0273-2289/89/2021—0181$03.00

Inhibition of Methane Formation from Municipal Refuse in Laboratory Scale Lysimeters

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ABSTRACT

Changes in chemical composition and population development of key groups of bacteria (hydrolytic, acetogenic, and methanogenic) were measured in a laboratory scale simulation of refuse decomposition from the time of initial incubation through the methane production phase. Inhibition of methane production appeared to be characteristic of refuse decomposition. It was observed in 20 of 32 leachate recycle containers and all 19 control containers. Inhibition was not owing to an absence of indigenous microorganisms, toxic concentrations of carboxylic acids or cations, or insufficient ammonia. Characteristics of inhibited and successful containers are compared.

Index Entries: Refuse; landfill; methane; anaerobic decomposition; bacteria.

INTRODUCTION

Methane produced in sanitary landfills represents a usable but underutilized source of energy. Energy recovery projects are frequently rejected because the onset of methane production is unpredictable and methane yields vary from 1 to 50% of potential yields based on refuse biodegradability data (*1,2*). Numerous studies on the enhancement of methane production (*3–6*) have not led to an understanding of the microbiology of refuse decomposition adequate to increase methane yields in sanitary landfills.

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Refuse conversion to methane is assumed to proceed on a pathway similar to that described for anaerobic sludge digestion. Three major groups of bacteria are involved (*7*): (1) the hydrolytic and fermentative bacteria that break down biological polymers such as cellulose and hemicellullose to sugars that are then fermented to carboxylic acids, alcohols, carbon dioxide, and hydrogen, (2) the obligate proton reducing acetogenic bacteria that convert carboxylic acids and alcohols to acetate and hydrogen, and (3) the methanogenic bacteria that convert primarily acetate and hydrogen plus carbon dioxide to methane.

Recently, Barlaz presented an updated characterization of refuse decomposition to include data on both microbial population development and chemical composition during decomposition (*8*). Characterization of refuse decomposition in four phases is summarized in Fig. 1 and below. Data for Fig. 1 were collected in laboratory scale landfills.

In the aerobic phase (1) both oxygen and nitrate are consumed and there is little change in the populations of cellulolytic, acetogenic, and methanogenic bacteria. Soluble sugars serve as the carbon source for microbial activity. In the anaerobic acid phase (2), carboxylic acids accumulate, the pH decreases, and there is some cellulose and hemicellulose decomposition. The methanogen population begins to increase and methane is detected in the landfill gas. In phase 3, the accelerated methane production phase, there is a rapid increase in the rate of methane production to some maximum value, a methane concentration of 50–60%, a decrease in carboxylic acid concentrations, an increase in the pH of the ecosystem, little solids hydrolysis, and increases in the populations of cellulolytic, acetogenic, and methanogenic bacteria. The fourth phase is termed the decelerated methane production phase. The methane concentration, pH, cellulolytic, and methanogenic populations remain at levels similar to those in phase three. Concurrently, the methane production rate decreases, the acetogen population increases, carboxylic acids are depleted, and there is an increase in the rate of cellulose plus hemicellulose hydrolysis. In the absence of leachate recycle and neutralization, the time required for the onset of each phase may be significantly longer than the times shown in Fig. 1.

The objective of the study cited above was to characterize microbial and chemical changes which occur during refuse decomposition. During the study, inhibition of refuse methanogenesis was observed which appeared to be characteristic of decomposition. The objectives of this paper are to describe the observed inhibition, compare successful and inhibited refuse decomposition and evaluate potential causes of inhibition.

MATERIALS AND METHODS

Materials and Equipment

Shredded domestic refuse from Madison, WI was sampled from the Madison Energy Recovery Plant. Refuse with a particle size of about 1.9

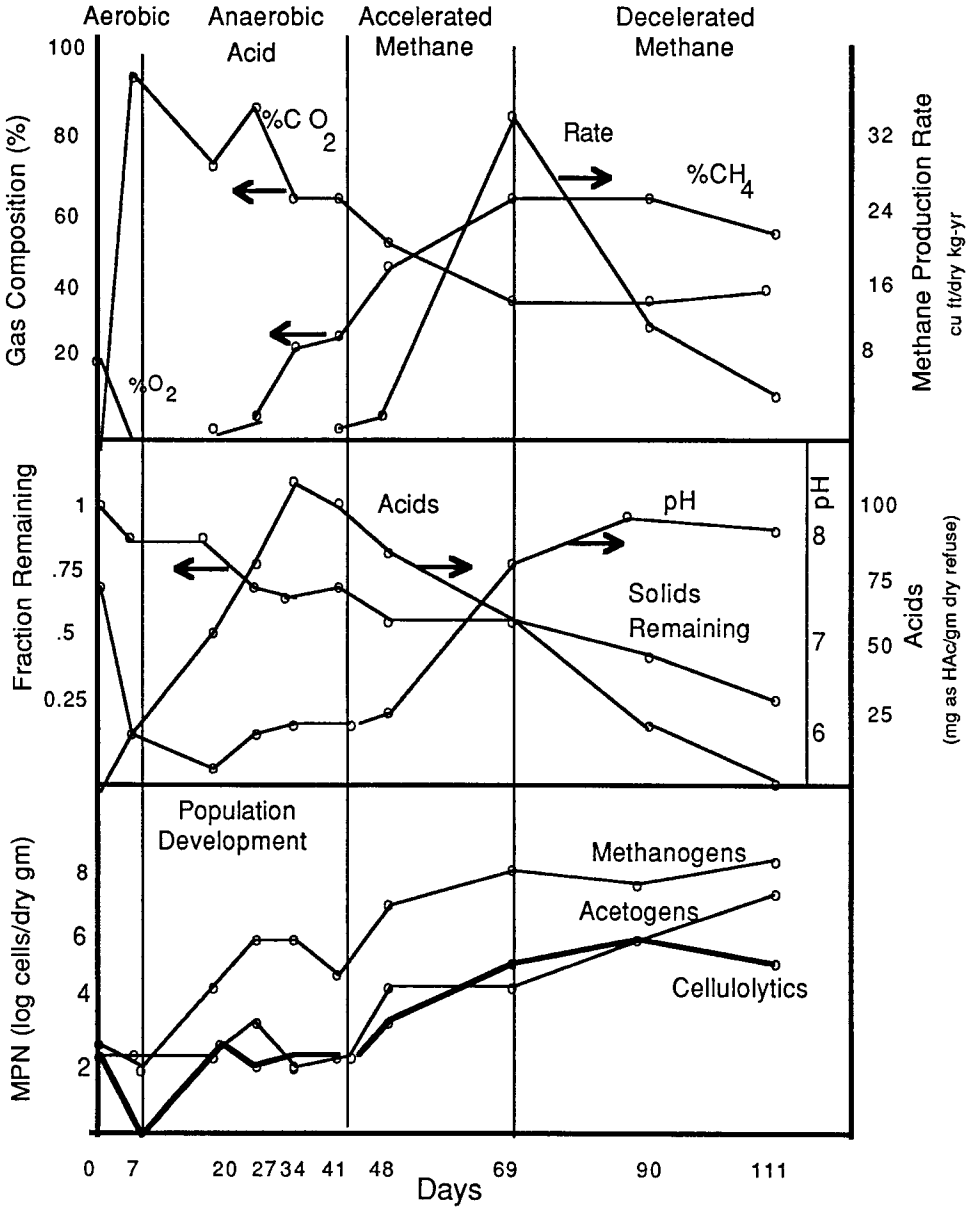

Fig. 1. Refuse decomposition with leacheate recycle. Arrows indicate the axis showing the appropriate units for each line. MPN data are expressed as the log of the population measured in cells/dry gram of refuse removed from a container. The methanogen MPN data is the log of the average of the acetate and hydrogen utilizing populations. Solids decomposition data is the ratio of the weight of cellulose plus hemicellulose removed from a container divided by the weight added to the container initially. The total carboxylic acids are expressed as acetic acid equivalents.

cm was used for all experimental work. The refuse was incubated in 2 L, wide mouth, Nalgene containers. The containers were modified for installation of a leachate collection port, a water addition port, a gas collection outlet, and a gas sampling port. All ports and the container lid were sealed with silicone caulk (Dow Corning 732 RTV) to provide a gas tight system. Leachate was collected in a one liter Viaflem container (Travenol, Deerfield, IL) connected to the Nalgene container with tygon tube.

Experimental Design

Fifty-six replicate 2-L containers were filled with shredded refuse to begin the experiment. Leachate recycle and neutralization was performed for 37 of the containers to enhance methane production. Leachate recycle and neutralization is an effective method for the enhancement of methane production (*3,6*) and in its absence refuse decomposition to methane may not have been observed for several years. Nineteen containers, termed the controls or unenhanced containers, were initiated to document refuse decomposition as it occurs in a sanitary landfill. All containers were incubated at 41°C, the optimal mesophilic temperature for refuse decomposition (*9*). This temperature accelerated decomposition, thus facilitating a short term study of a process that may require 2–10 y.

A complete microbial and chemical characterization required the entire contents of a container. Thus, once sampled a container could no longer be monitored. Containers that represented a logical progression in decomposition between fresh refuse and methane production were selected for sampling. Sample selection criteria included methane concentration and production data, and the leachate pH and 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 (*3*).

All of the containers producing measurable volumes of methane were in the enhanced category. Nine of these were sampled and used to characterize successful refuse decomposition, as presented in Fig. 1. Five containers that did not produce measurable volumes of methane were also sampled and these will be referred to as the unsuccessful containers.

The gas composition data suggested that there was little decomposition in the control containers. Three controls were sampled in order to compare their characteristics with the successful leachate recycle containers. Two leachate recycle containers in which there was no measurable methane production, termed the inhibited leachate recycle containers, were also sampled.

Incubation Conditions

Refuse in the leachate recycle containers was adjusted to 73% moisture (wet wt) with deionized water at the beginning of the experiment in order to generate ample leachate for neutralization and recycling. The control

containers were adjusted to 45% moisture and no leachate was generated. Leachate was neutralized and recycled 6 d/wk. Initially a 100 g/L sodium carbonate solution was used for neutralization. After seven weeks a potassium carbonate solution (171.6 g/L) was used for leachate neutralization to minimize the possibility of an inhibitory sodium concentration.

Procedure for Container Sampling and Inoculum Formation

On removal from a container, refuse was immediately placed in a plastic bag that was closed and all free air was removed by squeezing. Eighty percent by weight of the refuse in the bag was used for formation of an inoculum for Most Probable Number (MPN) enumerations. To form an inoculum the refuse was first adjusted to 88% moisture with sterile, anaerobic phosphate buffer (23.7 m*M*, pH 7.2). It was then blended for 1 min in a Waring blender (model CB-6) with a one gallon stainless steel jar. The blender jar and all other equipment used to process the refuse 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 aseptically under nitrogen and used as the inoculum. Dilutions of the inoculum were made in phosphate buffer (23.7 m*M*, pH 7.2) in the presence of glass beads (3 mm) to disrupt flocs. This procedure was validated by the addition of ruminal bacteria to refuse, and extraction and enumeration as described elsewhere (*10*).

Twenty percent by weight of the refuse was used for analysis of the soluble constituents. To form an extract for soluble constituent analysis the contents of the leachate collection bag, and additional deaerated, deionized water as needed, were added to the refuse to adjust its moisture content o 90%. In the case of the control containers, where there was no leachate accumulation, the moisture content was adjusted to 90% with water only. After a 60 s equilibration period a hand squeezed extract of the refuse was formed. The resulting liquid was then processed for measurement of the concentration of various soluble constituents such as carboxylic acids, phosphates, and ammonia. Concentrations were normalized to a per unit of dry refuse basis so that comparison of refuse with different moisture contents was possible.

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

Media Preparation and Enumeration Techniques

The total anaerobic population and the subpopulations of cellulolytic, hemicellulolytic, hydrogen producing acetogenic (based on butyrate catabolism) and acetate and H_2/CO_2 utilizing methanogenic bacteria were enumerated. The media and MPN method for each substrate were described previously (*8*) and are summarized here.

Five tube MPNs were used for enumerations. Tubes were incubated at 41°C and checked for growth after 30 d, except for the acetogen MPN tubes which were checked after 60 d.

The medium for enumeration of the total anaerobic population contained 10 soluble carbon sources (cellobiose, glucose, maltose, xylose, galactose, arabinose, mannose, starch, glycerol, and galacturonic acid), each at a concentration of 2.5 m*M*. Carbon sources were representative of refuse hydrolysis products. Microbial growth on cellulose was detected by visible disappearance of ball milled Whatman number 1 filter paper (*11,12*). Xylan from oat spelts (Sigma Chemical Co., St. Louis, MO, cat. # X-0376, lot number 105F-0276) was used for enumeration of the hemicellulolytic bacteria. Prior to use the xylan was soaked in distilled water for 24 h to remove the soluble and non-settleable material. The turbid water was removed by aspiration and the settled xylan was dried, weighed into test tubes (5g/L of medium) and autoclaved prior to the addition of sterile media. Tubes were counted as positive if their optical density (A_{600}) exceeded 0.4 (*10*). Methanogen MPN tests were performed with either 80 m*M* acetate or two atmospheres of hydrogen plus carbon dioxide. Acetogenic bacteria were enumerated based on conversion of butyrate (40 m*M*) to acetate and hydrogen (*13*). Butyrate was used in the acetogen MPN because of its prevalence in leachate (*3*).

Analytical Methods

Techniques for measurement of the soluble constituents of refuse, solids composition, and gas composition and production have been presented previously and are summarized here (*8*). Oxygen, nitrogen, carbon dioxide, and methane concentrations were measured weekly by gas chromatography, two days prior to the day on which a container was dismantled. Gas production was measured by a water displacement method (*14*). Carboxylic acids were measured by liquid chromatography (LC) with a differential refractometer detector (*15*). Cellulose, hemicellulose, and soluble sugars were measured by acid hydrolysis (in the case of cellulose and hemicellulose) followed by LC analysis (*16*). Moisture content, sulfates, ammonia and phosphates were measured, as described in references 17–20, respectively. Sulfides were measured by acidification and trapping in zinc acetate followed by a potassium bi-iodate/sodium thiosulfate titration (*21*). Total organic carbon (TOC) was determined with a Dohrmann DC 80 Analyzer.

RESULTS

A description of all of the sampled containers is presented in Table 1. Solids decomposition data, soluble constituent concentrations and carboxylic acid concentrations for the sampled containers are presented in Tables 2, 3, and 4, respectively. Microbial population data are presented in Table 5.

Table 1
Description of the Sampled Containers and Their Methane Production Data

Sample	Day sampled	Percent methane[a]	Methane production rate[b]	Cumulative methane production[c]	Description
Fresh Refuse	0	0	0	0	
			Unsuccessful Containers		
Control containers[d]					
1C	14	0	0		
11C	77	0.4	0		
16C	118	0.2	0		
Inhibited Leachate Recycle Containers[d]					
28L	62	0.9	0		
15L	83	22.0	0		
			Successful leachate recycle containers		
31L	7	0	0	0	anaerobic acid phase
16L	20	0.8	0	0	anaerobic acid phase
10L	27	3.8	0	0	anaerobic acid phase
35L	34	21.4	0	0	anaerobic acid phase
1L	41	27.4	0.52	0.01	accelerated methane production phase
9L	48	46.7	2.08	0.09	accelerated methane production phase
22L	69	64.9	32.8	1.13	accelerated methane production phase
24L	90	63.8	12.1	1.77	decelerated methane production phase
5L	111	58.1	4.5	3.07	decelerated methane production phase

[a]Methane concentration two days prior to sampling.
[b]Methane production rate, expressed in ft^3 CH_4 at STP/yr-dry kg of refuse used to fill the container, for the nine days prior to the day on which the container was sampled.
[c]Units of ft^3 CH_4 at STP/dry kg of refuse used to fill the container.
[d]The C designates a control container and the L designates a leachate recycle container.

Table 2
Solids Decomposition in the Sampled Containers

	Cellulose[a]	M_c[b]	Hemicellulose[a]	M_h[b]
Fresh Refuse	51.2	1.0	11.9	1.0
	Unsuccessful Containers			
Control containers				
1C	52.5	1.09	12.1	1.08
11C	53.2	1.04	11.4	0.96
16C	46.4	1.0	10.8	1.0
Inhibited leachate recycle containers				
28L	39.7	0.66	9.0	0.64
15L	41.9	0.68	7.6	0.54
	Successful Leachate Recycle Containers			
31L	53.0	0.89	13.4	0.96
16L	52.4	0.89	12.4	0.91
10L	48.7	0.7	12.0	0.75
35L	40.5	0.68	7.2	0.52
1L	46.6	0.72	10.3	0.68
9L	42.3	0.65	9.3	0.61
22L	40.5	0.64	9.1	0.62
24L	31.7	0.44	7.3	0.44
5L	24.5	0.29	4.4	0.23

[a]Data expressed as a percentage of the total dry mass of refuse removed from the container.

[b]The ratio of the cellulose (c) or hemicellulose (h) removed from a container divided by the weight of cellulose or hemicellulose in the container initially.

Methane Concentrations in the Control Containers

Traces of methane were detected in three of the control containers on d 53 and by d 81 there was methane in all 17 remaining control containers. However, the highest methane concentration on d 81 was 1.2%. There were no increases in the methane concentration of any of the control containers through day 222 and no measurable gas production.

Methane Concentrations in the Leachate Recycle Containers

Increasing methane concentration was the primary indicator that a container was in the process of population development and approaching an active state of methane production. Methane concentrations in the successful leachate recycle containers are presented in Table 1 and Fig. 1. There was a steady increase in the methane concentration of the successful containers through d 69 when the methane concentration was 64.9%. Thereafter methane concentrations remained nearly constant at values between 55% and 65%, as has been reported previously for refuse in an active state of methane production (*1–3*).

Table 3
pH and Soluble Constituent Concentrations in the Sampled Containers

	pH	Sugars[a]	Total acids as acetic[b]	Total organic carbon (TOC)	$\frac{TOC_a}{TOC}$[c]	Nitrate	Sulfate
Fresh Refuse mg/L[d]	7.5	16393[f]	0	46006	0	71.1	2071
mg/dry g[e]		3.46	0	9.71		0.015	0.44
			Unsuccessful Containers				
Control containers							
1C mg/L	5.8	1682	9060	18785	0.34	12.8	647
mg/dry g		1.5	8180	17.0		0.012	0.58
11C mg/L	5.2	1131	12660	16110	0.46	12.0	523
mg/dry g		1.3	14900	17.9		0.013	0.58
16C mg/L	5.3	585	21270	19595	0.66	36.2	471
mg/dry g		0.51	18420	17.0		0.03	0.41
Inhibited containers							
28L mg/L	6.4	0	11320	14648	0.47	0	4.4
mg/dry g		0	67920	87.9		0	0.026
15L mg/L	6.5	0	12950	12604	0.55	18.7	187
mg/dry g		0	60880	59.3		0.088	0.88

continued

Table 3 (Continued)

	pH	Sugars[a]	Total acids as acetic[b]	Total organic carbon (TOC)	$\frac{TOC_a[c]}{TOC}$	Nitrate	Sulfate
Successful Leachate Recycle Containers							
31L mg/L	6.1	511	4790	7643	0.43	0	276
mg/dry g		1.2	11410	18.2		0	0.66
16L mg/L	5.7	(87)[g]	7870	7614	0.55	0.5	179
mg/dry g		(0.57)	51960	50.3		0.003	1.18
10L mg/L	6.0	(105)[g]	14130	8668	0.94	0.6	235
mg/dry g		(0.58)	78250	48.0		0.003	1.3
35L mg/L	6.2	(97)[g]	14390	11924	0.69	0.5	0.01
mg/dry g		(0.63)	103630	85.9		0.004	1.4
1L mg/L	6.2		12850	11416	0.60	0.5	71
mg/dry g		0	95070	84.5		0.004	0.52
9L mg/L	6.3		13940	11805	0.68	0	51
mg/dry g		0	82270	69.6		0	0.30
22L mg/L	7.9		9510	7595	0.66	0	9.8
mg/dry g		0	55690	44.5		0	0.06
24L mg/L	8.4		4640	4091	0.55	0	6.9
mg/dry g		0	20520	18.1		0	0.03
5L mg/L	8.2		0	1976	0	0	0.5
mg/dry g		0	0	15.4		0	0.004

continued

Table 3 (Continued)

	Ammonia	Phosphate	Sulfide
Fresh Refuse mg/L	521	800	0
mg/dry g	0.11	0.17	0
		Unsuccessful Containers	
Control containers			
1C mg/L	648	32.5	0
mg/dry g	0.59	0.03	0
11C mg/L	692	16.6	8.4
mg/dry g	0.77	0.02	0.009
16C mg/L	2303	0	0
mg/dry g	2.0	0	0
Inhibited containers			
28L mg/L	245	1.7	4.5
mg/dry g	1.47	0.01	0.027
15L mg/L	426	0.5	1.3
mg/dry g	2	0.003	0.006

continued

Table 3 (Continued)

	Ammonia	Phosphate	Sulfide
	Successful Leachate Recycle Containers		
31L mg/L	249	20.9	2.7
mg/dry g	0.59	0.05	0.006
16L mg/L	295	32.5	2.2
mg/dry g	1.95	0.21	0.015
10L mg/L	1243	96.6	13.1
mg/dry g	6.88	0.53	0.07
35L mg/L	224	4.6	5.0
mg/dry g	1.61	0.03	0.036
1L mg/L	319	5.2	3.3
mg/dry g	2.36	0.04	0.024
9L mg/L	60	0.8	5.0
mg/dry g	0.35	0.005	0.03
22L mg/L	88	1.0	8.0
mg/dry g	0.52	0.006	0.047
24L mg/L	45	10.1	37.1
mg/dry g	0.2	0.04	0.16
5L mg/L	7.5	0	15.4
mg/dry g	0.06	0	0.12

[a]Total sugars including glucose, xylose, galactose, and arabinose.
[b]Total milligrams of acid, including, acetic, propionic, isobutyric, butyric, isovaleric, and valeric, expressed as acetic acid.
[c]Fraction of the measured total organic carbon accounted for by the carboxylic acid analyses.
[d]Units are mg/L of liquid in the refuse plus accumulated leachate after correction for dilution associated with formation of refuse extract.
[e]Units are mg/dry g of refuse as removed from a container.
[f]A large unidentified peak eluted at 53.82 min. This was between mannose (50.05 min) and the erythritol internal standard (53.89 min).
[g]Present below the limit, given in parentheses, at which a linear response of the instrument was verified.

Table 4
Carboxylic Acid Concentrations in the Sampled Containers[a]

	Lactate	Acetate	Propionate	Isobutyrate	Butyrate	Isovalerate	Valerate
Fresh Refuse			No acids detected				
			Unsuccessful Containers				
Control containers[b]							
1C mg/L[c]	1705	1544	<830[e]	<793	9364		
mg/dry g[d]	1.54	1.39	<0.75	<0.72	8.46		
11C mg/L	<703	6053	948	1493	7080		<739
mg/dry g	<0.78	6.74	1.06	1.66	7.88		<0.67
16C mg/L		9719	1625	2783	11130		1270
mg/dry g		8.42	1.41	2.41	9.64		1.1
Inhibited leachate recycle containers							
28L mg/L		5365	597	1697	5915	<488	
mg/dry g		32.2	3.58	10.2	35.5	<2.92	2.9
15L mg/L		8454	807	983	4145	<571	638
mg/dry g		39.74	3.79	4.41	19.48	<2.68	3.0

(continued)

Table 4 (Continued)

	Lactate	Acetate	Propionate	Isobutyrate	Butyrate	Isovalerate	Valerate
			Successful Leachate Recycle Containers				
31L mg/L	<775	1376			5015		
mg/dry g[d]	<1.84	3.27			11.93		
16L mg/L	<467	5139	<518	500	3513		<491
mg/dry g	<3.06	33.91	<3.41	3.30	23.18		<3.24
10L mg/L		7857	998	987	5882	700	634
mg/dry g		43.5	5.52	5.47	32.57	3.88	3.51
35L mg/L		7981	801	1408	6490	<488	647
mg/dry g		57.46	5.77	10.13	46.73	<3.51	4.66
1L mg/L		8253	1007	698	4168	<488	787
mg/dry g		61.07	7.45	5.17	30.84	<3.61	5.82
9L mg/L		7557	946	639	6956	<488	755
mg/dry g		44.59	5.58	3.77	41.04	<2.87	4.45
22L mg/L		5823	1700	903	2485	<274	<272
mg/dry g		34.1	9.95	5.29	14.54	<1.98	<1.59
24L mg/L		3206	1478	<162	<166	392	
mg/dry g		14.2	6.55	<1.72	<0.73	1.74	
5L mg/L		<137			<126		
mg/dry g		<1.07			<0.98		

[a]The average coefficient of variation for these data was 6%.
[b]Ethanol was present but not quantified in all three control containers.
[c]Units are mg/L of liquid in the refuse plus accumulated leachate after correction for dilution associated with formation of refuse extract.
[d]Units are mg/dry g of refuse as removed from a container.
[e]Acid detected at a level below the detection limit, expressed as less than the detection limit.

Table 5
Microbial Populations in the Sampled Containers[a]

Container	Day sampled	Total anaerobic	Hemicellulolytic	Cellulolytic	Methanogenic		Acetogenic
					Acetate	H_2/CO_2	Butyrate
Fresh refuse	0	4.1×10^6	5.7×10^5	2.5×10^1	1.1×10^3	1.9×10^2	1.9×10^2
			Unsuccessful Containers				
Control containers							
1C	14	4.7×10^7	1.6×10^5	0	19	0	1.6×10^2
11C	77	7.6×10^6	1.4×10^5	1.7	1.9×10^2	3.4×10^1	3.4×10^1
16C	118	2.2×10^7	6.0×10^4	1.3×10^1	9.7×10^2	1×10^3	1.7×10^2
Inhibited leachate recycle containers							
28L	62	2.9×10^8	2.9×10^6	1.4×10^1	6.3×10^1	0	8.1×10^1
15L	83	4.9×10^8	3.5×10^6	8.5×10^1	5.6×10^6	2.1×10^6	5.6×10^1
			Successful Leachate Recycle Containers				
31L	7	6.2×10^8	3.7×10^7	0	1.6×10^2	1.7×10^2	2.8×10^2
16L	20	8.1×10^8	2.1×10^6	4.4×10^2	1.7×10^4	3.7×10^4	1.7×10^2
10L	27	4.3×10^8	4.3×10^6	1.6×10^2	2.0×10^6	6.9×10^5	2.0×10^3
35L	34	2.7×10^8	9.1×10^6	1.8×10^2	1.6×10^6	1.6×10^6	$<10^2$[b]
1L	41	3.2×10^8	3.2×10^7	1.5×10^1	8.4×10^4	3.2×10^4	1.5×10^2
9L	48	1.8×10^8	1.8×10^7	3.5×10^3	1.8×10^7	7.7×10^6	2.9×10^4
22L	69	2.5×10^9	4.2×10^9	1.3×10^5	2.5×10^8	2.5×10^8	3.8×10^4
24L	90	9.8×10^8	6.1×10^8	7.6×10^5	1.7×10^8	3.8×10^7	1.7×10^6
5L	111	1.1×10^9	6.6×10^7	2.7×10^5	2.5×10^8	6.6×10^8	4.1×10^7

[a] Data expressed in cells per dry gram of refuse removed from the container.
[b] No positive tubes were detected in the 1:100 dilution and below. No tubes were inoculated at the 1:10 dilution.

Table 6
Distribution of Methane Concentrations in the Leachate Recycle Containers[a]

Methane concentration, %	0–10	11–20	21–30	31–40	41–50	>50[b]
Day						
25	29	1	3	1	1	0
32	20	5	4	3	2	0
39	10	6	8	7	0	2
46	4	0	19	5	2	2
53	1	1	15	9	1	4
60	1	0	13	8	3	6
67	0	0	10	8	3	9
74	0	3	11	4	2	9
81	0	2	9	7	1	10
88	0	3	8	6	1	10
95	0	1	10	4	2	10
102	0	1	8	4	2	12
109	0	1	9	2	1	14

[a]Presented as the number of containers in a methane concentration category. The total number of containers decreases over time as containers were sampled.
[b]Containers with a methane concentration of greater than 50% were in an active state of methane production.

Weekly methane concentration data for all leachate recycle containers are presented in Table 6. On d 25 there were 29 containers in which the methane concentration was between 0 and 10%, and 6 containers with methane concentrations greater than 10%. On d 32 there were 20 containers in which the methane concentration was between 0 and 10%, and 14 containers with methane concentrations greater than 10%. By d 46 the distribution of methane concentrations had spread out considerably and only 4 containers had methane concentrations between 0 and 10% with 19 of the containers between 21% and 30% methane.

With the decrease in the number of containers between 21% and 30% methane on d 53 through 81 (Table 6), there was an increase in the number of containers in which the methane concentration exceeded 50%. Concentrations above 50% corresponded to refuse actively producing methane. However, the rate at which containers progressed to greater than 50% methane decreased after d 67 and the methane concentration increased in only a few containers between days 81 and 109. There were still a number of containers in the 21–30% category on d 81–109 indicating an apparent bottleneck in methane concentration progression.

The failure of some containers to progress beyond 21–30% methane may be expressed in another way by establishing a working definition for inhibition. Inhibition was defined as the failure of the methane concentration in a container to progress for three consecutive weeks. For example,

one container progressed consistently to 33.2% methane on d 53 then stagnated and its methane concentration was 23.2% on d 109. Once a container exceeded 50% methane further concentration increases were not expected. Such containers were always in an active state of methane production and were not considered inhibited although their methane concentration may have been stagnant.

In summary, there was inhibition and no methane production in 11 leachate recycle containers, 5 were sampled before they could be classified, and 21 containers were successful in that they eventually reached an active state of methane production. Of the 21 successful leachate recycle containers, nine were classified as inhibited at some point prior to the onset of measurable methane production. That is, their methane concentration stagnated for at least three weeks and only thereafter, increased. This could suggest either microbial population shifts or adaptation of the population to an inhibitory environment. An additional 5 of the 21 successful containers were stagnant for two weeks. Only 7 of the 21 successful leachate recycle containers exhibited a steady increase in methane concentration on a weekly basis. Overall, 20 of 32 leachate recycle containers exhibited inhibition including 11 that did not produce methane and 9 which ultimately recovered and reached an active state of methane production.

DISCUSSION

The first part of this section addresses trends in refuse decomposition and causes of inhibition for the sampled control containers. Following that, characteristics and potential causes of inhibition in the inhibited leachate recycle containers are discussed. Finally, cation concentrations in all of the leachate recycle containers and nutrient limitations during refuse decomposition are discussed.

Characterization of Refuse Decomposition in the Control Containers

Containers 1C, 11C, and 16C were sampled early (d 14), near the middle (d 77) and at the end (d 118) of the experiment, respectively. Only traces of methane were detected in the headspace of containers 11C and 16C and there was no measurable gas production. All three controls were characterized by the absence of detectable cellulose and hemicellulose decomposition (Table 2).

Oxygen was depleted from the control containers within 6 d. There was a decrease in the pH of the refuse from an initial value of 7.5 to 5.8, 5.2, and 5.3, in containers 1C, 11C, and 16C, respectively. Mass balances which account for the weight of refuse, refuse composition, and container pore space indicated that there was insufficient oxygen and nitrate present

initially for complete oxidation of the sugars present in fresh refuse to carbon dioxide and water. After depletion of oxygen and nitrate these sugars would be fermented to carboxylic acids. Thus, the initial pH decrease can be attributed to the fermentation of sugars in fresh refuse and the low acid consuming activity of the methanogenic and acetogenic bacteria.

Acetate and butyrate were the major carboxylic acids present in all containers (Table 4) with lesser amounts of lactate, propionate, isobutyrate, and valerate. The soluble total organic carbon (TOC) of the control containers (Table 3), expressed as a fraction of the dry weight, remained constant between containers 1C, 11C, and 16C after the initial increase between fresh refuse and container 1C. Increases in the total carboxylic acid concentration were measured between containers 1C and 16C and the fraction of the TOC, which could be accounted for by carboxylic acids (TOC_a/TOC) increased from 34% in container 1C to 46% in container 11C and 66% in container 16C (Table 3). The increase in acids was a result of the conversion of organic carbon solubilized in the first 14 d of the experiment to carboxylic acids.

Microbial activity was inhibited in the control containers, as evidenced by the absence of both methane production and solids decomposition. Nevertheless, acid production and the increase in the TOC_a/TOC ratio between containers 1C and 16C is indicative of some microbial activity.

Nitrates were not depleted from the control containers as they were in the successful leachate recycle containers (Table 3). Rapid nitrate depletion was expected because its use as a terminal electron acceptor is energetically more favorable than sugar fermentation to carboxylic acids. The absence of a decrease in nitrate between fresh refuse and the control containers suggests inhibition of refuse decomposition prior to nitrate depletion. However, the accumulation of acids and the presence of hydrogen (date not shown) in the controls are indicative of fermentative activity expected after nitrate depletion. The presence of nitrates and acids in the control containers suggests that refuse decomposition was not uniform. This can be explained by the absence of water flux and mixing. Similarly, nonuniform refuse decomposition may also explain why sugars were not depleted in the control containers.

All of the trophic groups required for the conversion of refuse to methane were present in the fresh refuse (Table 5). However, no group multiplied in the controls relative to fresh refuse. There was a two order of magnitude increase for the acetate and H_2/CO_2 utilizing methanogenic bacteria between containers 1C and 16C, however, it was due to a methanogen population decrease between the fresh refuse and container 1C.

The increase in the methanogen population in the control containers occurred at a pH below 6. Methanogen population increases below pH 6.3 were also observed in the successful leachate recycle containers (Fig. 1). This is below the optimal pH for methanogenic activity, 6.8–7.4 (*7*). Perhaps assimilatory methanogen activity is not as severely inhibited by

low pH as is dissimilatory activity. Although the methanogen population increased under acidic conditions, there was no measurable methane production.

In summary, the rapid production of acids and subsequent pH decrease in the control containers appeared to inhibit most microbial activity. In the successful containers methane production was enhanced by external pH adjustment via leachate neutralization.

The increase in the methanogen population observed in the control containers suggests a mechanism by which methane may be produced in sanitary landfills in the absence of external neutralization. Assuming increases in the methanogen population were to continue beyond the time frame of this experiment, the methanogen population would eventually reach a critical level from which it could begin to consume acetate and reduce refuse acidity enroute to methane production. Given the low moisture contents typical of sanitary landfills and the absence of high water flux, it may be difficult for the methanogen population to spread throughout the refuse. Hence, only portions of the refuse may be converted to methane. This could explain the low conversions of refuse to methane in sanitary landfills.

Inhibition in the Control Containers

The 45% moisture used for the control containers left the refuse dry to the touch and raises the possibility that there was insufficient moisture for microbial activity. However, rapid oxygen depletion and the production of carboxylic acids suggests that moisture did not limit either aerobic or anaerobic activity.

A second potential explanation for inhibition in the control containers is a toxic carboxylic acid concentration. An acetate concentration of 9753 mg/L was measured in a leachate sample from container 22L on d 55, at which time its methane production rate was increasing. Similarly, a butyrate concentration of 6956 mg/L was measured in container 9L at takedown (Table 4). Here, too, the methane production rate was increasing. Thus, acetate and butyrate concentrations of at least 9753 mg/L and 6956 mg/L, respectively, occurred concurrently with increasing methane production rates. The acetate concentration did not exceed 9753 mg/L in any of the control containers. However, the butyrate concentration exceeded 6956 mg/L in all three controls. Inhibition due to butyrate in the control containers is possible, but not probable in that high acid concentrations corresponded with high rates of methane production (Tables 1 and 4).

As discussed previously, the acidic pH was the apparent cause for stagnation of refuse fermentation in the control containers. However, the presence of an inhibitory compound should also be considered. There was significantly less water in the control containers relative to the leachate recycle containers. Based on the different moisture levels, the concentra-

tion of a soluble material would have been about three times higher in the controls relative to the leachate recycle containers. Thus, any inhibitory compound would have been present at a higher concentration in the controls.

It is possible that an inhibitory compound was released from the refuse after incubation. Phenolic monomers from the degradation of plant material would be expected shortly after refuse burial. Phenolic compounds have been shown to inhibit the in vitro digestibility of cellulose by ruminal microorganisms (22). The value of TOC_a/TOC in containers 1C and 11C, 0.34 and 0.46, respectively, was low relative to the range of this ratio for the successful leachate recycle containers in the anaerobic acid and accelerated methane production phases (Table 3). This suggests a relatively high concentration of non-carboxylic acid, soluble organic carbon in containers 1C and 11C.

There was an increase in both the methanogen population and the TOC_a/TOC ratio in container 16C relative to container 11C, and the TOC_a/TOC ratio in container 16C (0.67), was similar to the ratios in the successful leachate recycle containers (Table 3). Thus, a decrease in the concentration of noncarboxylic acid, soluble organic carbon in container 16C corresponded with an increase in the methanogen population. The slow increase in the methanogen population and the absence of methane production in the control containers cannot be attributed to low pH with total certainty as methanogen population increases and increasing methane production rates were noted in container 9L at pH 6.3. The TOC, carboxylic acid, pH, and methanogen population data are consistent with the possibility that an inhibitory compound was converted to carboxylic acids by the time that container 16C was sampled. However, there is not sufficient data on the composition of the noncarboxylic acid TOC to conclude that an organic carbon compound was inhibitory.

Characteristics of Refuse Decomposition in the Inhibited Leachate Recycle Containers

Two inhibited leachate recycle containers were sampled (28L and 15L). The methane concentration in container 28L, 0.9%, was the lowest in any of the remaining leachate recycle containers on d 60. The methane concentration in container 15L was 34.9% on d 39, and only 22% on d 81, 2 d before it was sampled. There was no measurable methane production in either container.

The cellulose and hemicellulose decomposition measured in 28L and 15L (Table 2) was typical of that measured in the successful containers in the accelerated methane production phase (1L, 9L, and 22L). Similarly, sugars were also depleted in containers 28L and 15L. Acid production, dominated by acetate and butyrate, was typical of that in containers sampled in the anaerobic acid phase. The pH in containers 28L and 15L was 6.4 and 6.5, respectively. The carbon flow data indicate that there was significant microbial activity in these containers.

The microbial populations in container 28L (Table 5) were similar to those for refuse in an early stage of decomposition. Only the total anaerobic population increased relative to fresh refuse.

In container 15L only the total anaerobic and methanogenic populations increased relative to fresh refuse. The methanogen population increased by 3–4 orders of magnitude relative to fresh refuse, similar to that of successful containers sampled late in the anaerobic acid phase. This population development suggests that the methanogens at least partially adapted to the inhibitory condition in the container. However, there was no measurable methane production and there was a large acetate accumulation. As proposed for the successful and control containers, these data suggest that assimilatory methanogen activity may occur in the absence of dissimilatory activity. The acetogenic and cellulolytic populations in container 15L were typical of the successful containers prior to the onset of methane production in that there were no population development.

Potential Causes of Inhibition in Containers 28L and 15L

Neither low pH nor toxic accumulations of carboxylic acids is an explanation for inhibition in containers 28L and 15L. The pH in containers 28L and 15L was higher than the pH in container 1L at the onset of measurable methane production. Acetate and butyrate concentrations in 28L and 15L were below the maximum concentrations observed for the successful containers given earlier.

The TOC in container 28L was higher than that in the successful containers sampled in an early stage of decomposition (Table 3) and the TOC_a/TOC ratio in container 28L was 0.47. This indicates the presence of much noncarboxylic acid, soluble carbonaceous material. It is, therefore, possible that inhibition was related to a high concentration of an unidentified soluble organic compound or group of compounds.

The presence of nitrate in container 15L (Table 3) is not compatible with the presence of methane, carboxylic acids, and sulfides. Nitrate was rapidly depleted in the successful containers, indicating an adequate population of denitrifying bacteria in refuse. Container 15L was well mixed owing to leachate recycle, thus the presence of nitrate in microenvironments is unlikely. Carboxylic acids and sulfides are indicative of anaerobic activity expected after nitrate depletion. The data do not suggest that nitrate was the root cause of inhibition in container 15L although its presence is anomalous.

Sulfate reducing bacteria (SRB) are reported to outcompete methanogenic bacteria for hydrogen (23). Thus in a hydrogen limited ecosystem sulfate can inhibit methane production though some will occur (23). Significant fermentative activity, as evidenced by the accumulation of carboxylic acids, suggests that hydrogen is probably not limiting in the refuse ecosystem during the anaerobic acid phase.

It is unlikely that sulfate was inhibitory in container 28L. The sulfate concentration was relatively low, indicating that sulfate was either depleted or reduced to sulfide as it dissolved. Even if there were competition for hydrogen in container 28L, as a result of a high sulfate turnover rate, some methane production would have been expected (23). The decrease in the sulfate concentration relative to fresh refuse suggests that the sulfate reducing bacteria were not inhibited.

The possibility of sulfate inhibition in container 15L cannot be discounted. The sulfate concentration in this container was higher than that in the successful containers sampled in the accelerated and decelerated methane production phases. However, the complete inhibition of methane production by sulfate seems unlikely given the role of hydrogen in the refuse fermentation.

The ammonia concentrations in containers 28L and 15L were typical of those in the successful leachate recycle containers prior to the onset of methane production (31L, 16L, 35L). Thus inhibition cannot be attributed to an ammonia limitation. The 1.7 mg/L of phosphate in container 28L was greater than the concentration in containers 9L and 22L, which were sampled while their methane production rates were increasing. The phosphate concentration in container 15L, 0.5 mg/L, was less than in any successful container with the exception of 5L, sampled after production of a significant volume of methane. It is possible that phosphate limited the onset of methane production in container 15L.

In summary, identification of the inhibitory factor or factors in containers 28L and 15L has not been possible. There was hydrolytic and fermentative activity in both containers and methanogen population development in container 15L. Either the cause of the inhibition did not develop until after some fermentative activity, or the inhibition did not affect the hydrolytic and fermentative bacteria. In container 15L, the high sulfate and low phosphate concentrations were of concern. There was a large mass of unidentified soluble carbon in both containers, thus fueling speculation that the inhibitory compound or group of compounds contained soluble organic carbon. A more detailed description of soluble organic compounds in the refuse ecosystem is needed. The response of the refuse ecosystem to increased phosphate concentrations should also be explored.

Cation Toxicity and pH Inhibition

The preceeding discussion on inhibition addressed the sampled containers owing to the availability of extensive data. In this section, all of the leachate recycle containers are considered and the possibility that certain containers were inhibited owing to high cation concentrations or low pH is evaluated. The addition of sodium and potassium carbonate for leachate neutralization increased the concentrations of these cations in the leachate recycle containers.

Inhibitory cation levels have not been evaluated for the refuse ecosystem although Kugelman and Chin (*24*) have studied cation toxicity in anaerobic digestors. They suggested that sodium concentrations of 6900–8000 mg/L would not be inhibitory if the sodium accumulated slowly and other cations (antagonists) were present. They also recommended that potassium levels not exceed 5865–7820 mg/L under conditions where the potassium was applied in one dose with antagonists present. The case of a slowly increasing potassium concentration was not evaluated.

The calculated cation concentrations did not exceed the concentrations recommended by Kuelman and Chin (*24*) in any of the leachate recycle containers. The highest sodium concentrations in the successful containers were in 3L, 8L, 27L, and 30L, which had an average concentration of 5593 mg/L. The highest potassium concentrations in successful containers were in 27L, 29L, and 37L, which had an average concentration of 4628 mg/L. Cation concentrations were calculated from the mass of sodium and potassium carbonate added for leachate neutralization and do not include cations present in the fresh refuse.

The sodium and potassium concentrations in inhibited containers 28L and 15L were less than the concentrations in many of the successful leachate recycle containers. Thus, their inhibition cannot be attributed to cation toxicity. The sodium concentration in inhibited container 2L was 6197 mg/L by d 47, higher than that in all of the successful containers except 30L. The potassium concentration in 2L, 4534 mg/L, was higher than that in any of the successful containers. However, container 2L was first classified as inhibited on d 74, at which time its potassium concentration was 2884 mg/L, below the potassium concentration in several successful containers. Similarly, the potassium concentration in container 36L (5501 mg/L) was higher than that in any of the successful containers on d 110. However, when container 36L was first classified as inhibited on d 60, its potassium concentration of 2336 mg/L was below that in several successful containers. With the possible exception of the sodium concentration in container 2L, high cation concentrations are not an explanation for inhibition in any of the leachate recycle containers.

The average pH for all successful containers at the onset of methane production was 6.2 (stnd. dev. = 0.16). The average pH in the unsuccessful containers on d 118 was 6.3 (stnd. dev. = 0.12). Thus, low pH was not the cause of inhibition in the leachate recycle containers that did not produce methane.

Nutrient Concentrations in the Sampled Leachate Recycle Containers

The maximum rate of methane production for any container was measured for container 22L—32.8 ft^3 CH_4/ dry kg-y. Ammonia and phosphate concentrations of 88.8 mg/L and 1 mg/L, respectively, were mea-

sured concurrent with this rate of methane production. It is not possible to determine whether these nutrient concentrations limited the methane production rate.

A leachate sample from container 5L on d 41 contained 160 mg/L of ammonia and 3.1 mg/L of phosphate. At takedown on day 111 the ammonia concentration was 7.5 mg/L and no phosphate was detected. The decrease in nutrient concentrations in container 5L is consistent with the trend exhibited over containers 22L, 24L, and 5L (Table 3). At takedown, the methane production rate in container 5L was 18% of its maximum rate. The rate decrease was attributed to depletion of the accumulated carboxylic acids (*8*). Alternatively, ammonia and/or phosphate concentrations may have limited the rate of methane production in container 5L.

At field scale nutrient depletion in the decelerated methane production rate phase may not occur as observed in container 5L. Fresh refuse is typically buried over older refuse in full scale landfills and nutrients could potentially percolate from refuse in the anaerobic acid phase of decomposition, where nutrient concentrations are higher, to more decomposed, nutrient poor refuse.

Sulfate concentrations decreased as refuse decomposition progressed in the successful containers (Table 3). This suggests that the refuse ecosystem has the capacity to reduce sulfate to sulfide. Thus sulfate as well as sulfide can contribute to microbial sulfur requirements. Most of the sulfate was consumed while the pH of the refuse ecosystem was at or below 6.3. At a pH of 6.3, 75% of the dissolved sulfide will be in the form of H_2S (pk=6.9). The high levels of sulfate and the relatively low levels of sulfide in the successful containers suggests that most of the sulfide evolved from the refuse ecosystem as hydrogen sulfide. Sulfides may also have formed precipitates with metals. It is probable that there was sufficient flux of sulfide through the liquid phase for microorganisms to meet their sulfur requirements.

The rate of methane production in containers 9L and 22L was increasing at takedown when sulfide concentrations were 5 and 8 mg/L, respectively. Though lower than the concentrations used by Boone (*25*) for the growth of methogens (9.6 to 19.2 mg/L), there is no evidence to suggest that sulfide was limiting the rate of methane production in containers 9L and 22L.

In summary, measurable methane production occurred in 21 of 32 containers enhanced by leachate recycle and neutralization and 0 of 19 unenhanced containers. Inhibition in the control containers appeared to be related to low pH or an unidentified soluble carbon compound. Methane concentration data suggested that inhibition was characteristic of refuse decomposition with leachate recycle. Inhibition of methane production in the leachate recycle containers was not due to an absence of indigenous microorganisms, low pH, toxic carboxylic acid or cation concentrations, or insufficient ammonia. An unidentified soluble carbon compound and low phosphates may be significant in inhibition.

ACKNOWLEDGEMENTS

This work was supported by the American Public Power Association, Wisconsin Power and Light, and the Wisconsin Alumni Research Foundation. Marilyn Effland of the US Forest Products Laboratory performed the cellulose, hemicellulose and sugar analyses. The expertise of Deirdre Barlaz in editing the manuscript is appreciated.

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