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MICROBIAL, CHEMICAL AND METHANE PRODUCTION CHARACTERISTICS OF ANAEROBICALLY DECOMPOSED REFUSE WITH AND WITHOUT LEACHATE RECYCLING

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Microbial and chemical characteristics of refuse in an active state of methane production, incubated in the laboratory with and without leachate recycle, were compared. There were no significant differences in the total anaerobic population or the sub-populations of cellulolytic, hemicellulolytic, acetogenic or methanogenic (based on acetate or H_2/CO_2 utilization) bacteria in refuse incubated with or without leachate recycle. Therefore, leachate recycle may be used to accelerate refuse decomposition in laboratory-scale test lysimeters without changing the microbial composition of the aforementioned trophic groups. Differences in soluble constituent concentrations and methane production patterns between leachate recycle and non-leachate recycle containers were attributed to the mixing associated with leachate recycle. Under certain circumstances, leachate recycle is a useful technique for acceleration of refuse decomposition in the laboratory, thus reducing the period of time required to study the effect of an addition to the refuse ecosystem on methane production.

Key Words—Municipal solid waste, refuse, methane, landfill, anaerobic digestion.

1. 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 1–30% of potential yields based on refuse biodegradability data (Ham *et al.* 1979, Halvadakis *et al.* 1983). Research on enhancement of methane production (Barlaz *et al.* 1987, Kinman *et al.* 1987, Buivid *et al.* 1981, Pohland 1975) has not led to an understanding of refuse decomposition adequate to predict and increase methane yields in sanitary landfills. Research on the microbiology of refuse decomposition was recently reviewed (Barlaz *et al.* 1990). Several studies have measured enzyme activities in refuse incubated under laboratory conditions and excavated from landfills. Others have measured microbial populations in leachate and refuse from full-scale landfills.

Cellulose and hemicellulose are the principal biodegradable constituents of refuse accounting for 91% of the total methane potential (Barlaz *et al.* 1989*a*). Three major groups of bacteria are involved in the conversion of cellulosic material to methane (Zehnder 1978): (1) the hydrolytic and fermentive bacteria which break down biological polymers such as cellulose and hemicellulose to sugars which are then fermented to carboxylic acids, alcohols, carbon dioxide and hydrogen, (2) the obligate proton

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reducing acetogenic bacteria which convert carboxylic acids and alcohols to acetate and hydrogen, and (3) the methanogenic bacteria which convert primarily acetate and hydrogen plus carbon dioxide to methane. Sulfate reducing bacteria (SRB) may also play a role in the anaerobic mineralization of cellulosic material (Widdel 1988). In the presence of sulfate, electrons may be directed towards sulfate reduction by SRB with the production of hydrogen sulfide and carbon dioxide. In electron donor limited ecosystems, sulfate reducing and methanogenic bacteria compete for electrons (Robinson & Tiedje 1984). Barlaz *et al.* (1989*b*) suggested that the excess of carboxylic acids in the refuse ecosystem may lessen the significance of competition between sulfate-reducing and methanogenic bacteria.

It is difficult to study refuse decomposition under closely controlled laboratory conditions because, in the absence of stimulation, several years may elapse prior to the onset of methane production. Leachate recycle has been shown to accelerate refuse decomposition at laboratory scale (Barlaz *et al.* 1987, Pohland 1975), making it possible to sample well decomposed refuse in as little as 111 days (Barlaz *et al.* 1989*a*). Pohland & Gould (1986) used leachate recycle to accelerate refuse decomposition, thus reducing the time required to assess the effects of co-disposal of industrial waste sludge with municipal refuse. However, leachate recycle is not typically practiced in full-scale landfills and the effects of leachate recycle on microbial and chemical characteristics of refuse in an active state of methane production are unknown.

Our objective here is to compare microbial and chemical characteristics of refuse actively producing methane, incubated with and without leachate recycle and neutralization. The overall objective of our research was to measure changes in microbial populations and chemical characteristics of refuse between the time of initial incubation in laboratory-scale lysimeters and well decomposed refuse. These results have been reported previously (Barlaz *et al.* 1989*b*) and are summarized here.

2. Experimental methods

2.1 Materials and equipment

Shredded domestic refuse from Madison, WI, U.S.A. was incubated in two liter Nalgene containers. The refuse particle size was less than 1.9 cm. Containers were modified for installation of a leachate collection port, a water addition port, a gas collection outlet and a gas sampling port.

As described below, anaerobically decomposed refuse was used to seed two containers. This refuse came from Drum S of a previous study (Barlaz *et al.* 1987). The refuse had been stored in plastic bags at 4°C for 2 years prior to use here. Its effectiveness as a seed was verified in preliminary work.

2.2 Experimental design

The objective of this study was to compare microbial and chemical characteristics of refuse actively producing methane, incubated with and without leachate recycle. Refuse in an active state of methane production was required for this study. Thus, enhancement techniques were used to be certain that such refuse would be available in a reasonable period of time. Thirty-seven containers were initiated with leachate recycle and neutralization. Two containers were seeded with old, anaerobically degraded refuse. Both leachate recycle and neutralization and seed addition have been shown to accelerate refuse decomposition (Barlaz *et al.* 1987).

A complete microbial and chemical characterization required destructive sampling. Thus, once sampled, a container could no longer be monitored. Nine leachate recycle containers were sampled to characterize refuse decomposition under conditions of leachate recycle. These results are presented in Fig. 1 and discussed in the following section. Data for containers sampled on days 90 and 111, 24L and 5L respectively, are used here to characterize refuse in an active state of methane production under conditions of leachate recycle. Two seeded containers, 1S and 2S, were sampled to characterize refuse actively producing methane in the absence of leachate recycle.

2.3 Incubation conditions

Refuse in the leachate recycle containers was adjusted to 73% moisture (wet weight) with deionized water at the beginning of the experiment in order to generate ample leachate

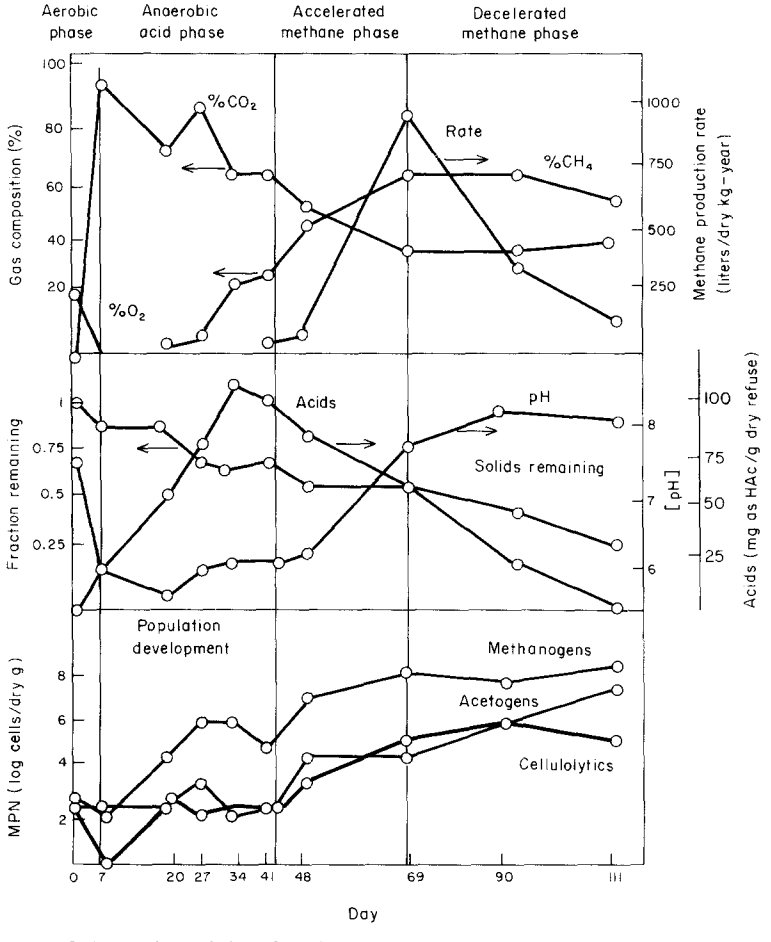

Fig. 1. Summary of observed trends in refuse decomposition with leachate recycle. The total carboxylic acids (acetate, propionate, isobutyrate, butyrate, iso-verate and valerate) are expressed as acetic acid equivalents [(mg acid/l) × (eq. wt. acetate) × (eq. wt. acid) $^{-1}$]. Methanogen MPN data are the log of the average of the acetate and H_2CO_2 utilizing populations. Solids remaining is the ratio of the mass of cellulose plus hemicellulose removed from a container divided by the weight of cellulose plus hemicellulose added to a container initially. Gas volume data were corrected to dry gas at standard temperature and pressure (STP). (Reprinted by permission of the American Society for Microbiology.)

for neutralization and recycling. Leachate was collected in one liter Viaflem intravenous bags (Travenol Laboratories, Morton Grove, IL, U.S.A.) placed below each refuse container. Leachate was neutralized and recycled 6 days per week. Initially a 100 g/l sodium carbonate solution was used for neutralization. After 7 weeks a potassium carbonate solution (171.6 g/l) was used for neutralization to minimize the possibility of an inhibitory sodium concentration. Cation concentrations did not influence the results of this study (Barlaz *et al.* 1989*c*). Leachate was recycled by raising the leachate collection bag above the refuse container and allowing leachate to flow through the flexible connecting tube into the container.

The seeded containers were adjusted to 48.5% moisture and no leachate was generated. These containers were filled with equal proportions of old, anaerobically decomposed refuse and fresh refuse on a dry weight basis. All containers were incubated at 41°C, the optimal mesophilic temperature for refuse decomposition (Hartz *et al.* 1982).

2.4 Container sampling, inoculum formation and microbial enumeration

Procedures for container sampling, inoculum formation and microbial enumeration have been described elsewhere (Barlaz *et al.* 1989*b*) and are summarized here. At container takedown, refuse was immediately placed in a plastic bag which 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 blended at 88% moisture in phosphate buffer (23.7 mM, pH 7.2) under nitrogen. The phosphate buffer was prepared by autoclaving, then cooling while sparging and gassing with nitrogen. 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. All equipment used for inoculum formation was autoclaved prior to sample processing. The inoculum formation procedure was validated in preliminary experiments (Barlaz *et al.* 1989*d*).

The remaining 20% of the refuse was used for formation of an extract for soluble constituent analyses. To form this extract, the contents of the leachate collection bag, and additional deaerated, deionized water as needed, were added to the refuse to adjust its moisture content to 90%. In the case of the seeded 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 centrifuged, filtered (0.45 μm), acidified and refrigerated or frozen prior to measurement of the various soluble constituents such as carboxylic acids, sulfates, phosphates and ammonia. Samples for sulfate analysis were not acidified.

The total anaerobic population and the sub-populations of cellulolytic, hemicellulolytic, hydrogen producing acetogenic (based on butyrate catabolism) and acetate- and H_2/CO_2-utilizing methanogenic bacteria were enumerated. Five tube MPNs were used for enumerations. Tubes were incubated at 41°C and checked for growth after 30 days, except for the acetogen MPN tubes which were checked after 60 days.

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 mM. 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

(Varel *et al.* 1984, Warshaw *et al.* 1985). Xylan from oat spelts (Sigma Chemical Co., St. Louis, Mo. cat. no. 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. Acetogenic bacteria were enumerated based on conversion of butyrate (40 mM) to acetate and hydrogen (Mackie & Bryant 1981). Butyrate was used in the acetogen MPN because of its prevalence in leachate (Barlaz *et al.* 1987). Methanogen MPN tests were performed with either 80 mM acetate or two atmospheres of hydrogen plus carbon dioxide.

2.5 Analytical methods

Techniques for measurement of the soluble constituents of refuse, solids composition, and gas composition and production have been presented previously (Barlaz *et al.* 1989*b*) and are summarized here. Methane concentration was measured weekly by gas chromatography. Gas production was measured by water displacement of an acidified sodium chloride solution. Carboxylic acids were measured by liquid chromatography with a differential refractometer detector. Soluble sugars were measured by liquid chromatography (Pettersen *et al.* 1984). Sulfate, ammonia and nitrate, and phosphate were measured as described by Hoeft *et al.* (1973), Keeney & Nelson (1982) and Bray & Kurtz (1945), respectively.

3. Results

As part of our overall study, an updated characterization of refuse decomposition was developed to include data on both microbial population development and chemical composition during decomposition (Barlaz *et al.* 1989*b*). 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, 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 second phase of decomposition, termed the anaerobic acid phase, 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, and a methane concentration of 50–60% is attained. This is accompanied by 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 and 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. Under field conditions, a period of constant methane production is sometimes observed between phases 3 and 4.

Two leachate recycle containers, 24L and 5L, which are represented by the data points for days 90 and 111 in Fig. 1, are used to characterize refuse in an active state of methane

production incubated with leachate recycle and neutralization. Two seeded containers (1S and 2S) were sampled to characterize refuse actively producing methane in the absence of leachate recycle and neutralization. The sampled containers and their methane yields are listed in Table 1. In that the sampled containers were producing methane at takedown, the methane yields presented in Table 1 do not represent the maximum methane potential of refuse; estimates of which are addressed elsewhere (Barlaz *et al.* 1989*a*). Methane production rate data for the sampled leachate recycle and seeded containers are presented in Fig. 2.

Microbial population and soluble constituent data for the sampled containers are presented in Tables 2 and 3, respectively. Soluble constituent data are presented in both mg/l and mg/dry g. The former units represent the concentration in the refuse ecosystem to which microbial populations were exposed. The latter units make it possible to compare concentrations of soluble constituents in refuse of different moisture contents.

4. Discussion

Refuse decomposition in the seeded containers is characterized in this section. In addition, trends in decomposition between the leachate recycle and seeded containers are discussed to evaluate whether there are differences in refuse decomposed with and without leachate recycle.

Containers 1S and 2S were sampled on days 55 and 99, respectively, and cannot be considered as precise replicates. Nonetheless, the carboxylic acid concentration data coupled with the trend in the methane production rate data suggest that each of these containers was in the decelerated methane production phase at the time of sampling. The seeded containers are expected to represent a range of microbial and chemical characteristics which may be present in refuse decomposing in the decelerated methane production phase, in the absence of leachate recycle. Similarly, although containers 24L and 5L were both sampled in the final phase of refuse decomposition, they cannot be treated as precise replicates. Refuse in container 5L was more completely decomposed than that in container 24L. However, containers 24L and 5L represent a range of conditions likely to be present in actively decomposing refuse incubated with leachate recycle.

TABLE 1
Description of the sampled containers and their methane production data

Sample*	Day sampled	Percent methane†	Methane production rate‡	Cumulative methane production§
Leachate recycle containers				
24L	90	63.8	342.7	50.1
5L	111	58.1	127.4	86.9
Seeded conainers				
1S	55	55.7	184.1	36.2
2S	99	58.6	205.6	51.0

* The L and S designate leachate recycle and seeded containers, respectively.

† Methane concentration two days prior to sampling.

‡ Methane production rate, expressed in liters CH_4 at standard temperature and pressure (STP)/year-dry kg of refuse used to fill the container, for the 9 days prior to the day on which the container was sampled.

§ Liters CH_4 at STP/dry kg of refuse used to fill the container. The yield is per kg of fresh refuse added to the seeded containers.

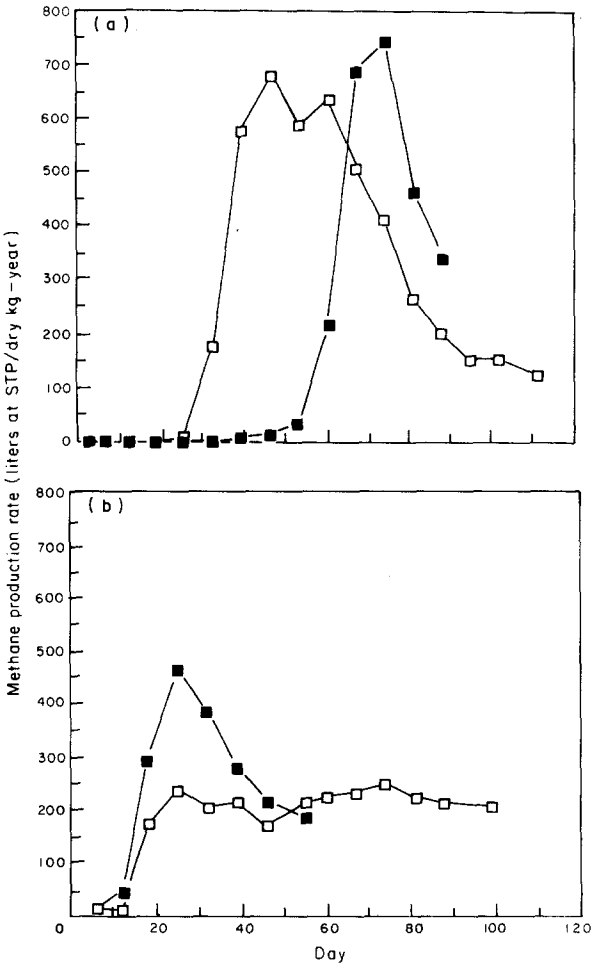

Fig. 2. Rate of methane production versus time in (a) leachate recycle containers (□, 5L; ■, 24L) and in the (b) seeded containers (■, 1S; □, 2S). Leachate recycle containers were sampled in the decelerated methane production phase (see Fig. 1). Data are the weekly average methane production rate, corrected to dry gas at standard temperature and pressure (STP). Data were normalized to the weight of fresh refuse used to fill a container.

4.1 Microbial populations in the sampled containers

The total anaerobic population in both seeded containers was between 10^8–10^9 cells/dry g. With the exception of the cellulolytic bacteria, there were no significant differences ($P=0.05$) in any of the sub-populations between containers 1S and 2S. The cellulolytic population measured for container 1S was higher than that measured for any of the sampled containers and the fraction of the total anaerobic population represented by cellulolytics was atypically high.

With the exception of the cellulolytic population in 1S, the fraction of the total anaerobic population represented by each trophic group in the seeded containers was similar to the corresponding fraction in the leachate recycle containers (Table 2). Thus, for the trophic groups measured, microbial population composition was similar between seeded and leachate recycle containers actively producing methane.

TABLE 2
Microbial populations in the sampled containers*

Sample	Day sampled	Total anaerobic	Hemi-cellulocytic	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
Fraction of total population†			1.4×10^{-1}	6.0×10^{-6}	2.6×10^{-4}	4.6×10^{-5}	4.6×10^{-5}
Leachate reycle containers							
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
Fraction of total population			6.2×10.1^{-1}	7.7×10^{-4}	1.8×10^{-1}	3.8×10^{-2}	1.8×10^{-3}
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
Fraction of total population			6.2×10^{-2}	2.5×10^{-4}	2.3×10^{-1}	6.2×10^{-1}	3.8×10^{-2}
Seeded containers							
1S	55	8.3×10^8	6.1×10^7	6.3×10^6	1.7×10^8	9.9×10^7	3.8×10^6
Fraction of total population			7.3×10^{-2}	7.5×10^{-3}	2.1×10^{-1}	1.2×10^{-1}	4.5×10^{-3}
2S	99	2.4×10^8	2.4×10^7	3.0×10^4	1.8×10^8	4.0×10^7	6.4×10^6
Fraction of total population			1.0×10^{-1}	1.3×10^{-4}	7.7×10^{-1}	1.7×10^{-1}	2.7×10^{-2}

* Data expressed in cells per dry gram of refuse removed from the container.

† The fraction of the total anaerobic population represented by each sub-population. That the sum of this fraction does not add up to 1.0 is expected. The total anaerobic population includes many anaerobic heterotrophic bacteria not enumerated as a separate sub-population. In addition, the 95% confidence limits of the MPN data presented here are, on average, plus or minus 300%.

TABLE 3
pH and soluble constituent concentrations in the sampled containers

Sample	Units	pH	Sugars*	Total acids†	Nitrate	Sulfate	Phosphate	Ammonia
Fresh refuse	mg/l‡	7.5	16393§	0	71.1	2071	800	521
	mg/dry g‖		3.46	0	0.015	0.44	0.17	0.11
Old refuse¶	mg/l	7.0	0	0	219.6	439	1.1	0
	mg/dry g		0	0	0.22	0.45	0.0034	0
Leachate recycle containers								
24L	mg/l	8.4		4640	0	6.9	10.1	45.0
	mg/dry g		0	20.5	0	0.03	0.04	0.2
5L	mg/l	8.2		0	0	0.5	0	8.0
	mg/dry g		0	0	0	0.004	0	0.06
Seeded containers								
1S	mg/l	6.8	0	2850	0	258	4.3	168
	mg dry g		0	3.11	0	0.28	0.005	0.18
2S	mg/l	6.1	0	3888	13.4	72.5	41.2	226
	mg/dry g		0	4.77	0.02	0.09	0.05	0.27

* Total sugars including glucose, xylose, galactose and arabinose.

† Total acids including acetic, propionic, butyric, isobutyric, valeric and isovaleric, expressed as acetic acid equivalents.

‡ Units are mg/l of liquid in the refuse plus accumulated leachate after correction for dilution associated with formation of a refuse extract. There was no leachate associated with the fresh refuse, old refuse or seeded containers.

§ A large unidentified peak eluted at 53.82 min. This was between mannose (50.05 min) and the erythritol internal standard (53.89 min)

‖ Units are mg/dry g of refuse as removed from a sampled container.

¶ Old refuse used to seed containers 1S and 2S.

4.2 Gas production in the sampled containers

Both seeded containers produced methane within a few days of initiation of the experiment, as expected from previous work (Barlaz *et al.* 1987). Methane production did not begin in the leachate recycle containers until there was growth of the methanogen population and partial neutralization of the refuse ecosystem.

Container 1S was sampled relatively early and it is not possible to determine whether its methane production rate was going to decrease asymptotically or maintain a constant value. The shape of the methane production rate curve for container 2S was different from that in container 1S and the leachate recycle and neutralization containers (Fig. 2). In container 2S the methane production rates reached a maximum and then stayed at 70–100% of its maximum for 10 weeks, at which time it was sampled. Container 1S and all 12 leachate recycle containers in which there was measurable methane production, reached a maximum rate and then decreased (Barlaz 1988).

Substrate availability may explain the difference in methane production rate patterns between containers 1S and 2S. Carboxylic acids were present in both seeded containers at takedown, although from the trend exhibited in the leachate recycle containers (Fig. 1), depletion of acids would have been expected. Given the absence of mixing by leachate recycle in containers 1S and 2S, it is likely that refuse decomposition was not uniform and there could have been localized acid accumulations in the seeded containers. In the leachate recycle containers, the methane production rate decreased as acids were depleted and polymer hydrolysis limited the rate of methane production. That there was not a sustained decrease in the methane production rate in container 2S after it reached its maximum rate, suggests that carboxylic acids were diffusing to the methane producing parts of the container where they were consumed. The sustained decrease in the methane production rate for container 1S suggests that although present, carboxylic acids did not diffuse to the methane producing parts of the container and polymer hydrolysis controlled the methane production rate.

In addition to the carboxylic acid data, several other soluble constituent parameters suggest that the refuse did not undergo uniform decomposition in the seeded containers. The pH in containers 1S and 2S, 6.8 and 6.1 respectively, were more acidic than would be expected from refuse beyond its period of maximum methane production (Fig. 1). Similarly, sulfates were present in both seeded containers at concentrations higher than would be expected based on the sulfate depletion exhibited in the leachate recycle containers (Table 3). Finally, nitrate was present in container 2S, though it was rapidly depleted in the leachate recycle containers. Methane production is severely inhibited in the presence of nitrate (Bollag & Czlonkowski 1973) providing additional evidence that refuse decomposition was not uniform in container 2S.

4.2.1 Nutrient limitations

It is not possible to judge whether the seeded containers were nutrient limited at takedown. Both phosphate and ammonia were present in containers 1S and 2S at concentrations which sustained relatively high rates of methane production in the leachate recycle containers. However, as discussed above, it is unlikely that these nutrients were distributed evenly throughout containers 1S and 2S, so the methane producing parts of the refuse could have been nutrient limited.

4.2.2 Methane production rates in the sampled containers

The lower rates of methane production in the seeded containers relative to the leachate

recycle containers (Fig. 2) may be attributed to differences in the initial pattern of refuse decomposition. In the leachate recycle containers, there were large accumulations of carboxylic acids prior to the onset of methane production (Fig. 1). At the time when methanogenic and acetogenic activity increased and methane production began in the leachate recycle containers, there was a large accumulation of carboxylic acids. This high concentration of soluble substrate stimulated the methane production rate (Barlaz *et al.* 1989*b*). In the seeded containers, an acclimated population of acetogenic and methanogenic bacteria was present initially and some of the acids were converted to methane as they were generated. Rapid acid consumption is inferred by the immediate onset of methane production in containers 1S and 2S. The methane production rate in the seeded containers was not stimulated by large accumulations of carboxylic acids, which never materialized, as in the leachate recycle containers.

5. Conclusions

(1) The total anaerobic population, and the sub-populations of cellulolytic, hemicellulolytic, acetogenic (based on butyrate catabolism) and acetate- and hydrogen plus carbon dioxide-utilizing methanogenic bacteria in refuse in an active state of methane production, were the same whether the refuse was incubated with leachate recycle at 72% moisture, or without leachate recycle at 48% moisture.
(2) Leachate recycle causes a well mixed soluble constituent pool which is atypical of both laboratory-scale lysimeters operated in the absence of water flux, and field-scale landfills. If the effects of a well-mixed soluble constituent pool can be addressed, then leachate recycle is a useful technique for acceleration of refuse decomposition and expeditious investigations of the refuse ecosystem at laboratory-scale.

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