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AEROBIC AND ANAEROBIC TREATMENT OF HIGH-STRENGTH HAZARDOUS LIQUID WASTES

E.S. VENKATARAMANI*

Merck & Co., Inc., P.O. Box 2000, Rahway, NJ 07065 (U.S.A.)

R.C. AHLERT

Rutgers, The State University of New Jersey, College of Engineering, Department of Chemical and Biochemical Engineering, Piscataway, NJ 08855-0909 (U.S.A.)

and P. CORBO

DuPont Corp., Waynesboro, VA 22980 (U.S.A.)

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Summary

Leachate was obtained from a disposal site that had received residential, industrial and hazardous wastes. After separation of an oily floating layer, the remaining aqueous phase contained very high concentrations of dissolved salts and soluble organic matter, together with dispersed oil. Mixed microbial populations were obtained from sewage treatment plants and acclimated to this leachate. Both aerobic and anaerobic systems were studied; bacterial growth rates and hazardous chemical utilization rates were measured. Large reductions in Dissolved Organic Carbon (DOC) were achieved with both types of cultures, however, neither was quantitative. Control of pH is important in all cases; added nutrients and the presence of a preferred carbon source appeared to be less critical. These laboratory observations suggest that sequential anaerobic/aerobic reactions will be required to achieve total destruction of dissolved organic matter.

Introduction

High-strength wastes are a by-product of modern industrial societies everywhere. Industrial development has increased the volume of wastewater to be disposed of, whereas the capacity of receiving waters to accept and neutralize increasing loads of organic and inorganic pollutants is limited. The pollution of groundwater by high-strength hazardous waste residues, leaching from landfills adds a new dimension to existing pollution problems. Many landfills have inadvertently accepted chemical waste for disposal. Over a period of time, waste containers corrode and release solvents, oils and synthetic chemicals, that con-

*Also with the Department of Chemical and Biochemical Engineering, Rutgers, The State University of New Jersey.

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stitute potential problems of enormous magnitude and complexity. The list of abandoned hazardous waste sites to be cleaned up under CERCLA could grow to between 1500 and 2500 [1]. Possible contamination of groundwater by landfill leachate is most serious in the Northeast (U.S.A.).

Depending on the wastes deposited at a hazardous landfill site, the leachate produced can contain various synthetic and toxic chemicals, including heavy metals and known or suspected carcinogens [2]. Identification and evaluation of the fate of organic compounds in hazardous landfill leachate is hindered by diverse solute compositions and concentrations. Many solutes represent classes of molecules that biologists and biochemists have not investigated in detail. Shukrow et al. [3] obtained composition data on leachates, contaminated aquifers and surface waters in the proximity of twenty seven sites containing hazardous wastes. Ghassemi et al. [4] developed a data base for thirty different leachates from eleven landfills. Hill et al. [5] investigated relationships between BOD, COD and TOC using a significantly larger data file, for some industrial waste categories.

Recently, Kosson developed an efficient, cost-effective, in-situ bio-reclamation technique for clean-up of hazardous leachates and soil contaminated with waste residues [6]. An extensive discussion of biological treatment of high-strength leachates and hazardous industrial residues can be found in a review article by the authors [7]. The present work was designed to establish that aerobic, as well as anaerobic, biological treatment can be used effectively to stabilize organic compounds found in high-strength hazardous waste residues originating from an industrial landfill.

In the aerobic treatment step, an activated sludge biomass was used to develop microbial populations capable of metabolizing the organic compounds present in the leachate under study. A research scheme was designed to identify the mode of removal of leachate derived organic compounds. Kinetics of the process were elucidated by following microbial responses to organic species in the high-strength waste during batch biological treatment studies. In the anaerobic treatment step, an anaerobic population was developed from an industrial seed acclimated to two feeds: (i) one containing the hazardous industrial residue, and (ii) a second, synthetic feed simulating the volatile fatty acid content of the untreated hazardous industrial residue. The seed cultures were maintained for nine months. Toxicity and kinetics of volatile fatty acid uptake and methane production from hazardous residue containing other contaminants were compared with those of the synthetic waste to assess the feasibility of application of anaerobic treatment for waste stabilization.

Research scheme

The research scheme for aerobic treatment studies consisted of four phases. In Phase I, mixed microbial seed obtained from the Somerset-Raritan (NJ) sewage treatment plant was grown on glucose and growth parameters esti-

mated. In Phase II, mixed microbial populations were acclimated to landfill leachate in the presence of glucose. In Phase III, the mixed cultures from Phase II were grown on the leachate dilutions alone, i.e., leachate was the sole source of carbon for growth and energy. Phase IV was similar to Phase III; however, no nutrients (nitrogen, phosphorus and trace elements) were added. After acclimation, growth and degradation studies were performed in each phase with varying concentrations of leachate.

Materials and methods

(a) High-strength industrial waste residue

The high-strength, complex hazardous liquid residue (landfill leachate) used in these studies is the aqueous phase of an oil/water leachate mixture. The leachate was obtained from a disposal site that had received hazardous and industrial wastes for several decades. This waste was obtained by the U.S. EPA Oil and Hazardous Materials Spills Branch (Edison, NJ). Leachate samples were obtained at different points and times at the same landfill and were used in aerobic and anaerobic biological treatment studies.

All studies utilized pretreated leachate. Lime flocculation followed by recarbonation and pH adjustment, using sulfuric acid, was used to clarify the wastewater. Time average values of inorganic elements and gross properties of pretreated wastewater are given in Table 1. Ultrafiltration analyses revealed that about 80–90% of the organic matter present in pretreated leachate had a molecular weight of 500 or less [8]. The organic solutes were extremely numerous and could not be identified by practical means. However, it was observed that about 40% of leachate DOC was due to volatile fatty acids [9]; ranges in concentration are cited for acetic, propionic and butyric acids.

(b) Acclimated mixed culture

An activated sludge was developed by aerating secondary sludge obtained from the Somerset-Raritan (NJ) sewage treatment plant. This plant treats a mixture of domestic and industrial wastewater. A mixed culture was grown on glucose and ammonium sulfate in a mineral nutrient medium, free of added chloride ion. Acclimation of the heterogeneous culture was accomplished by the addition of high-strength wastewater, increasing concentration with time. The progress of acclimation was monitored daily as DOC removal. A highly stable population was developed in the bioreactor in about three weeks.

For the anaerobic studies, two methanogenic cultures were selected. A leachate digesting culture was selected directly with the leachate as the feed. A volatile fatty acid (VFA) digesting culture was selected using acetic, propionic and butyric acids in equivalent concentrations, as found in the untreated leachate. The anaerobic seed was obtained from the Berkeley Heights (NJ) sewage treatment plant.

TABLE 1

Typical properties of pretreated leachate

Parameter	Range of values	
DOC	8,000–12,000	mg/l
COD	23,000–30,000	mg/l
TKN	1450	mg/l
NH_3-N	1000	mg/l
Total P	14	mg/l
DOC of Fatty Acids	4,000– 5,000	mg/l
Acetic Acid	7,000– 8,000	mg/l
Propionic Acid	1,000– 1,500	mg/l
Butyric Acid	4,000– 5,000	mg/l
TDS	15,000–17,000	mg/l
Conductivity	13,000–18,000	μmhos/cm
Sulfate	3400	mg/l
Sulfide	not detected	
Nitrate	11	mg/l
Nitrite	2	mg/l
Na,Ca	1,700–17,000	mg/l
Mg,Fe	17–170	mg/l
B,Mn	1.7–17	mg/l
Ni	0.17–1.7	mg/l
Pb,Cr,Si,Al,Cu,Ag	0.017–0.17	mg/l
pH	7.5–9.0	mg/l
Color	Yellowish brown	
	Not detected at levels listed (mg/l)	
Hg	17	
As,Te,P,Ti,Cd,Li,Zn,Sr, Ba,Sb,Ca,In,Bi,Sn,Mo	1.7	
V,Nb,Ti,Co,Zr	0.17	
Be,Ce	0.017	

(c) Analytical procedure

Most of the analytical tests performed on the leachate were described in Standard Methods for the Examination of Wastes and Wastewater [10]. Sugar analyses were performed using the DNS method [11] and Somogyi's method [12]. Cell mass analyses were performed gravimetrically as well as by optical density measurements at a wavelength of 540 nm. Organic carbon analyses were performed with an Oceanography International apparatus, employing an ampule sealing module and a Horiba PIR 2000 IR analyzer. A 40 ml sample of mixed liquor was centrifuged at 10,000 rpm for fifteen minutes; the clear supernatant was used for DOC and sugar analyses. Due to the complex nature of the wastewater, TOC and DOC were chosen as the main performance parameters. Volatile fatty acids and low molecular weight compounds were assayed

using a Hewlett–Packard Model 5880A gas chromatograph equipped with a flame ionization detector. Gas analysis was performed on a gas chromatograph equipped with a thermal conductivity detector.

(d) Experimental set-up

All the aerobic biostabilization experiments in Phases I, II and III were carried out in wide mouth bottles of 4000 ml capacity, equipped with carborundum diffusers [13]. The working volume in all experiments was two liters. Air flow was maintained at 1.5 vvm (volume of air/volume of reactor fluid, minute). Reactor contents were mixed with the sparged air. Air to the reactor was saturated with water to prevent excessive evaporation. Temperature and pH were maintained at $20 \pm 2°C$ and 6.5–7.5, respectively. All the experiments of Phase IV were carried out in a Micro-Ferm Fermentor (New Brunswick Scientific Co., Edison, NJ) with pH, temperature, air flow and agitation controls. Carbon dioxide evolution was measured with a Horiba Infrared Analyzer. Dissolved oxygen concentration was measured with a DO analyzer (New Brunswick Scientific Co., Edison, NJ).

For the anaerobic studies, two liter glass jars, operated in a batch mode and maintained at 37° C by passing water through immersed copper coils, were used as the mother culture reactors. These reactors were mounted on magnetic stirrers. The evolved gas was collected over 1 N sulfuric acid. Due to inherent merits [14] batch toxicity experiments were performed using the culture selected for the volatile fatty acids (VFA culture) and the method developed by Miller for the cultivation of anaerobes [15]. Fifty ml culture aliquots were transferred to 100 ml serum bottles filled with gas consisting of 70% nitrogen and 30% CO_2, capped with rubber stoppers and aluminum seals. Five ml of full-strength leachate, with appropriate amounts of yeast extract and phosphorus were injected into duplicate bottles. This corresponds to approximately one-tenth of leachate DOC cencentration, six times higher than that at which the culture was adapted. Samples were taken twice daily and frozen for later analysis. Duplicate control bottles were dosed with synthetic waste composed of acetic, propionic and butyric acids at equivalent amounts present in the test with appropriate amounts of nitrogen, phosphorus and yeast extract.

Anaerobic batch experiments using leachate were performed in duplicate 500 ml bottles with a rubber stopper containing a glass stopcock, through which a hypodermic needle was inserted to remove samples and measure gas. A medium containing (per liter) yeast extract (2 g), 20 ml of 1 *M* phosphate buffer (pH 7.0), resazurin (1 mg) and 1 ml of trace metal solution [16] was boiled to remove oxygen and cooled under nitrogen. After cooling, 65 ml of leachate, 2 g $NaHCO_3$, 0.25 g cystine HCl and 0.25 g $Na_2S \cdot 9H_20$ were added to the medium and pH adjusted to 7.1 ± 0.1. The 500 ml serum bottles, previously purged with nitrogen, were filled to 350 ml with the final medium described above and inoculated with 50 ml of either leachate culture or VFA culture.

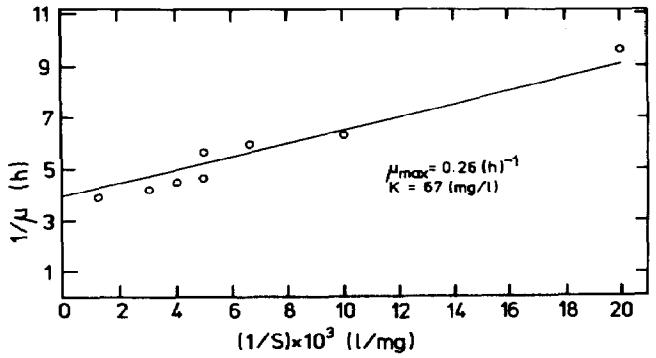

Fig. 1. Inverse of specific growth rate as a function of inverse of glucose concentration.

Results and discussion

(a) Aerobic biological studies

Experimental results of Phase I substantiated Monod growth kinetics [17]. Figure 1 is a typical Lineweaver–Burk plot; the reciprocal of specific growth rate is plotted against the reciprocal of glucose concentration. The maximum specific growth rate of the mixed microbial population, a composite of the growth rates of different organisms, is 0.26 h^{-1}; the half-saturation constant is 67 mg/l. Glucose was used as it is one of the easily metabolizable substrates that would serve as a source of carbon for growth and energy for many organisms in the heterogeneous population. Establishment of mixed microbial kinetic parameters with glucose would serve as the control and provide intrinsic parameter estimates that can be compared to the ones obtained with growth on leachate derived substrates, both in the presence and absence of glucose.

Examples of experimental results from Phase II are shown in Figs. 2 and 3. Figure 2 depicts growth rates of acclimated organisms at high concentrations of leachate (30 and 35% by volume), in the presence of glucose (2.5 g/l). The maximum specific growth rate, 'μ_{max}' of the mixed microbial population is 0.06 h^{-1}. Figure 3 illustrates the fate of dissolved organic carbon with time, during the course of growth of an acclimated mixed microbial population on leachate. Table 2 summarizes the result of growth and biodegradation studies performed; from 72 to 92% removal of dissolved organic carbon was observed. The specific growth rate of the acclimated mixed microbial population lies in the range of 0.08–0.1 h^{-1}, for leachate concentrations below 7.5% by volume. For leachate concentrations from 12.5 up to 35% the specific growth rate falls in the range of 0.06–0.07 h^{-1}, compared to 0.26 h^{-1} for glucose.

Low net growth may indicate co-metabolism and high maintenance energy requirements. Also, natural selection and/or genetic alteration that involves rearrangement in the metabolic pathways of the organisms may have taken

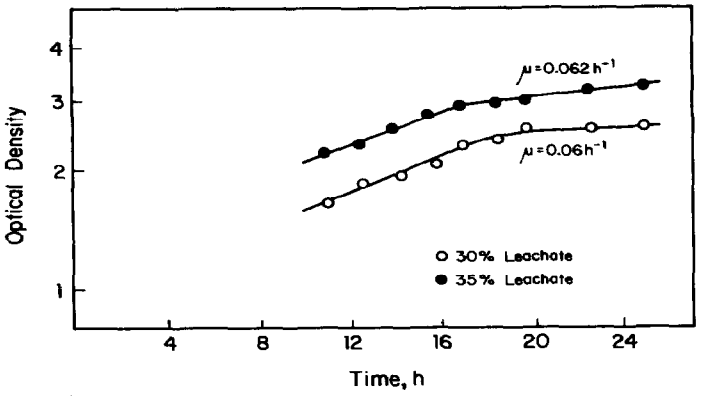

Fig. 2. Effect of leachate concentration on the growth rate of an acclimated mixed microbial population with glucose present.

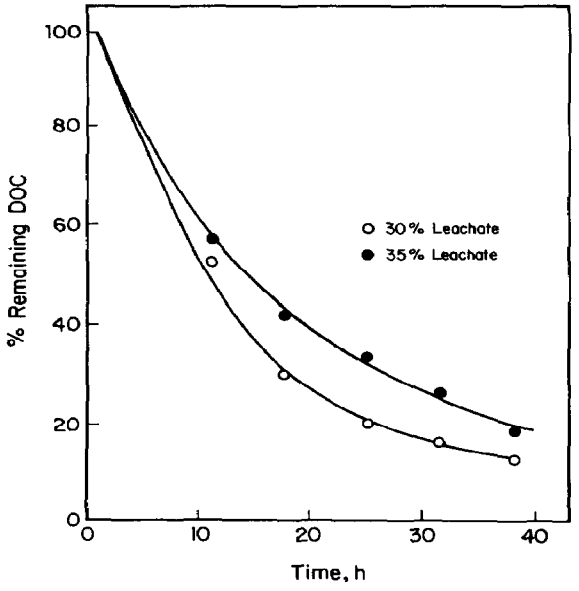

Fig. 3. Fate of dissolved organic carbon during the growth of an acclimated mixed microbial population with glucose present.

place during acclimation and are reflected in the altered specific growth rate of the microorganisms. The acclimated population is capable of degrading the organic carbon present in leachate. The remaining dissolved organic carbon represents less biodegradable organic matter present in the leachate and/or metabolic intermediates and end-products. One possible reason for enhanced degradation of leachate derived organic carbon may be co-metabolism.

TABLE 2

Specific growth rates and efficiency of DOC removals obtained in Phase II of aerobic biostabilization studies

Percent pretreated leachate	Time of batch operation, h	Specific growth rate 'μ', h^{-1}	Percent DOC removal
2.0	42.5	0.098	88.4
5.0	42.5	0.10[a]	90.7
7.5	42.5	0.082	92.8[c]
10.0	42.5	NM	87.2
12.5	42.5	0.07	88.8
15.0	42.5	0.07	84.1
17.5	42.5	0.068	86.8
20.0	35.5	0.07	72.3[d]
22.5	35.5	0.062	79.0
25.0	35.5	0.07	80.6
30.0	38.0	0.06[b]	87.1
35.0	38.0	0.062	80.0

NM: not measured.
[a]Maximum specific growth rate observed.
[b]Minimum specific growth rate observed.
[c]Maximum DOC removal.
[d]Minimum DOC removal.

In Phase III, the acclimated population was subjected to growth and degradation of organic carbon derived solely from leachate. Figures 4 and 5 illustrate results obtained with 15 and 30% leachate, respectively. These plots depict the fate of organic carbon with respect to time. DOC represents the dissolved organic carbon (leachate derived organic carbon) in the medium and TOC represents the total organic carbon in the system. TOC is the sum of DOC plus the organic carbon in cellular mass and organic carbon adsorbed onto the cell mass (sorption), if any. The data suggest that leachate derived carbon can be used as the sole source of carbon for growth and energy. Co-metabolism is not the sole mode of oxidation of the organic matter present in leachate. The decrease in TOC and DOC with time indicates that removal of organic carbon from the system is due to biological oxidation and not to sorption effects. Also, the difference between TOC and DOC values represents the organic carbon associated with cellular mass. Stripping experiments conducted at neutral pH demonstrated negligible loss of DOC due to air stripping or evaporation [18]. Table 3 summarizes growth rates and efficiency of DOC removal obtained in Phase III of the research. From 70 to 88.4% removal of DOC was achieved. The specific growth rate of the mixed culture fall in the range of 0.05–0.06 h^{-1}. The

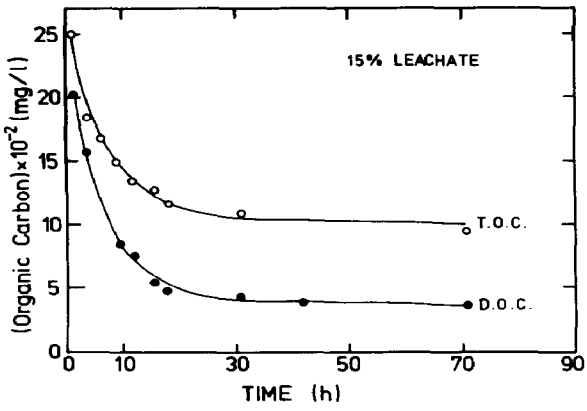

Fig. 4. Fate of organic carbon during the course of biodegragation of leachate only.

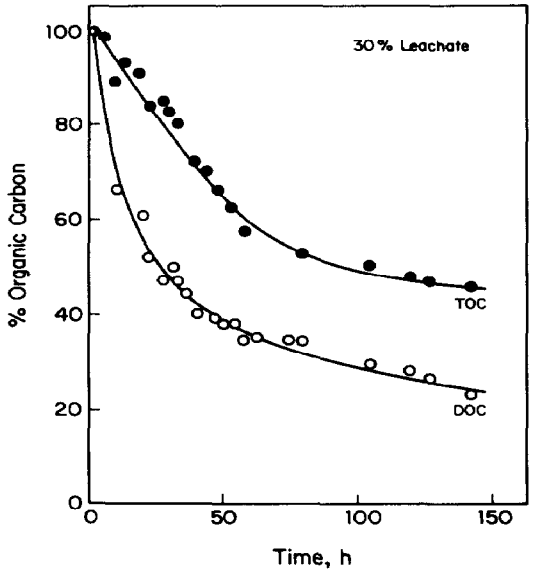

Fig. 5. Fate of organic carbon during the course of biodegradation of leachate only.

absence of highly fluctuating specific growth rate values indicates stable and well acclimated mixed microbial populations. The presence or absence of glucose does not appear to influence the growth rates of the mixed microbial population, since similar specific growth rates were obtained in Phases II and III.

In Phase IV, the acclimated population used carbon, nitrogen and phosphorus derived solely from leachate. Figure 6 represents results obtained with a leachate concentration of 20%. Figure 6 includes five plots that describe variations of pH, cell mass, DOC, TOC and cumulative carbon in evolved carbon

TABLE 3

Specific growth rates and efficiencies of organic carbon removal obtained in Phase III of aerobic biostabilization studies

Percent pretreated leachate	Time of batch operation, h	Specific growth rate 'μ', h^{-1}	Percent DOC removal
1.0	42.5	NM	82.4
2.0	42.5	NM	80.7
5.0	42.5	NM	86.6
10.0	42.5	NM	82.8
15.0	70.5	NM	83.0
20.0	70.5	NM	88.4[c]
25.0	142.0	0.052	70.2
30.0	142.0	0.05[a]	77.5
35.0	142.0	0.058	72.0
40.0	142.0	0.06[b]	70.0[d]

NM: Not Measured.
[a]Minimum specific growth rate observed.
[b]Maximum specific growth rate observed.
[c]Maximum DOC removal.
[d]Minimum DOC removal.

dioxide with respect to batch time. The pH increases to a certain point after which it remains steady. It appears that the culture utilizes the fatty acids first, before utilizing other compounds present in the leachate. This result can be inferred from the cell mass plot, also. The latter suggests a diauxic type of growth, with two distinct growth phases. The point at which the growth shifted coincides with the time at which the pH value became steady. The specific growth rate of the first exponential phase is 0.14 h^{-1} and that of the second exponential phase is 0.02 h^{-1}. The dissolved oxygen concentration, during the study, was maintained above 80% of saturation.

Carbon balance calculations carried out for the system are presented in Fig. 7. The solid straight line is the initial total organic carbon value. By conservation of mass, the cumulative carbon content of the system should add up to the total organic carbon with which the system was started. The experimental points shown are the sum of DOC, carbon in cell mass and carbon evolved as carbon dioxide. It has been assumed that 50% of the dry cell weight is organic carbon. The solid curved line is the difference between TOC and DOC values during the course of the experiment. The experimental points shown are carbon in cell mass. A reasonable agreement between the theoretical and experimental values provide evidence for the biological oxidation of organic species present in the leachate.

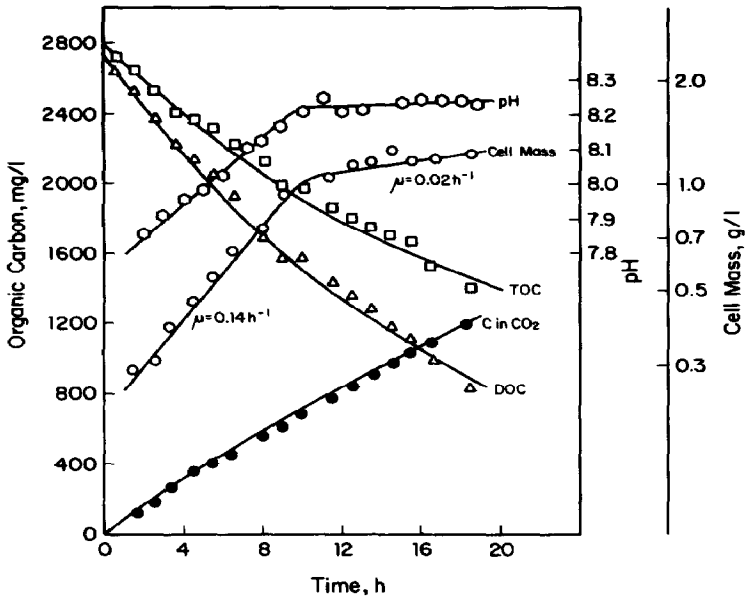

Fig. 6. Fate of organic carbon and microbial responses observed during a study with 20% leachate (no pH control).

The effect of pH control on responses of the acclimated culture was also studied. pH was controlled by the addition of 1 N sulfuric acid; pH was maintained at 7.5. The experiment with a leachate concentration of 20% was repeated under these conditions; results are illustrated in Fig. 8. Enhanced specific substrate uptake rate was observed under conditions of controlled pH. Hence, all further experiments were conducted at a controlled pH of 7.5.

Table 4 describes the experimental results obtained in Phase IV of biodegradation studies. It was observed that yield and specific growth rate decreased with increasing leachate concentrations, indicating substrate inhibition. It was evident from various batch experiments that the maximum DOC removal was 90% of the initial DOC; about 50% was removed in the first exponential phase and the rest was removed in the second exponential phase. Data obtained with 20, 30, 50 and 100% leachate concentrations was used to obtain kinetic parameters for the system. Using the values of specific growth rates and corresponding substrate concentrations, the value of the inhibition constant and maximum specific growth rate was determined by nonlinear parameter estimation [8]. Kinetic parameters of the acclimated heterogeneous population, obtained from these plots, are summarized in Table 5. If it is assumed that the maintenance [M] requirements are negligible, as has been observed for wastewater systems [19–21], it is possible to quantify the role of co-metabolism [C] in biological oxidation of xenobiotic compounds [8].

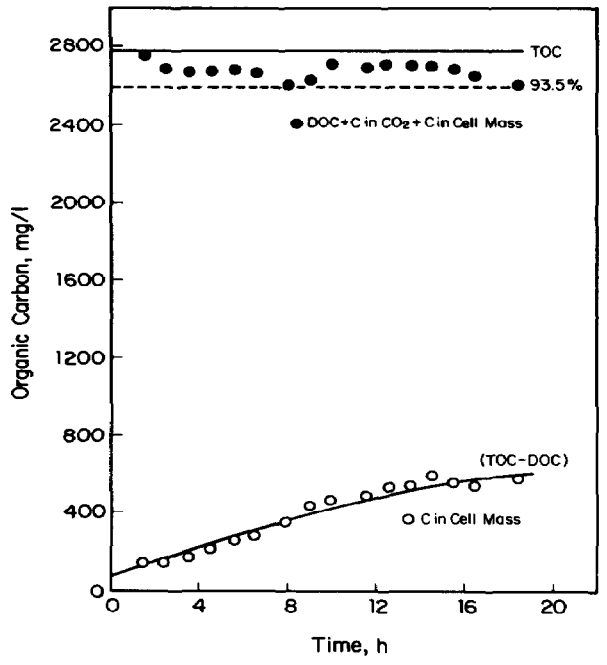

Fig. 7. Carbon balance for the experiment with 20% leachte (no pH control).

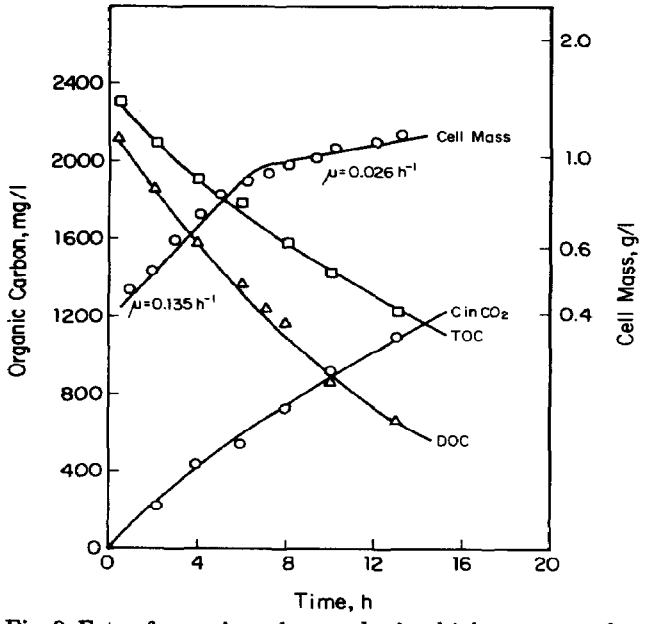

Fig. 8. Fate of organic carbon and microbial responses observed during a study with 20% leachate (with pH control, pH = 7.5 ± 0.1).

TABLE 4

Results obtained in Phase IV of aerobic biostabilization studies

DOC	Specific growth rate, μ, h^{-1}	Overall microbial yield, Y (g cell mass / g leachate mass)	Y_E (g cell mass / g leachate mass utilized for energy)	Specific substrate uptake (g leachate carbon / g cell, h)	Percent DOC removal
(i) First exponential phase					
785[a]	0.12	0.228	0.32	97.5	55.8
1360	0.14	0.254	0.372	79.0	44.2
1060	0.135	0.252	0.37	113.0	45.4
1710	0.11	0.222	0.31	100.5	40.2
3350	0.085	0.161	0.20	103.5	60.1
4675	0.05	0.111	0.13	95.0	50.2
(ii) Second exponential phase					
628[a]	0.018	0.26	0.385	77	33.0
1088	0.020	0.129	0.154	182.5	40.8
848	0.026	0.146	0.179	229	54.0
1368	0.022	0.123	0.145	163	54.3
2680	0.018	0.128	0.152	149	59.8
3740	0.0175	0.120	0.141	142	28.0

[a]Experiments run with no pH control.

(b) Anaerobic biological studies

In spite of the present significance and future potential, anaerobic waste treatment processes have not enjoyed favorable reputations. Though anaerobic treatment of wastewaters has been practiced with good success by a number of food processing and related industrial categories, practical feasibility of direct treatment of other industrial discharges that are mainly composed of synthetic organic compounds is yet to be proven [22]. In the present study,

TABLE 5

Kinetic parameters of the heterogenous microbial populations as estimated from the results of Phase IV

Parameters	First exponential growth phase	Second exponential growth phase
μ_{max}, h^{-1}	0.22	0.03
K_i, mg C/l	1770.06	4620.0
M+C	0.37	0.077
	0.38[a]	0.08[a]

[a]from 1/Y vs. $1/\mu$.

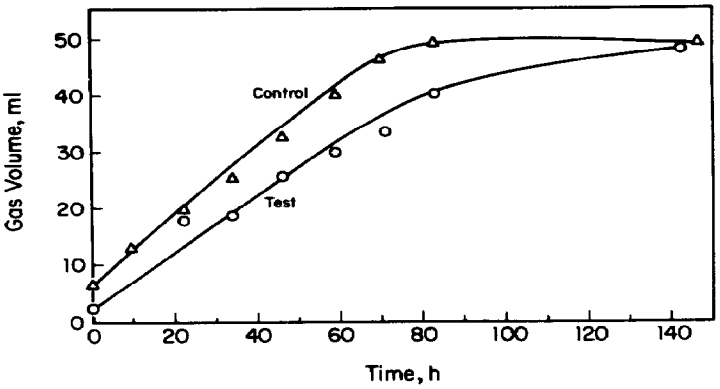

Fig. 9. Gas production for control and test reactors with a volatile fatty acid (VFA) digesting culture.

anaerobic treatment is of particular interest because an appreciable fraction of organic matter present in the leachate includes precursors to anaerobic methanogenesis. These contaminants, short chain, volatile fatty acids are present due to the activity of acid formers at the landfill site. Anearobic studies were performed by selecting a culture for acetate, propionate and butyrate at concentrations found in the leachate (VFA culture) and a culture acclimated to leachate (leachate culture). These cultures were maintained in the laboratory for nine months. The average gas composition was 71% methane and 29% carbon dioxide; pH was about 7.3 ± 0.1.

(c) Toxicity study

The objective of this experiment was to compare the rates of utilization of acetate, propionate and butyrate, in leachate containing other organic species, to a control using synthetic leachate with no other contaminants present. Figure 9 shows the effect of diluted (one-tenth) leachate on gas production, compared to a control receiving synthetic leachate. Since the aim was to quantitate any toxicity effects, the VFA culture was used for the experiments. The control experiment produced a larger total volume of gas; it received a higher dose of volatile fatty acids than the test experiment. However, gas production rates were virtually the same, indicating that inhibition due to non-volatile fatty acid contaminants in the leachate is not a factor in methanogenesis. Butyrate and propionate removals exhibited a lag of about 20 h, followed by rapid uptake, at rates comparable to the controls [9]. The rates of acetate removal were approximately the same, despite higher concentration in the control. Overall, no detrimental toxicity effects were observed at a leachate dilution of 10% using VFA (unadapted to leachate) culture.

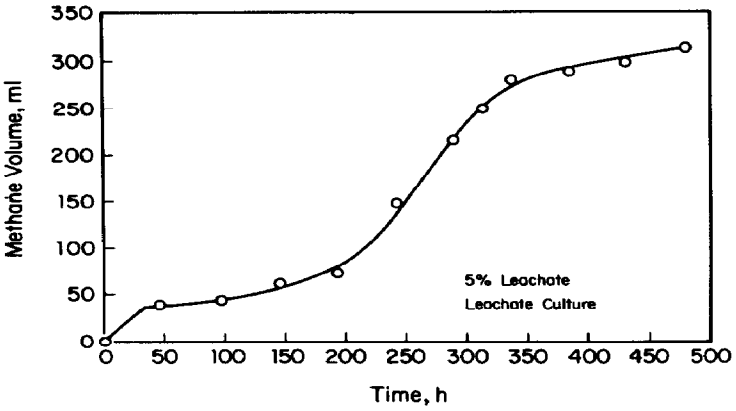

Fig. 10. Methane production as a function of batch time.

(d) Batch studies

The objective was to determine leachate derived dissolved organic carbon (DOC) removal, methane production and breakdown or appearance of specific compounds to assess rate limiting kinetics. The leachate digesting culture and the volatile fatty acids (VFA) digesting culture were used in the experiments with 5, 10 and 20% leachate. Averaged DOC removals were 64.3% by the leachate digesting culture and 69.1% by the volatile fatty acid digesting culture. These values were in excess of 40% removal, that could be expected if only the volatile fatty acids DOC are removed. Thus, an additional 25–30% of leachate derived DOC is removed by these cultures. The insignificant difference in DOC removals between VFA and leachate cultures may indicate the similarity of the population selected by using different media compositions. The observation that the VFA culture did not experience detrimental toxicity effects when subjected to degradation studies with leachate will support the claim. In general, it is easier to obtain a stable and viable culture by adapting it to specific compounds, then to a complex waste, rather than by adapting it directly to the waste.

The rate of methane production for a 5% leachate batch experiment with leachate culture is shown in Fig. 10. Methane production was very slow during the initial 200 h, after which it increased at an appreciable rate. This is likely a result of sulfate-reducing bacteria competing for hydrogen and acetate. The extended lag period followed by rapid methane production may not be attributed to cell growth, since methanogens are known for extremely slow growth. Also, cell mass analyses revealed negligible cell growth during the experimental period. The volume of methane produced was 0.99 and 0.95 l/g DOC removed for leachate and fatty acid digesting cultures, respectively. This value is slightly higher than typical reported values of 0.91–0.93 l/g (m^3/kg) DOC

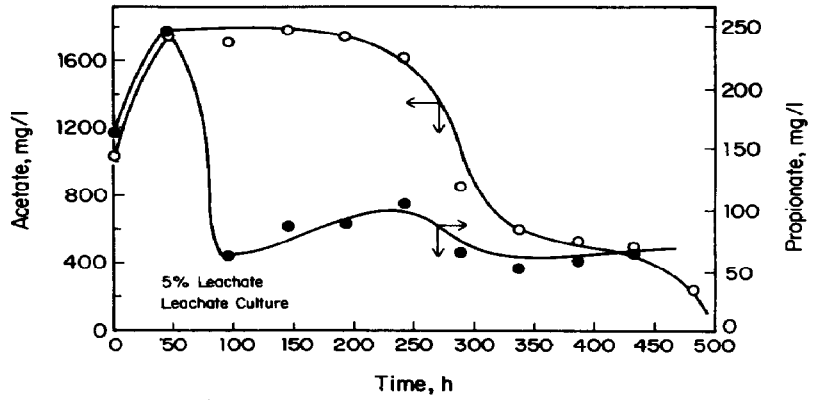

Fig. 11. Acetate and propionate concentrations as a function of batch time: ○ acetate, ● propionate.

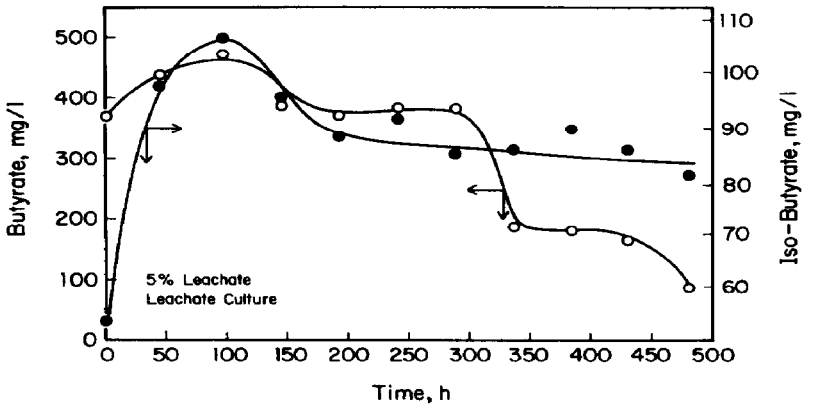

Fig. 12. Butyrate and iso-butyrate concentrations as a function of batch time: ○ butyrate, ● iso-butyrate.

removed [23]. The fatty acids profiles for the 5% leachate experiment with leachate culture are illustrated in Figs. 11 and 12. The acetate concentration reached the highest level, nearly 1750 mg/l, in the first 50 h and stayed steady until about 200 h; after, acetate metabolism took place. Since acetate serves as the major precursor for methane formation, the onset of rapid methane production and the metabolism of acetate coincide at about 200 h. Acetate is completely metabolized in 500 h. The butyrate profile showed responses similar to acetate; the concentration built up quickly and stayed steady until 250 h before it was metabolized quantitatively. The propionate concentration also reached a maximum value in about 50 h; it was metabolized to a large extent in the next 50 h. No further metabolism of propionate took place, as evidenced by a steady concentration of about 75 mg/l until the end of the batch experiment. Iso-

TABLE 6

Results of anaerobic treatment studies

Culture in the reactor	Slope, mg DOC/h	Correlation[a] coefficient	Average dry cell weight, mg/l	Specific DOC removal rate, day^{-1}
Leachate	2.344	0.93	344	0.163
Culture	1.953	0.98	324	0.144
VFA	2.290	0.96	300	0.182
Culture	2.954	0.98	283	0.240

[a]Linear regression of $y = mx + b$.

butyrate concentration built up in the first 100 h and was metabolized, to some extent, in the next 100 h; after, it stayed steady at about 85 mg/l. Iso-butyrate formation was probably due to butyrate metabolism during the initial phase of the batch. The major volatile fatty acids contributing to methanogenesis are acetate and butyrate. The data suggests that of the non-volatile fatty acid contaminants that are utilized by acid formers are converted to acetate with a small fraction being converted to butyrate, propionate and iso-butyrate.

Dry cell weight was measured as optical density as well as by a gravimetric method and correlated with a standard curve. The dry cell weight remained relatively constant over the experimental period at around 334 and 291 mg/l for leachate and VFA cultures, respectively. The leachate digesting culture and the volatile fatty acid digesting culture exhibited an average specific DOC utilization rate of 0.154 and 0.211 day^{-1}, respectively. These results are shown in Table 6. Experimental results with 10% leachate showed similar responses as those observed with 5% leachate. However, at 20% leachate concentration, reactor failures occurred, a likely result of one or both of the following effects: (i) inhibitors to methanogenesis present at appreciable concentrations, and-/or, (ii) too high total volatile fatty acid concentration in the reactors, leading to toxicity due to the unionized portion. The second phenomena seems more likely because at about 50 h the concentration of acetic acid had increased dramatically and the pH was dropping, driving the system towards failure [23]. Also, the low bicarbonate alkalinity value may not have provided adequate buffering capacity. The lag observed with 5 and 10% dilutions can probably be explained by removal of acetate that acts as the driving force for the removal of other compounds [24].

Conclusions

Aerobic biological studies revealed that a mixed microbial population, acclimated to landfill leachate, degraded 80–90% of the organic species present in

the hazardous industrial waste liquor, with or without the addition of glucose and other nutrients. Loss of dissolved organic carbon (DOC) is not due to stripping, evaporation and/or sorption; it is due to biological oxidation. Biostabilization was rapid. Mixed microbial cultures exhibited a diauxic type of growth. As signaled by the increase in pH, during the first exponential growth phase, it is likely that the mixed culture utilizes the fatty acid fraction of the organic solutes in the first exponential phase. Further, it is likely there are at least two groups of organisms and that fatty acid metabolizing organisms have a higher specific growth rate than the others and, hence, show a diauxic response. Reasonable agreement in the carbon balance provides clear evidence for biodegradation of the organic species present in the leachate. Low sludge yield was observed in this study; this reduces the sludge disposal problem associated with aerobic treatment. The oxygen requirements of the system are quite nominal, also [8]. If it is assumed that microbial maintenance requirements are negligible, as has been observed for wastewater systems, it is possible to quantify the role of cometabolism in the biological oxidation of anthropogenic compounds. The possibility of oxidative assimilation (non-proliferation) is ruled out because quantitative evolution of carbon dioxide, increase in cell mass and protein content were observed. The ability of the acclimated population to utilize organic carbon and other nutrients solely from leachate further improves process prospects. It was possible to treat highly concentrated waste liquor, i.e., up to 10,000 mg/l of organic carbon. The absence of highly fluctuating DOC values indicates a stable and well-acclimated microbial population.

Anaerobic biological studies demonstrate a DOC reduction of 64.3% for a culture selected with leachate and a reduction of 69.1% for a culture selected for the degradation of acetate, propionate and butyrate. Specific DOC utilization rates of 0.154 and 0.211 day^{-1} were observed for the leachate and volatile fatty acid digesting cultures, respectively. Cell growth was not observed to any extent during batch experiments. Leachate effects on the cultures were studied through examination of individual volatile fatty acids during batch experiments. Large concentrations of acetate were built up before overall removal was observed. The butyrate profile shows responses similar to that of acetate removal. Propionate and iso-butyrate were more difficult to remove as both left an appreciable amount of unmetabolized acid. However, their concentrations were small relative to acetate and butyrate. Acetate and butyrate are the major volatile fatty acids contributing to methanogenesis. Also, the fraction of the non-volatile fatty acids contaminants of the leachate that are converted to volatile fatty acids by the acid formers mostly end up as acetate with a small fraction being converted to butyrate, propionate and isobutyrate. Reactor failures were observed for studies with 20% leachate. The failure was likely the result of overloading the system with volatile fatty acids. At 5 and 10% leachate concentrations, no toxicity effects due to non-volatile fatty acid contaminants

were observed. Methane was produced at levels of 0.95 to 0.99 l/g (m^3/kg) DOC removed.

The information obtained in this study clearly demonstrates that aerobic as well as anaerobic biological treatment can be used effectively to stabilize organic contaminants found in high-strength hazardous waste residues. The successes in this study and in in-situ biodegradation studies [25], conducted by the authors' research group, further substantiates the significant potential for biological degradation of organic contaminants of industrial origin, and the application of microbial treatment as a primary means of residual liquid renovation and disposal. Since about 60–70% of the leachate derived DOC can be removed by anaerobic treatment, a process scheme involving anaerobic pretreatment of the waste residue followed by final aerobic polishing would provide the optimal approach. This option would drastically reduce the cost associated with energy requirements and sludge disposal. A number of field-scale studies, based on the concept of combined anaerobic and aerobic porcesses, have proven it is a viable treatment approach.

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