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Respirometric Analysis of the Biodegradation of Organic Contaminants in Soil and Water

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ABSTRACT

Client-funded bench-scale investigations concerning the likelihood of successfully applying biological remediation to hazardous wastes must be cost-effective, and they usually need only determine if biodegradation is likely to occur on site. To assess the potential for stimulating biodegradation, biochemical oxygen demand (BOD) was used to continuously monitor bacterial respiration during growth on mixed organic wastes from contaminated water and soil. Continuously collected oxygen-consumption data provided information on the overall metabolic activity of the resident bacterial population and permitted direct observation of the cessation of microbial respiratory activity and, thus, the termination of aerobic degradation. The correlation of biological oxygen utilization with biodegradation was confirmed using independent analytical methods. Continuous, long-term BOD analysis was applied to bench-scale studies to assess the biodegradation of mixed organic wastes from contaminated sites and industrial waste effluents. This information was used to make an initial determination regarding the need to further explore bioremediation as a potential remedial-action technology using on-site, pilot-scale testing.

Index Entries: Respirometer; oxygen demand; organic; pollutant; biodegrade.

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INTRODUCTION

Enhanced biodegradation of hazardous organic compounds contaminating soils, ground water, and waste streams is an important technology for remediating and preserving natural resources (*1–3*). One of the critical issues regarding bioremediation of organic pollutants is the ability to effect remediation of a complex set of organic compounds. Rarely is site contamination restricted to a single compound. The common case is that a site is contaminated with many different compounds. This fact alone greatly complicates the demonstration and effective implementation of a bioremediation program that will treat all the target compounds found on the site. Even though all the organic contaminants found at a specific site may have been shown to be individually biodegradable, the demonstration of biodegradation of a complex mixture in a nonideal matrix, such as soil, is an important priority before a treatment scheme can be implemented.

An important first step in evaluating the efficacy of a bioremediation program for a contaminated site or waste stream is to determine the ability of the offending compounds to biodegrade. The most successful and the most rapid demonstrations of biodegradation of organic pollutants, in general, have occurred under aerobic conditions (*4–6*). Anaerobic biodegradation of a number of organic chemicals has been demonstrated (*4*); however, the list of degradable compounds is limited, as are actual field implementations of *in situ* anaerobic bioremediation. Aerobic metabolism can be quantified by the consumption of molecular oxygen; therefore, respirometry permits analytical measurement of microbial activity based on oxygen consumption (*4*).

This report describes the application of respirometry to the measurement of biodegradation of complex mixtures of organic compounds found at an inactive coal-coking plant and in a chemical-plant waste stream. Both scenarios represent actual hazardous waste sites. The experimental designs described were constructed to address specific client and regulatory needs. Although the experimental approaches have definite and obvious limitations, they represent the kind of protocol that is currently acceptable from cost, timing, and regulatory vantages. The fundamental issue behind this work was to determine if bioremediation was worth more detailed evaluation as a remedial action for the two sites described. Because of the motivation behind his work, it is important to realize that experiments were performed once, usually in duplicate or triplicate. Even though fundamental scientific reasons exist for pursuing additional investigations and for clarifying results, client interest, and thus funding, is usually not available for additional experimentation. An interpretation must be made from the results of the first set of experimental data. This paper describes the use of respirometry to address some of the pertinent questions that arise during the assessment of the potential for bioremediation of hazardous wastes.

METHODS

Microorganisms

Biodegradation studies were conducted with bacterial populations native to the contaminated sites. Organisms native to a coal-coking waste site were taken from the soil and groundwater in an inactive, partially drained waste lagoon at a coking plant. Organisms acclimated to chemical-plant wastes were collected from a treatment lagoon that received plant effluent. Contaminated groundwater and soil slurries or chemical-plant effluent was used to provide organic carbon in biodegradation studies. Nitrogen, as ammonia, and phosphate were supplied to the microbial cultures as a solution of the proprietary microbial nutrient Restore 375® (IT Corporation, Knoxville, TN). The pH was monitored and adjusted as needed to maintain a pH between 6 and 8.

Respirometry

A computerized respirometer (N-Con Systems, Computox computerized respirometer, Larchmont, NY) was used to measure oxygen consumption continuously. The respirometric study of coking-waste biodegradation was run for 500 h. The study of chemical-plant effluent was run for 400 h. The data-collection interval was 2 h. The respirometer supplied pure oxygen to eight independent reactors in response to the pressure differential created by oxygen consumption. The gas composition in each reactor approximated that of air for the duration of the study. The mass of oxygen added per delivery to active reactors was approx 0.2 mg. The actual amount of oxygen delivered to each reactor was determined by calibrating the delivery volume according to the manufacturer's instructions. Calibration consisted of averaging between 100 and 200 deliveries and converting volume to mg O_2. Carbon dioxide was removed from the gas phase by adsorption with 5*N* sodium hydroxide. The temperature of the reactors was maintained at 25°C. The reactors were continuously stirred to facilitate gas equilibration. Inactive reaction mixtures did not show oxygen consumption for 500 h of continuous monitoring. Once the reactor vessels were sealed, there was no evidence of leakage.

Each reactor contained 500 mL of contaminated water from the coke plant. Soil slurries were made by adding either 10 or 50 gm (wet wt) of contaminated soil to 500 mL of contaminated water. Slurries with a water-to-soil ratio greater than 10:1 were not used, because the mass of soil stalled the magnetic stir bars. A total of 675 mL of chemical-plant effluent was used during respirometric analysis of oxygen consumption in plant effluent.

Analytical Measurements

The nitrogen as ammonia and ortho-phosphate content of the various samples were determined using the Nesslerization method, Standard

Table 1
Experimental Design for Assessing Oxygen Demand During Biodegradation of Organic Pollutants a Coking Plant Waste Lagoon

Treatment[a]	Reactor Contents		Inhibitor[b]	Nutrient Addition[c]		
	Water (mL)	Soil (g)		Time(h)	Time(h)	Time(h)
1-Water	500	0	---	0	212	336
2-Water, abiotic	500	0	$HgCl_2$	0	212	336
3-50:1[d]	500	10	---	0	189	336
4-10:1	500	50	---	0	212	336
5-10:1, abiotic	500	50	$HgCl_2$	0	212	336
6-10:1, untreat	500	50	$HgCl^2$	---	---	---

[a]Data represent the mean of duplicate samples in Treatments 1, 3, and 4.

[b]Saturated $HgCl_2$ was added to treatments 2 and 5 to inhibit biological oxygen consumption. The final $HgCl_2$ concentration was 100 $\mu g \cdot mL^{-1}$ (ppm).

[c]A sterile solution of Restore 375® was used to provide nutrient amendments. The first nutreint amendment supplied 200 mg/L Restore 375®, subsequent additions were of 500 mg/L Restore 375®.

[d]Water to soil ratio.

Method #418B (*7*), and the ascorbic acid method, Standard Method #425F (*7*). Total dissolved organic carbon was determined on filter-clarified samples using a Dohrmann total-carbon analyzer, Standard Method #505B (*7*). Chemical oxygen demand was quantitated spectroscopically using the acidic persulfate oxidation method, Standard Method #508C (*7*). Specific organic compounds were detected and quantitated using gas chromatography/mass spectroscopy.

RESULTS

Oxygen consumption was used to determine microbial activity in soil and water slurries containing organic wastes. Oxygen consumption was also used to assess nutrient limitations and metabolic inhibition resulting from high concentrations of waste compounds. Contaminant reduction was determined by measuring the concentration of specific contaminants or the total carbon and chemical oxygen demand in treated samples. Nutrient limitations were determined by observing increased oxygen consumption following the addition of nutrients to treatments. Potential inhibitory effects of organic wastes on biological oxygen consumption were evaluated by comparing respiration under different concentrations of organic wastes.

Coking Waste

The treatment scheme for respirometric evaluation of coking-waste biodegradation is shown in Table 1. Figure 1 shows oxygen consumption

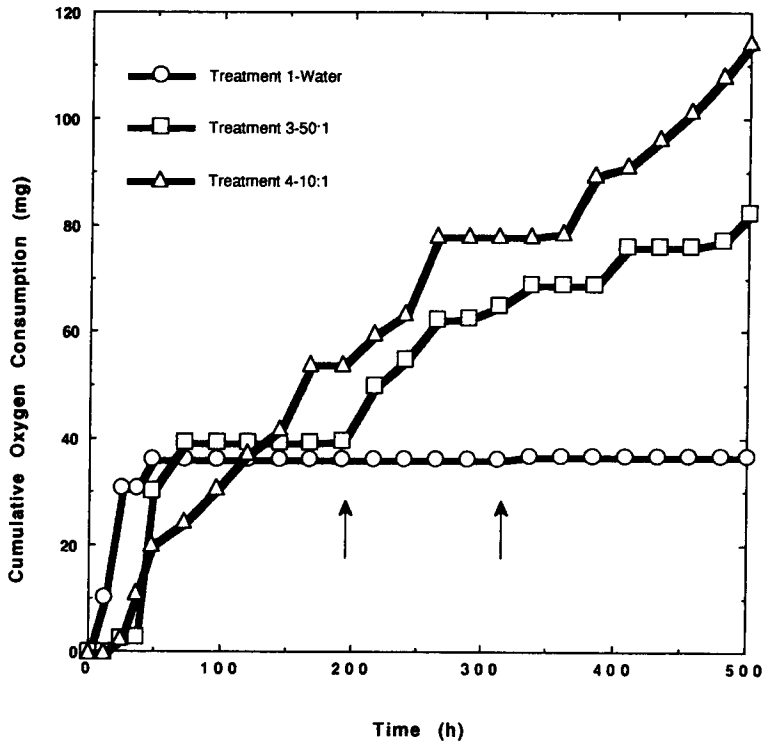

Fig. 1. Cumulative oxygen consumption during biodegradation of coal-coking wastes. Treatment 1 is the biological oxygen demand for water taken from the laboratory composite and supplemented with nutrients. Treatment 3 is the biological oxygen demand imposed by a 50:1 water-to-soil mixture supplemented with nutrients. Treatment 4 is the biological oxygen demand imposed by a 10:1 water:soil mixture supplemented with nutrients. Arrows indicate points of addition of 500 ppm Restore 375® to amend the nutrient content of the treatments.

by three different treatments. Treatment 1 contained only ground water. The oxygen-consumption curve suggested that usable carbon was quickly depleted. The addition of nutrients did not stimulate further oxygen consumption, indicating that nutrients were not limiting microbial activity. Usable organic carbon appeared to be the limiting factor in this treatment.

Treatment 3, Fig. 1, shows oxygen consumption by a composite of 50 parts groundwater to 1 part contaminated soil. Treatment 4 is a composite of 10 parts groundwater to 1 part soil. In both treatments, nutrient limitation was observed. Nutrients were added initially and then during the experiment, as indicated by the arrows. Following each addition of nutrient, oxygen consumption was stimulated. The total amount of oxygen consumed by Treatment 4 was not five times that of Treatment 3, even though the carbon content was five times greater. This observation suggested that the higher concentration of organic contaminants had an inhibitory effect on microbial respiration. However, time constraints imposed by contractual agreements did not permit the process to go to

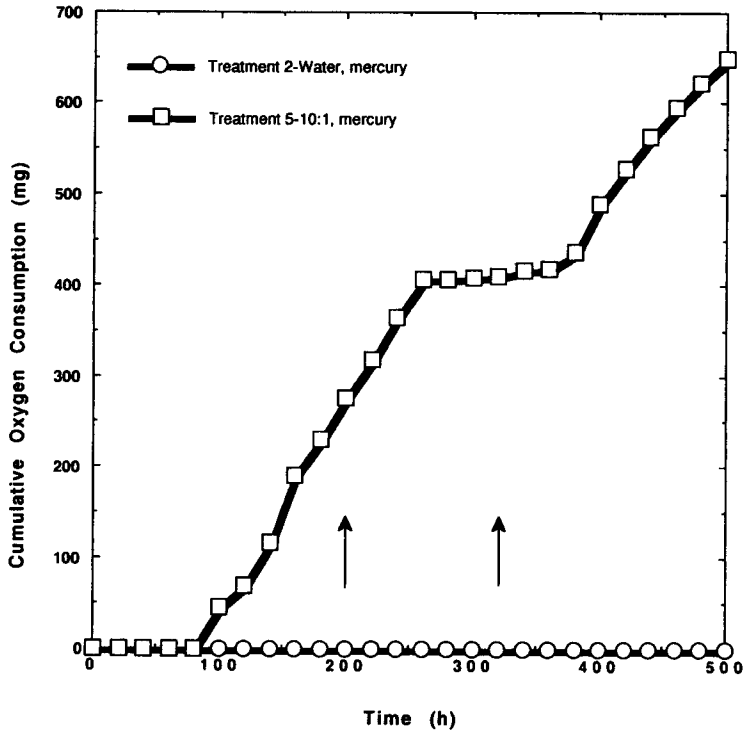

Fig. 2. Cumulative oxygen consumption during biodegradation of coal-coking wastes by mercury-treated controls. Treatment 2 demonstrates the lack of oxygen consumption in response to mercury addition. Viable microorganisms were not recovered from this treatment. The response of this treatment also demonstrates the stability of the respirometer during long-term measurements. Treatment 5 is a mercury-amended 10:1 water:soil treatment. Note the 80-h lag time prior to the beginning of oxygen consumption. Viable microorganisms were recovered from this treatment. Arrows indicate the addition of nutrients to the treatments.

completion; therefore, the true difference between diluted and undiluted samples cannot be determined.

In order to demonstrate that the observed oxygen consumption was attributable to microbial activity, biologically inhibited controls were examined for oxygen consumption. Figure 2 shows the results of two treatments amended with 100 mg/L mercury.

Oxygen consumption in Treatment 2 (water only) was effectively inhibited by mercury. The results of this treatment indicate that microbial activity was the source of oxygen consumption. These results also demonstrate the long-term stability of the respirometer. No leakage, artifactual oxygen deliveries, or other experimental or mechanical anomolies were detected in this biologically inhibited treatment. The response of this treatment is in contrast to that of Treatment 1, Fig. 1.

Table 2
The Initial and Final Concentration
of Priority Pollutants in the 50:1 Water:Soil Treatment

Compound	Initial Concentration Mass (ng)	Final Concentration Mass (ng)
Benzene	578	159
Ethylbenzene	271	30
Toluene	478	30
Acenaphthene	553800	30
Acenaphthylene	1068500	1770
Anthracene	1659000	2360
Benzo(a)anthracene	2072000	2655
Benzo(b)fluoranthene	1422000	2242
Benzo(k)fluoranthene	2014500	2006
Benzo(a)pyrene	1777000	2449
Benzo(g,h,i)perylene	394400	1888
Chrysene	1717000	2301
Fluoranthene	5869500	8555
Fluorene	1609500	1977
Indeno(1,2,3-cd)pyrene	1003000	1357
Naphthalene	3559500	5133
Phenanhtrene	7116000	7080
Pyrene	475300	6785

In the mercury-treated soil/water composite, Treatment 5, a high level of oxygen consumption was observed. Examination of the oxygen-consumption curve for this treatment distinguished it from the non-mercury-amended treatments. First, the lag period was 80 h for the mercury-amended treatment, whereas it was only 24 h for the other treatments. Second, total oxygen consumption was much greater in the mercury-amended treatment; compare the 650 mg of oxygen used in Treatment 5 to the 80 and 115 mg used in Treatments 3 and 4, respectively. These differences suggest that a mercury-resistant strain with a high respiration rate was selected in the mercury-amended soil/water composite. The absence of such a response in the groundwater control may be a result of the level of mercury in the water-only treatment compared to that in the soil/water treatment. The high concentration of carbon particles in the soil/water treatment may have bound a significant portion of the added mercury so that the effective concentration was lower than the 100 mg/L added.

The reduction in the concentration of specific organic compounds present in coke waste was determined by quantitating the concentration of target pollutants in each treatment before and after respirometric analysis. Tables 2 and 3 indicate that 100- to 10,000-fold reductions in specific compounds were achieved during the respirometric study. Total dissolved carbon analysis and total petroleum hydrocarbon analysis were not reliable tools for determining the effectiveness of biodegradation because of

Table 3
Initial and Final Concentration of Priority Pollutants in 10:1 Water:Soil Treatments

Compound	Initial Concentration Mass (ng)	Final Conc. (Tmt 4) Mass (ng)	Final Conc. (Tmt 5) Mass (ng)	Final Conc. (Tmt 6) Mass (ng)
Benzene	2891	37	8	74
Ethylbenzene	1357	7	8	74
Toluene	2389	37	8	74
Acenaphthene	2725000	1239	144	2950
Acenaphthylene	5316500	9440	1164	9588
Anthracene	8267000	5531	781	7375
Benzo(a)anthracene	10332000	16225	1196	11948
Benzo(b)fluoranthene	7086000	12206	1100	16078
Benzo(k)fluoranthene	10038500	9366	1260	13423
Benzo(a)pyrene	8857000	12132	1435	11726
Benzo(g,h,i)perylene	1952000	8739	1068	8555
Chrysene	8561000	11616	1084	9956
Fluoranthene	29233500	33188	3508	36875
Fluorene	7981500	1881	287	6933
Indeno(1,2,3-cd)pyrene	5015000	6711	909	7375
Naphthalene	17719500	7633	1021	29500
Phenanhtrene	35436000	9698	1021	28763
Pyrene	2292500	22863	2870	23600

the complex nature of coking wastes. The soil contained coal and coke particles, cinders, ash, tar, bits of rubber, and metal fragments. Since many of the carbon-containing materials found on site were not biodegradable, general analytical techniques for total carbon gave potentially misleading data. Therefore, analysis for specific target compounds was used to predict the potential for bioremediation.

Based on the amount of priority pollutants found in the untreated soil and groundwater, about 150 mg of organic waste were present as priority pollutants in a respirometer reactor containing a slurry of 10 parts ground water to 1 part soil. Considering that the organic wastes were primarily hydrocarbons, about 95% of the mass of the contaminants was carbon. Initially each 10:1 slurry contained about 140 mg of carbon; by the end of the respirometer experiment, each compound was reduced by 100- to 10,000-fold, leaving less than 1 mg of contaminant in the reactor. The total oxygen utilized in the 10:1 slurry was 115 mg of O_2. The ratio of oxygen (O_2) consumed to carbon consumed was 0.82. In the 50:1 water:soil slurry, the waste-product content of the treatment was about 30 mg. The carbon content was 28.5 mg and the oxygen consumed was 80 mg. In this treatment, the ratio of oxygen (O_2) consumed to carbon consumed was 2.8 to 1. For the mercury-treated soil:water slurry, the carbon content was 140 mg with 650 mg of oxygen consumed, giving an oxygen-to-carbon ratio of 4.6 to 1.

Table 4
Treatment Composition for Chemical Plant Waste Water and Contaminated Ground Water

Treatment[a]	Components				
	Trench area %	Lagoon %	Peripheral %	Nutrients ppm	Lime (μg/g)
1	11.1	44.4	44.4	1000	no
2	11.1	44.4	44.4	0	no
3	11.1	44.4	44.4	1000	yes
4	11.1	44.4	44.4	2000	yes
5	11.1	44.4	44.4	0	yes
6	2.0	8.2	89.8	1000	no
7	2.0	8.2	89.8	1000	yes

[a]Treatments 1, 3, 4, 6, and 7 were connected to the respirometer to measure biological oxygen demand. The total volume of each treatment was 675 mL.

Table 5
Total Dissolved Organic Carbon, Chemical Oxygen Demand, and Biological Oxygen Demand of Treatments Following Respirometric Analysis of Oxygen Consumption

Treatment	TOC (mg/L)			COD (mg/L)			Oxygen Consumed[a] (mg/L)
	Initial	Final	%Removal	Initial	Final	%Removal	
1a	1582	900	43	2200	1300	41	1153
1b	1582	490	69	2200	1150	48	994
3a	1429	830	42	2200	1200	46	910
3b	1429	745	48	2220	1350	39	1155
4a	1429	815	43	2220	1400	37	954
6a	120	31	74	180	25	86	290
6b	120	27	78	180	100	44	314
7a	118	31	74	177	25	86	319

[a]Value represents the amount of oxygen consumed per L during the experiment.

Chemical-Plant Effluent

Respirometric analysis of the biodegradation of chemical-plant effluent was used to determine the potential for reducing the chemical and biological oxygen demand of the plant's waste stream to meet discharge requirements. The treatment schedule is given in Table 4. Oxygen consumption was monitored for 400 h, at which time samples were tested for residual total dissolved organic carbon (TOC) and chemical oxygen demand (COD). The initial and final TOC, COD, and BOD are given in Table 5. Respirometry data for undiluted samples are presented in Fig. 3. Data

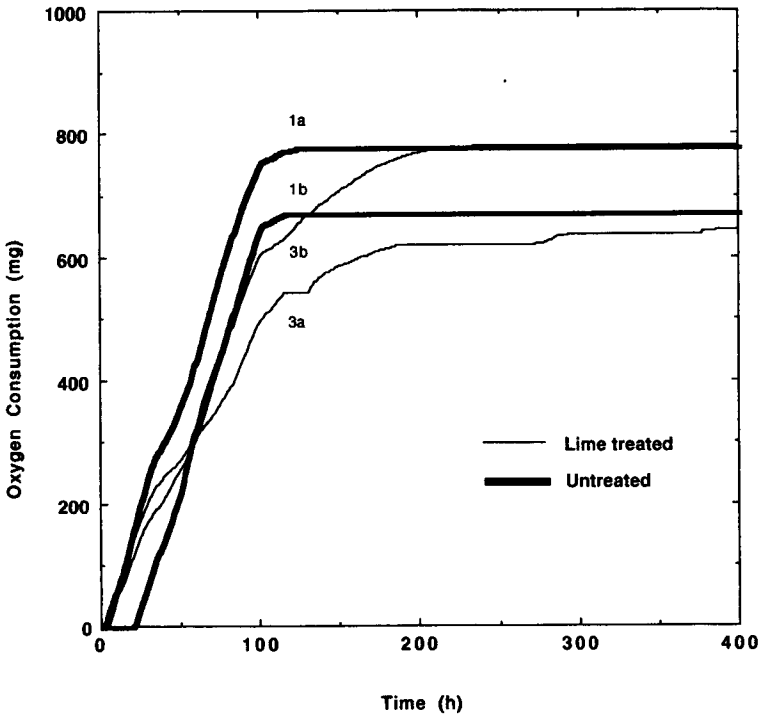

Fig. 3. Biological oxygen consumption by treatments 1a, 1b, 3a, and 3b. Heavy lines represent cumulative oxygen consumption by treatments 1a and 1b, 2.5:10:10 mixtures with 1000 ppm Restore 375® without lime treatment. Light lines represent cumulative oxygen consumption by treatments 3a and 3b, 2.5:10:10 mixtures with 1000 ppm Restore 375® and lime treatment. The total volume of liquid in each treatment was 675 mL. Oxygen-consumption data is shown for 675-mL batches.

for 10-fold dilutions of the primary treatments are given in Fig. 4. The ratio of oxygen consumed per COD and TOC is given in Table 6. The diluted treatments gave substantially higher ratios than the undiluted treatments. The reason for the discrepancy between the two treatment schemes is unclear. The percentage of carbon removed averaged 45% in undiluted treatments and 74% in diluted treatments. This difference suggested that concentrated plant effluent at least partially inhibited microbial metabolism. The approximately twofold difference in oxygen-to-carbon ratios calculated using TOC and COD probably results from the fact that only dissolved carbon is measured in TOC data, whereas total carbon is derived from COD data.

Respirometric analysis revealed that the undiluted treatments responded in similar fashions. Initially, the diluted treatments displayed similar oxygen-consumption patterns; however, later in the incubation,

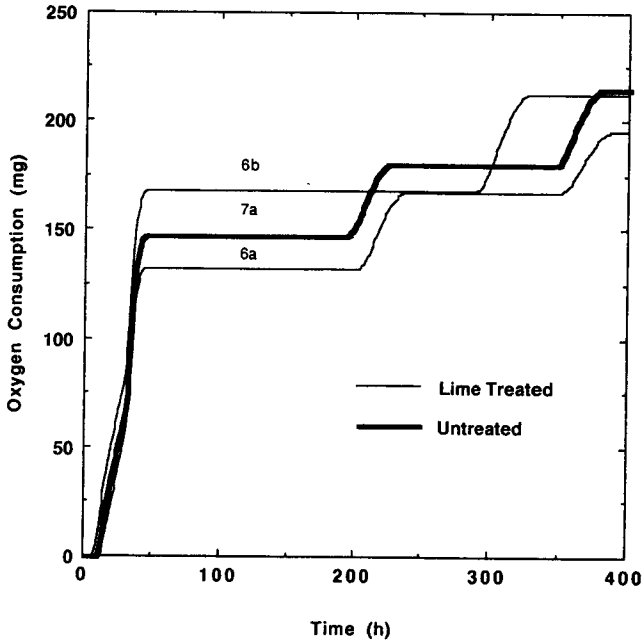

Fig. 4. Biological oxygen consumption by treatments 6a, 6b, and 7a. Light lines represent cumulative oxygen consumption by treatments 6a and 6b, 2;5:10:10 mixtures diluted 10-fold with peripheral groundwater with 1000 ppm Restore 375® without lime treatment. The heavy line represents cumulative oxygen consumption by treatment 7a, 2.5:10:10 mixture diluted 10-fold with peripheral groundwater with 1000 ppm Restore 375® and lime treatment. The total volume of liquid in each treatment was 675 mL. Oxygen consumption data is shown for 675-mL batches.

Table 6
Ratio of Oxygen Consumed Per Total Dissolved Carbon Consumed and Reduction in Chemical Oxygen Demand

Treatment[a]	BOD/ΔTOC	BOD/ΔCOD
1a	0.6	1.3
1b	0.3	0.9
3a	0.6	1.1
3b	0.6	1.7
4a	0.6	1.2
6a	1.2	1.9
6b	1.2	3.9
7a	1.4	2.1

[a]Treatments are as described in Table 3.

the treatments diverged, and two different patterns became obvious. This observation indicated that the bacterial populations in each treatment had the potential to respond to prevailing conditions. The fact that two different responses occurred indicate the flexibility inherent in microbial populations and the complexity of predicting the response of a population to a given set of conditions.

DISCUSSION

Many organic compounds have been demonstrated to biodegrade in laboratory settings using pure microbial strains or specially selected consortia of bacteria. Evidence for biodegradation of compounds in natural soils, ground water, and waste streams, in combination with other organic compounds, salts, and heavy metals, is not as abundant. Currently, the first obstacle to implementing a biological treatment program for physically amenable contaminated sites or waste streams is proof of biodegradation. To address this question, contaminated samples are brought into the laboratory for evaluation of their potential for biodegradation.

Respirometric analysis of microbial oxygen consumption during aerobic metabolism provides an analytical tool for determining the ability of native or exogenous bacteria to respire in the presence of organic contaminants. These organic compounds serve as potential carbon and energy sources for bacterial growth and metabolism. Respirometry gives a view of microbial activity that is difficult and expensive to obtain using other analytical approaches. When coupled to other measures of degradation, such as contaminant removal or intermediate product formation, demonstration of biological oxygen consumption using only the carbon available in contaminated samples suggests that biodegradation is an operative mechanism of contaminant destruction.

By continuously monitoring oxygen consumption, interesting aspects of the biodegradation process were observed in two cases presented here. Nutrient limitations were detected during respirometric analysis of coking-waste degradation by simply adding nutrients to respirometer reactors after oxygen consumption ceased. Increased oxygen consumption was a clear indication of nutrient limitation.

Aspects of bacterial population dynamics during growth on organic contaminants were also observed. Surprising characteristics of bacterial activity were revealed in both coking-waste degradation and chemical-waste-stream treatment. The appearance of an apparent mercury-resistant population with a high respiratory rate would have been very difficult to observe using periodic "sample and analyze" methodology; however, continuous examination of oxygen consumption provided crucial information concerning lag periods and respiration rates. The patterns of oxygen consumption observed in the later stages of chemical waste treatment (Fig. 4) would have been obscured without continuous oxygen-consumption data.

In the case of coal-coking wastes, respirometry coupled with contaminant-removal data indicated that bioremediation was a potentially useful method for treating the contaminated site. The concentration of polynuclear aromatic hydrocarbons was apparently reduced in biologically active treatments during the bench-scale respirometer study. The level of reduction of many of the priority pollutants was comparable. Published evidence indicates that many of these compounds should have degraded at significantly different rates (*8–11*). Based on this information, greatly different residual concentrations among the tested compounds would be expected unless degradation had proceeded to completion. The performance criteria for this study did not include determination of the biodegradation rate constants for individual compounds. Respirometric analysis of crude mixtures of organic contaminants cannot be used to make rate measurements for individual components of the mixture.

Results from the respirometric analysis of chemical-plant wastewater treatment indicated that about 40–60% of the TOC and COD could be removed by aerobic microbial activity in undiluted samples. A simple three-stage aerobic pilot-scale bioreactor was constructed on-site and seeded with bacteria from a waste-treatment lagoon. The results from the bioreactor indicated that about 60% of the total dissolved organic carbon was removed by the bioreactor. These results were in accordance with those obtained in bench-scale studies using respirometry. In this case, the 60% reduction was not sufficient to meet discharge criteria; therefore, other treatment alternatives are currently being addressed. Nevertheless, the reliability of respirometry to measure the inherent capability of a population of bacteria to biodegrade a complex mixture of compounds was confirmed.

The purpose of this report has been to describe the use of respirometry in actual treatability studies designed to examine the potential utility of bioremediation. The details of the microbiological, chemical, and physical processes that interacted during these studies have not been elucidated. From a purely scientific perspective, client-funded studies often raise more questions than they answer; however, the only question of merit in the client's mind is the potential for successful bioremediation of the waste contaminating his or her site. Therefore, the primary purpose of treatability studies, such as those described, is to provide enough information to make an informed decision on whether to continue pursuing bioremediation as a treatment alternative.

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