Journal of Environmental Sciences Vol. 13, No. 4, pp. 508—513, 2001

ISSN 1001—0742
CN11—2629/X

Article ID: 1001-0742(2001)03-0508-06 **CLC number**: X131.1 **Document code**: A

Effect of leachate recycle and inoculation on microbial characteristics of municipal refuse in landfill bioreactors

SHEN Dong-sheng, HE Ruo, REN Guo-ping, I. Traore, FENG Xiao-shen

(Department of Environment and Resource, Zhejiang University, Hangzhou 310029, China. E-mail: shends@mail.hz.zj.cn)

Abstract: Population development of key groups of anaerobic and aerobic bacteria involved in municipal refuse decomposition under laboratory landfill bioreactors with and without leachate recycle and inoculation was measured since modeling municipal refuse was landfilled in bioreactors for about 210 days. Hydrolytic fermentative bacteria (HFB), hydrogen-producing acetogenic bacteria (HPAB), methane-producing bacteria (MPB), sulfate-reducing bacteria (SRB), anaerobic and aerobic cellulolytic bacteria and denitrabacteria were enumerated by the most probable number technique. The results showed that the dominant microorganism groups were the methanogenic bacteria including hydrolytic fermentative, hydrogen-producing acetogenic and methane-producing bacteria. They were present in fresh refuse but at low values and positively affected by leachate recycle and refuse inoculation. The amounts of HFB or HPAB in digesters D4 and D5 operated with inoculation and leachate recycle reached their maximum values of 10^{10} – 10^{12} cells/g dry refuse for HFB or 10^5 – 10^6 cells/g dry refuse for HPAB on day 60, in digester D3 operated with leachate recycle on day 120 for HFB (10^9 cells/g dry refuse) or on day 90 for HPAB (10^5 cells/g dry refuse), and in digesters D1 and D2 on day 210 for HFB (10^9 cells/g dry refuse) or on day 90 for HPAB (10^4 – 10^6 cells/g dry refuse). The population of methane-producing bacteria in digesters D4 and D5 sharply increased on days 60 and 90 respectively, however in digesters D1, D2 and D3 on day 120. Leachate recycle and inoculation changed the cellulolytic microorganisms composition of refuse ecosystem, the higher amounts of anaerobic cellulolytic bacteria were measured in digesters D4 and D5 (10^7 cells/g dry refuse), followed by digesters D3 (10^6 cells/g dry refuse), D2 or D1(10^4 cells/g dry refuse). However, the amounts of aerobic cellulolytic bacteria were much lower than that of anaerobic cellulolytic bacteria. And it was higher in digester D3 than those in digesters D1, D2, D4 and D5. The amounts of SRB and denitrabacteria were also higher in digester D5 than those in digesters D1, D2, D3 and D4. Refuse decomposition could be accelerated by leachate recycle and inoculation in the view of microorganism development.

Keywords: municipal refuse; landfill bioreactor; leachate recycle; inoculation; anaerobic bacteria

Introduction

The disposal of waste to landfill is the dominant method of disposal for domestic and most other waste streams. However, non landfilling which has previously been the method of choice for many cities is at a crisis point for the reasons of shorter life, space availability, cost and pollution, in particular, landfilling of domestic solid waste gives rise to a highly polluting liquor known as leachate. The attainment of sustainable waste management, and the development of sustainable landfilling practices will be an important step in sustainable development recognizes.

Understanding the microbiological processes occurring in landfill site is of paramount importance both to controlling pollution problems resulting from the accumulation of carboxylic acids and inorganic ions and to the commercial exploitation of landfill bioreactors (Jones, 1983; Barlaz, 1989). Substrate decomposition to methane in sanitary landfill follows the patterns established for habitats such as sediments (Abram, 1978), sludge digestion and the rumen, can be regarded as being concerned first with hydrolysis and fermentation of polymers such as starch, protein, cellulose, hemicellulose and lignin to volatile fatty acids (Bryant, 1977). In the absence of nitrate and sulphate, the terminal products of refuse decomposition are carbon dioxide and methane, which represents a usable but underutilized source of energy, but research on enhancement of methane production has not led to an understanding of refuse decomposition adequate to predict and increase methane yields in sanitary landfills (Barlaz, 1987; Kinman, 1987; Buivid, 1981). Pohland and Gould (Pohland, 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 are little known.

Table 1 Composition of the mixed refuse

Components	Percent by wet weight
Vegetables	45.0
Fish	2.50
Meat	1.00
Fruit	8.95
Paper	7.47
Plastics and leather rubber	11.92
Cellulose	3.60
Brick sand and soil	8.46
Metals and glasses	7.57
Woods	3.53

The objective of this paper is firstly to compare microbial characteristics of refuse, incubated with and without leachate recycle and inoculation in laboratory-scale landfill bioreactors, and secondly to evaluate the significance of the five process condition.

1 Materials and methods

1.1 Materials

Synthetic municipal solid waste mixture was man-made in our laboratory, composition of this material is shown in Table 1.

The refuse mixture was shredded into 2 to 4 cm in length or width; the water content of mixture was about 50% of the water holding capacity.

1.2 Experimental equipment

Each simulated bioreactor landfill was assembled as shown in Fig. 1. The

Foundation item: The National Natural Science Foundation of China (No. 59808012) and Zhejiang Province (No. 599127)

refuse mixture was compacted in each of equipments until a specific weight of 0.5 ton per cubic meter was attained.

1.3 Experimental design

The objective of this study was to study and compare microbial characteristics of refuse during the refuse decomposition, incubated with and without leachate recycle and inoculation, thus five equipments were employed, among them, the first one (D1) was used as control, the second (D2) was adjusted to 75% moisture (wet weight) and no leachate recycle, the third (D3) was adjusted to 75% moisture and initiated with leachate recycle but no inoculation, the fourth (D4) was adjusted to 75% moisture and initiated with leachate recycle and inoculated with high-effective microorganisms selected from old refuse in general landfill, the fifth (D5) was nearly the same as the fourth but double inoculation. All leachate was not neutralized and recycled through the top of the bioreactor on a daily basis. All stimulated landfill bioreactors were incubated at 28 ± 1℃.

1.4 Procedure for container sampling inoculum formation and microbial enumeration

Approximately 100g of the refuse mixture were withdrawn periodically from the each of bioreactors at bioreactors take down. The refuse sample was immediately placed in a plastic bag, which was closed and all free air was removed by squeezing. 50g of the refuse sample in the bag was used to form an inoculum for most probable number (MPN) enumeration. To form an inoculum, the refuse was blended at 88% moisture in distilling and autoclaving water under 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 inoculums. The remaining was used to monitored for moisture content by measuring the loss of sample weight after drying in an oven at 105℃ for approximately 16 – 24h to a constant weight.

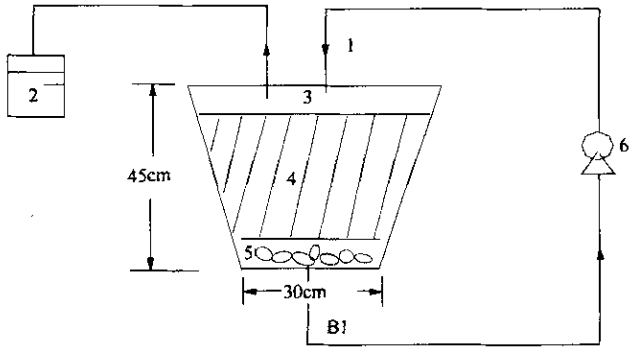

Fig.1 Schematic representation of digesters used during the experiment
1. water injection port; 2. gas bag; 3. sand layer; 4. refuse (solid waste); 5. stone; 6. pump; BI. bottom of digester

The hydrolytic fermentative bacteria, hydrogen-producing acetogenic bacteria, methanogens, sulfate-reducing bacteria, aerobic and anaerobic cellulolytic bacteria and denitrobacteria were enumerated. Three MPN tubes were used for enumeration. Tubes were incubated at 35 ± 1℃ and checked for growth after 30 days except for the acetogen and methanogen MPN tubes which were checked after 60 days.

1.5 Medium preparation and enumeration techniques

Medium composition for determination of hydrolytic fermentative bacteria is as follows (g/L): glucose 10, beef extract 3, peptone 5, NaCl 3, cystein 0.5, resazurin 0.002, pH 7.2 – 7.4.

Medium composition for determination of hydrogen-producing acetogenic bacteria is as follows (per liter): CH_3CH_2COONa 30 mmole, $CH_2(CH_2)_2COONa$ 30 mmole, sodium lactate 30 mmole, sodium succinate 30 mmole, CH_3CH_2OH 30 mmole, yeast extract 2g, $MgCl_2$ 0.1g, NH_4Cl 1.0g, K_2HPO_4 0.4g, KH_2PO_4 0.4g, cystein 0.5g, resazurin 0.002g, trace element solution 10 ml, soil extract solution 300 ml, pH 7.0 – 7.3.

Composition of the medium used for determination of methanogens is as follows (g/L): NH_4Cl 1.0, $MgCl_2$ 0.1, KH_2PO_4 0.4, K_2HPO_4 0.4, yeast extract 1.0, cystein 0.5, HCOONa 5.0, CH_3COONa 5.0, CH_3OH 5.0 ml, H_2/CO_2 80/20 (v/v), soil extract 300 ml, trace element solution 10 ml.

Composition of the medium used for determination of sulfate-reducing bacteria as follows (per liter): 10% NH_4Cl + 1% $MgCl_2$ 10 ml, 4% K_2HPO_4 + 4% KH_2PO_4 10 ml, yeast extract 1.0 ml, cysteine 0.5g, Na_2SO_4 1.14g, lactate 0.5 ml, NaOH 0.5g, trace element solution 10 ml, resazurin 1.0ml, $FeSO_4$ 0.25g, agar 20g, pH 7.2.

Composition of the medium used for determination of anaerobic cellulolytic bacteria is as follows (g/L): KH_2PO_4 1.0, $Na(NH_4)HPO_4$ 2.0, peptone 1.0, $MgSO_4 \cdot 7H_2O$ 0.5, $CaCl_2 \cdot 6H_2O$ 0.3, $CaCO_3$ 5.0, filter paper without starch 7cm × 1cm.

Composition of the medium used for determination of aerobic cellulolytic bacteria is as follows (g/L): KH_2PO_4 1.0, $MgSO_4 \cdot 7H_2O$ 0.3, $FeCl_3$ 0.01, NaCl 0.1, $NaNO_3$ 2.5, $CaCl_2$ 0.1, filter paper without starch 7cm × 1cm, pH 7.2.

Composition of the medium used for determination of denitrobacteria is as follows (g/L): KNO_3 2.0, $MgSO_4 \cdot 7H_2O$ 0.2, K_2HPO_4 0.5, pH 7.2.

Preparation of soil extract was as follows: taking several paddy soil and adding tap water (soil/water = 1:1.5), stir it and then let it stand still for 24h, filter the supernatant with filter paper, sterilize the filtrate and store it in a refrigerator.

Composition of trace element solution was as follows (g/L): $N(CH_2COOH)_3$ 4.5, $FeCl_2 \cdot H_2O$ 0.4, $MnCl_2 \cdot 6H_2O$ 0.12, $CoCl_2 \cdot 6H_2O$ 0.12, $ZnCl_2$ 0.1, $AlK(SO_4)_2$ 0.01, NaCl 1.0, $CaCl_2$ 0.02, Na_2MoO_4 0.01, H_2BO_4 0.01.

Enumeration techniques: The population of each group was measured by MPN method with triplicate. The growth, the formation of hydrogen, methane, nitrogen and H_2S, and the degradation of filter paper were used as the indexes of population for hydrolytic, fermentative, hydrogen-producing acetogenic, methanogenic bacteria, denitrobacteria, sulfate-reducing and aerobic (anaerobic) cellulolytic bacteria.

2 Results and discussion

2.1 Population of methanogenic flora

2.1.1 General trend of methanogenic population

The amounts of methanogenic microorganisms including hydrolytic fermentative, hydrogen-producing acetogenic and methane-producing bacteria were measured during different times of refuse decomposition which was buried for 210 days. The results of a MPN determination in triplicate for all the methanogenic bacteria were presented in Table 2. There was a common trend that all the methanogenic groups were present in fresh refuse but at low values and their amounts increased suddenly in all the digesters during the first two weeks, then increased progressively up to the highest values and decreased gradually.

2.1.2 Effect of leachate recycle and inoculation on methanogenic bacteria population during refuse decomposition

There were differences in the population of methanogenic bacteria between digesters operated with or without leachate recycle and inoculation. The variation with time in the population of methanogenic bacteria including hydrolytic fermentative, hydrogen-producing and methane-producing bacteria in all digesters was different (Table 2). The population of hydrolytic fermentative bacteria in digesters D4 and D5 operated with inoculation and leachate recycle reached the maximum (10^{10} – 10^{12} cells/g dry refuse, respectively) on day 60, in digester D3 operated with leachate recycle on day 120 (10^{10} cells/g dry refuse) and for D1 and D2 on day 210.

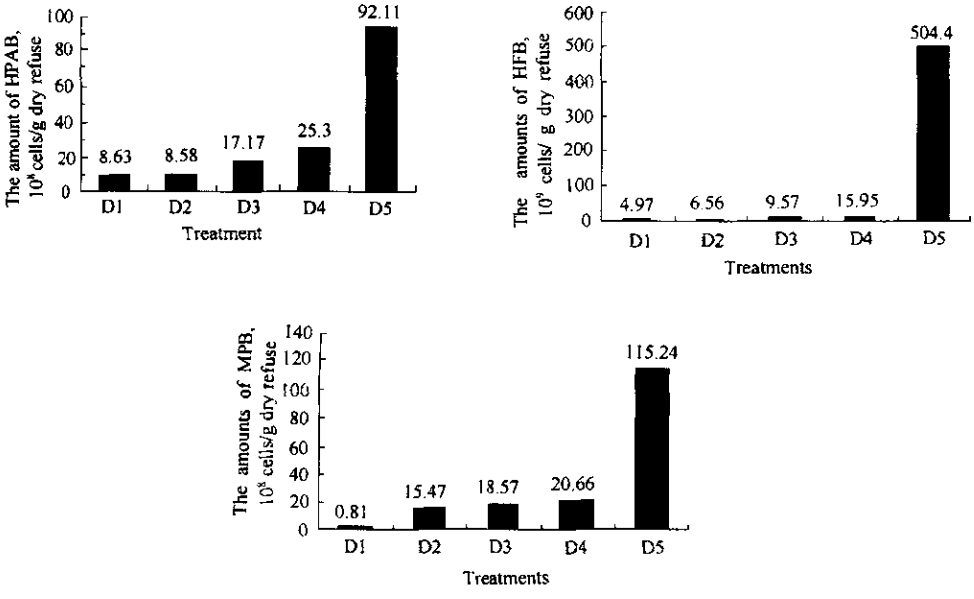

Fig.2 Total average (MPN) of methangenic bacteria during refuse decomposition

Table 2 The amounts of methanogenic bacteria in different digesters during refuse decomposition (10^4 cells/g dry refuse)

Microbial group	Digester identity	Sampling time, d							
		0	7	14	30	60	90	120	210
Hydrolytic fermentative bacteria (HFB)	D1	6.4×10^{1}	2.18×10^{3}	9.83×10^{4}	9.83×10^{4}	2.55×10^{4}	2.46×10^{5}	7.72×10^{4}	3.43×10^{6}
	D2	6.4×10^{1}	3.8×10^{4}	3.1×10^{4}	1.87×10^{4}	2.18×10^{4}	2.18×10^{5}	3.43×10^{5}	4.58×10^{6}
	D3	6.4×10^{1}	1.8×10^{5}	4.98×10^{5}	1.8×10^{4}	3.8×10^{5}	1.8×10^{6}	4.4×10^{6}	3.8×10^{5}
	D4	6.4×10^{1}	5.6×10^{6}	4.98×10^{5}	3.8×10^{4}	6.0×10^{6}	3.8×10^{5}	1.8×10^{5}	6.0×10^{4}
	D5	6.4×10^{1}	1.0×10^{6}	1.31×10^{5}	3.8×10^{5}	4.0×10^{8}	1.8×10^{5}	1.8×10^{6}	2.8×10^{4}
Hydrogen-producing acetogenic bacteria (HAB)	D1	9.16×10^{-1}		4.37	5.46×10^{1}	1.62×10^{2}	2.46×10^{4}	1.63×10^{4}	1.55×10^{4}
	D2	9.16×10^{-1}		4.96	4.91×10^{2}	2.18×10^{4}	1.03×10^{6}	1.03×10^{4}	2.06×10^{4}
	D3	9.16×10^{-1}		2.36	6.0×10^{4}	1.8×10^{5}	6.0×10^{5}	3.8×10^{5}	8.0×10^{4}
	D4	9.1×10^{-1}		8.35×10^{1}	1.0×10^{5}	6.0×10^{5}	6.0×10^{5}	1.0×10^{5}	4.6×10^{4}
	D5	9.16×10^{-1}		8.38×10^{2}	3.8×10^{5}	3.8×10^{6}	3.8×10^{6}	6.0×10^{4}	2.6×10^{4}
Methane-producing bacteria (MPB)	D1	9.0×10^{-4}		9.0×10^{-4}	2.18×10^{-1}	7.66	6.56×10^{-1}	6.04×10^{5}	2.1×10^{2}
	D2	9.0×10^{-4}		1.24×10^{-1}	1.96×10^{-1}	3.43	4.58	6.0×10^{5}	3.0×10^{2}
	D3	9.0×10^{-4}		2.36×10^{-1}	1.2×10^{1}	2.6×10^{2}	6.0×10^{2}	1.2×10^{6}	9.5×10^{2}
	D4	9.0×10^{-4}		1.05	1.2×10^{1}	1.0×10^{2}	4.4×10^{4}	1.72×10^{6}	7.0×10^{3}
	D5	9.0×10^{-4}		1.57×10^{-1}	6	2.6×10^{5}	1.8×10^{5}	6.0×10^{6}	7.8×10^{3}

Table 2 indicated that leachate recycle and inoculation affected refuse methanogenic population. Leachate recycle could increase anaerobic condition by creating suitable condition for methanogenic bacteria. Total or average amounts of each group of methanogenic bacteria were higher in digester D5 followed by digesters D4, D3, D2 and D1 respectively (Fig.2). The differences in methanogenic population between D4 and D5 also confirmed that the methanogenic bacteria in refuse ecosystem might be affected by inoculation.

2.2 Population of cellulolytic bacteria

Cellulose is the principal constituent of refuse. Both aerobic and anaerobic bacteria are capable of cellulose degradation. The complete conversion product of cellulose is methane under anaerobic condition or carbon dioxide under aerobic condition. But the fungi are more successful than bacteria in the degradation of cellulose embedded in lignin.

2.2.1 Anaerobic cellulolytic bacteria

2.2.1.1 General trend

The amounts of anaerobic cellulolytic bacteria were measured during different periods of refuse decomposition. The results are shown in Table 3. It was obvious that the population of anaerobic cellulolytic bacteria was lower in fresh refuse and increased to its maximum on day 120 in all digesters. Contrary to the population of methanogenic bacteria, the population of anaerobic cellulolytic bacteria did not decreased obviously after reaching its maximum. The population of anaerobic cellulolytic bacteria was at $10^4 - 10^7$ cells/g dry refuse, about 10^4 times less than hydrolytic fermentative bacteria, 100 times less than hydrogen-producing acetogenic bacteria and about 10 times less than methane-producing bacteria.

2.2.1.2 Effect of leachate recycle and inoculum on anaerobic cellulolytic bacteria

The difference between digesters D1 and D2 suggested the stimulatory effect of moisture content. The population of anaerobic cellullytic bacteria in digesters D1 and D2 was significantly higher than that in digesters D3, D4 and D5 operated with leachate recycle and inoculation during the first two months, because leachate recycle and inoculation stimulated degradation of refuse and led to the decrease of pH. Low pH restrained the growth of anaerobic cellulolytic bacteria. With the degradation of refuse and increase of pH, leachate recycle and inoculation began to affect about two months later, therefore, the total or average amounts of anaerobic cellulolytic bacteria were higher in digester D5 followed by digesters D4, D3, D2 and D1 (Fig. 3).

Table 3 The amounts of anaerobic and aerobic cellulolytic bacteria during the decomposition of refuse with different treatments

(cells/g dry refuse)

Microbial group	Digester identity	Sampling time, d							
		0	7	14	30	60	90	120	210
Anaerobic cellulolytic bacteria (AeCB)	D1	1	3.5×10^1	2.07×10^2	4.37×10^2	1.19×10^4	2.13×10^4	6.0×10^4	6.0×10^4
	D2	1	3.6×10^1	1.03×10^2	5.89×10^2	1.79×10^4	3.66×10^4	4.58×10^4	8.0×10^4
	D3	1	4.0×10^1	1.4×10^2	1.0×10^4	6.6×10^2	5.2×10^4	8.0×10^6	3.6×10^6
	D4	1	4.4×10^1	3.1×10^1	8.0×10^2	8.0×10^4	6.4×10^6	1.0×10^7	1.4×10^6
	D5	1	8.0×10^1	1.2×10^1	2.4×10^2	2.8×10^4	1.0×10^6	4.6×10^7	4.0×10^5
Aerobic cellulolytic bacteria (ACB)	D1	0	2.2×10^1	2.0×10^1	2.4×10^1	4.3×10^1	5.7×10^2	6.0×10^2	6.0×10^2
	D2	0	4.0×10^1	1.44×10^2	2.0×10^1	2.6×10^2	1.37×10^3	8.0×10^2	3.66×10^2
	D3	0	4.0×10^1	1.05×10^2	4.0×10^1	6.6×10^1	1.4×10^2	4.6×10^2	6.4×10^2
	D4	0	4.0×10^1	1.83×10^2	4.0×10^1	1.2×10^2	1.4×10^3	1.0×10^3	1.0×10^3
	D5	0	4.0×10^1	8.34×10^2	4.0×10^1	1.2×10^1	3.8×10^2	1.0×10^2	1.0×10^3

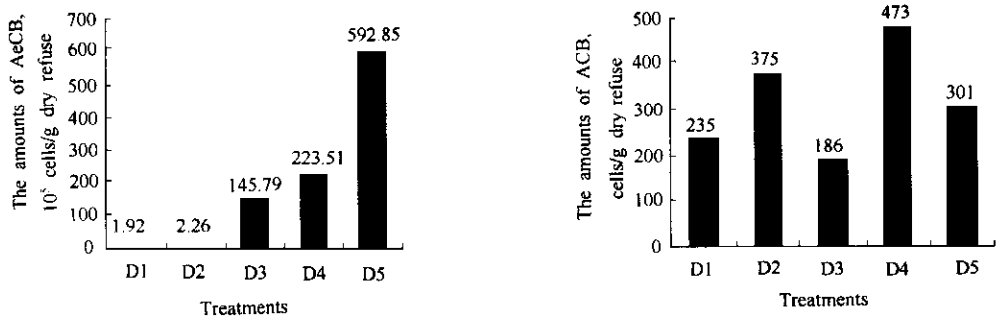

Fig.3 Total average (MPN) of cellulolytic bactria during refuse decomposition

2.2.2 Aerobic cellulolytic bacteria

2.2.2.1 General trend

There was a common trend that the population of aerobic cellulolytic groups was at low value after initiation of the digesters and did not shown significant increase or decrease before day 60. After then, the population of aerobic cellulolytic bacteria in all digesters showed a little increase ($10^1 - 10^3$ cells/g dry refuse, less than the amounts of anaerobic cellulolytic bacteria during refuse decomposition). This is contrary to the general trend that the population of aerobic bacteria must decrease with time according to the condition of refuse ecosystem. However, its increase can be explained by the fact that

facultative cellulolytic bacteria might be more dominant with the time during refuse incubation periods.

2.2.2.2 Effect of leachate recycle and inoculation

The average or total amounts of aerobic cellulolytic bacteria growing in digesters D2 and D3 were significantly higher than that in digesters D4 and D5 operated with leachate recycle and inoculation and that in digester D1 was the lest (Table 3 and Fig.3), which showed that inoculation did not affect the amounts of aerobic cellulolytic bacteria and moisture content might be more important factor affecting the amounts of aerobic cellulolytic bacteria in refuse decomposition.

2.3 Sulfate-reducing bacteria

Sulfate-reducing bacteria (SRB) participates in interspecies hydrogen transfer in the complex fermentation leading to methanogenesis and leading to massive and spectacular accumulation of sulfide. In addition to the control of methane gas production, sulfate reduction can also enhance the stabilization of waste.

2.3.1 General trend

The amounts of SRB were measured during different periods of refuse incubation, the results of a MPN determination in triplicate for all digesters are presented in Table 4. There was a common trend that all the groups of SRB were presented in the fresh refuse, but at low level (less than 100 cells/g dry refuse) compared to the population of mathanogenic bacteria. With the incubation of refuse in landfill bioreactors, the amounts of SRB increased gradually to the maximum, then decreased again.

Table 4 The amounts of sulfate-reducing bacteria (SRB) in refuse with different treatments during the refuse decomposition (cells/g dry refuse)

Digester identity	Incubation time, d							
	0	7	14	30	60	90	120	210
D1	1.0×10^{2}	2.07×10^{2}	1.97×10^{2}	4.37×10^{2}	3.4×10^{3}	—	7.73×10^{5}	4.29×10^{4}
D2	1.0×10^{2}	1.0×10^{2}	5.24×10^{2}	3.93×10^{4}	2.29×10^{2}	—	3.2×10^{6}	2.52×10^{5}
D3	1.0×10^{2}	1.2×10^{3}	3.93×10^{3}	4.4×10^{2}	4.0×10^{2}	—	6.4×10^{5}	3.6×10^{4}
D4	1.0×10^{2}	1.2×10^{5}	4.7×10^{6}	1.0×10^{3}	4.0×10^{1}	—	1.6×10^{5}	3.0×10^{4}
D5	1.0×10^{2}	1.8×10^{6}	1.05×10^{7}	6.0×10^{3}	4.0×10^{2}	—	1.8×10^{6}	6.0×10^{4}

2.3.2 Effect of leachate recycle and inoculation

The population of SRB was positively affected with refuse inoculation and leachate recycle during the early stage of refuse incubation. It increased up to the maximum (10^{5} – 10^{6} cells/g dry refuse) in digesters D4 and D5 on day 14, after then, because of the decrease of sulfate and increase of organic acids concentration, the amounts of SRB sharply decreased until day 120. Because of the decrease of organic matters and re-oxidation of H_2S in leachate by air, the amounts of SRB increased again in some degree. However, the amounts of SRB in digesters D1, D2 and D3 increased gradually with incubation time and reached the maximum (10^{5} – 10^{6} cells/g dry refuse) on day 120. This explained that inoculation and leachate recycle accelerated refuse decomposition into simple organic matters which can be used by SRB, MPB and other fermentative bacteria, and SRB can compete with methanogens for substrate due to their high affinity for acetate and hydrogen in hydrogen limited environment and in the presence of SO_4^{2-}, but SRB could not compete with methanogens at high organic concentration (Min, 1993).

2.4 Denitrabacteria

The amounts of denitrabacteria measured during refuse incubation are presented in Table 5. The results of a MPN determination in quadruplicate showed that they were much more than that of methanogenic and sulfate-reducing bacteria

Table 5 The amounts of denitrabacteria in refuse with different treatments during the decomposition of organic matter (cells/g dry refuse)

Digester identity	Incubation time, d							
	0	7	14	30	60	90	120	210
D1	7.3×10^{5}	1.31×10^{6}	1.31×10^{6}	5.46×10^{4}	5.12×10^{2}	3.66×10^{3}	4.29×10^{2}	2.4×10^{3}
D2	7.3×10^{5}	6.4×10^{5}	4.75×10^{6}	1.18×10^{4}	8.01×10^{3}	4.1×10^{4}	3.78×10^{3}	2.4×10^{3}
D3	7.3×10^{5}	1.4×10^{6}	6.03×10^{6}	4.0×10^{4}	4.4×10^{3}	8.0×10^{4}	8.0×10^{3}	4.0×10^{3}
D4	7.3×10^{5}	6.6×10^{6}	6.6×10^{6}	4.0×10^{4}	6.6×10^{5}	8.0×10^{4}	5.2×10^{3}	2.4×10^{3}
D5	7.3×10^{5}	1.4×10^{6}	1.4×10^{6}	4.0×10^{4}	6.4×10^{7}	6.6×10^{4}	8.0×10^{3}	6.4×10^{4}

during the early stage of refuse decomposition, because nitrate may be reduced by denitrabacteria much more easily than sulfate reduced by SRB and carbon dioxide reduced by methane-producing bacteria, denitrabacteria can compete with methane-producing bacteria and SRB (Fig.4 and Fig.5). However, with the refuse decomposition and the decrease of nitrate, the population of denitrabacteria decreased slowly with time in all digesters up until day 210, the population of denitrabacteria in digesters D4 and D5 decreased much faster than that in digesters D1, D2 and D3 since leachate recycle and inoculation accelerated refuse decomposition and provided more hydrogen and reducing power for nitrate reduction.

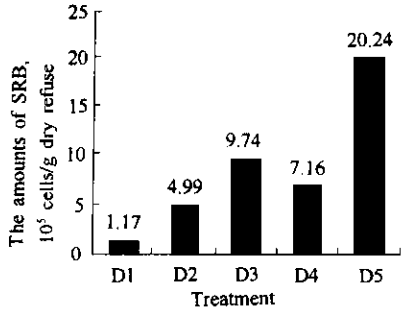

Fig.4 Total average (MPN) of sulfate-reducing bacteria during refuse decomposition

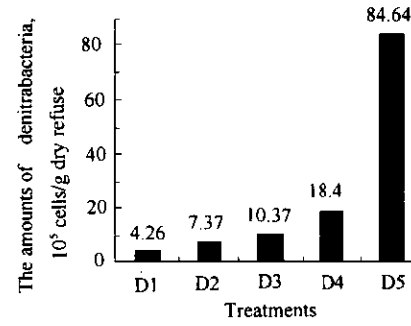

Fig.5 Total average (MPN) of denitrabacteria during refuse decomposition

References:

Abram J W, Nedwell D B, 1978. Inhibition of methanogenesis by sulfate reducing bacteria competing for transferred hydrogen[J]. Arch Microbial, 117:89—92.

Barlaz M A, Milk M W, Ham R K, 1987. Cas production parameters in sanitary landfill simulator[J]. Waste management and research, 5: 27—39.

Barlaz M A, Ham R K and Schaefer D M, 1989. Bacterial population development and chemical characteristics of decomposition in a simulated sanitary landfill[J]. Appl Environ Microbial, 55:55—65.

Bryant M P *et al*., 1977. Growth of desulfovibrio in lactate or ethanol media low in sulfate association with H_2-utilizing bacteria[J]. Appl Environ Microbial, 33:1162—1169.

Buivid M G *et al*., 1981. Fuel gas enhancement by controlled landfill of municipal solid waste[J]. Resource Recovery and Conservation, 6: 3—20.

Jones K L *et al*., 1983. Methane general and microbial activity in a domestic refuse landfill site[J]. Eur J Appl Microbial Biotech, 18:242—245.

Kinman R N *et al*., 1987. Gas enhancement techniques in landfill simulator[J]. Waste Management and Research, 5:13—25.

Min H, 1993. Anaerobic microbiology[M]. Hangzhou: Zhejiang Agricultural University Press.

Pohland F G, Gould J P, 1986. Co-disposal of municipal refuse and industrial waste sludge in landfills[J]. Wat Sci Technol, 18:177—192.

(Received for review June 12, 2000. Accepted October 19, 2000)