Enumeration of fermentative and hydrolytic micro-organisms from three sanitary landfills

A. C. PALMISANO,* D. A. MARUSCIK and B. S. SCHWAB

Environmental Safety Department, The Procter & Gamble Co., Ivorydale Technical Center, Cincinnati, OH 45217, USA

(Received 14 April 1992; revised 7 September 1992; accepted 21 October 1992)

Fermentative bacteria were isolated from refuse excavated from municipal solid waste landfills in New York, Florida and Arizona. Anaerobic bacteria cultured on enriched solid media ranged from 10^5 to 10^8 c.f.u. (g dry wt of refuse)⁻¹. A significant correlation ($P < 0.03$) was found between numbers of anaerobes cultured at 37 °C and moisture content of refuse. Bacteria hydrolysing starch and protein represented 0–15% of the total anaerobes cultured; no anaerobic bacteria hydrolysing ball-milled cellulose were isolated. Aerobic bacteria isolated on enriched medium ranged between 10^4 and 10^7 c.f.u. (g dry wt)⁻¹. Direct microscopic counts of total bacteria associated with refuse were in the order of 10^{10} bacteria (g dry wt)⁻¹. These data suggest that, despite relatively high densities of bacteria in landfills, polymer hydrolysis is mediated by a small percentage of the fermentative population.

Introduction

Public concern with the disposal of municipal solid waste (MSW) in landfills has escalated in recent years as existing landfills close and new landfills become difficult to site (Carra & Cossu, 1990). In the US, an estimated 73% of MSW (118 million metric tons) was landfilled in 1988 (USEPA, 1990). Materials generated in the MSW stream in the US were of approximately 40% paper and paperboard, 17.6% yard waste, 7% glass, 8.5% metals, 7.4% food wastes, 8% plastics, and 11.6% other refuse, by weight (USEPA, 1990).

Components of the organic fraction of refuse may serve as substrates for micro-organisms in landfill environments (Grainger *et al.*, 1984; Archer & Robertson, 1986; Sleat *et al.*, 1987). The chemical composition of refuse includes polymers such as cellulose, starch, protein and lignin (Jones & Grainger, 1983). Aerobic bacteria may have a minor role in refuse decomposition prior to burial and the establishment of anoxic conditions. Anaerobic consortia within landfills mediate the processes of polymer hydrolysis, fermentation to organic acids, and mineralization by methanogenesis (Senior & Balba, 1990). Methanogenesis, the final step of decomposition of refuse in landfills, has received con-

siderable attention (Barlaz *et al.*, 1987; Archer & Peck, 1989) driven in part by interest in methane as an alternative fuel source (Campbell *et al.*, 1985). For high molecular mass polymeric materials in MSW, however, polymer breakdown to monomers and their subsequent fermentation to organic acids must occur prior to methanogenesis.

Laboratory scale landfills or lysimeters have been widely used to study the degradation of refuse by anaerobic microbial consortia (Bookter & Ham, 1982; Barlaz *et al.*, 1989a, b; Pohland *et al.*, 1985). These laboratory models have proven useful for hypothesis testing about landfill processes, however, extrapolation of models to full-scale facilities can be limited, because of diversity among and heterogeneity within landfills (Parkes & Senior, 1988). To date, microbiological data from full-scale, operating landfills are few (Campbell *et al.*, 1985) owing to the logistical difficulties inherent in their excavation.

The purpose of this study was (i) to culture and enumerate populations of fermentative anaerobic and aerobic bacteria; and (ii) to estimate populations of polymer-degrading micro-organisms in refuse excavated from three MSW landfills.

Methods

Study sites. Fresh Kills Landfill in Staten Island, New York was excavated 6–10 October, 1989 (Sufliya *et al.*, 1992); the area typically receives about 109 cm of rain and 63 cm of snow annually. Upper

* Author for correspondence. Present address: Office of Naval Research, Code 1141, 800 N. Quincy St, Arlington, VA 22217–5660, USA. Tel. (703) 696 2660; fax (703) 696 1212.

layers of refuse were relatively dry, however, refuse collected from below the water table was saturated, creating a dark grey slime. Los Reales Landfill in Tucson, AZ was excavated on 21 November, 1989; Tucson receives an average of 28 cm of rain per year. The Naples Landfill in Naples, FL, which receives about 135 cm of rain annually, was excavated on 12–14 March, 1990. All landfill excavations were conducted in collaboration with The Garbage Project, University of Arizona.

Sample collection. Refuse samples from Fresh Kills and Naples Landfill were collected with a bucket auger (Kellett's Well Boring, Simpsonville, SC). Refuse samples from the Los Reales Landfill were collected with a back hoe. All samples were collected from depths > 3 m to avoid contamination with surface refuse. Samples were dated from newspapers found in the excavated refuse. Prior to microbiological analysis, refuse samples were passed through a 5 cm mesh screen to remove larger debris. Exposure of samples to air was minimized as much as possible during sample collection.

Immediately upon collection, refuse samples from each site (Fresh Kills Landfill, 5 samples; Los Reales Landfill, 2 samples; Naples Landfill, 5 samples) were placed in sterile Petri dishes, then enclosed in anaerobic bags (GasPak, BBL, Becton Dickinson) with an atmosphere of 5–10% (v/v) CO₂. The anaerobic bags were kept cold on blue ice during transport to the Environmental Safety Department where they were placed in an anaerobic chamber (Forma Scientific Model 1024, Marietta, OH) with an atmosphere of 85% N₂, 10% H₂, and 5% CO₂, by vol.

Sample preparation. Inside an anaerobic chamber, 10 g of refuse was removed from the centre of each sample and added to a stomacher bag (Tekmar, Cincinnati, OH) containing 300 ml of pre-reduced, anaerobically sterilized (PRAS) buffer (Scott Laboratories) and mixed for approximately 1 min. This extract was serially diluted in PRAS buffer prior to inoculation.

Viable counts of fermentative and hydrolytic anaerobes. To culture fermentative bacteria, 0.1 ml diluted extract was inoculated under a continuous stream of N₂ into each medium using a VPI Anaerobic Culture System (Bellco). Roll tubes were prepared immediately upon inoculation on a Bellco tube spinner. Parallel sets of roll tube cultures were incubated at 22 °C and 37 °C for approximately 1–2 weeks or until the number of c.f.u. remained unchanged. C.f.u. were normalized per gram dry weight of refuse samples.

Fermentative anaerobes were isolated on Medium 10 (Carr Scarborough), which is a non-selective, enriched medium for isolation and enumeration of carbohydrate-fermenting bacteria (Caldwell & Bryant, 1966). Briefly, 1 l of Medium 10 contained: glucose, 0.05 g; cellobiose, 0.05 g; soluble starch, 0.05 g; yeast extract, 0.05 g; trypticase, 0.2 g; haemin; mineral salts; a mixture of volatile fatty acids (acetic acid, propionic acid, *n*-butyric acid, *n*-valeric acid, isovaleric acid, isobutyric acid, DL- α -methylbutyric acid), resazurin, and cysteine sulphide.

Polymer-degrading anaerobes from Fresh Kills and Tucson Landfills were cultured on peptone yeast glucose (PYG) agar amended with the polymer of interest. Differential staining or clearing of the medium identified polymer-degrading microbes. Anaerobic starch-degrading bacteria were identified by adding approximately 0.5 ml saturated KI solution to colonies growing on PYG medium with 0.2% soluble starch. Starch hydrolysis was indicated by clear zones around colonies. Proteolytic degraders were determined by adding several drops of 0.05% naphthol blue black (amido black, Sigma) in 7% (v/v) acetic acid solution to colonies growing on PYG agar amended with 0.4% gelatin. Colonies producing extracellular proteases were surrounded by clear zones. To isolate cellulolytic bacteria, ball-milled cellulose was added (Gerhardt *et al.*, 1981) to either PYG medium or to minimal medium (L-salts medium, Leadbetter & Foster, 1960). Minimal medium was used to avoid potential catabolite repression of cellulase

activity by glucose in PYG medium (Khan & Patel, 1990). Roll tubes were examined for clearing of the cellulose.

Viable counts of aerobic bacteria. Aerobic bacteria were estimated by serial dilution in phosphate buffer (0.0175 M-Na₂HPO₄; 0.033 M-KH₂PO₄; pH 6.8) and culturing with pour plates of Standard Methods Agar (Difco). Parallel sets of cultures were incubated at 22 °C and 37 °C.

Direct microscopic counts of bacterial numbers. Between 0.5 and 1.0 g refuse were fixed in a final concentration of 3.7% (w/v) formaldehyde. Samples for direct microscopic counts of acridine orange-stained cells were prepared using a modification of the methods of Ellery & Schleyer (1984) and Velgji & Albright (1986). Briefly, all samples were brought up to a known volume with filtered (0.2 μ m) 10% (w/v) sodium pyrophosphate and incubated for 5–10 min. The samples were cooled to 0 °C and alternately sonicated and cooled (30 s each) for 3 min. These samples were then stained with acridine orange and filtered on to 0.2 μ m porosity Nuclepore filters; the bacteria were counted as described by Hobbie *et al.* (1977), and bacterial numbers were normalized to dry weight of refuse.

Results

Refuse samples used for culturing bacteria from Fresh Kills Landfill dated from 1962/1965 (the sample contained a mixture of refuse from two years) to 1988 (Table 1). Moisture levels for these samples ranged from 23.4% in the 1980 sample to 74.7% in the 1971 sample. *In situ* temperatures were lowest for older refuse samples, ranging from 18.3 ° (1962/1965) to 43.3 °C (1988) at five Fresh Kills Landfill sites sampled for culturing bacteria. All Fresh Kills Landfill sites were characterized by relatively high densities of bacteria, as determined by direct microscopic counts (1.1 – 5.8×10^{10} bacteria (g dry wt)⁻¹ using acridine orange stain. Despite predominantly anaerobic conditions in the landfill, aerobic bacteria were culturable at numbers comparable to anaerobes. Anaerobic bacteria ranged from 0.2 – 32.8×10^6 c.f.u. (g dry wt)⁻¹ at 22 °C and 0.5 – 842×10^6 c.f.u. (g dry wt)⁻¹ at 37 °C; aerobic bacteria ranged from 0.5 – 23.2×10^6 c.f.u. (g dry wt)⁻¹ at 22 °C and 0.2 – 17.4×10^6 c.f.u. (g dry wt)⁻¹ at 37 °C. The lowest numbers of anaerobic and aerobic bacteria were found in the oldest refuse (1962/1965). A positive correlation was found between total numbers of anaerobic bacteria cultured at 37 °C and percent moisture ($r = 0.92$; $P < 0.03$; Systat). No significant correlation, however, was found between moisture and anaerobes cultured at 22 °C, or moisture and aerobes cultured at 22 ° or 37 °C.

Refuse collected from Los Reales and Naples Landfills dated from the 1980s. Two refuse samples excavated from Los Reales Landfill were characterized by lower moisture than Fresh Kills Landfill; *in situ* temperature data were not collected from Los Reales Landfill. Despite lower moisture, direct microscopic counts for Los Reales Landfill (2.82 – 4.17×10^{10} bacteria (g dry wt)⁻¹ were similar in magnitude for counts from Fresh Kills Landfill,

Table 1. Enumeration of bacteria from three landfills by direct microscopic counts and by culturing on solid media at 22 °C and 37 °C

Landfill	Site	Year†	Water (%)	Site temperature (°C)	AODC [10 ⁻¹⁰ × bacteria (g dry wt) ⁻¹]	Anaerobes [10 ⁻⁶ × c.f.u. (g dry wt) ⁻¹]*		Aerobes [10 ⁻⁶ × c.f.u. (g dry wt) ⁻¹]*	
						22 °C	37 °C	22 °C	37 °C
Fresh Kills	8-2	1988	40.1	43.3	4.6 ± 3.0*	20.5 ± 1.8	23.0 ± 3.5	9.68 ± 0.15	8.1 ± 0.5
Fresh Kills	1-2	1984	28.9	29.4	1.10 ± 0.10*	1.5 ± 0	2.91 ± 0.52	7.02 ± 0.74	4.44 ± 0.14
Fresh Kills	3-2	1980	23.4	21.6	5.8 ± 0.9*	0.65 ± 0.06†	2.79 ± 0.22†	11.5 ± 0.4†	7.04 ± 1.21†
Fresh Kills	9-2	1971	74.7	21.6	5.1 ± 2.7*	32.8 ± 1.5†	842 ± 54†	23.2 ± 0.6†	17.4 ± 0.4†
Fresh Kills	5-3	1962/65	43.3	18.3	2.4 ± 0.9*	0.2 ± 0.3	0.543 ± 0.011	0.490 ± 0.301	0.217 ± 0.005
Los Reales	1	1986	26.2	ND	2.82	3.1 ± 0.4†	0.725 ± 0.015†	0.354 ± 0.015†	0.0154 ± 0.0070†
Los Reales	2	1986	6.1	ND	4.17	0.229 ± 0.038†	2.86 ± 0.13†	1.88 ± 0.06†	1.50 ± 0.06†
Naples	1-2	1987	16.1	49.4	4.88	ND	50.3 ± 0.9	ND	50.8 ± 0.5
Naples	2-4	1987	35.4	57.2	2.81	ND	0.0789 ± 0.0080	ND	0.020 ± 0.008
Naples	2-6	1987	22.0	60.0	3.00	ND	0.632 ± 0.002	ND	0.683 ± 0.007
Naples	1-3	1987	12.3	62.8	3.05	ND	0.26 ± 0.01	ND	0.486 ± 0.008
Naples	2-8	1982	29.5	54.4	4.05	ND	4.70 ± 0.38	ND	9.15 ± 0.15

* Means ± SD of 3 subsamples.

† Significant difference between two temperatures at $P \leq 0.05$.

‡ Dated year of sample.

§ AODC, acridine orange direct count.

ND, no data.

Table 2. Fermentative bacteria degrading protein or starch in refuse excavated from Fresh Kills and Los Reales landfills

Figures are mean percentages ± SD from 3 replicates. Absolute figures [10⁻⁶ × c.f.u. (g dry wt)⁻¹] representing 100% are in parentheses.

Landfill	Site	Year*	Proteolytic bacteria		Amylolytic bacteria	
			22 °C	37 °C	22 °C	37 °C
Fresh kills	8-2	1988	15 ± 4 (53.3 ± 1.6)	0.5 ± 0.8 (43.6 ± 11.0)	12 ± 7 (4.01 ± 0.50)	2 ± 2 (8.94 ± 0.39)
Fresh kills	1-2	1984	11 ± 4 (22.1 ± 1.8)	4 ± 2 (2.97 ± 0.27)	4.0 ± 0.5 (1.63 ± 1.16)	2 ± 9 (2.69 ± 0.15)
Fresh kills	3-2	1980	2.0 ± 0.5 (7.91 ± 0.47)	2.0 ± 0.1 (7.32 ± 0.18)	3.0 ± 0.4 (3.36 ± 1.55)	2.0 ± 0.5 (6.54 ± 0.55)
Fresh kills	9-2	1971	10 ± 3 (25.2 ± 0.8)	0.9 ± 0.3 (14.1 ± 0.4)	0.2 ± 0.3 (708 ± 176)	2 ± 2 (230 ± 5)
Fresh kills	5-3	1965	7 ± 2 (0.35 ± 0.04)	0 ± 0 (0.90 ± 0.02)	0.4 ± 0.4 (1.30 ± 0.03)	4 ± 1 (3.88 ± 0.09)
Los Reales	1	1986	0.6 ± 0.3 (0.84 ± 0.02)	0.2 ± 0.4 (6.88 ± 0.08)	0.2 ± 0.4 (0.67 ± 0.07)	0.7 ± 0.8 (8.40 ± 0.42)
Los Reales	2	1986	5.5 ± 1.2 (2.87 ± 0.07)	3.4 ± 1.2 (3.43 ± 0.08)	13.8 ± 7.2 (2.57 ± 0.35)	5.3 ± 1.0 (3.99 ± 0.26)

* Dated year of sample.

and numbers of culturable fermentative bacteria were within the range found for samples from Fresh Kills Landfill.

The Naples Landfill was characterized by moderate moisture (12.3–35.4%) and higher temperatures (49.4–62.8 °C). Refuse samples from Naples Landfill were only cultured at 37 °C because of the higher *in situ* tempera-

tures. Both direct microscopic counts and c.f.u. per g dry wt of refuse were, in general, within the range found for refuse from Fresh Kills Landfill.

Bacteria from refuse samples were cultured anaerobically on media enriched with specific polymers to screen for hydrolytic activity (Table 2). Proteolytic colonies represented 0.6 to 15% and 0 to 4% of the

fermentative populations in refuse samples from Fresh Kills and Los Reales Landfills incubated at 22 °C and 37 °C, respectively. Amylolytic bacteria comprised 0.2–14% and 0.7–5.3% of the fermentative population cultured from refuse samples incubated at 22 °C and 37 °C, respectively. No consistent trend in the percentage of hydrolytic bacteria was evident with age of refuse. Cellulolytic organisms, which were to be identified by clearing of ball-milled cellulose in an agar matrix, were not cultured from samples from Fresh Kills, Los Reales or Naples Landfills.

Discussion

Although relatively high numbers of fermentative bacteria [10^5 – 10^8 c.f.u. (g dry wt) $^{-1}$] were cultured from landfilled refuse, only a small percentage (0–15%) were capable of producing extracellular hydrolytic enzymes for starch or protein degradation. While hydrolysis is a prerequisite for utilization of complex biopolymers by micro-organisms, initial enzymic attack may depend on a select few organisms (Colberg, 1988). Enumeration of c.f.u. on solid media is undoubtedly a very conservative estimate of the total number of fermentative bacteria in a landfill, since only some bacteria will be culturable on the medium provided. Moreover, the isolation of a viable organism from a landfill does not imply that the organism is active *in situ*; it may be merely surviving under landfill conditions. By contrast, the direct microscopic counts of acridine orange stained bacteria [10^{10} bacteria (g dry wt) $^{-1}$] may be an overestimate of viable bacteria, because non-viable cells may be inadvertently counted by this method.

Microbiological data from full-scale landfills is very limited, and many earlier studies focused on aerobic rather than anaerobic populations (Cook *et al.*, 1967). This emphasis on aerobic populations is somewhat surprising, since aerobic micro-organisms undoubtedly play only a minor role in refuse decomposition and gas production in landfills. In the present study, approximately 10^4 to 10^7 aerobic bacteria (g dry wt) $^{-1}$ were cultured from landfilled refuse samples on solid media. Campbell *et al.* (1985) suggested that aerobes may contribute to heat production prior to the development of anoxic conditions in buried refuse. Cook *et al.* (1967) isolated aerobic fungi, streptomycetes, and photosynthetic bacteria from a sanitary landfill, fresh household refuse, and seepage from the landfill. Public health considerations were the impetus for several studies of landfill microbiology which focused on the isolation of indicator organisms such as coliforms and faecal streptococci from model and full scale landfills which received both municipal and hospital waste (Donnelly & Scarpino, 1984; Pahren, 1987). MSW that had been incubated

for 20 months in a model landfill contained an estimated 10^5 to 10^6 fungi and 10^6 actinomycetes by plate counts, and 10^6 to 10^8 aerobic proteolytic bacteria (g dry wt) $^{-1}$ by MPN counts using liquid media (Filip & Kuster, 1979).

Our screening of colonies for polymer hydrolysis suggested that only a small proportion (<15%) of the fermentative population produced extracellular enzymes capable of hydrolyzing protein and starch. Jones *et al.* (1983) examined hydrolytic bacteria in refuse from Aveley Landfill, Essex, Great Britain, as a function of depth. Proteolytic and amylolytic bacteria were each approximately 1% of the total anaerobic population. In a separate lysimeter study, Jones & Grainger (1983) used MPN counts to estimate changes in bacterial numbers during a 120 d laboratory incubation of water-saturated, freshly pulverized domestic refuse. Proteolytic organisms decreased from approximately 10^8 to 10^6 organisms (g dry wt) $^{-1}$ during the course of the incubation while amylolytic organisms were approximately 10^6 (g dry wt) $^{-1}$.

To date, very few cellulolytic bacteria from landfills have been isolated and characterized (Bagnara *et al.*, 1985; Westlake, 1989). Our inability to isolate cellulolytic bacteria may be the result of (i) their low abundance in landfills (Jones *et al.* 1983); (ii) difficulty in extracting bacteria from refuse; (iii) inadequacy of cellulase detection (Robson & Chambliss, 1989); or most likely, (iv) their inability to grow on the solid media provided. Since <1% of the total bacteria determined by microscopic counts were culturable, cellulose-degrading bacteria may have easily been missed.

Logistic constraints resulted in analysis of relatively few refuse samples from the very heterogeneous landfill environment. Clearly, it is not appropriate to generalize from three sites to the approximately 7000 landfills in the United States which vary considerably in mode and efficiency of operation. However, we believe that our data on landfill microbiology will provide a baseline for future studies on a relatively poorly understood community of decomposers. An understanding of the establishment and maintenance of anaerobic communities in landfills is critical to improving leachate quality and thereby reducing the risk of environmental impacts of landfills such as groundwater contamination (Stegmann, 1983; Harper & Pohland, 1988).

Dr W. L. Rathje and The Garbage Project, Dr C. P. Gerba, Dr R. K. Ham, Dr J. S. Robinson, Dr J. M. Suffita, Mr W. Hughes, Mr L. W. King, and Mr B. Kellet collaborated on the Fresh Kills Landfill excavation. Moisture data were supplied by Dr J. M. Suffita. The research program was funded in its entirety by contributions from the Council for Solid Waste Solutions, E. I. DuPont de Nemours & Co., Exxon Chemical Co., Hercules, Inc., Hoechst Celanese, Jefferson Smurfit/Container Corporation of America, National Council of the Paper Industry on Air and Stream Improvement, The Perseco Co., The Procter & Gamble Co., Scott Paper Co., and Sonoco Products Co. The

authors thank Dr T. W. Federle and Ms B. A. Nuck for helpful discussions.

References

- ARCHER, D. B. & PECK, M. W. (1989). The microbiology of methane production in landfills. In *Microbiology of Extreme Environments and its Potential for Biotechnology*, pp. 187–204. Edited by M. S. da Costa, J. C. Duarte & R. A. D. Williams. London & New York: Elsevier Applied Science.
- ARCHER, D. B. & ROBERTSON, J. A. (1986). The fundamentals of landfill microbiology. In *Energy from Landfill Gas*, pp. 116–122. Edited by J. R. Emberton & R. F. Emberton. Oxfordshire: Harwell Laboratory.
- BAGNARA, C., TOCI, R., GAUDIN, C. & BELAICH, J. P. (1985). Isolation and characterization of a cellulolytic micro-organism *Cellulomonas fermentans* sp. nov. *International Journal for Systematic Bacteriology* **35**, 502–507.
- BARLAZ, M. A., MILKE, M. W. & HAM, R. K. (1987). Gas production parameters in sanitary landfill simulators. *Waste Management and Research* **5**, 27–39.
- BARLAZ, M. A., HAM, R. K. & SCHAEFER, D. M. (1989a). Mass-balance analysis of anaerobically decomposed refuse. *Journal of Environmental Engineering* **115**, 1088–1102.
- BARLAZ, M. A., D. M. SCHAEFER & HAM, R. K. (1989b). Bacterial population development and chemical characteristics of refuse decomposition in a simulated sanitary landfill. *Applied and Environmental Microbiology* **55**, 55–65.
- BOOKER, T. J. & HAM, R. K. (1982). Stabilization of solid waste in landfills. *Journal of Environmental Engineering Division, Proceedings of the American Society of Civil Engineers* **108**, 1089–1100.
- CALDWELL, D. R. & BRYANT, M. P. (1966). Medium without rumen fluid for nonselective enumeration and isolation of rumen bacteria. *Applied Microbiology* **14**, 794–801.
- CAMPBELL, D. J. V., FIELDING, E. R., & ARCHER, D. B. (1985). Understanding refuse decomposition practices to improve landfill gas energy potential. In *Proceedings of Energy from Biomass, Third EC Conference*, pp. 1151–1155. Edited by W. Palz. London: Elsevier Science Publishing.
- CARRA, J. S. & COSSU, R. (1990). *International Perspectives on Municipal Solid Wastes and Sanitary Landfilling*. San Diego: Academic Press.
- COLBERG, P. J. (1988). Anaerobic microbial degradation of cellulose, lignin, oligolignols, and monoaromatic lignin derivatives. In *Biology of Anaerobic Microorganisms*, pp. 333–372. Edited by A. J. B. Zehnder. New York: John Wiley and Sons.
- COOK, H. A., CROMWELL, D. L. & WILSON, H. A. (1967). Micro-organisms in household refuse and seepage water from sanitary landfills. *Proceedings of the West Virginia Academy of Sciences* **39**, 107–114.
- DONNELLY, J. A. & SCARPINO, P. V. (1984). Isolation, characterization, and identification of micro-organisms from laboratory and full-scale landfills. *EPA Project Summary, EPA600/S2-84-119*, pp. 1–7. Cincinnati: Municipal Environmental Research Laboratory.
- ELLERY, W. N. & SCHLEYER, M. H. (1984). Comparison of homogenization and ultrasonication as techniques in extracting attached sedimentary bacteria. *Marine Ecology – Progress Series* **15**, 247–250.
- FILIP, Z. & KUSTER, E. (1979). Microbial activity and the turnover of organic matter in a municipal refuse disposed of in a landfill. *European Journal of Applied Microbiology & Biotechnology* **7**, 371–379.
- GERHARDT, P., MURRAY, R. G. E., COSTILOW, R. N., NESTER, E. W., WOOD, W. A., KRIEG, N. R. & PHILLIPS, G. B. (1981). *Manual of Methods for General Bacteriology*. Washington, DC: American Society for Microbiology.
- GRAINGER, J. M., JONES, K. L. & HOTTEN, P. M. (1984). Estimation and control of microbial activity in landfill. In *Microbiological Methods for Environmental Biotechnology*, pp. 259–273. Edited by J. M. Grainger & J. M. Lynch. Orlando: Academic Press.
- HARPER, S. R. & POHLAND, F. G. (1988). Design and management strategies for minimizing environmental impact at municipal solid waste landfill sites. *Proceedings of Joint American Society of Civil Engineers–Canadian Society of Civil Engineers National Conference on Environmental Engineering, Vancouver*, pp. 669–688.
- HOBBIE, J. E., DALEY, R. J. & JASPER, S. (1977). Use of Nuclepore filters for counting fluorescence microscopy. *Applied and Environmental Microbiology* **33**, 1225–1228.
- JONES, K. L. & GRAINGER, J. M. (1983). The application of enzyme activity measurements to a study of factors affecting protein, starch and cellulose fermentation in domestic refuse. *European Journal of Applied Microbiology and Biotechnology* **18**, 181–185.
- JONES, K. L., REES, J. F. & GRAINGER, J. M. (1983). Methane generation and microbial activity in a domestic refuse landfill site. *European Journal of Applied Microbiology & Biotechnology* **18**, 242–245.
- KHAN, A. W. & PATEL, G. B. (1990). Cellulose degradation by mesophilic anaerobic bacteria. In *Synthesis and Biodegradation of Cellulose*, pp. 355–375. Edited by C. D. Haigler & P. J. Weimer. New York & Basel: Marcel Dekker.
- LEADBETTER, L. R. & FOSTER, J. W. (1960). Bacterial oxidation of gaseous alkanes. *Archives of Microbiology* **35**, 92–104.
- PAHREN, H. R. (1987). Microorganisms in municipal solid waste and public health implications. *CRC Critical Reviews in Environmental Control* **17**, 187–228.
- PARKES, R. J. & SENIOR, E. (1988). Multistage chemostats and other models for studying anoxic ecosystems. In *Handbook of Laboratory Model Systems for Microbial Ecosystems*, vol. 1., pp. 51–71. Edited by J. W. T. Wimpenny. Boca Raton: CRC Press.
- POHLAND, F. G., HARPER, S. R., CHANG, K.-C., DERTIEN, J. T. & CHIAN, E. S. K. (1985). Leachate generation and control at landfill disposal sites. *Water Pollution Research Journal of Canada* **20**, 10–24.
- ROBSON, L. M. & CHAMBLISS, G. H. (1989). Cellulases of bacterial origin. *Enzyme and Microbial Technology* **11**, 626–644.
- SENIOR, E. & BALBA, M. T. M. (1990). Refuse decomposition. In *Microbiology of Landfill Sites*, pp. 18–57. Edited by E. Senior. Boca Raton: CRC Press.
- SLEAT, R., HARRIES, C., VINEY, I. & REES, J. F. (1987). Activities and distribution of key microbial groups in landfill. *Proceedings of the ISWA Symposium on Process, Technology and Environmental Impact of Sanitary Landfills, Cagliari, Sardinia*, pp. 1–14.
- STEGMANN, R. (1983). New aspects on enhancing biological processes in sanitary landfill. *Waste Management and Research* **1**, 201–211.
- SUFLITA, J. M., GERBA, C. P., HAM, R. K., PALMISANO, A. C., RATHJE, W. L. & ROBINSON, J. A. (1992). Microbiological, chemical, and archaeological investigation of the Fresh Kills Landfill. *Environmental Science and Technology* **26**, 1486–1494.
- United States Environmental Protection Agency (1990). Characterization of Municipal Solid Waste in the United States: 1990 Update. EPA Report no. 530-SW-90-042. US Department of Commerce, National Technical Information Service.
- VELGYI, M. I. & ALBRIGHT, L. J. (1986). Microscopic enumeration of attached marine bacteria of seawater, marine sediment, fecal matter, and kelp blade samples following pyrophosphate and ultrasound treatments. *Canadian Journal of Microbiology* **32**, 121–126.
- WESTLAKE, K. (1989). Cellulolytic bacteria in sanitary landfill. In *Proceedings in Landfill Microbiology Research and Development Workshop*, pp. 20–33. Edited by P. S. Lawson & Y. R. Alston. Oxfordshire: Harwell Laboratory.