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Bacterial Population Development and Chemical Characteristics of Refuse Decomposition in a Simulated Sanitary Landfill

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Population development of key groups of bacteria involved in municipal refuse conversion to methane was measured from the time of initial incubation through the onset of methane production. Hemicellulolytic bacteria, cellulolytic bacteria, hydrogen-producing acetogens, and acetate- and H_2-plus-CO_2-utilizing methanogens were enumerated by the most-probable-number technique with media containing oat spelt xylan, ball-milled cellulose, butyrate, acetate, and H_2 plus CO_2, respectively. Refuse decomposition was monitored in multiple replicate laboratory-scale sanitary landfills. A laboratory-scale landfill was dismantled weekly for microbial and chemical analysis. Leachate was neutralized and recycled to ensure methanogenesis. The methane concentration of the sampled containers increased to 64% by day 69, at which time the maximum methane production rate, 929 liters of CH_4 per dry kg-year, was measured. Population increases of 2, 4, 5, 5, and 6 orders of magnitude were measured between fresh refuse and the methane production phase for the hemicellulolytic bacteria, cellulolytic bacteria, butyrate-catabolizing acetogens, and acetate- and H_2-CO_2-utilizing methanogens, respectively. The cellulolytic bacteria and acetogens increased more slowly than the methanogens and only after the onset of methane production. The initial decrease in the pH of the refuse ecosystem from 7.5 to 5.7 was attributed to the accumulation of acidic end products of sugar fermentation, to the low acid-consuming activity of the acetogenic and methanogenic bacteria, and to levels of oxygen and nitrate in the fresh refuse sufficient for oxidation of only 8% of the sugars to carbon dioxide and water. Cellulose and hemicellulose decomposition was most rapid after establishment of the methanogenic and acetogenic populations and a reduction in the initial accumulation of carboxylic acids. A total of 72% of these carbohydrates were degraded in the container sampled after 111 days. Initially acetate utilization, but ultimately polymer hydrolysis, limited the rate of refuse conversion to methane. Microbial and chemical composition data were combined to formulate an updated description of refuse decomposition in four phases: an aerobic phase, an anaerobic acid phase, an accelerated methane production phase, and a decelerated methane production phase.

Methane is the terminal product of a series of biologically mediated reactions involved in refuse decomposition in sanitary landfills. The methane produced in sanitary landfills represents a usable, but underutilized, form of energy. Projects to recover landfill gas are frequently rejected because the onset of methane production is unpredictable and yields are typically 1 to 50% of the volumes calculated from refuse biodegradability data. Several studies on parameters which may enhance methane production have been reported (2, 6, 26, 32), but this work has not led to increased yields in sanitary landfills or an understanding of the microbiology of refuse decomposition.

Municipal refuse typically contains 40 to 50% cellulose, 10 to 15% lignin, 12% hemicellulose, and 4% protein (4; this study). Refuse conversion to methane is assumed to proceed on a pathway similar to that described for anaerobic sludge digestion (44). Three major groups of bacteria are assumed to be involved in methane production from refuse: (i) the hydrolytic and fermentative bacteria, which convert biological polymers such as cellulose and hemicellulose to sugars which are then fermented to carboxylic acids, alcohols, carbon dioxide, and hydrogen; (ii) the obligate proton-reducing acetogenic bacteria, which convert longer-chain carboxylic acids and alcohols to acetate, hydrogen, and carbon dioxide; and (iii) the methanogenic bacteria, which convert primarily acetate and hydrogen plus carbon dioxide to methane.

In an early study of landfill microbiology, Cook et al. (9) concentrated on isolation of aerobic bacteria. In later work (7, 11, 15, 16, 21, 22, 34–36, 38a), researchers began to enumerate populations and measure enzyme activities of the hydrolytic bacteria. Recently, researchers have begun to enumerate methanogens and measure levels of the electron carrier unique to methanogenic bacteria, F_{420}, in landfill samples (7, 15, 38a). Farquhar and Rovers (14) were the first to characterize refuse decomposition to methane, and they relied almost solely on gas composition data. Others (33, 34) have updated their description to include trends in carboxylic acid production and cellulose decomposition but not microbial population development.

The objective of this study was to characterize the microbial and chemical changes which occur in refuse during its anaerobic conversion to methane.

MATERIALS AND METHODS

Materials. Shredded domestic refuse was sampled from the Madison Energy Recovery Plant. Refuse with a particle size of 1.9 cm and below was used for all experimental work.

Experimental equipment. Refuse was incubated in 2-liter wide-mouth Nalgene containers. A polyethylene male adapter (0.64 by 0.64 cm) was installed in the bottom of each container as a leachate drainage port. Two such adapters

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were installed in the lid of each container for water addition and gas collection. Adapters were held in place with nylon hex nuts. All fittings and the container lid were coated with silicone caulk (732 RTV; Dow Corning Corp., Midland, Mich.) prior to tightening to provide a gas-tight system. The headspace of the container was sampled through a hole drilled in the side of the container and sealed with a rubber stopper. Leachate was collected in a 1-liter Viaflem bag (Travenol Laboratories, Morton Grove, Ill.).

Experimental design. Refuse decomposition was monitored in laboratory-scale simulated sanitary landfills. Thirty-seven 2-liter containers were filled to capacity with shredded refuse at the beginning of the experiment, and a container was dismantled weekly for microbiological and chemical analyses. Leachate recycle and neutralization was initiated in all containers to ensure methane production. Leachate recycle and neutralization is an effective method for enhancement of methane production; in its absence, it is difficult to observe methane production under laboratory conditions (2).

A complete microbial and chemical characterization required the entire contents of a container. Thus, once sampled, a container could no longer be monitored. Containers were selected for sampling to represent a logical progression in decomposition between fresh refuse and methane production. Sample selection criteria included methane concentration and production data, leachate pH, and the leachate volume recycled. A container was randomly selected for sampling from a subset in which behavior conformed to typical trends in methane concentration as reported previously (2).

Incubation conditions. Deionized water was added to the refuse to adjust its moisture content to 73% (wt/wt) to ensure the availability of ample free liquid for leachate recycle. All leachate in the leachate collection bag below each container was neutralized and recycled through the top of the container on a daily basis, 6 days per week. Leachate generation rates ranged from 300 to 700 ml/day. Initially, a solution of 100 g of sodium carbonate per liter was used for leachate neutralization. After 7 weeks, potassium carbonate (171.6 g/liter) was used to minimize the possibility of an inhibitory sodium or potassium concentration. Guidelines for maximum permissible cation concentrations in anaerobic digestors (27) were followed, and there was no evidence of cation toxicity (M. A. Barlaz, D. M. Schaefer, and R. K. Ham, Appl. Biochem. Biotechnol., in press). Leachate neutralization was discontinued once a container reached pH 7 or began to produce measurable volumes of methane. The experimental containers were incubated at 41°C, the optimal temperature for mesophilic refuse decomposition (17).

Procedure for container sampling and inoculum formation. Refuse was removed from a container and immediately placed in a plastic bag. All free air was quickly squeezed from the bag, and the refuse was mixed by shaking and hand manipulation without opening the bag. Approximately 20% of the wet weight of the refuse in the bag was removed and processed for measurement of pH, carboxylic acids, sulfide, sulfate, sugar, ammonia, nitrate, phosphate, and total organic carbon. All of the leachate which had accumulated in the leachate collection bag and additional deaerated, deionized water as needed were added to the refuse aliquot to form a slurry of at least 85% moisture (wt/wt). The slurry was mixed by hand for 1 min, after which 100 ml was poured off for analysis of dissolved sulfide. A hand-squeezed extract was formed from the remainder of the slurry. The pH and alkalinity were measured on a portion of the free liquid. The remainder of the liquid was centrifuged and filtered through a 0.45-μm-pore-size filter. Samples for analysis of the total organic carbon (TOC) and sugars were frozen. Samples for sulfate analysis were stored at 4°C. Samples for phosphate, ammonia, organic nitrogen, and nitrate analyses were acidified to pH 2 with sulfuric acid and stored at 4°C. Samples for carboxylic acid analysis were acidified as above and frozen.

The remaining 80% of the refuse removed from a container was used to process a sample for formation of an inoculum for microbial enumeration. Experiments performed to develop and validate the inoculum formation procedure are presented elsewhere (3). The moisture content of the refuse was adjusted to 88% with sterile anaerobic phosphate buffer (23.7 mM, pH 7.2). This buffer was made anaerobic by sparging it with nitrogen after autoclaving. The refuse was then blended for 1 min in a Waring blender (model CB-6; 20,000 rpm free running) with a 1-gal (3.8-liter) stainless-steel jar. The blender jar and all other equipment used for inoculum formation were autoclaved and purged with nitrogen prior to use. After blending, an extract of the refuse was formed by hand squeezing. The free liquid from hand squeezing (filtrate) was collected in a sterile flask under nitrogen. To blend 80% of the refuse removed from a container, it was necessary to blend the refuse in four batches. The filtrate from each batch was combined, mixed, and used as the inoculum for microbial enumerations.

The solids remaining after preparation of the inoculum and soluble constituent extracts were recovered, dried, and used for determination of the moisture, cellulose, hemicellulose, and lignin content of the refuse.

Medium preparation and enumeration techniques. The total anaerobic population as well as the populations of cellulolytic bacteria, hemicellulolytic bacteria, hydrogen-producing acetogens (based on butyrate catabolism), and acetate- and H_2-CO_2-utilizing methanogens were enumerated by the most-probable-number (MPN) technique with five tubes per dilution. Media are described in Table 1. All gases except 80:20 H_2-CO_2 were passed through a hot copper column to remove traces of oxygen. The mixture of hydrogen (99.99% pure) and carbon dioxide (99.8% pure) was supplied by Matheson Scientific, Inc. (Joliet, Ill.). Passage of this mixture through a hot copper column was not necessary as evidenced by the resazurin indicator and absence of a problem with the growth of *Methanobacterium formicicum* in pure culture.

The MPN and 95% confidence intervals were either determined from the tables of deMan (10) or calculated as described by Parnow (30). Dilution series were formed in phosphate dilution solution containing the phosphate solution described in Table 1, footnote *b*, resazurin, and cysteine, all at the same concentrations as in Table 1. Serum bottles used for inoculum dilution contained glass beads (3 mm) to disturb flocs (8).

All MPN tests were checked for growth after 30 days, except for the acetogen MPN test, which was incubated for 60 days. Tubes were incubated at 41°C, the same temperature at which the refuse was incubated. Tests were performed in anaerobic culture tubes (18 by 150 mm) sealed with butyl rubber stoppers that were held in place with an aluminum crimp (Bellco Biotechnology, Inc., Vineland, N.J.).

Medium for enumeration of the total anaerobic population contained 10 carbon sources, each at a concentration of 2.5 mM. Carbon sources were representative of refuse hydrolysis products. Tubes were counted as positive if their optical density at 600 nm (OD_{600}) was greater than 0.1.

TABLE 1. Media used for MPN tests

Addition (per liter of medium)	Medium					
	Total anaerobe	Xylan	Cellulose	Acetogen	H_2-CO_2	Acetate
Phosphate solution (ml)	100[b]	100[b]	100[b]	100[b]	0.2[c]	0.1[c]
M3 solution[d] (ml)	100	100	100	100	100	100
Mineral solution[e] (ml)	10	10	10	10	10	10
Vitamin solution[f] (ml)	10	10	10	10	10	10
Resazurin (0.1%) (ml)	2	2	2	2	2	2
Volatile fatty acids[g] (ml)	10	10	10			
Yeast extract (g)	1	0.25	1		2	2
Trypticase peptones (g)	2	0.25	2		2	2
Hemin (0.01%)[h] (ml)	10	10	10			
Sodium bicarbonate (g)					1.0	0.5
Carbon source[a]						
Distilled water (ml)[a]	588	698	420	698	868	868
Boiled under:	CO_2	CO_2	CO_2	N_2-CO_2 (80:20)	N_2	N_2
Na_2CO_3 (8%)[i,j] (ml)	50	50	50			
$NaHCO_3$ (5%)[j,k] (ml)				70		
Cysteine hydrochloride (5%)[j] (ml)	10	10	10	10	10	10
Final pH	6.6	6.6	6.6	7.0	6.7	7.2

[a] After adding distilled water, adjust pH to 7.2 with NaOH. After adding remaining constituents, dispense 9 ml into pressure tubes and autoclave for 20 min at 121°C. The carbon source for each medium follows. For total anaerobes, add 100 ml of a stock carbohydrate solution that contained the following (per liter): cellobiose, 9.01 g; glucose, 4.51 g; maltose, 9.01 g; xylose, 3.75 g; galactose, 4.51 g; arabinose, 3.75 g; mannose, 4.51 g; and galacturonic acid, 5.3 g before boiling. The stock carbohydrate solution was filter sterilized and handled as a sterile solution to minimize chances for growth. It was stored under nitrogen at 4°C. Soluble starch (0.41 g) and 10 ml of a 1.8% glycerol solution were added directly to the medium before boiling. Cellulose medium contained 278 ml of a 1.8% solution of Whatman no. 1 filter paper porcelain ball milled for 18 h and added before boiling. Xylan medium contained 0.05 g of heat-treated xylan per tube as described in the text. Butyrate medium contained 0.1 ml of a filter-sterilized 4 M butyric acid solution added to each tube after autoclaving. Acetate medium, sodium acetate (6.56 g/liter) was added directly to the medium prior to boiling. For H_2-CO_2 medium, tubes were prepared under nitrogen. After inoculation, tubes were flushed with a stream of 80:20 H_2-CO_2 and pressurized to 2 atm (202.6 kPa).

[b] The phosphate solution contained 16.1 g of KH_2PO_4 and 20.7 g of K_2HPO_4 per liter. It was prepared with carbonate-free water and stored under nitrogen at 4°C.

[c] A concentrated phosphate solution containing 15 g of KH_2PO_4 and 22.14 g of K_2HPO_4 per 100 ml was used to supply the phosphates. It was prepared under nitrogen and autoclaved separately.

[d] The M3 solution contained (per liter): NH_4Cl, 10 g; NaCl 9 g; $MgCl_2 \cdot 6H_2O$, 2 g; and $CaCl_2 \cdot 2H_2O$, 1 g. It was stored at 4°C under nitrogen.

[e] As described by Kenealy and Zeikus (24).

[f] As described by Wolin et al. (42).

[g] Modified from that described by Leedle and Hespell (28) by the addition of phenylacetic acid (0.0068 g) and 3-phenylpropionic acid (0.0075 g), based on work by Stack et al. (39) and Hungate and Stack (20).

[h] Prepared by dissolving 0.01 g in 100 ml of distilled water which contained 0.1 g of NH_4Cl and 0.1 g of NaOH.

[i] As described in reference 28.

[j] Added after adjusting solution containing the other ingredients to pH 7.2 and boiling under specified gas phase.

[k] The 5% sodium bicarbonate solution was boiled under an 80:20 N_2-CO_2 gas phase and stored under the same prior to use.

Growth on cellulose was detected by visible disappearance (40, 41). Tubes were considered positive if there was greater than 50% disappearance of ball-milled Whatman no. 1 filter paper. The cellulolytic MPN was performed in duplicate as two five-tube MPNs. The results were merged and interpreted as a 10-tube MPN.

Xylan from oat spelts (catalog no. X-0376, lot 105F-0276; Sigma Chemical Co., St. Louis, Mo.) was used for enumeration of hemicellulolytic bacteria. As purchased, the xylan formed a turbid suspension in water. Assessment of microbial growth by OD required removal of the nonsettleable and soluble fractions of the xylan. This was accomplished by soaking the xylan in distilled deionized water for 24 h, removing the supernatant by aspiration, and drying the remaining xylan at 65°C. After drying, the xylan was ground in a Wiley mill and 0.05 g was dispensed into each tube. Tubes were sparged with carbon dioxide, stoppered, and autoclaved. The liquid component of the xylan medium was autoclaved separately, and the liquid and solid fractions were combined aseptically after cooling. To add the liquid medium to the sterilized xylan, it was necessary to remove the rubber stoppers from the culture tubes and replace them aseptically. A tool was constructed to handle the rubber stoppers aseptically (18). To verify that the tubed medium was sterile, tubes were incubated for 3 days prior to use and checked for growth. As purchased, the xylan included 5.3% glucose, 70.5% xylose, 8.6% arabinose, and 2.2% galactose. After heat fixing, the levels of glucose, xylose, arabinose, and galactose were 5.8, 75.2, and 6.2% and not detectable, respectively. Thus, heat fixing did not alter the substrate significantly. The heat fixing process also efficiently removed soluble sugars as the medium contained only 10 mg of glucose, 60 mg of xylose, and 10 mg of arabinose per liter.

Uninoculated sterile controls were incubated with the hemicellulolytic MPN test to measure the OD of fine xylan particles suspended when tubes were vortexed prior to OD measurement. Tubes were counted as positive if their OD exceeded 0.4.

Acetogenic bacteria were enumerated as described by Mackie and Bryant (29). A 1-ml sample of a pure culture of *M. formicicum* isolated from the whey digester of Chartrain and Zeikus (8) was used as the hydrogen scavenger. Butyrate (40 mM) was used as the carbon source based on its prevalence in leachate samples (2). The methane concentration in tubes containing butyrate was compared with the methane concentration in control tubes lacking butyrate at each dilution. Tubes in which the methane concentration was significantly greater than that of the controls (P = 99%) were counted as positive.

Methanogen MPN tests were performed with either 80 mM acetate or 2 atm (202.6 kPa) of hydrogen plus carbon dioxide. Tubes were counted as positive if they contained greater than 0.5% methane (8).

Analytical methods. Gas volume was measured daily by

displacement of acidified (pH 2) saline water. If daily gas production exceeded the 1-liter capacity of the gas collection container, gas volumes were extrapolated from that produced in a 4- to 8-h period.

Gas concentration (CO_2, O_2, N_2, and CH_4) in the refuse containers was measured with a gas partitioner (model 1200; Fisher Scientific Co., Pittsburgh, Pa.) with a thermal conductivity detector. Gases were separated by two columns in series: (i) aluminum tubing (198 by 3.2 mm) with 80/100-mesh Column Pak TQ and (ii) aluminum tubing (335 by 4.8 mm) with 60/80-mesh Molecular Sieve 13x. The column was operated at 75°C with helium as the carrier gas. When detected, the presence of hydrogen was noted qualitatively. Under the conditions described here, hydrogen was not detected at a concentration of less than 3%.

Methane concentration in MPN tubes was measured with a gas chromatograph (model 438; Packard Instrument Co., Inc., Rockville, Md.) with a thermal conductivity detector and a silica gel (80/100-mesh) column (1.83 m by 2 mm) operated at 52°C.

Carboxylic acids were measured by liquid chromatography (13). The system included a Waters M-45 pump, a Waters R401 differential refractometer, a Waters U6K injector, a Bio-Rad HPX-87H column with a Bio-Rad Cation H guard column, and a Fiatron column heater. Peaks were integrated and reported on a Shimadzu C-R3A integrator. The system was operated at a mobile-phase flow rate of 0.7 ml/min, a column temperature of 45°C, a column pressure of 1,000 lb/in^2 (6,891 kPa), and a detector attenuation of 2. The mobile phase was 0.015 N H_2SO_4 plus 0.15 g of EDTA per liter. The injection volume was 50 μl.

Samples for carboxylic acid analysis were filtered through a 0.45-μm-pore-size filter, acidified to pH 2 with sulfuric acid, and frozen. Samples were then thawed, refrozen, and thawed. Material precipitated from solution by this process was removed by filtration through a 0.45-μm-pore-size filter. Samples were concentrated by less than 0.5% as a result of this step.

To produce cleaner chromatograms, we removed sugars from the samples (38). A 1-ml portion of sample was transferred to a plastic centrifuge tube. One milliliter of a calcium hydroxide solution (3.57 M) and 0.5 ml of a cupric sulfate solution [0.4 M as $Cu(SO_4) \cdot 5H_2O$] were added to the sample. The sample was vortexed and refrigerated at 4°C. After at least 30 min, the sample was centrifugated at 4°C and 9,820 × *g* for 10 min. The supernatant was then decanted into a clean centrifuge tube, 25 μl of concentrated H_2SO_4 was added, and the centrifuge tube was capped, vortexed, and frozen. Tubes were then thawed, refrozen, and rethawed. Finally, the sample was centrifuged as described above, decanted into another vial for storage, and frozen prior to liquid chromatographic analysis. In preliminary work, it was found that samples were diluted by a factor of 2.63 as a result of this procedure.

Cellulose, hemicellulose, and soluble sugars were measured by acid hydrolysis (in the case of cellulose and hemicellulose) followed by liquid chromatographic analysis (31). Acid-insoluble lignin was measured by the sulfuric acid digestion technique (12). Moisture content was measured by drying to constant weight at 75°C (1a). Sulfates were measured by barium chloride precipitation (19). Ammonia was measured by sulfuric acid titration (23), and phosphates were measured by a colorimetric ammonium molybdate method (5). Sulfides were measured by acidification followed by trapping in zinc acetate and a potassium bi-iodate-sodium thiosulfate titration (1). Total organic carbon (TOC) was measured on a Dohrmann total organic carbon DC 80 analyzer.

RESULTS

In addition to the fresh-refuse analysis, nine containers were sampled for the population development data set. The

TABLE 2. Soluble constituent concentrations in the sampled containers[a]

Container	Day sampled	Sugars[b]	TOC	TOC_a/TOC[c]	Nitrate	Sulfate	Phosphate	Ammonia	Sulfide
Fresh refuse	0	16,393	46,006	0	71.1	2,071	800	521	0
		3.46	9.71		0.015	0.44	0.169	0.11	0
31L	7	511	7,643	0.43	0	276	20.9	249	2.7
		1.46	18.19		0	0.66	0.05	0.59	0.006
16L	20	<87	7,614	0.55	0.5	179	32.5	295	2.2
		<0.59	50.25		0.003	1.18	0.21	1.95	0.015
10L	27	<105	8,668	0.94	0.6	235	96.6	1,243	13.1
		<0.58	48.0		0.003	1.3	0.53	6.88	0.07
35L	34	<87	11,924	0.69	0.5	1.4	4.6	224	5.0
		<0.63	85.9		0.004	0.01	0.03	1.61	0.036
1L	41		11,416	0.60	0.5	71	5.2	319	3.3
		0	84.5		0.004	0.52	0.04	2.36	0.024
9L	48		11,805	0.68	0	51	0.8	60	5.0
		0	69.7		0	0.30	0.005	0.35	0.03
22L	69		7,595	0.66	0	9.8	1.0	89	8.0
		0	44.5		0	0.06	0.006	0.52	0.047
24L	90		4,091	0.55	0	6.9	10.1	45	37.1
		0	18.1		0	0.03	0.04	0.2	0.16
5L	111		1,976	0	0	0.5	0	7.5	15.4
		0	15.41		0	0.004	0	0.06	0.12

[a] The top number shows milligrams per liter of liquid in the refuse plus accumulated leachate. The bottom number shows milligrams per gram (dry weight) of refuse removed from a container.

[b] Total sugars including glucose, xylose, galactose, and mannose. A large unidentified peak eluted at 53.82 min. This was between mannose (50.05 min) and the erythritol internal standard (58.89 min). Data preceded by a less than sign (<) indicate that sugar was detected below the concentration at which a linear response of the instrument was verified, as given parenthetically.

[c] The fraction of the soluble TOC accounted for by carboxylic acid analyses.

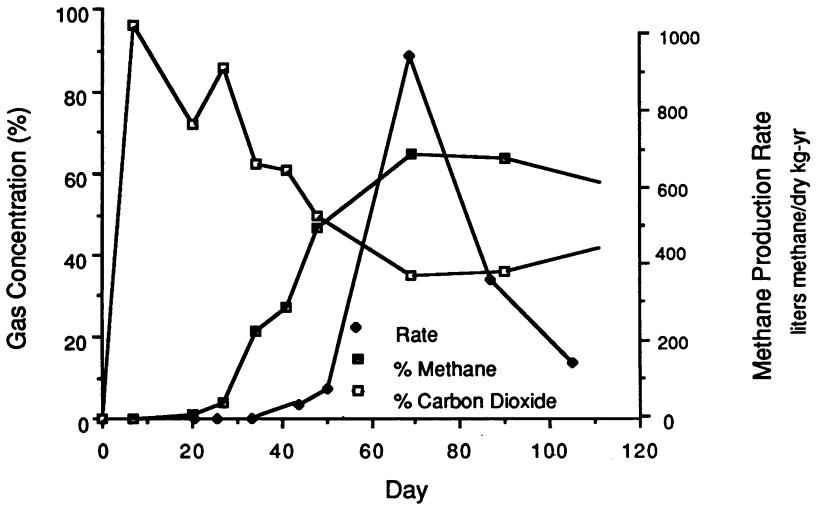

FIG. 1. Gas composition in each container 2 days before the container was sampled and the average rate of methane production in each container for the 9-day period prior to the time at which the container was sampled. Gas volume data were corrected to dry gas at standard temperature and pressure. Methane production rates were normalized to the dry weight of refuse used to fill a container.

container names and the time to sampling are given in Table 2. Methane and carbon dioxide concentrations in the sampled containers 2 days prior to dismantling are presented in Fig. 1. The average rate of methane production in each container for the 9 days prior to sampling is also presented in Fig. 1. Measurable methane production was first observed on day 41 (container 1L), and the methane production rate increased through day 69 (container 22L). Four containers were sampled prior to the onset of measurable methane production (31L, 16L, 10L, 35L), two containers were sampled while their methane production rates were increasing (1L and 9L), container 22L was sampled at what was believed to be its maximum rate of methane production, container 24L was sampled 14 days after its maximum methane production rate, and container 5L was sampled at a point when its methane production rate was decreasing slowly.

The methane production rates for containers 22L, 24L, and 5L are presented in Fig. 2. These three containers all followed the same trend of a rapid increase in methane production rate followed by a decrease for containers 24L and 5L. This gives credence to the use of several distinct containers for characterization of the refuse ecosystem. The rapid onset of methane production in 5L made it possible to sample a container late in its decomposition cycle after 111 days. The trend in the methane production rate data exhibited by containers 22L, 24L, and 5L was typical of that observed in containers which were not sampled (data not shown).

Population development data are illustrated in Fig. 3. The decrease in the acetate-utilizing methanogen population in the first week of the experiment was also observed in a container in which leachate was not recycled (Barlaz et al., in press). Thus, this decrease may not have been due simply to inherent differences between containers. A temperature increase from the waste heat of aerobic metabolism is typical of the first phase of refuse decomposition (16a). This temperature increase may have reduced the viability of the methanogen population. Other possible explanations include the release of a toxic material from the refuse, the shock associated with initiation of leachate recycle, or the pH decrease. Population development of acetate and H_2-CO_2-utilizing methanogens paralleled each other in every sample. There was no apparent reason for decreases in the hemicellulolytic population between days 7 and 20 and the methanogen population between days 34 and 41. The acetogenic and cellulolytic bacteria were the slowest trophic groups to develop. No significance is given to the small increases and decreases in the acetogenic and cellulolytic populations prior to day 48 (container 9L).

The decomposition of cellulose, hemicellulose, and lignin in the sampled containers is illustrated in Fig. 4. The hemicellulose concentration on day 34 was lower than that on days 41, 48, and 69 and was probably a result of inherent differences between containers.

Soluble TOC and sugar concentrations in the sampled containers are presented in Table 2. The concentrations of acetate, propionate, isobutyrate, butyrate, and valerate in the sampled containers are presented in Fig. 5. Acetate and butyrate concentrations increased prior to the onset of methane production and decreased thereafter. Propionate

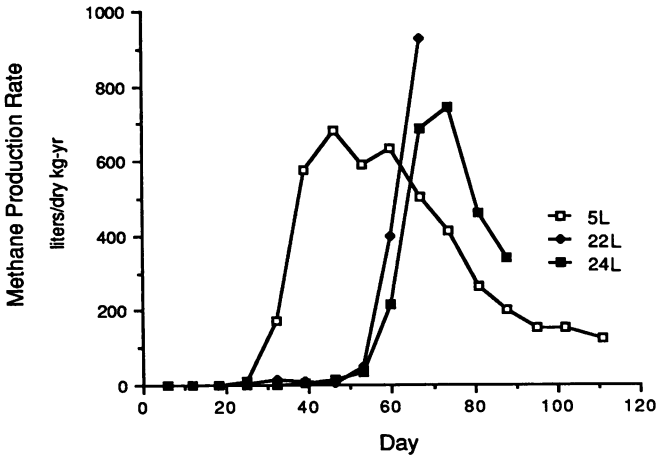

FIG. 2. Rate of methane production versus time in containers 22L, 24L, and 5L. These containers provided samples at maximum, decreasing, and low methane production rates, respectively. Data were corrected to dry gas at standard temperature and pressure.

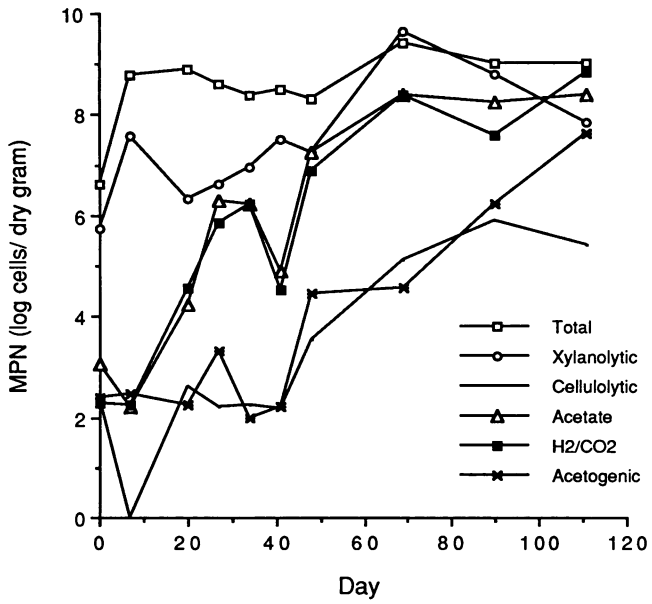

FIG. 3. Population development in the sampled containers at takedown as the log cells per g (dry weight) of refuse. The data for day 0 are for the fresh refuse used to fill the containers.

was not detected until day 27, and its concentration continued to increase after concentrations of other acids decreased. This may be attributed to the decrease in valerate, which is converted to acetate and propionate. The fraction of the soluble TOC accounted for by the carboxylic acids (TOC_a/TOC) is given in Table 2. This fraction was 0 on days 0 and 111 when no carboxylic acids were detected.

The pH of the sampled containers at takedown is presented in Fig. 6. These data include the effect of leachate neutralization.

Nitrate and sulfate concentrations in the sampled containers are presented in Table 2. Nitrates were rapidly depleted in the refuse ecosystem. The sharp decrease in the sulfate

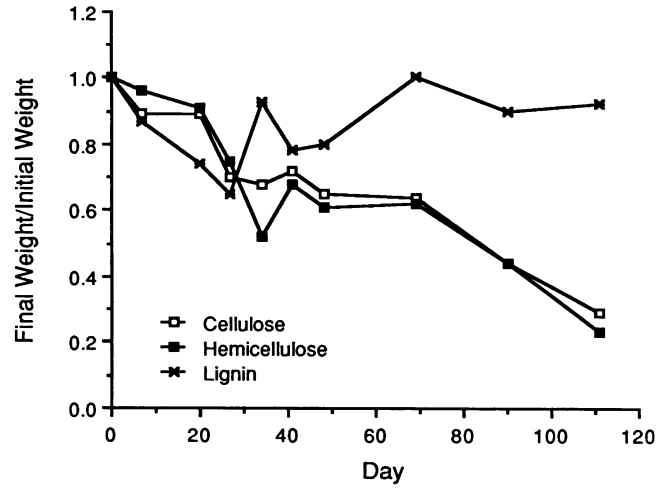

FIG. 4. Solids degradation in the sampled containers at takedown expressed as the ratio of the weight of solids (cellulose, hemicellulose, or lignin) removed from a container divided by the weight of solids added to the container at the beginning of the experiment.

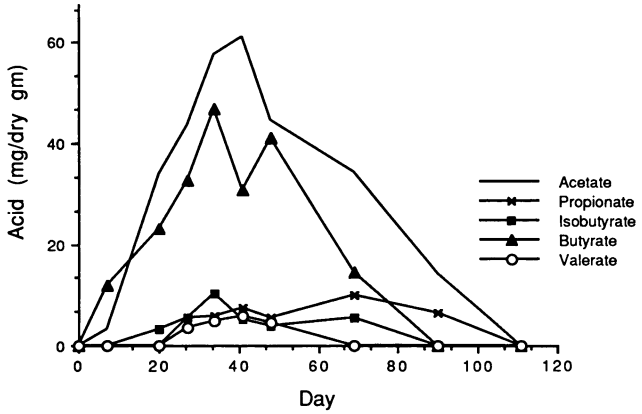

FIG. 5. Concentrations of carboxylic acids in the sampled containers at takedown (milligrams of acid per gram [dry weight] of refuse). The concentrations of acetate, propionate, isobutyrate, butyrate, and valerate in fresh refuse were less than 0.7, 0.65, 0.62, 0.63, and 0.61 mg/g (dry weight), respectively.

concentration in container 35L (day 34) appears to be anomalous.

Ammonia, phosphate, and sulfide concentrations are presented in Table 2. Ammonia and phosphate concentrations decreased after the onset of methane production. The ammonia, phosphate, and sulfide concentrations were unusually high in container 10L (day 27) relative to the other containers, suggesting that there was a nutrient-rich material in this container at the outset. Sulfide concentrations were relatively high in containers 24L (day 90) and 5L (day 111). The pH was greater than 8 in these containers; thus, most of the sulfide was in the form of HS^-, from which it cannot leave the system as a gas. The lower sulfide concentration in container 22L (day 69, pH 7.9) is not consistent with the trend of increasing sulfide concentration as a function of pH. Interpretation of sulfide data is complicated in that reduced sulfur may be released into the refuse ecosystem from amino acids and then be taken up in anabolic reactions. Sulfides may also form metal precipitates. There was no evidence that nutrients limited refuse methanogenesis (Barlaz et al., in press).

DISCUSSION

As observed here, refuse decomposition with leachate recycle may be described in four phases: aerobic phase,

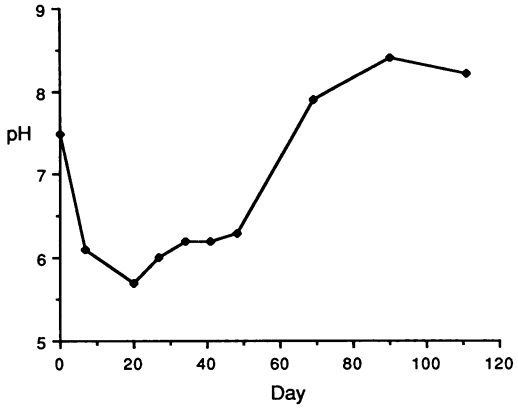

FIG. 6. pH of the sampled containers at takedown. Note that the leachate from each container was neutralized to pH 7 and recycled daily prior to the onset of measurable methane production.

TABLE 3. Microbiological and chemical characteristics of refuse decomposition

Day (container)	pH	Gas composition (%)			Methane production rate[a]	Cumulative methane[b]	Acids[c]	Solids decomposed[d]	Microbial populations		
		CH_4	CO_2	O_2					Cellulolytic[e]	Acetogen[e]	Methanogens[e,f]
0 (fresh refuse)	7.5	0	0	21	0	0	0	1	2.4	2.4	2.8
7 (31L)	6.1	0	96	0	0	0	11.41	0.90	ND[g]	2.45	2.2
20 (16L)	5.7	0.8	72.1	0	0	0	51.96	0.90	2.6	2.2	4.4
27 (10L)	6.0	3.8	86.0	0	0	0	78.25	0.71	2.2	3.3	6.1
34 (35L)	6.2	21.4	62.5	0	0	0	103.63	0.66	2.3	2.0	6.2
41 (1L)	6.2	27.4	61.2	0	14.72	0.28	95.07	0.71	2.2	2.2	4.8
48 (9L)	6.3	46.7	49.8	0	58.91	2.55	82.27	0.64	3.5	4.5	7.1
69 (22L)	7.9	64.9	34.1	0	928.6	32.0	55.69	0.63	5.1	4.6	8.4
90 (24L)	8.4	63.8	36.2	0	342.7	50.1	20.52	0.44	5.9	6.2	8.0
111 (5L)	8.2	58.1	41.9	0	126.0	86.94	0	0.28	5.4	7.6	8.7

[a] Methane production rate, expressed as liters of CH_4 per year-kilogram (dry weight) of refuse at standard temperature and pressure as constructed, for the 9 days prior to the day on which a container was sampled.
[b] Liters of CH_4 per kilogram (dry weight) of refuse at standard temperature pressure as constructed.
[c] Total carboxylic acids as acetic acid in milligrams of acid per gram of dry refuse removed from a container at the time of sampling. Acid concentrations were measured in a water extract of the refuse.
[d] Ratio of the weight of cellulose plus hemicellulose removed from a container divided by the weight of cellulose plus hemicellulose aded to the container initially.
[e] Log of the cellulolytic, acetogenic, and methanogenic populations in cells per gram (dry weight) removed from a container at the time of sampling.
[f] Log of the average of the acetate- and H_2-CO_2-utilizing methanogen populations.
[g] ND, Not detected.

anaerobic acid phase, accelerated methane production phase, and decelerated methane production phase. Trends in refuse decomposition are summarized in Table 3 and Fig. 7.

In the aerobic phase, both the oxygen and nitrate were depleted and the sugars present in fresh refuse were converted to carbon dioxide and water. Considering the sugar and nitrate concentrations in fresh refuse, an assumed porosity of the refuse (25%), and an assumed headspace of the experimental containers (100 ml), the amount of oxygen and nitrate present in a container initially was sufficient for oxidation of about 8% of the sugars to carbon dioxide. The reactions used to make this calculation are:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O \quad (1)$$

$$5C_6H_{12}O_6 + 24H^+ + 24NO_3^- \rightarrow 42H_2O + 12N_2 + 30CO_2 \quad (2)$$

All the trophic groups necessary for the conversion of refuse to methane were present in fresh refuse.

The second phase of refuse decomposition, characterized by containers 31L, 16L, 10L, and 35L (days 7 to 34), is termed the anaerobic acid phase. Oxygen and nitrate were depleted as described above, so only anaerobic biological activity was possible in phase two. The anaerobic acid phase probably began in container 31L prior to its sampling (day 7) as its oxygen concentration was 1.7% on day 2 and likely depleted before day 6 when no oxygen was detected. Sugars were present at the beginning of phase two, and the mixing resulting from leachate recycle suggests that they were well distributed. Assuming that all sugars were used before insoluble substrates were, all cellulose and hemicellulose hydrolysis occurred under anaerobic conditions.

The anaerobic acid phase was characterized by an accumulation of carboxylic acids and a decrease in pH from 7.5 in fresh refuse to between 5.7 and 6.2. The accumulation of acidic fermentation intermediates can be attributed to the insufficient levels of oxygen and nitrate in fresh refuse for the complete sugar oxidation, carbon dioxide dissolution, and low acid-consuming activities of the acetogenic and methanogenic bacteria.

The TOC_a/TOC was relatively low on day 7 (container 31L) and increased thereafter, suggesting production of a variety of non-carboxylic acid TOC initially and conversion to carboxylic acids with time. Although carboxylic acids increased throughout the anaerobic acid phase, the pH of the ecosystem stabilized between 6.0 and 6.2 as a result of leachate neutralization.

Cellulose and hemicellulose hydrolysis was not consistent in phase 2, although it was observed early in the acid phase at pH 5.7 and a cellulolytic population of 440 cells per g (dry weight). Perhaps feedback inhibition owing to the accumulation of carboxylic acids inhibited polymer hydrolysis. The cellulose and hemicellulose hydrolysis which did occur exacerbated the acid accumulation.

Carbon dioxide concentrations above 90%, indicative of fermentative activity, were observed early in the anaerobic acid phase. This CO_2 bloom has been reported previously (14). Methane was first detected on day 20 and increased to 21.4% at the end of the acid phase (day 34).

The total anaerobic and hemicellulolytic populations increased by 2 and 1 orders of magnitude, respectively, between fresh refuse and the anaerobic acid phase, while no increases were measured for the cellulolytic or acetogenic bacteria. The methanogen population increased by 4 orders of magnitude between day 7 and day 34, the beginning and end of the acid phase. This increase occurred over a pH range of 5.7 to 6.2, although the pH optimum for methanogenic bacteria is between 6.8 and 7.4 (44). Perhaps assimilatory methanogen activity is not as severely inhibited by low pH as is dissimilatory activity. Measurable methane production was not detected in the acid phase. However, methane concentration increases on days 20, 27, and 34 indicate some methane production. Thus, some energy was available for methanogen population development.

The total anaerobic population exceeded the methanogenic and acetogenic populations by a factor of 10^6 at the beginning of the anaerobic acid phase. This caused an imbalance in the refuse fermentation which manifested itself in the accumulation of acids (Fig. 5) and hydrogen (data not shown). The increase in the methanogen population in phase 2 was evidently sufficient to consume hydrogen. This allowed the conversion of other, more reduced organic inter-

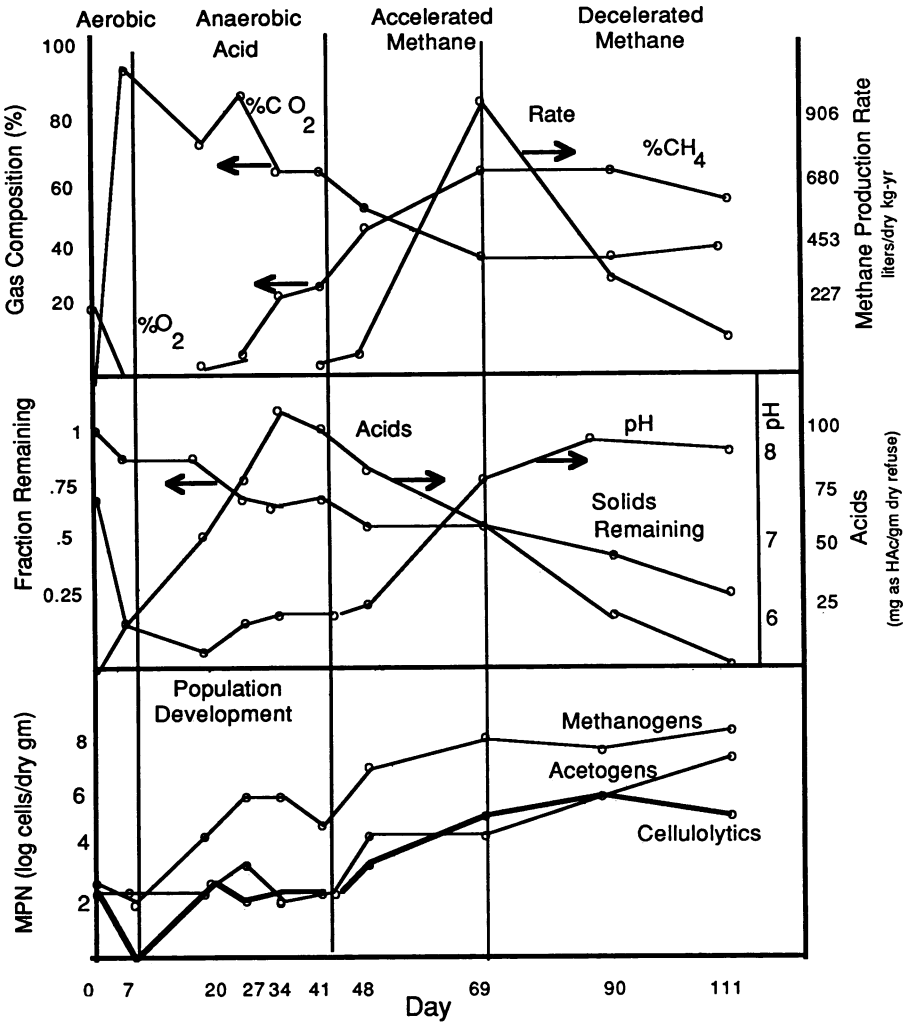

FIG. 7. Summary of observed trends in refuse decomposition with leachate recycle. The total carboxylic acids are expressed as acetic acid equivalents. Methanogen MPN data are the log of the average of the acetate- and H_2-CO_2-utilizing populations. Solids remaining is the ratio of cellulose plus hemicellulose removed from a container divided by the weight of cellulose plus hemicellulose added to the container initially. Gas volume data were corrected to dry gas at standard temperature and pressure.

mediates to carboxylic acids as evidenced by the increase in the TOC_a/TOC ratio (Table 2).

External neutralization of the refuse ecosystem enabled decomposition to proceed through the anaerobic acid phase to the third or accelerated methane production phase (2; Barlaz et al., in press). Description of phase 3 is based on the behavior of containers 1L, 9L, and 22L (days 41, 48, and 69) in which the methane production rate increased consistently to the maximum rate exhibited in the successful leachate recycle containers. As the methane production rate increased, its concentration approached 60%.

Phase 3 was characterized by a decrease in the accumulation of carboxylic acids concurrent with increasing rates of methane production. The pH of the refuse increased from 6.2 to 7.9, and the methanogen population increased. Most notably, the cellulolytic and acetogen populations increased in the third phase of refuse decomposition above the numbers measured in fresh refuse.

The sulfate concentration increased prior to the onset of measurable methane production and decreased thereafter. The increase may have been due to its solubilization from the recycling of acidic leachate. The sulfate data suggest that the refuse ecosystem has the capacity to reduce high concentrations of sulfate (Table 2). The near depletion of sulfate after container 22L (day 69) does not necessarily mean that sulfate was depleted, only that it was reduced as rapidly as it was solubilized.

Sulfate-reducing bacteria are reported to outcompete methanogens for hydrogen (37). Thus, in a hydrogen-limited environment, sulfate will inhibit methane production, although some will occur (37). The high production of carboxylic acids suggests that hydrogen is not limiting in the refuse ecosystem. Thus, it is unlikely that sulfate inhibited methane production. The methane production rate was increasing at the time that container 9L, in which the sulfate concentration was 51 mg/liter, was sampled.

There was little solids hydrolysis in phase 3. As in phase 2, feedback inhibition owing to the acid accumulation may have been responsible.

In the accelerated methane production phase, the methanogenic bacteria appeared to reach a critical level. They began to pull the refuse fermentation by consumption of

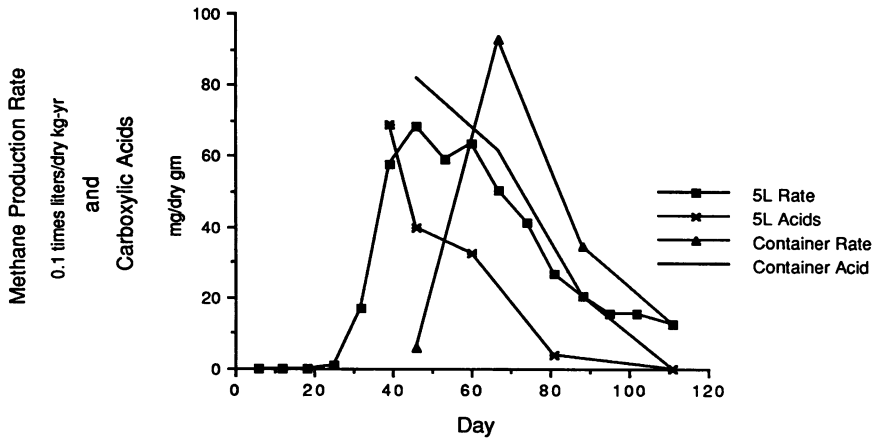

FIG. 8. Relationship of the methane production rate and the carboxylic acid concentrations. Data denoted as Container Rate and Container Acid reflect data for containers 9L, 22L, 24L, and 5L at the time that they were dismantled. Data based on leachate samples and the methane production rate for container 5L only are denoted as 5L Rate and 5L Acids. Gas volume data were corrected to dry gas at standard temperature and pressure.

acetate and hydrogen. Hydrogen consumption improved conditions for conversion of butyrate and propionate to acetate. Acetate consumption allowed the pH of the ecosystem to increase. As the pH increased, the acetogen population increased, which allowed for additional carboxylic acid consumption and a further increase in the methane production rate. At the end of this phase, the methanogenic bacteria comprised 10% of the total anaerobic population.

The decelerated methane production phase was characterized by containers 24L and 5L (days 90 and 111). In phase 4, the rate of methane production decreased, although the methane concentration remained at about 60%. Carboxylic acid concentrations decreased to levels below the detection limit for these containers, 137 and 126 mg/liter for acetate and butyrate, respectively. The pH of the ecosystem increased to values above 8.0.

The maximum rate of solids decomposition was observed in the decelerated methane production phase. Only 28% of the cellulose plus hemicellulose present in fresh refuse remained at the end of phase 4 compared with 63% remaining at the end of phase 3. There was no consistent, measurable weight loss of the insoluble lignin in refuse, which is in agreement with a recent summary of anaerobic lignin decomposition (43).

The change in the total anaerobic, hemicellulolytic, cellulolytic, and methanogenic populations in phase 4 was not significant considering the trends exhibited by these populations previously. However, the acetogen population continued to increase.

In earlier phases of refuse decomposition, rapid cellulose and hemicellulose hydrolysis contributed to acid accumulations. In phase 4, in which the rate of polymer hydrolysis exceeded that exhibited earlier, there was no acid accumulation. The difference may be explained by increases of 5 and 6 orders of magnitude for the acetogen and methanogen populations, respectively, between fresh refuse and the decelerated methane production phase.

The methane production rates and carboxylic acid concentrations for containers 9L, 22L, 24L, and 5L are presented in Fig. 8. After a rapid increase in the methane production rate, there was a consistent decrease as acid concentrations decreased. In the second and third phases of refuse decomposition, it was acid utilization which limited the onset and rate of methane production. There were no accumulations of sugars or acids in container 5L (day 111). After consumption of carboxylic acids, polymer hydrolysis limited refuse methanogenesis.

The continuous decrease in the methane production rate during phase 4 suggests a concurrent decrease in the rate of polymer hydrolysis. This could be explained by the preferential utilization of cellulose and hemicellulose, which are less heavily lignified, thus more degradable. As discussed above, there was significant cellulose and hemicellulose decomposition, but little lignin decomposition (Fig. 4). Thus, with time, the remaining refuse became enriched in its lignin content. Khan (25) demonstrated that both the extent and rate of cellulose degradation increased in cellulosic substrates which contained decreasing amounts of lignin.

Characteristics of refuse decomposition described here represent events which would occur under ideal circumstances. Under field conditions, methane production at a constant rate is typically observed between phases 3 and 4. Throughout the above characterization, data were presented with time as the abscissa. These times should not be applied to other conditions since they were influenced by the refuse particle size, the frequency of leachate recycle and neutralization, and the incubation conditions. Neither leachate recycle nor shredded refuse are typically used in full-scale landfills. However, no external additions of bacteria were made to the refuse. The major differences in refuse incubated under different conditions would be the length of time required for observation of all four phases of refuse decomposition and, without leachate recycle, the magnitude of the methane production rate.

This characterization of refuse decomposition has demonstrated the severe impact of sugars. The slowest trophic groups to develop were the cellulolytic and acetogenic bacteria. Initially acetate utilization, but ultimately polymer hydrolysis, limited the rate of methane production. The techniques developed for use in this study will be useful for assessment of the effects of enhancement parameters on the refuse ecosystem.

ACKNOWLEDGMENTS

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