0, Applied Maths, Sint-Martens-Latem, Belgium) and analysed using the Dice similarity coefficient by constructing unweighted pair group method with arithmetic mean (UPGMA) dendrograms (optimization 1·0%, tolerance 0·5%). Principal components analysis (PCA) was performed using spss (SPSS Inc., Chicago, IL, USA) to obtain two-dimensional plots showing relatedness of populations. PCA is a data reduction technique which takes into account all the variables in a given set, determines the patterns of similarities and differences between the variables and expresses the results as a two or three-dimensional plot. For fingerprint patterns such as those obtained by DGGE or RFLP, bands are classified as present or absent (binary) and compared by constructing a band-matching table (Boon *et al.* 2002; Caddick *et al.* 2006). Microbial concentrations (direct microscopic cell counts) were compared by paired *t*-tests (GraphPad Instat, La Jolla, USA). Shannon diversity index is a measure of the richness (number of species present in a community) and abundance of any

ecological habitat. Shannon diversity index (H) was calculated by using the formula:

$$H = -\sum p_i \log p_i$$

calculated as $p_i = n_i/N$ where n_i is the height of a peak and N is the sum of the peak heights of all bands in the densitometric curve (Eichner *et al.* 1999; Ogino *et al.* 2001; Haack *et al.* 2004).

Shannon indices were calculated for the community profile of each given time point of individual bioreactors and an average of these was reported.

Results

Total cell concentrations

Total cell concentrations (measured by epifluorescence microscopy) in the first month of bioreactor operation increased about an order of magnitude, from 3×10^8 cells ml^{-1} to 3×10^9 cells ml^{-1} in MSW bioreactors and co-disposal bioreactors. Cell concentrations dropped after 50 days, then stabilized in all bioreactors except MSW1. Cell concentrations varied only about four-fold from bioreactor start-up to the termination of the experiment. There was a significant difference between the mean cell concentrations of the two MSW bioreactors calculated over the course of the study (paired t-test; $P < 0{\cdot}0001$). The difference was attributable largely to the increased cell concentrations in the second half of the experiment in MSW1. In contrast, the difference in cell concentrations for the two co-disposal bioreactors was not significant ($P > 0{\cdot}95$). Cell concentrations in leachate from MSW bioreactors were significantly greater than those from co-disposal bioreactors ($P = 0{\cdot}048$).

pH of leachate

The initial increase in microbial numbers in all bioreactors corresponded with an initial decrease in pH from neutral to 5·5 by day 25. The pH returned to neutral by day 50 and remained neutral till the conclusion of the experiment.

DGGE analysis of microbial communities

An initial experiment was performed to test the reproducibility of DGGE in the complex matrix of the leachate. Triplicate leachate samples taken within several minutes of one another were analysed from a selected bioreactor (Co-disposal 2). The similarity of the DGGE patterns for triplicate analyses of both archaeal and bacterial community structure based on 16S rRNA genes was greater than 95%, showing high reproducibility of the method (data not shown).

Leachate samples collected on 12–15 dates over a period of 8 months were subjected to DGGE. The first incidence of detection of *Archaea* by PCR using 16S rRNA genes corresponded to the occurrence of negative oxidation-reduction potentials and the presence of volatile acids, indicating anaerobic conditions (Cardoso *et al.* 2006), on day 25. Inhibition of the PCR was not responsible for the absence of archaeal PCR products in the early leachate samples, as positive control DNA spiked into these leachates was amplified.

Archaeal community structure

Analysis of archaeal DGGE patterns indicates that in all the bioreactors, the initial population changed substantially between start-up (day 25) and maturation (day 50) (Fig. 1). The data from a representative bioreactor for each treatment (MSW or co-disposal) are shown in Fig. 1a,b, respectively. In the MSW bioreactors archaeal community fingerprints over a 2-month period (day 50–78, designated cluster I) clustered together followed by a substantial change in the community at day 99 (Fig. 1a). Cluster II, which includes patterns from day 120 to day 218, denotes a cluster of comparatively similar patterns towards the end of the study. In the co-disposal bioreactors, archaeal DGGE patterns after bioreactor start-up indicated a more gradual shift in the community structure with less well-defined clusters (Fig. 1b). Principal components analysis (PCA) of the DGGE patterns also demonstrated temporal shifts in communities (Fig. 1a,b).

Bacterial community structure

Analysis of bacterial DGGE patterns revealed that the patterns in MSW bioreactors were clustered in discrete groups of high (>75%) similarity. Cluster I included day 1–25, cluster II day 50–99 and cluster III day 169–218 (Fig. 2a). In contrast, the patterns in co-disposal bioreactors shifted in a more gradual manner (Fig. 2b). The data from one bioreactor per treatment are shown in Fig. 2; relationships among the patterns of duplicate bioreactors were similar for both MSW and co-disposal treatments. PCA results for bacterial community structure also corresponded to the results obtained from the UPGMA dendrograms (Fig. 2a,b).

Microbial community structure was more similar within bioreactors than between bioreactors of the same treatment (data not shown). This grouping was consistently observed for *Archaea* and *Bacteria* in MSW and co-disposal bioreactors. Comparison of communities in MSW *vs* co-disposal bioreactors did not reveal grouping by treatment (waste type); thus, factors other than the

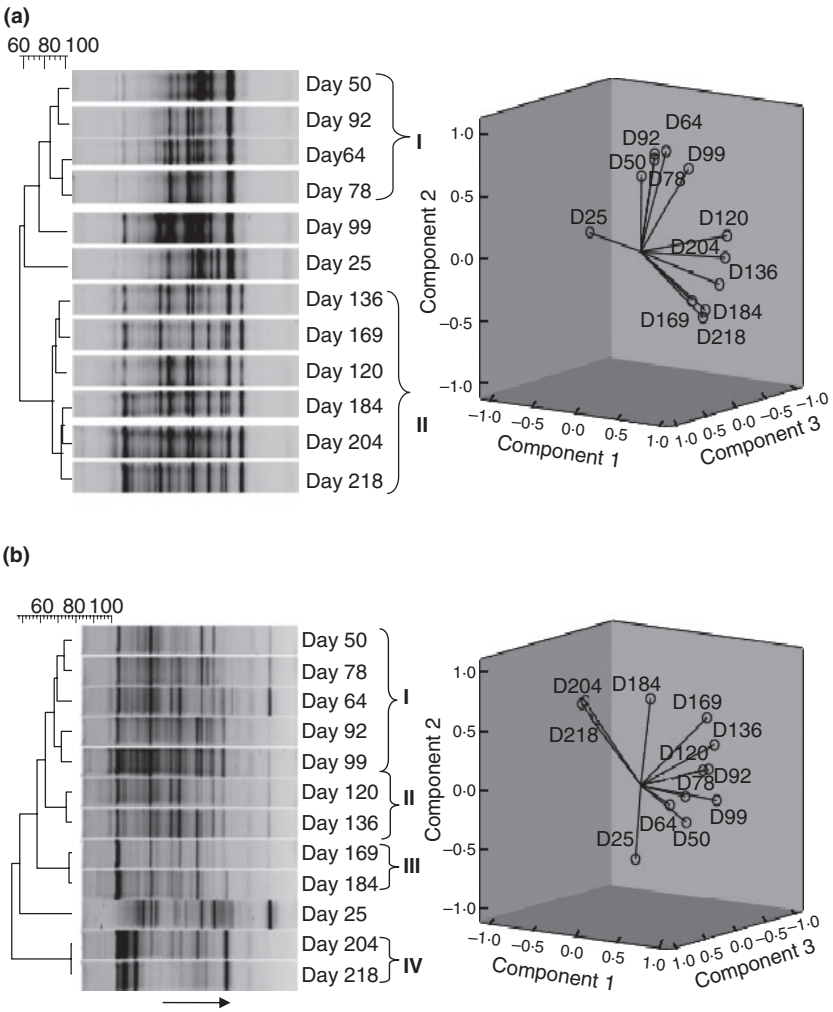

Figure 1 Dendrograms and corresponding principal components analysis of archaeal population structure in (a) MSW and (b) co-disposal bioreactors. I, II, III and IV denote clusters of similar patterns (>75% similarity). Note that *Archaea* were not detected in the leachate before day 25. (Clustering patterns of duplicate bioreactors for both MSW and co-disposal were similar). Arrow indicates increasing direction of denaturant and acrylamide gradient from lower to higher concentration.

waste composition were most instrumental in determining community structure.

Calculation of the Shannon diversity indices revealed a higher apparent diversity of observed archaeal populations (average $H = 1{\cdot}37$) in the bioreactors as compared to the bacterial populations (average $H = 1{\cdot}24$) (Table 1).

Sequence analysis of methanogens

Seventeen unique *mcrA* gene sequences were found out of the 37 clones analysed for the day 50 leachate sample of the co-disposal bioreactor (Table 2). The most numerically dominant clone was closely related to the uncultured methanogen clone RS-ME43 isolated from rice field soil (Lueders *et al.* 2001). Methanogen clones from the day 50 leachate sample were closely related to the members of *Methanosarcinales*, *Methanobacteriales* and *Methanomicrobiales*. Twelve unique *mcrA* gene sequences were found out of the 32 clones analysed for the day 218 leachate sample (Table 2). The most dominant clone was closely related to the uncultured methanogen clone MidMcrA114 isolated from the sediment of the Pearl River Estuary (Jiang, L.J., Xiao, X. and Chen, J.Q., unpublished data). All the methanogen clone sequences from the day 218 leachate sample were related to members of the Order *Methanomicrobiales*.

Discussion

The various cells of a landfill generally contain waste that is at different stages of decomposition depending upon waste composition, residence time, moisture, etc., making it difficult to study the progression of microbial population development in the highly heterogeneous landfill environment. Compared to landfills, bioreactors are simpler systems that allow better control over the variables that play a role in waste degradation. As a simplified system, bioreactors have been employed in other studies to study microbial community dynamics (Barlaz *et al.* 1989a), cellulose degrading *Clostridium* populations

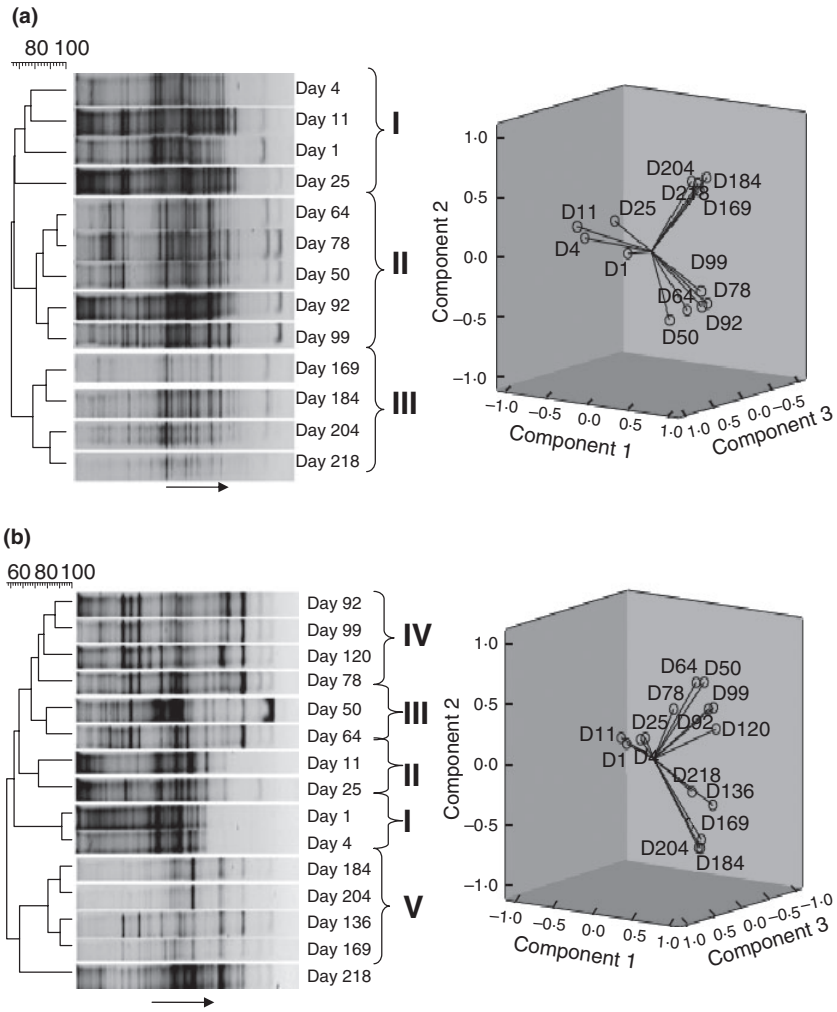

Figure 2 Dendrograms and corresponding principal components analysis of bacterial population structure in (a) MSW and (b) co-disposal bioreactors. I, II, III, IV and V denote clusters of similar patterns (>75% similarity). (Clustering patterns of duplicate bioreactors for both MSW and co-disposal were similar). Arrow indicates increasing direction of denaturant and acrylamide gradient from lower to higher concentration.

Table 1 Shannon diversity indices calculated for the DGGE patterns of *Archaea* and *Bacteria*

	Treatment	Archaea	Bacteria
Shannon diversity index (*H*)	MSW1	1·374	1·195
	MSW2	1·384	1·218
	Co-disposal1	1·361	1·241
	Co-disposal2	1·367	1·296

(Burrell *et al.* 2004), methanogen communities (Griffin *et al.* 1997) and chemical composition of leachate (Pohland and Kim 2000; Ledakowicz and Kaczorek 2004).

In spite of the attempt to construct parallel (duplicate) bioreactors in this study, cell concentrations in duplicate MSW bioreactors varied significantly over the course of the experiment, while these values in co-disposal bioreactors, which included biosolids, were similar. The variable cell concentrations between duplicate MSW bioreactors may be attributable to the heterogeneous nature of the waste, which was shredded municipal waste obtained from a landfill in Palm Beach County, FL. This waste contained paper, plastic, food and other common components of garbage, and was not standardized. This heterogeneity probably also contributed to the dissimilarity of community profiles in duplicate bioreactors. These results underscore the complexity of determining the factors that influence processes such as clogging, which can lead to landfill failures and management problems (Rohde and Gribb 1990; Fleming *et al.* 1999).

A common trend seen in all bioreactors was a decrease in pH, which was consistently accompanied by a rise in cell concentrations over the first 25–30 days of the study. This trend reflects the successive processes that typically occur during solid waste degradation. During the early stages, aerobic and facultative anaerobic heterotrophs decompose organic substrates and quickly exhaust oxygen and nitrate (Barlaz *et al.* 1989a). Cellulose and hemicellulose comprise a high percentage (45–60%) of landfilled material and are easily biodegradable (Barlaz *et al.* 1989b). Cellulose degradation is performed by hydrolytic

Table 2 Frequency of methanogen clones observed in the day 50 and day 218 samples obtained from the co-disposal bioreactor

Clone*†	Putative group (Order)	Closest relatives	Clone library (%)
JME1 (FJ435818)	*Methanobacteriales*	*Methanobacterium* sp. MB4 (DQ677519)	3
JME2, 5–7 (FJ435819, FJ435822, FJ435823, FJ435824)	*Methanomicrobiales*	*Methanocorpusculum parvum* (AY260445)	11
JME3 (FJ435820)	*Methanosarcinales*	Rice field soil clone RS-ME28 (AF313863)	3
JME4,8 (FJ435821, FJ435825)	*Methanomicrobiales*	Biogas plant clone ATB-EN-5737-M022 (FJ226633)	5
JME11–13,15,36 (FJ435826, FJ435827, FJ435828, FJ435830, FJ435849)	*Methanosarcinales*	*Methanosarcina mazei* strain TMA (AB300778)	14
JME14,22–27,30,37,43 (FJ435829, FJ435836, FJ435837, FJ435838, FJ435839, FJ435840, FJ435841, FJ435844, FJ435850, FJ435854)	*Methanosarcinales*	Rice field soil clone RS-ME43 (AF313876)	27
JME16,18,19 (FJ435831, FJ435833, FJ435834)	*Methanosarcinales*	*Methanosarcina* sp. HB-1 Subsurface groundwater clone (AB288266)	8
JME17,32 (FJ435832, FJ435846)	*Methanosarcinales*	*Methanosarcina barkeri* mcrBCDGA (Y00158)	5
JME20 (FJ435835)	*Methanosarcinales*	Nankai trough marine sediment core clone NANK-ME73121 (AY436550)	3
JME28 (FJ435842)	*Methanobacteriales*	Anaerobic digester clone ME_dig80_2_15 (DQ680456)	3
JME29 (FJ435843)	*Methanobacteriales*	Bovine rumen clone unfaunated-mcrA-13 (AB244709)	3
JME31 (FJ435845)	*Methanosarcinales*	UASB bioreactor clone GranMCR7M10 (AY937278)	3
JME34 (FJ435847)	*Methanomicrobiales*	Cattle manure clone G4INMC365 (DQ262403)	3
JME35 (FJ435848)	*Methanobacteriales*	Human fecal sample clone DC_clone-mcrA-2 (AM921682)	3
JME38 (FJ435851)	*Methanosarcinales*	Upland pasture soil clone SI_18 (DQ994847)	3
JME40 (FJ435852)	*Methanosarcinales*	*Methanosarcina mazei* strain LYC (AB300782)	3
JME41 (FJ435853)	*Methanomicrobiales*	Cattle manure clone G4INMC365 (DQ274999)	3
DME1,35 (FJ435855, FJ435885)	*Methanomicrobiales*	Biogas plant clone ATB-EN-9759-M148 (FJ226741)	6
DME2,3,8,31 (FJ435856, FJ435857, FJ435862, FJ435881)	*Methanomicrobiales*	*Methanocorpusculum parvum* (AY260445)	13
DME4,5,7 (FJ435858, FJ435859, FJ435861)	*Methanomicrobiales*	Biogas plant clone ATB-EN-5737-M022 (FJ226633)	9
DME6,19,30 (FJ435860, FJ435871, FJ435880)	*Methanomicrobiales*	Biogas plant clone MARMC548 (DQ260615)	9
DME9 (FJ435863)	*Methanomicrobiales*	*Methanocorpusculum labreanum* Z (CP000559)	3
DME10,20–22 (FJ435864, FJ435872, FJ435873, FJ435874)	*Methanomicrobiales*	Lake sediment clone Beu4ME-34 (AY625600)	13
DME11,16–18,25,33,34,38 (FJ435865, FJ435868, FJ435869, FJ435870, FJ435877, FJ435883, FJ435884, FJ435886)	*Methanomicrobiales*	Estuary sediment clone MidMcrA114 (EU681946)	25
DME13 (FJ435866)	*Methanomicrobiales*	*Methanocorpusculum* sp. MSP (AY260446)	3
DME15,27 (FJ435867, FJ435879)	*Methanomicrobiales*	Biogas plant clone F10RTCR23 (DQ261495)	6
DME23 (FJ435875)	*Methanomicrobiales*	Sewer clone (EF628141)	3
DME24,26 (FJ435876, FJ435878)	*Methanomicrobiales*	Gas condensate-contaminated aquifer clone L44B (EU364876)	6
DME32 (FJ435882)	*Methanomicrobiales*	Biogas plant clone G8RTCR50 (DQ260503)	3

*All isolates labelled JME denote clones obtained from day 50 sample.
†All isolates labelled DME denote clones obtained from day 218 sample.

and fermentative bacteria and fungi in the primarily anaerobic conditions of the landfill (Pourcher *et al.* 2001; Van Dyke and McCarthy 2002; Burrell *et al.* 2004; Lockhart *et al.* 2006; McDonald *et al.* 2008). Fermentative bacteria use monosaccharides and amino acids to produce alcohols, organic acids, carbon dioxide and hydrogen

(Barlaz *et al.* 1989a), which causes more acidic conditions (lower pH). Methanogens utilize carbon dioxide, hydrogen, acetate and formate resulting in an increase in pH (Mormile *et al.* 1996). Removal of acetate by iron reducing bacteria (Frenzel *et al.* 1999; Lin *et al.* 2007) and bicarbonates produced by sulfate-reducing bacteria (SRB) (Elliott *et al.* 1998) could also cause increase in pH of leachates. After the initial decrease in pH at day 25, pH values increased to near neutral and remained at that level through the conclusion of the study.

Another common trend among bioreactors was that, irrespective of waste composition (MSW *vs* co-disposal), archaeal populations exhibited higher apparent diversity as assessed by DGGE than the bacterial populations. To the best of our knowledge, no previous study has compared the diversity in 16S rRNA sequences of archaeal *vs* bacterial populations in decomposing solid waste. The emergence of a diverse population of *Archaea* about 25 days after inoculation and its maintenance throughout the study reflects the process of succession and ultimately maturation of the microbial community in the bioreactors. Although DGGE reflects a broad community structure, like any other molecular technique, it is subject to biases and errors such as selective amplification, heteroduplex formation and co-migration of DNA fragments (Muyzer and Smalla 1998). However, DGGE is widely employed in ecological studies because it enables quick and convenient comparison of temporal and spatial distributions of the predominant members in a population as compared to other molecular methods such as cloning and sequencing.

Analysis of microbial community structure in the laboratory bioreactors revealed temporal shifts of archaeal and bacterial populations in bioreactors regardless of the waste content, suggesting a succession process from an immature to a mature community. These results concur with other studies that demonstrated succession during solid waste decomposition. For example, a study on composting of MSW using phospholipid fatty acid analysis (PLFA) to identify operational taxonomic units suggested four stages of waste degradation (Herrmann and Shann 1997). Two studies using culture-based methods of microbial identification also found population shifts that suggested succession processes (Barlaz *et al.* 1989a; Boothe *et al.* 2001). Interestingly, microbial populations in MSW bioreactors were clustered into groups with high similarity, whereas a gradual change was detected in microbial populations from co-disposal bioreactors. The diverse inoculum provided by the biosolids may have contributed to the gradual change in community structure for the co-disposal bioreactors.

Methanogens belong to the archaean kingdom *Euryarchaeota* (Winker and Woese 1991; Barns *et al.* 1996), and are divided into five orders: *Methanosarcinales*, *Methanomicrobiales*, *Methanobacteriales*, *Methanococcales* and *Methanopyrales* (Bapteste *et al.* 2005). Our study found representatives from the *Methanosarcinales*, *Methanobacteriales* and *Methanomicrobiales* in the initial stages of waste degradation (day 50 sample). Interestingly, the later stages (day 218 sample) were exclusively dominated by members of the *Methanomicrobiales*, which include genera such as *Methanocorpusculum* and *Methanoculleus*. Several of the sequences obtained in this study were most similar to cultured methanogens, for example *Methanobacterium* sp. MB4, *Methanosarcina mazei* and *Methanocorpusculum parvum* while others were most similar to sequences obtained from uncultured organisms found in a variety of environments, including biogas plants, rice field soil, subsurface sediments and the bovine rumen. Previous studies of methanogens in leachate from bioreactors or landfills identified a number of phylogenetic groups including *Methanosaeta* and *Methanobacteriaceae* (Calli *et al.* 2003), *Methanomicrobiales* (*Methanoculleus* and *Methanofollis*) and *Methanosarcinales* (*Methanosaeta* and *Methanosarcina*) (Uz *et al.* 2003). *Methanosarcina* sp., *Methanobacterium* sp. and *Methanocorpusculum* sp. were isolated from anaerobic sewage sludge digestors (Bryant and Boone 1987; Raskin *et al.* 1994; Griffin *et al.* 1997; Whitehead and Cotta 1999).

Despite the great variability in leachate microbial community profiles, the temporal trend in microbial community structure was consistently observed for archaeal and bacterial populations in the simulated solid waste bioreactors. Gaining an understanding of the environmental factors that influence these high-diversity communities will require extensive research, but this effort is justified by the potential for using this knowledge to improving landfill management practices.

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References

Bano, N., Ruffin, S., Ransom, B. and Hollibaugh, J.T. (2004) Phylogenetic composition of Arctic Ocean archaeal assemblages and comparison with Antarctic assemblages. *Appl Environ Microbiol* **70**, 781–789.

Bapteste, E., Brochier, C. and Boucher, Y. (2005) Higher-level classification of the Archaea: evolution of methanogenesis and methanogens. *Archaea* **1**, 353–363.

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

Barlaz, M.A., Schaefer, D.M. and Ham, R.K. (1989b) Mass balance analysis of decomposed refuse in laboratory scale lysimeters. *ASCE J Environ Eng* **115**, 342–349.

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

Boon, N., De Windt, W., Verstraete, W. and Top, E.M. (2002) Evaluation of nested PCR-DGGE (denaturing gradient gel electrophoresis) with group-specific 16S rRNA primers for the analysis of bacterial communities from different wastewater treatment plants. *FEMS Microbiol Ecol* **39**, 101–112.

Boothe, D.D.H., Smith, M.C., Gattie, D.K. and Das, K.C. (2001) Characterization of microbial populations in landfill leachate and bulk samples during aerobic bioreduction. *Adv Environ Res* **5**, 285–294.

Braker, G., Zhou, J., Wu, L., Devol, A.H. and Tiedje, J.M. (2000) Nitrite reductase genes (nirK and nirS) as functional markers to investigate diversity of denitrifying bacteria in pacific northwest marine sediment communities. *Appl Environ Microbiol* **66**, 2096–2104.

Bryant, M.P. and Boone, D.R. (1987) Isolation and Characterization of *Methanobacterium formicicum* Mf. *Int J Syst Bacteriol* **37**, 171.

Burrell, P.C., O'Sullivan, C., Song, H., Clarke, W.P. and Blackall, L.L. (2004) Identification, detection, and spatial resolution of *Clostridium* populations responsible for cellulose degradation in a methanogenic landfill leachate bioreactor. *Appl Environ Microbiol* **70**, 2414–2419.

Caddick, J.M., Hilton, A.C., Armstrong, R.A., Lambert, P.A., Worthington, T. and Elliott, T.S. (2006) Description and critical appraisal of principal components analysis (PCA) methodology applied to pulsed-field gel electrophoresis profiles of methicillin-resistant *Staphylococcus aureus* isolates. *J Microbiol Methods* **65**, 87–95.

Calli, B., Mertoglu, B., Tas, N., Inanc, B., Yenigun, O. and Ozturk, I. (2003) Investigation of variations in microbial diversity in anaerobic reactors treating landfill leachate. *Water Sci Technol* **48**, 105–112.

Calli, B., Mertoglu, B., Inanc, B. and Yenigun, O. (2005) Methanogenic diversity in anaerobic bioreactors under extremely high ammonia levels. *Enz Microb Technol* **37**, 448–455.

Cardoso, A.J., Levine, A.D., Nayak, B.S., Harwood, V.J. and Rhea, L.R. (2006) Lysimeter comparison of the role of waste characteristics in the formation of mineral deposits in leachate drainage systems. *Waste Manag Res* **24**, 560–572.

Delong, E.F. (1992) Archaea in coastal marine environments. *Proc Natl Acad Sci USA* **89**, 5685–5689.

Dhillon, A., Lever, M., Lloyd, K.G., Albert, D.B., Sogin, M.L. and Teske, A. (2005) Methanogen diversity evidenced by molecular characterization of methyl coenzyme M reductase A (mcrA) genes in hydrothermal sediments of the Guaymas Basin. *Appl Environ Microbiol* **71**, 4592–4601.

Earl, J., Hall, G., Pickup, R.W., Ritchie, D.A. and Edwards, C. (2003) Analysis of methanogen diversity in a hypereutrophic lake using PCR-RFLP analysis of mcr sequences. *Microb Ecol* **46**, 270–278.

Eichner, C.A., Erb, R.W., Timmis, K.N. and Wagner-Dobler, I. (1999) Thermal gradient gel electrophoresis analysis of bioprotection from pollutant shocks in the activated sludge microbial community. *Appl Environ Microbiol* **65**, 102–109.

Elliott, P., Ragusa, S. and Catcheside, D. (1998) Growth of sulfate-reducing bacteria under acidic conditions in an upflow anaerobic bioreactor as a treatment system for acid mine drainage. *Water Res* **32**, 3724–3730.

Ferrari, V.C. and Hollibaugh, J.T. (1999) Distribution of microbial assemblages in the Central Arctic Ocean Basin studied by PCR/DGGE: analysis of a large data set. *Hydrobiologia* **401**, 55–68.

Ferris, M.J., Muyzer, G. and Ward, D.M. (1996) Denaturing gradient gel electrophoresis profiles of 16S rRNA-defined populations inhabiting a hot spring microbial mat community. *Appl Environ Microbiol* **62**, 340–346.

Fleming, I.R., Rowe, R.K. and Cullimore, D.R. (1999) Field observations of clogging in a landfill leachate collection system. *Can Geotech J* **36**, 685–707.

Frenzel, P., Bosse, U. and Janssen, P.H. (1999) Rice roots and methanogenesis in a paddy soil: ferric iron as an alternative electron acceptor in the rooted soil. *Soil Biol Biochem* **31**, 421–430.

Griffin, M.E., McMahon, K.D., Mackie, R.I. and Raskin, L. (1997) Methanogenic population dynamics during start-up of anaerobic digesters treating municipal solid waste and biosolids. *Biotechnol Bioeng* **57**, 342–355.

Haack, S.K., Fogarty, L.R., West, T.G., Alm, E.W., McGuire, J.T., Long, D.T., Hyndman, D.W. and Forney, L.J. (2004) Spatial and temporal changes in microbial community structure associated with recharge-influenced chemical gradients in a contaminated aquifer. *Environ Microbiol* **6**, 438–448.

Hales, B.A., Edwards, C., Ritchie, D.A., Hall, G., Pickup, R.W. and Saunders, J.R. (1996) Isolation and identification of methanogen-specific DNA from Blanket Bog Peat by PCR amplification and sequence analysis. *Appl Environ Microbiol* **62**, 668–675.

Halet, D., Boon, N. and Verstraete, W. (2006) Community dynamics of methanotrophic bacteria during composting of organic matter. *J Biosci Bioeng* **101**, 297–302.

Herrmann, R.F. and Shann, J.F. (1997) Microbial community changes during the composting of municipal solid waste. *Microb Ecol* **33**, 78–85.

Huang, L.N., Zhou, H., Chen, Y.Q., Luo, S., Lan, C.Y. and Qu, L.H. (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.

Huang, L.N., Chen, Y.Q., Zhou, H., Luo, S., Lan, C.Y. and Qu, L.H. (2003) Characterization of methanogenic archaea in the leachate of a closed municipal solid waste landfill. *FEMS Microbiol Ecol* **46**, 171–177.

Huang, L.N., Zhu, S., Zhou, H. and Qu, L.H. (2005) Molecular phylogenetic diversity of bacteria associated with the leachate of a closed municipal solid waste landfill. *FEMS Microbiol Lett* **242**, 297–303.

Junca, H. and Pieper, D.H. (2004) Functional gene diversity analysis in BTEX contaminated soils by means of PCR-SSCP DNA fingerprinting: comparative diversity assessment against bacterial isolates and PCR-DNA clone libraries. *Environ Microbiol* **6**, 95–110.

Kjeldsen, P., Barlaz, M.A., Rooker, A.P., Baun, A., Ledin, A. and Christensen, T.H. (2002) Present and long-term composition of MSW landfill leachate: a review. *Crit Rev Environ Sci Technol* **32**, 297–336.

Ledakowicz, S. and Kaczorek, K. (2004) Laboratory simulation of anaerobic digestion of municipal solid waste. *J Environ Sci Health A Tox Hazard Subst Environ Eng* **39**, 859–871.

Lin, B., Braster, M., Roling, W.F.M. and van Breukelen, B.M. (2007) Iron-reducing microorganisms in a landfill leachate-polluted aquifer: complementing culture-independent information with enrichments and isolations. *Geomicrobiol J* **24**, 283–294.

Lockhart, R.J., Van Dyke, M.I., Beadle, I.R., Humphreys, P. and McCarthy, A.J. (2006) Molecular biological detection of anaerobic gut fungi (Neocallimastigales) from landfill sites. *Appl Environ Microbiol* **72**, 5659–5661.

Lueders, T., Chin, K.J., Conrad, R. and Friedrich, M. (2001) Molecular analyses of methyl-coenzyme M reductase alpha-subunit (mcrA) genes in rice field soil and enrichment cultures reveal the methanogenic phenotype of a novel archaeal lineage. *Environ Microbiol* **3**, 194–204.

McDonald, J.E., Lockhart, R.J., Cox, M.J., Allison, H.E. and McCarthy, A.J. (2008) Detection of novel *Fibrobacter* populations in landfill sites and determination of their relative abundance via quantitative PCR. *Environ Microbiol* **10**, 1310–1319.

Mori, K., Sparling, R., Hatsu, M. and Takamizawa, K. (2003) Quantification and diversity of the archaeal community in a landfill site. *Can J Microbiol* **49**, 28–36.

Mormile, M.R., Gurijala, K.R., Robinson, J.A., McInerney, M.J. and Suflita, J.M. (1996) The Importance of hydrogen in landfill fermentations. *Appl Environ Microbiol* **62**, 1583–1588.

Muyzer, G. and Smalla, K. (1998) Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. *Antonie Van Leeuwenhoek* **73**, 127–141.

Nercessian, D., Upton, M., Lloyd, D. and Edwards, C. (1999) Phylogenetic analysis of peat bog methanogen populations. *FEMS Microbiol Lett* **173**, 425–429.

Ogino, A., Koshikawa, H., Nakahara, T. and Uchiyama, H. (2001) Succession of microbial communities during a biostimulation process as evaluated by DGGE and clone library analyses. *J Appl Microbiol* **91**, 625–635.

Pearson, A., Huang, Z., Ingalls, A.E., Romanek, C.S., Wiegel, J., Freeman, K.H., Smittenberg, R.H. and Zhang, C.L. (2004) Nonmarine crenarchaeol in Nevada hot springs. *Appl Environ Microbiol* **70**, 5229–5237.

Pohland, F.G. and Kim, J.C. (2000) Microbially mediated attenuation potential of landfill bioreactor systems. *Water Sci Technol* **41**, 247–254.

Pourcher, A., Sutra, L., Hebe, I.I., Moguedet, G., Bollet, C., Simoneau, P. and Gardan, L. (2001) Enumeration and characterization of cellulolytic bacteria from refuse of a landfill. *FEMS Microbiol Ecol* **34**, 229–241.

Raskin, L., Poulsen, L.K., Noguera, D.R., Rittmann, B.E. and Stahl, D.A. (1994) Quantification of methanogenic groups in anaerobic biological reactors by oligonucleotide probe hybridization. *Appl Environ Microbiol* **60**, 1241–1248.

Rohde, J.R. and Gribb, M.M. (1990) *Biological and Particulate Clogging of Geotextile/Soil Filter Systems. Geosynthetic Testing for Waste Containment Applications*. Philadelphia: American Society for Testing and Materials.

Senior, E., Watson-Craik, I.A. and Kasali, G.B. (1990) Control/promotion of the refuse methanogenic fermentation. *Crit Rev Biotechnol* **10**, 93–118.

U.S. EPA (1999) *U.S. Methane Emissions 1990-2020: Inventories, Projections, and Opportunities for Reductions; EPA 430-R-99-013*. Washington, DC: Environmental Protection Agency, Office of Air and Radiation.

Uz, I., Rasche, M.E., Townsend, T., Ogram, A.V. and Lindner, A.S. (2003) Characterization of methanogenic and methanotrophic assemblages in landfill samples. *Proc R Soc Lond B Biol Sci* **270**, 202–205.

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

Whitehead, T.R. and Cotta, M.A. (1999) Phylogenetic diversity of methanogenic archaea in swine waste storage pits. *FEMS Microbiol Lett* **179**, 223–226.

Winker, S. and Woese, C.R. (1991) A definition of the domains Archaea, Bacteria and Eucarya in terms of small subunit ribosomal RNA characteristics. *Syst Appl Microbiol* **14**, 305–310.

