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# ACCLIMATED MIXED MICROBIAL RESPONSES TO ORGANIC SPECIES IN INDUSTRIAL LANDFILL LEACHATE

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## Summary

Acclimated mixed microbial cultures utilize organic carbon and other nutrients solely from leachate and exhibit a diauxic type of growth, implying the presence of two groups of organic "substrates" in the leachate under study. Carbon balance calculations provide clear evidence that the loss of dissolved organic carbon is due to biological oxidation and not to sorption, stripping or evaporation. Substrate inhibition and low sludge yield are observed. The oxygen requirements of the mixed culture are nominal. It is possible to treat high-strength hazardous wastewater at an overall organic carbon removal of about 80%. Application of reverse osmosis improves the quality of effluents from biological degradation.

## Introduction

Contamination of groundwater resources by landfill leachate is relatively recent and creates serious pollution control problems. Of the 76,000 identified landfill sites in the nation, from 1,000 to 2,000 pose significant risks [1]. About 160 million tons of hazardous wastes, i.e., about 0.7 ton per person, are generated each year. Hazardous wastes are the by-product of modern industrial societies everywhere. Industrial landfill leachates are more complex than sanitary landfill leachates; industrial residues are diverse in terms of composition and concentration [2—4]. Much of the data on biological degradation of industrial wastes has been obtained with pure compound—pure culture and/or pure compound—mixed culture systems. Microbial responses in multicomponent mixed culture systems vary substantially from case to case. Corbo and Ahlert [5] reported the microbial removal mechanisms of organic matter from industrial landfill leachate by activated sludge. Shahalam addressed the important topic of the self-purifying capacity of a natural stream that receives industrial wastes containing cyanide, phenol and carbohydrates [6]. An extensive discussion of biological treatment of landfill leachates can be found in a review article by the authors and co-workers [7].

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This work was designed to establish that an activated sludge biomass can be acclimated to develop microbial populations capable of metabolizing the spectrum of organic species present in industrial landfill leachate. Treatment of one or more organic pollutants leads to responses not normally encountered in simple systems. Hence, a research scheme was designed to establish the efficiency of removal of dissolved organic carbon from landfill leachate and the mode of removal of hazardous organic compounds by an acclimated population: complete assimilation, physical association with the sludge, stripping and/or evaporation. The kinetic parameters of the acclimated populations have been measured to quantify the responses to synthetic organic compounds during biological oxidation.

## Research scheme

The research scheme consisted of four phases; see Fig. 1. In Phase I, mixed microbial seed obtained from a municipal (Somerset-Raritan, N.J.) sewage treatment plant was grown on a synthetic waste and growth parameters estimated. In Phase II, these mixed microbial populations were acclimated to leachate in the presence of glucose. After acclimation to leachate, growth and degradation studies were performed with leachate, in the presence of glucose, at leachate concentrations ranging from 1 to 35% by volume. In Phase III, mixed cultures from Phase II were grown on leachate alone, as the sole source of carbon for growth and energy. Phase III growth and degradation studies were performed at leachate concentrations ranging from 1 to 40% by volume. Phase IV was similar to Phase III, except that no nutrients (nitrogen, phosphorous and/or trace elements) were added. Growth and degradation studies were performed at leachate concentrations up to 100% by volume. In addition, the specific oxygen uptake rate of acclimated cultures were measured in the presence and absence of leachate.

## Materials and methods

*a. Industrial landfill leachate*

The high-strength, complex industrial landfill leachate used in these studies is the aqueous phase of a raw oil—water leachate mixture. This was provided by the USEPA Oil and Hazardous Materials Spill Branch (Edison, N.J.). This waste contains a spectrum of hazardous organic compounds [3]. All studies utilized pretreated leachate. Pretreatment with lime, followed by recarbonation, was used to clarify the wastewater and remove dispersed oil [8]. Typical properties of pretreated leachate are summarized in Table 1. Investigations of pretreated leachate composition suggested the presence of alcohols, aldehydes, aromatic hydrocarbons, alkyl and aryl halides, aromatic hydroxyl groups, and amines [9]. It has been observed that leachate can contain up to 40% volatile fatty acids [10].

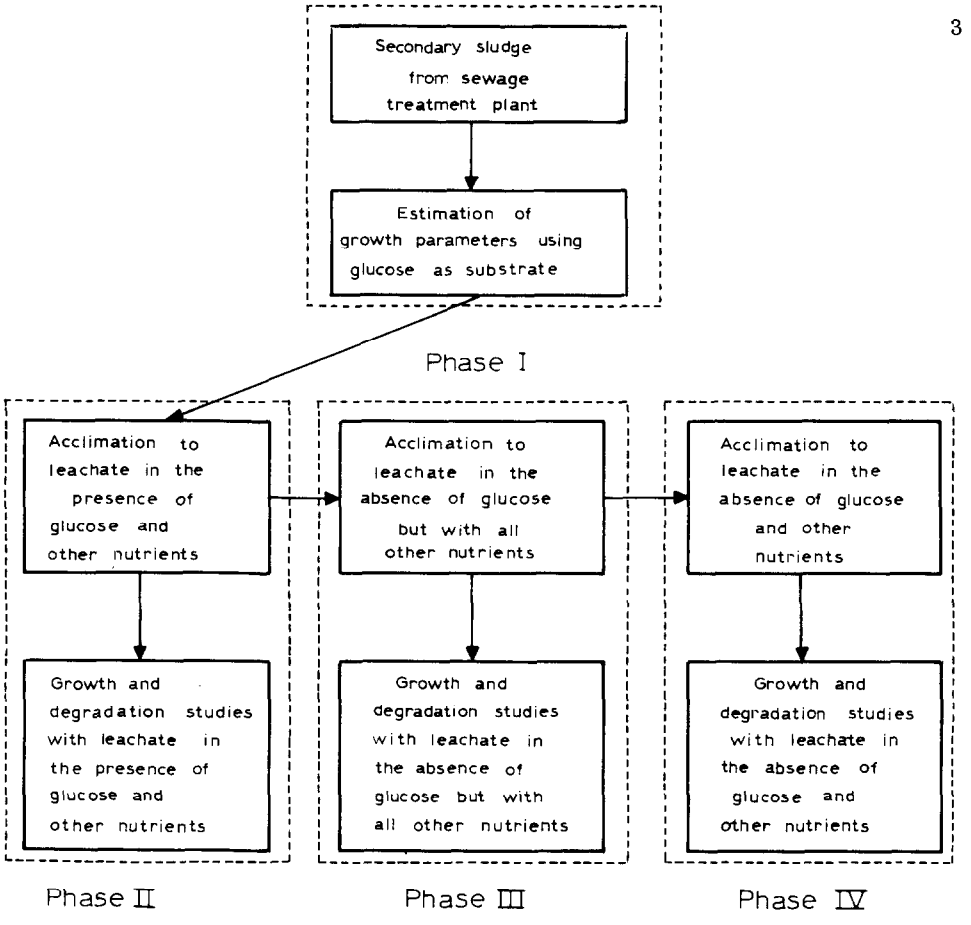


Fig. 1. Research scheme.

It is probable that anaerobic digestion takes place at landfill sites; acid formers can be active even when methanogens, which are very sensitive to a toxic environment, are not. Hence, a higher concentration of fatty acids in raw leachate is reasonable. Ultrafiltration analyses revealed that about 80% of the organic matter present in pretreated leachate has a molecular weight of 500 or less. This confirms the highly synthetic nature of the organic constituents of this waste liquor.

*b. Acclimated mixed microbial culture*

Acclimation of the heterogeneous culture was accomplished by adding high-strength leachate in increasing concentration with time to the mother culture. Glucose and other nutrients were added along with leachate to facilitate cometabolic degradation, together with adaptation to the organic species in the leachate. The progress of acclimation was estimated by the

TABLE 1

Leachate properties

| | |
|---|---|
| Residual turbitity | 1—3 NTU |
| DOC | 8—12,000 mg/l |
| COD | 23—30,000 mg/l |
| TKN | 1450 mg/l |
| $NH_3$—N | 1000 mg/l |
| Total P | 14 mg/l |
| Fatty acids | 4—5,000 mg/l |
| TDS | 15—17,000 mg/l |
| Conductivity | 13—18,000 mg/l |
| Sulfate | 3400 mg/l |
| Sulfide | not detected |
| Nitrate | 11 mg/l |
| Nitrite | 2 mg/l |
| Na, Ca | 1,700—17,000 mg/l |
| Fe | 4 mg/l |
| Mg | 0.5 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 |
| Color | yellowish brown |

daily DOC removal. A highly stable population was developed in the bioreactor in about three weeks.

*c. Analytical procedure*

Most of the analytical tests performed on the leachate are described in Standard Methods for the Examination of Wastes and Wastewater [11]. Cell mass estimates 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, utilizing 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 and the clear supernatant used for DOC analyses. TOC analyses were performed on mixed liquor samples. In this study, TOC and DOC were chosen as the main performance parameters.

*d. Experimental set-up*

All batch experiments of Phases I, II and III were carried out in wide-mouth bottles of 4000 ml capacity, equipped with carborundum diffusers. All the experiments conducted in Phase IV were carried out in a fermentor with pH, temperature, airflow and agitation controls. Carbon dioxide evolution was measured using a Horiba infrared absorption system. Dissolved oxygen concentration was measured using a D.O. analyser. The experimental lay-out is shown in Fig. 2.

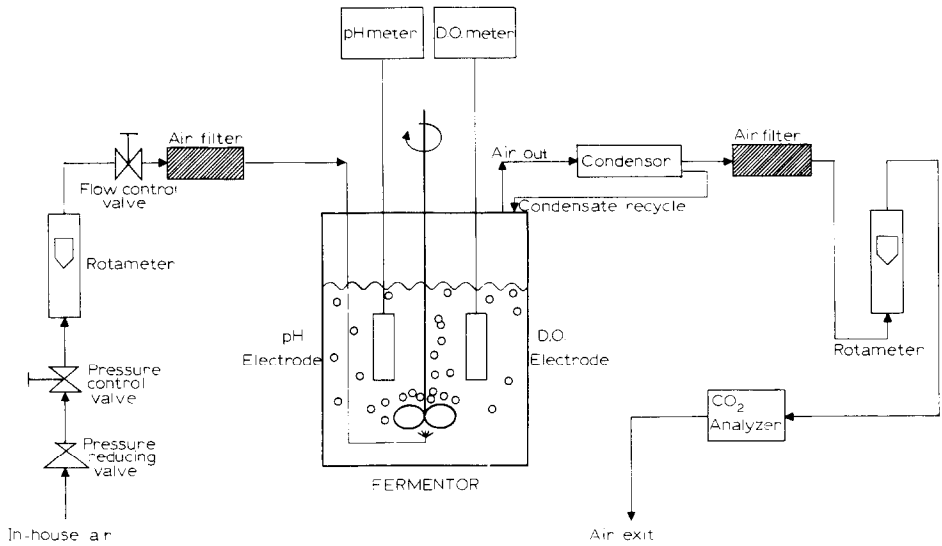


Fig. 2. Experimental layout.

## Results and discussion

It has been assumed in the design of these studies that the mixed microbial population follows Monod grow5h kinetics [12]. The underlying assumption in the analysis is that the functional population can be described in terms of an average growth rate and half-saturation constant. Experimental results substantiated the validity of these assumptions. Here, we will not discuss the results of Phases I, II and III in any depth. A detailed discussion of these results can be found elsewhere [13]. For continuity, a few important points are highlighted. The maximum specific growth rate of the mixed microbial population in Phase II was in the range of 0.06 to 0.07 $h^{-1}$, as compared to 0.26 $h^{-1}$ for synthetic sewage in Phase I. Since the leachate is deemed toxic, probably only organisms that can tolerate and utilize leachate carbon propagate. Low net growth can be attributed to cometabolism and/or high maintainance energy requirements that result in catabolism of large amounts of substrate carbon to $CO_2$ and smaller levels of assimilation into new cell mass. Another possibility is natural selection and/or genetic alteration that may have taken place during acclimation and is reflected in the decrease of $\mu_{max}$ of the system.

The data from Phase III of the study suggests that leachate-derived carbon can be used as the sole source of carbon for energy and growth and that removal of organic carbon is due to biological oxidation and not to sorption or stripping or evaporation. Quantitative proof of the above can be seen in the results of Phase IV. In Phase IV, the acclimated populations used carbon, phosphorous and nitrogen (the three most important elements required for growth) derived solely from the leachate.

Figure 3 represents the results obtained with an acclimated culture with a leachate concentration of 20%. Figure 3 includes five plots describing variations of pH, cell mass, DOC, TOC and cumulative carbon in carbon dioxide produced with respect to time. pH increases to a certain point after which it remains steady. The increase in pH can be attributed to the biological removal of fatty acids and reduction of sulfate and other compounds. It appears that the culture utilizes the fatty acids first, before utilizing other compounds present in the leachate. This result can be inferred also from the cell mass plot. The latter suggests a diauxic type of growth, with two distinct growth phases. The time at which the growth shifts almost coincides with the time at which the pH value becomes 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 reaction was maintained above 90% of saturation at all times, except in oxygen uptake rate studies of only minutes in duration.

Carbon balance calculations carried out for the system are presented in Fig. 4. The solid straight line at the top is the initial total organic carbon value. Because of 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 as-

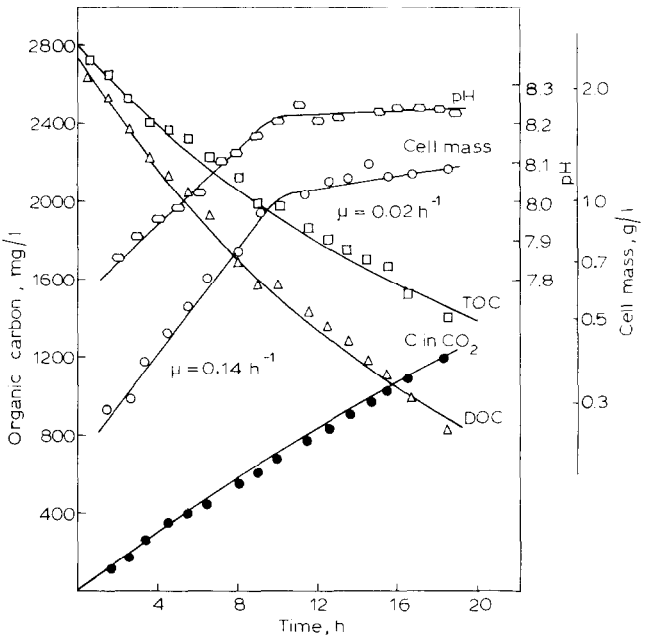


Fig. 3. Fate of organic carbon and mixed microbial responses observed during a study with 20% leachate (without pH control).

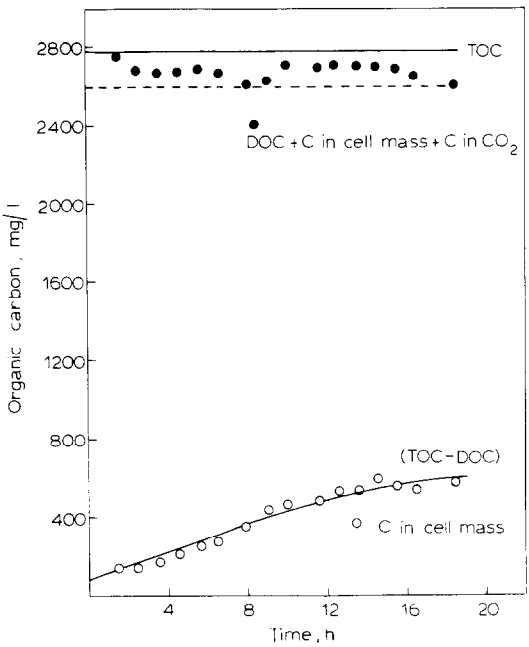


Fig. 4. Carbon balance for the study with 20% leachate.

sumed that 50% of the dry weight is due to the organic carbon of the cell mass. The solid curved line at the bottom is the difference between TOC and DOC values during the course of the experiment. The experimental points shown are the carbon in cell mass. A reasonable agreement between carbon dioxide evolution and DOC removal provides clear evidence for the biodegradation of the organic species present in the leachate. The specific substrate uptake of the first and second exponential phases are 158 and 365 mg of DOC per gram cell carbon per hour, respectively. The overall biomass yield of the first and second exponential phases are 0.317 and 0.161 grams of cell carbon per gram leachate organic carbon, respectively.

The effect of controlled pH on the responses of the acclimated culture was also studied. pH was controlled by the addition of 1 *N* sulfuric acid; pH was maintained at pH 7.5. The experiment with a leachate concentration of 20% was repeated under these conditions; the results are illustrated in Fig. 5. Under controlled pH the specific substrate uptake increased from 158 to 226 mg leachate organic carbon per gram cell carbon per hour. This implies that biodegradation is faster.

All further experiments were conducted at a controlled pH of 7.5. Table 2 describes the experimental results obtained in Phase IV of biodegradation studies. From Table 2 can be seen that both the yield and specific growth rate decrease with increasing leachate concentrations. The decrease in specific growth rate with increasing leachate concentration is attributed to substrate inhibition. Inhibition may be due to the toxic nature of the

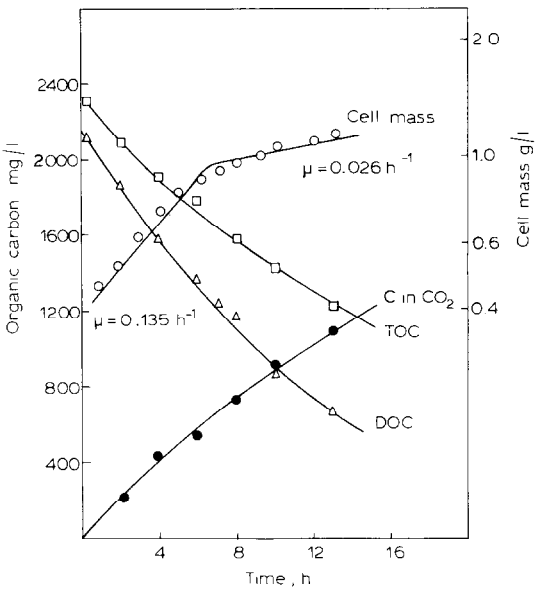


Fig. 5. Fate of organic carbon and mixed microbial responses observed during a study with 20% leachate (with pH control).

TABLE 2

Results obtained in Phase IV of aerobic biostabilization studies

| Pretreated leachate concentration (%) | Specific growth rate ($\mu$) ($h^{-1}$) | Overall microbial yield ($Y$) (gcc $glc^{-1}$)[a] | Specific DOC uptake rate (glc $gcc^{-1}$ $h^{-1}$)[a] | Overall DOC removal (%) |
|---|---|---|---|---|
| *First exponential phase* | | | | |
| 10[b] | 0.12 | 0.205 | 195 | 55.8 |
| 20[b] | 0.14 | 0.317 | 158 | 44.2 |
| 20 | 0.135 | 0.315 | 226 | 45.4 |
| 30 | 0.11 | 0.277 | 201 | 40.2 |
| 50 | 0.085 | 0.201 | 207 | 60.1 |
| 100 | 0.05 | 0.139 | 190 | 50.2 |
| *Second exponential phase* | | | | |
| 10[b] | 0.018 | 0.325 | 154 | 74.0 |
| 20[b] | 0.020 | 0.161 | 365 | 72.0 |
| 20 | 0.026 | 0.182 | 458 | 73.0 |
| 30 | 0.022 | 0.154 | 326 | 89.0 |
| 50 | 0.018 | 0.16 | 298 | 91.0 |
| 100 | 0.0175 | 0.15 | 284 | 73.0 |

[a]gcc = gram cell carbon, glc = gram leachate carbon (DOC).
[b]Experiments run with no pH control.

organic species in the leachate, high conductivity, high TDS and/or a combination of these. The variability in yield may also be due to these factors. The decrease in specific substrate uptake rate is a consequence of decrease in specific growth rate with increasing leachate concentration. It was evident from various batch experiments that the average overall DOC removal was about 80% of the initial; 50% is removed in the first exponential phase and the rest in the second exponential phase. The average specific substrate uptake rate in the first and second exponential phases are 196 and 314 mg DOC per gram cell carbon per hour, respectively.

The response of the mixed microbial population with regard to oxygen uptake rate was monitored. Experiments were conducted to determine the specific oxygen uptake rate when the cells were grown on (i) leachate with no nutrients added and (ii) glucose with all required nutrients.

In a batch culture, the oxygen uptake rate per unit volume of the culture is given by:

$$\frac{dC}{dt} = K_L a\,(C_s - C) - Q_{O_2}\,(X) \qquad (1)$$

where: $C_s$ is the concentration of dissolved oxygen in equilibrium with the partial pressure, $P$, in bulk gas phase; $C$ is the concentration of dissolved oxygen in bulk liquid; $K_L a$ is the volumetric oxygen-transfer coefficient; $Q_{O_2}$ is the specific rate of oxygen uptake (microbial respiration); and $X$ is the cell mass concentration. The term $-Q_{O_2}\,(X)$ in the equation can be determined from the decrease of $C$ with time if the oxygen (air) supply is turned off and if the rate of oxygen consumption by the culture is un-

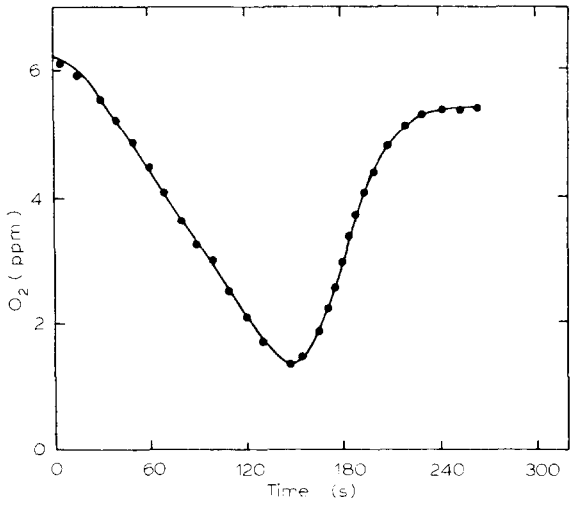


Fig. 6. Dissolved oxygen profile of a mixed microbial population grown on leachate (20%); $X$ = 1.3 g/l, $T$ = 25° C, $V$ = 1.55 vvm, $\omega$ = 1000 rpm, $Q_{O_2}$ = 0.03 mg $O_2$ per second per gram cell.

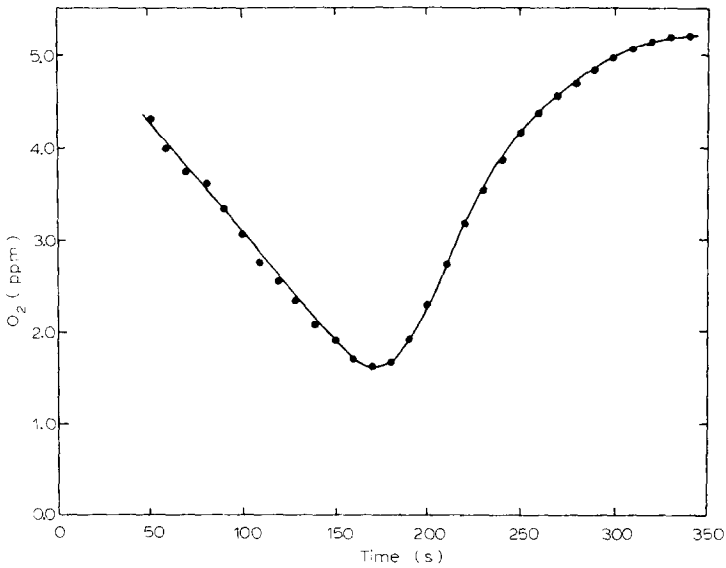


Fig. 7. Dissolved oxygen profile of a mixed microbial population grown on glucose (5 g/l); $T$ = 25°C, $V$ = 1.5 vvm, $\omega$ = 700 rpm, $X$ = 0.8 g/l, $Q_{O_2}$ = 0.0276 mg $O_2$ per second per gram cell.

affected by the suspension of this air supply [14]. When aeration is begun again, the value of $C$ increases and levels off ($dC/dt = 0$) once the oxygen supply and consumption rates are balanced.

Results of the experiment run specially for specific oxygen uptake rate studies at a leachate concentration of 20% are depicted in Fig. 6. The specific rate of oxygen uptake (microbial respiration) is 0.03 mg $O_2$ per gram cell mass per second. The results of experiments with 5% glucose plus all nutrients are illustrated in Fig. 7. The specific rate of oxygen uptake (microbial respiration) under these conditions is 0.027 mg $O_2$ per gram cell mass per second. From these results, the specific rate of oxygen uptake by the culture with leachate is about 10% greater than that observed with glucose. Generally, the sensor response characteristics must be considered in the dynamic technique. The response time of the oxygen sensor used is 5 s, which is quite rapid. In addition, it is assumed that the oxygen transferred from the head space of the fermentor to the reactor fluid is negligible. The experimental results substantiate the validity of the assumptions. The increased respiration rate in the presence of leachate may be due to the adverse environment and/or cometabolism.

## Conclusions

The mixed microbial populations in batch cultures utilize organic carbon and other nutrients solely from leachate and exhibit diauxic type or growth.

This implies that there are at least two groups of organic species present in the leachate. With the assumption that 50% of dry cell weight is due to organic carbon in cell mass, a reasonable agreement in the carbon balance provides clear evidence of the biodegradability of the organic species present in the leachate. Hence, the loss of DOC is due to biological oxidation and not to sorption, stripping or evaporation.

An advantage of the system studied is low sludge yield. It was possible to treat highly concentrated hazardous wastewater (up to 10,000 mg/l of organic carbon). The absence of highly fluctuating DOC values indicated a stable and well acclimated microbial population in growth experiments. In addition, the oxygen requirements of the mixed culture were quite nominal.

Aerobic degradation reduces soluble wastewater organic carbon by about 80% in one day or less. The remaining DOC is a combination of refractory organic leachate solutes and metabolic intermediates/end products that may render the resulting solution hazardous to some degree. Greater time and/or physical chemical separation may be appropriate to eliminate residual DOC. The application of reverse osmosis, after biological assimilation, led to removal of 80% of the remaining organic carbon. These results suggest that reverse osmosis can be used effectively to improve the quality of effluents from biological treatment.

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## References

1 Current developments, Environmental Reporter, No. 269 (June 20, 1980).
2 J.C. Lamb III, W.C. Westgarth, J.L. Rogers and A.P. Virnimmen, A technique for evaluating the biological treatability of industrial wastes, J. Water Pollut. Control Fed., 36(10) (1964) 1263.
3 A.J. Shuckrow, A.P. Pajak and J.W. Osheka, Concentration technologies for hazardous aqueous waste treatment, Prepared for Municipal Environmental Research Lab, Cincinnati, Ohio, PB 81-150583, EPA 600/2-81-019, February 1981.
4 M. Ghassemi, S. Quinlivan, M. Haro, I. Metzger, L. Scinto and H. White, Final Report on Compilation of Hazardous Waste Leachate Data, EPA 68-02-3174, Work Assignment No. 101, PP 113, April 1983.
5 P. Corbo and R.C. Ahlert, Microbial responses to organic species in industrial landfill leachate, 38th Annual Purdue Industrial Waste Conference, May 10—13, 1983.

6 A.M. Shahalam, Mixed culture biological activity in water containing low concentration of cyanide, phenol and BOD, 38th Annual Purdue Industrial Waste Conference, May 10—13, 1983.
7 E.S. Venkataramani, P. Corbo and R.C. Ahlert, Biological treatment of landfill leachates, Crit. Rev. Environ. Control (in press).
8 C.S. Slater, C.G. Uchrin and R.C. Ahlert, Physiochemical pretreatment of landfill leachates using coagulation. J. Environ. Sci. Health, A18 1 (1983) 125.
9 C.S. Slater and S. Houthuysen, Application of functional group testing methods in determination of landfill leachate constituents, Progress Report No. 18 to USEPA, Edison, NJ, May 1982.
10 P. Corbo, Doctoral dissertation, Rutgers University, New Brunswick, NJ 08854, 1984.
11 Standard Methods for the Examination of Water and Wastewater, 15th Edn., APHA, AWWA, WPCF, Washington, DC, 1981.
12 J. Monod, The growth of bacterial cultures, Ann. Rev. Microbiol., 3 (1949) 371.
13 E.S. Venkataramani, Aerobic microbial treatment of high strength industrial landfill leachate, AIChE Diamond Jubilee Meeting, Washington, D.C., November 1983.
14 B. Bandopadhyay, A.E. Humphrey and H. Taguchi, Dynamic measurement of the volumetric oxygen transfer coefficient in fermentation systems, Biotechnol. Bioeng., 9 (1967) 553.