73-07-2

April 1977
Report No. Env. E. 57-77-3

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Prepared under the sponsorship of the
Massachusetts Division of Water Pollution Control Grants 73-7(2) and 76-10(2)
and the U.S. Forest Service Grant USDA 23-591.

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ENVIRONMENTAL ENGINEERING PROGRAM
DEPARTMENT OF CIVIL ENGINEERING
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Acknowledgements

We wish to thank Dr. Jinnque Rho for his suggestions in designing the experiment and Dr. Otto L. Stein for photomicrographs of the test algae. We wish to acknowledge the efforts of Ronald Lavigne, Robert Pease, Frank Tirsch, George Lombardo, Sandra Ferry, and Aaron Jennings who performed physical-chemical analyses. Dr. David and Trina Hosmer helped conduct statistical analyses. We express our appreciation to Miss Dorothy Blasko for her patience in typing the manuscript.

This project was funded by the Massachusetts Division of Water Pollution Control Grant Numbers MDWPC 73-7(2) and 76-10(2) and the U. S. Department of Agriculture, Northeastern Forest Experiment Station through grant 23-591.

Introduction

Recently, leachate generated at sanitary landfills has been recognized as a carrier of high concentrations of pollutants. Under pressure from state and Federal regulatory agencies the solid waste industry has been searching for low cost treatment methods for leachate. A multitude of schemes, both physical-chemical and biological, have been proposed. As Chian and DeWalle (1) pointed out, treatability of leachate is related to its chemical composition, especially the nature of the organic matter. Based upon their findings, it was anticipated that leachate generated at young (i.e., less than five years old) landfill sites, in which the organic matter consists mainly of free volatile fatty acids, could be readily degraded by biological means; whereas leachate from old fills would be more amenable to physical-chemical treatment. Boyle and Ham (2) evaluated a variety of biological treatment schemes. Processes included anaerobic and aerobic biological treatment of leachate, aerobic treatment of selected combinations of leachate and domestic wastewater in a simulated activated sludge treatment plant, and anaerobic followed by aerobic polishing treatment of leachate. Preliminary laboratory scale tests indicated that biological treatment of sanitary landfill leachate was effective in removing organics. Cook and Foree (3) determined the susceptibility of sanitary landfill leachate to treatment by aerobic biological methods. Greater than 97.6 percent COD removal efficiencies were obtained.

Field observations by University of Massachusetts environmental engineers of algal blooms in dilute leachate pools prompted the authors to study leachate treatment using algal lagoons. Design of treatment lagoons requires knowledge of an appropriate range of dilution factors to avoid toxicity. A modified version of the Algal Assay Procedure Bottle Test, AAPBT (4), was adapted to study the effects of sanitary landfill leachate on algal growth. Objectives of this study were:

1. to adapt the AAPBT to the problem of evaluating sanitary landfill leachate;
2. to determine whether various dilutions of leachate are stimulatory or inhibitory to algae;
3. to provide some basis for design of algal lagoons for leachate treatment.

Materials and Methods

Unialgal cultures of the green alga Scenedesmus dimorphous were used in this study. These cultures were maintained in 3.78ℓ (one gallon) glass culture vessels containing AAPBT synthetic algal nutrient medium. Because several recommended nutrients were unavailable, the following substitutions were made: 12.170 mg/ℓ $MgCl_2 \cdot 6H_2O$ for 5.700 mg/ℓ $MgCl_2$, 415.543 μg/ℓ $MnCl_2 \cdot 4H_2O$ for 264.264 μg/ℓ $MnCl_2$, 1.429 μg/ℓ $CoCl_2 \cdot 6H_2O$ for 0.780 μg/ℓ $CoCl_2$, and 0.01073 μg/ℓ $CuCl_2 \cdot 2H_2O$ for 0.009 μg/ℓ $CuCl_2$. Cultures were incubated in a constant temperature control room at a temperature of 24 $\pm$ 2°C. Illumination was provided by two ceiling lamps, each equipped with two 48-inch cool white fluorescent bulbs. Light intensity adjacent to the vessels at liquid level was approximately 400 foot-candles*, as recommended by Trainor (5). The light cycle was 14 hours of light and 10 hours of darkness, as suggested by Myers and Graham (6). Culture vessels were rotated daily in an attempt to correct for any local differences in light intensity. All other test procedures are outlined in the Algal Assay Procedure Bottle Test.

Samples of sanitary landfill leachate were taken from the Barre, Massachusetts landfill site. A test vessel was made up for each of four dilutions of leachate. Three controls were prepared in a manner similar to that used for the test vessels except that no leachate was added. Therefore, controls contained only AAPBT synthetic algal nutrient medium in the same concentration as that of the leachate dilutions.

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*Measured with a Weston Illumination Meter, Model 756.

A complete physical-chemical analysis of the leachate was made immediately before the test period. The following analyses were performed:

1. total solids
2. volatile solids
3. chemical oxygen demand (COD)
4. pH
5. alkalinity
6. chloride
7. phosphate-phosphorus
8. ammonia-nitrogen
9. nitrate-nitrogen
10. specific conductance

Two liter volumes of each of the test vessels, together with the control vessels, were inoculated with an appropriate volume of *Scenedesmus dimorphous* cells to result in a starting concentration of 1×10^4 cells/mℓ. All vessels were incubated in a climate control room under test conditions described above. To permit adequate gas exchange, each of the vessels was hand swirled twice daily, 20 times in the morning and 20 times in the evening. Growth of algae was monitored over a two week test period. The Sedgewick Rafter counting procedure was adapted from *Standard Methods* (7). Counts were obtained at a magnification of 100X using a 10X ocular and a 10X objective and a Whipple disc placed in the eyepiece. Algal cells were counted in 10 or more random fields of the Whipple grid, as recommended by Moore (8). For selecting locations of fields to be counted, a procedure similar to that of Ingram and Palmer (9) was followed. Cell counts were made on days 3, 5, 7, 9, 11, and 14, as suggested by the Inter-Laboratory Precision Test (10). Typical growth curves are pictorially defined in Figure 1.

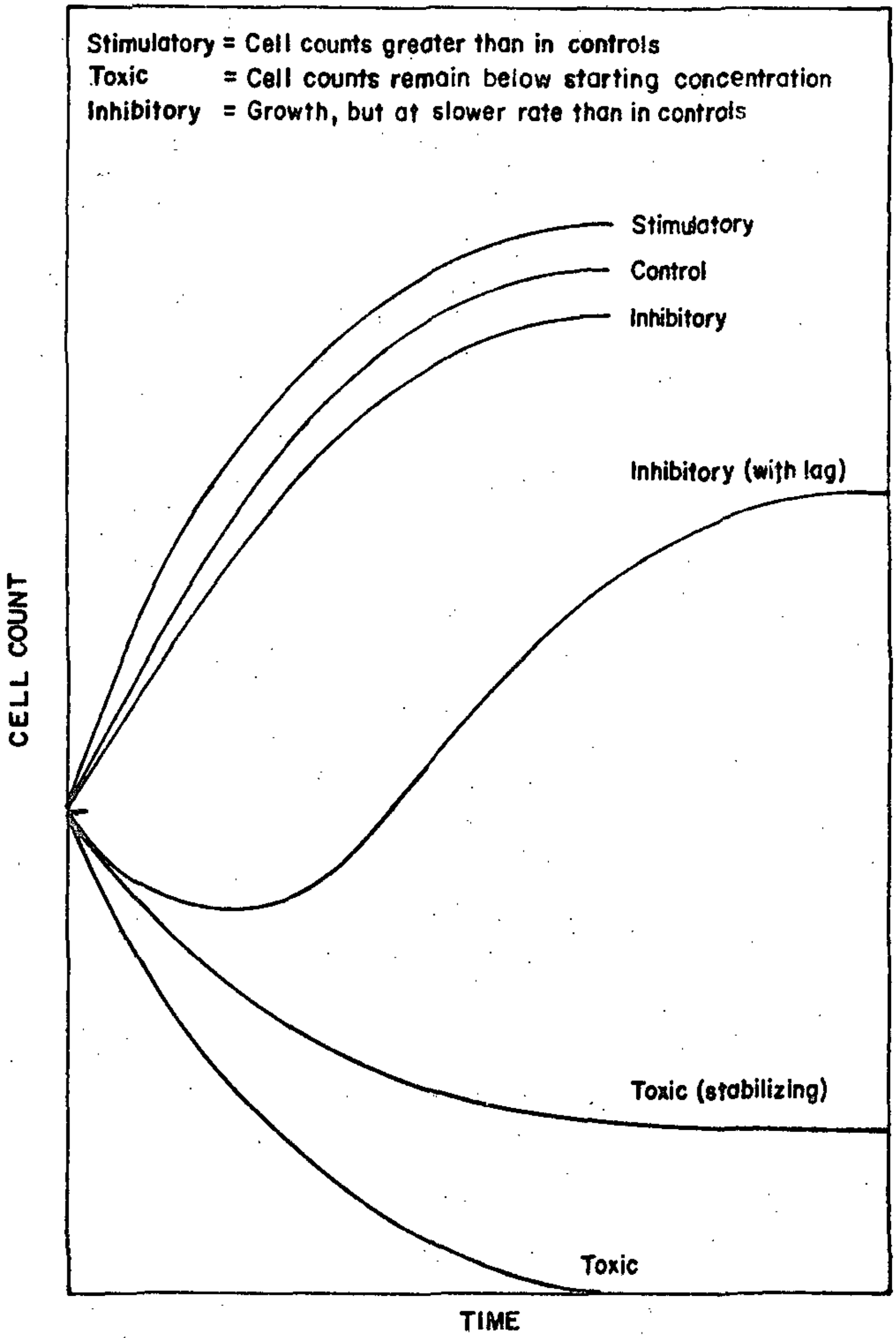

FIGURE I. DEFINITIONS OF TYPICAL GROWTH CURVES.

Two preliminary assays were run to provide introductory information about the effects of leachate on algal growth. Run #1 was made using undiluted leachate, 1/2 leachate (i.e., 1 part leachate and 1 part dilution water, or 2 parts total volume), 1/10 leachate, and 1/100 leachate; Run #2, 1/100 leachate, 1/500 leachate, 1/1000 leachate, and 1/10,000 leachate.

Four subsequent assays were conducted. The objective of each was to determine the effects of leachate on algal growth. A different sample of leachate for each test run was collected from the Barre landfill site. In effect, parameter concentrations and their relationship to one another were varied over a wider range than those afforded by the exploratory assays.

Results and Discussion

Exploratory Assays

For the two preliminary assays, samples of pure leachate were taken at the Barre landfill site during the weeks of January 5, and January 9, 1976. Results of physical-chemical analyses of these samples are shown in Table 1. A comparison of constituent materials shows that, with the exception of chloride, the samples were similar. Constituent materials of leachate dilutions for Runs #1 and #2 immediately before the start of the two week test period appear in Table 2.

Growth curves for Runs #1 and #2 are presented in Figures 2 and 3, respectively. As noted in Figure 2, both undiluted leachate and 1/2 leachate were toxic to algae and resulted in a zero cell count by day 3.

1/10 leachate and 1/100 leachate were also toxic to algae. The cell concentration first decreased then appeared to stabilize significantly below the starting concentration. There was no evidence of algal growth recovery during the two week test period.

In Run #2, 1/100 leachate again proved toxic to algae. The growth curve was similar to the one for 1/100 leachate in Run #1. Number of cells decreased during the first week from the starting concentration, then stabilized at approximately 200 cells/mℓ by day seven.

In both 1/1000 leachate and 1/10,000 leachate, the number of cells increased over a period of 14 days to numbers comparable to the controls. 1/500 leachate, however, was inhibitory to algal growth. Maximum standing crop, in terms of cell number, was only on the order of 229,000 cells/mℓ, significantly lower than those of the controls.

Table 1. Analyses of Pure Leachate for Run #1 and #2

Parameter	Run #1	Run #2
Total Solids (mg/ℓ)	10,800	13,200
Volatile Solids (mg/ℓ)	3930	4480
COD (mg/ℓ)	16,300	17,000
pH	5.30	5.75
Alkalinity (mg/ℓ $CaCO_3$)	4000	6500
Chloride (mg/ℓ)	575	10.2
Phosphate-Phosphorus (mg/ℓ)	0.0280	0.0500
Ammonia-Nitrogen (mg/ℓ)	362	488
Nitrate-Nitrogen (mg/ℓ)	2.42	4.47
Specific Conductance (μmho/cm)	-	>8000

Table 2. Constituent Materials of Leachate Dilutions for Run #1 and #2

Parameter	Run #1				Run #2			
Dilution Ratio	Undiluted	1/2	1/10	1/100	1/100	1/500	1/1000	1/10,000
Total Solids (mg/ℓ)	10,700	5390	1110	178	158	61.0	33.0	19.0
Volatile Solids (mg/ℓ)	3910	1990	415	62.0	60.0	33.0	26.0	10.0
COD (mg/ℓ)	16,200	8240	1690	269	214	150	11.9	0.0
pH	5.30	5.35	5.35	5.05	5.75	6.00	6.20	6.55
Alkalinity (mg/ℓ $CaCO_3$)	3850	1950	293	32.5	65.0	20.5	15.0	13.0
Chloride (mg/ℓ)	525	275	67.5	12.0	8.00	7.00	7.0	6.00
Phosphate-Phosphorus (mg/ℓ)	0.00700	0.00800	0.275	0.288	0.165	0.0550	0.140	0.0300
Ammonia-Nitrogen (mg/ℓ)	370	115	27.5	2.70	3.52	0.820	0.550	0.220
Nitrate-Nitrogen (mg/ℓ)	3.07	0.182	1.92	2.77	1.94	1.82	1.98	1.92
Specific Conductance (μmho/cm)	-	-	-	-	450	200	160	140

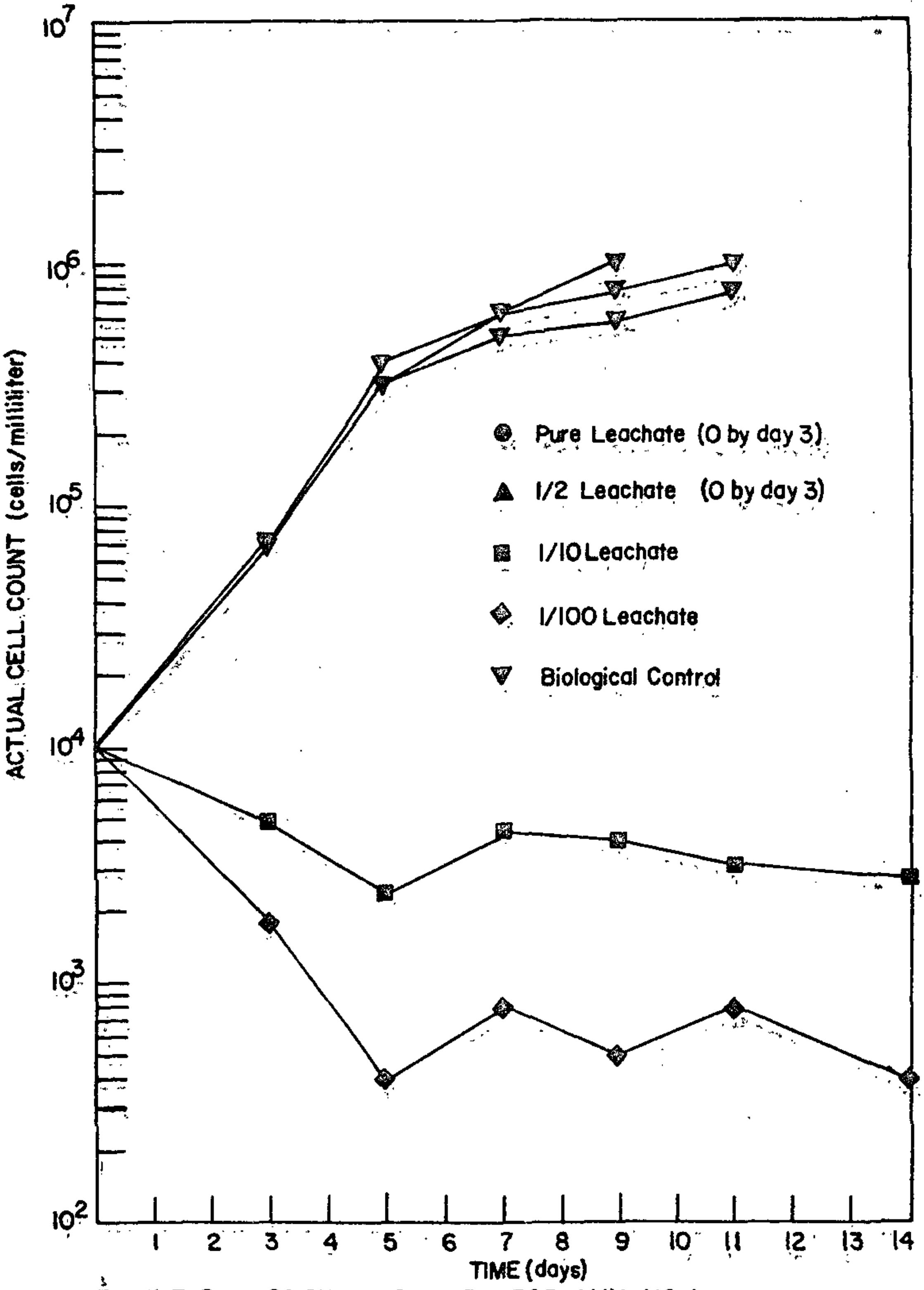

FIGURE 2. GROWTH CURVES FOR RUN NO. 1

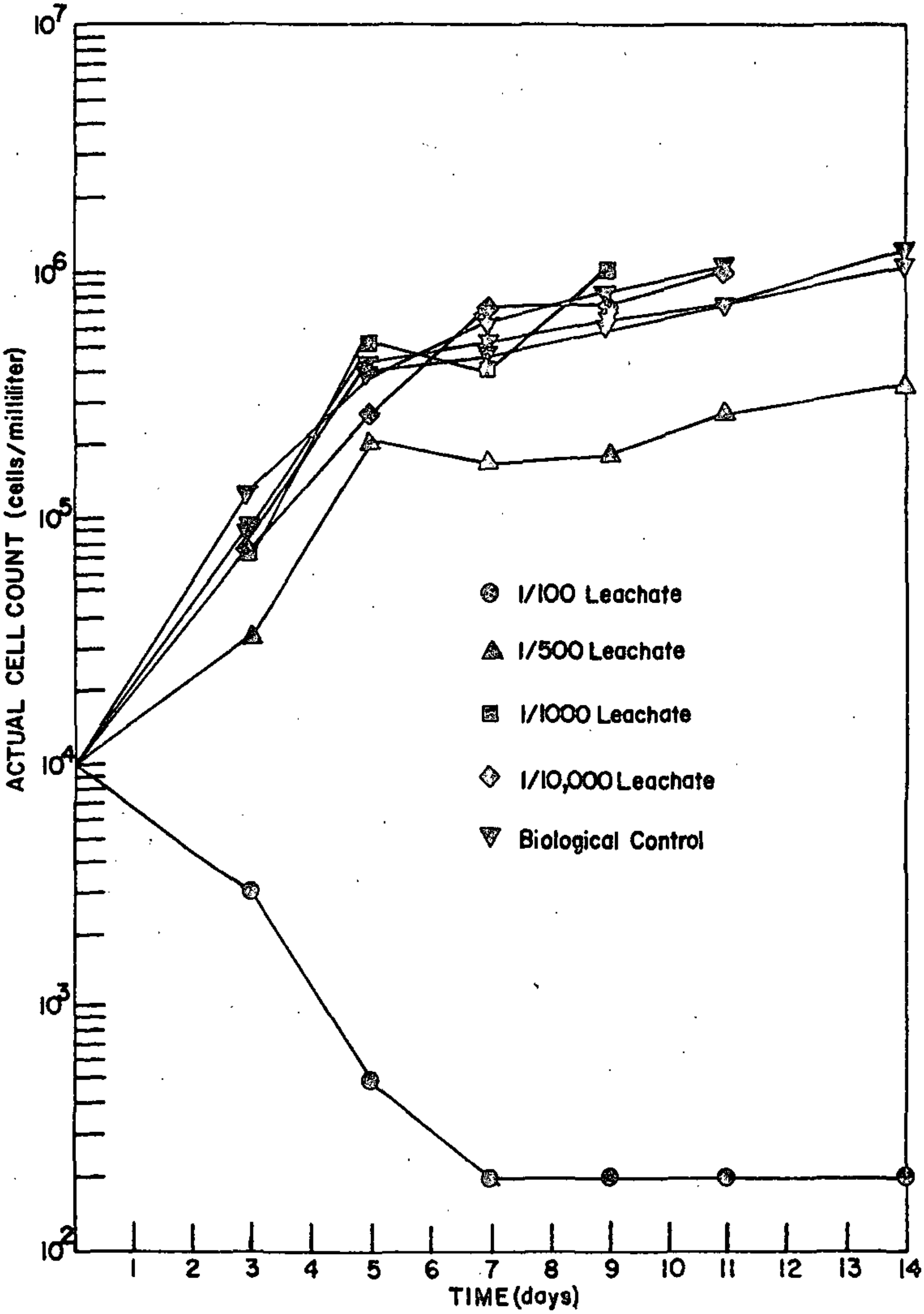

FIGURE 3. GROWTH CURVE FOR RUN NO. 2

Based upon the results of these two exploratory assays, it was decided that the range of dilutions of leachate which would yield the most meaningful data for determining the potential effects of leachate on algal growth in treatment lagoons should be from 1/100 to 1/2000. Consequently, dilution factors of 1/100, 1/500, 1/1000, and 1/2000 were used to make up leachate dilutions for most of the subsequent assays.

Subsequent Assays

Run #3

For Run #3, a sample of pure leachate was taken during the week of February 9, 1976. Constituent materials, which appear in Table 3, were significantly different than those of the samples used in the exploratory assays. Total and volatile solids concentrations were less than half those of both Runs #1 and #2. Chemical oxygen demand (COD) was slightly more than one-third that of the first two runs. The concentration of phosphate-phosphorus was nearly five times greater. Both ammonia-nitrogen and nitrate-nitrogen contents were lower, especially the former.

A look at the constituent materials of leachate dilutions for Run #3 (see Table 3) shows that total solids and volatile solids concentrations were similar, in contrast to those of Runs #1 and #2. COD and specific conductance decreased with an increase in dilution factor. The pH seemed to increase, as would be expected since less leachate was added. The disproportionately low value of alkalinity in 1/500 leachate (5.50 mg/ℓ $CaCO_3$) and the high nitrate-nitrogen concentration (4.97 mg/ℓ) were apparently due to experimental error. The remaining constituent materials seemed to decrease in concentration with increasing dilution factor.

Table 3. Constitutent Materials of Leachate for Run #3

Parameter	Concentration				
Dilution	1/1	1/100	1/500	1/1000	1/2000
Total Solids (mg/ℓ)	4330	85.0	80.0	87.0	72.0
Volatile Solids (mg/ℓ)	1610	63.0	62.0	54.0	52.0
COD (mg/ℓ)	5760	40.0	12.0	4.00	0.0
pH	5.75	5.80	5.20	6.75	6.80
Alkalinity (mg/ℓ $CaCO_3$)	1300	25.0	5.50	13.5	12.5
Chloride (mg/ℓ)	57.6	9.50	7.35	7.50	7.25
Phosphate-Phosphorus (mg/ℓ)	0.220	0.360	0.110	0.0800	0.0450
Ammonia-Nitrogen (mg/ℓ)	85.0	1.50	0.800	0.0420	0.0560
Nitrate-Nitrogen (mg/ℓ)	1.69	2.03	4.97	2.65	2.49
Specific Conductance (μmho/cm)	8000	230	170	150	140

The growth curves for Run #3 are shown in Figure 4. The 1/100 leachate was definitely inhibitory to algae as manifested by a lag period of three days. Inhibitory effects declined with time, and a fairly normal growth pattern was established except for an apparent die-off between day 11 and 14. Number of cells per mℓ in 1/100 leachate never reached the concentrations in other leachate dilutions. Maximum standing crop was approximately 100,000 cells/mℓ.

The 1/500 and 1/1000 leachates exhibited similar growth patterns. In fact, between day 7 and 11, the number of cells was almost identical. In contrast to 1/1000 leachate, maximum standing crop in 1/500 leachate was some 800,000 cells/mℓ, much lower than those of the controls.

The growth curve in 1/2000 leachate followed the normal growth pattern of the controls. On day 14, cell density was so high that clumping occurred, making an accurate count impossible. A similar phenomenon was observed in each of the controls. This suggested that the growth potential of the 1/2000 leachate was as great as that of the controls.

Run #4

The sample of pure leachate for Run #4 was taken during the week of February 23, 1976. Analyses of constituent materials, which are shown in Table 4, revealed that concentrations of total solids (18,000 mg/ℓ) and volatile solids (6150 mg/ℓ) were significantly higher than those of the previous three samples. COD was higher, implying that this leachate possessed greater pollutional strength. Specific conductance (>16,000 μmho/cm) was also higher. Because of the greater strength of this sample, a leachate dilution of 1/10,000 was included in Run #4.

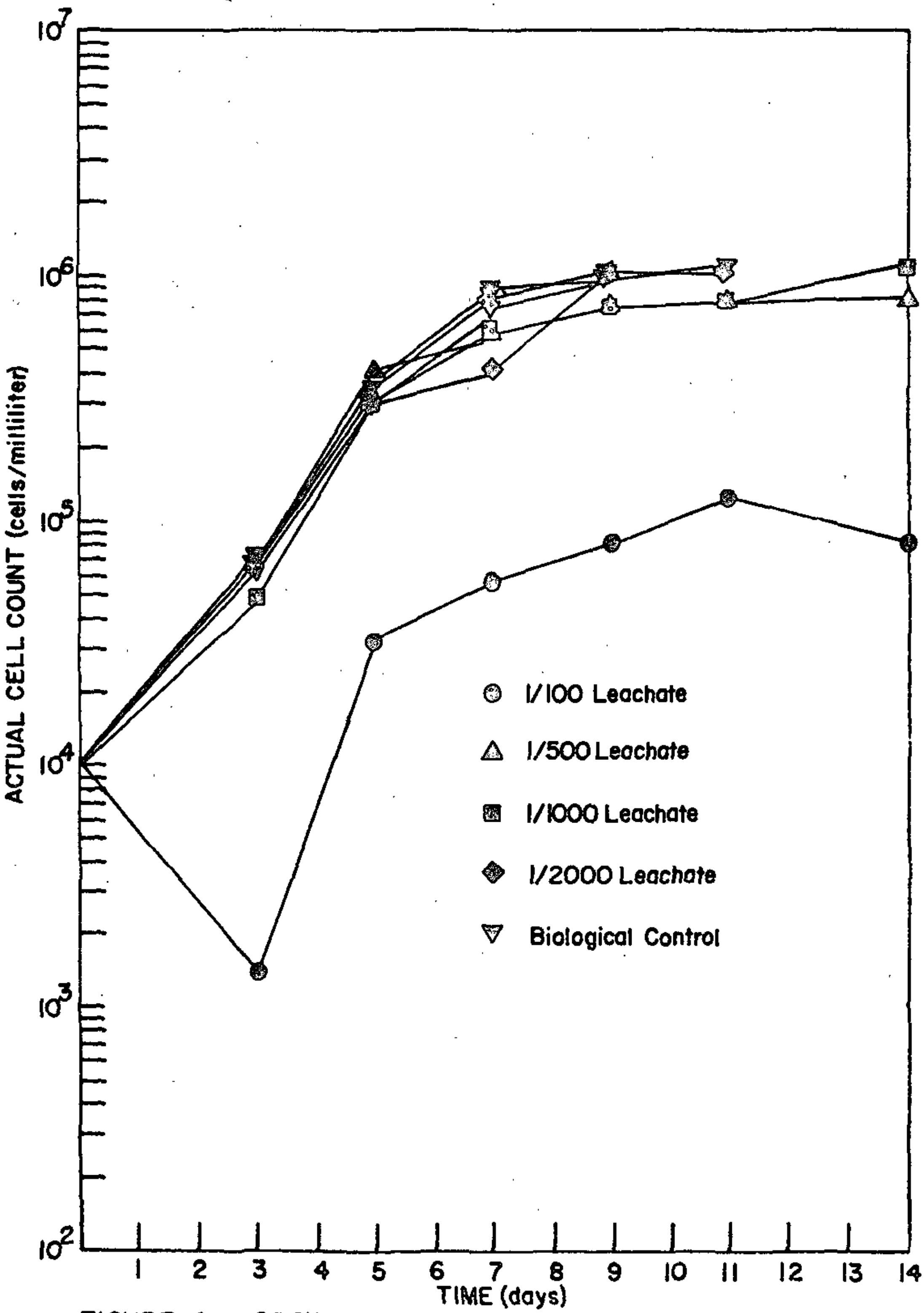

FIGURE 4. GROWTH CURVE FOR RUN NO. 3

Table 4. Constitutent Materials of Leachate Dilutions for Run #4

Parameter	Concentration				
Dilution	1/1	1/100	1/500	1/1000	1/10,000
Total Solids (mg/ℓ)	18,800	159	127	103	104
Volatile Solids (mg/ℓ)	6150	87	45	54	53
COD (mg/ℓ)	24,900	252	51.2	19.7	3.90
pH	5.40	5.60	5.90	6.25	6.60
Alkalinity (mg/ℓ $CaCO_3$)	5900	66.0	19.5	16.0	12.0
Chloride (mg/ℓ)	800	14.0	8.80	7.80	6.00
Phosphate-Phosphorus (mg/ℓ)	0.220	0.215	0.330	0.0390	0.187
Ammonia-Nitrogen (mg/ℓ)	340	4.00	0.800	0.420	0.200
Nitrate-Nitrogen (mg/ℓ)	3.19	1.84	1.80	1.84	1.87
Specific Conductance (μmho/cm)	16,000	540	225	185	160

The growth curves for each dilution are presented in Figure 5. As indicated in previous runs, 1/100 leachate was toxic to algae. Number of cells decreased from the starting concentration of 1×10^4 cells/mℓ to 1200 cells/mℓ by day 3 and 0 cells/mℓ by day 5.

Algal growth in 1/500 leachate demonstrated inhibition as manifested by a lag period of three days. After this initial lag period, a fairly normal growth pattern was observed as in the case of 1/100 leachate in Run #3. Again, a slight die-off took place toward the end of the two week test period. Maximum standing crop was significantly lower than those of the controls.

1/1000 and 1/10,000 leachate followed very similar growth patterns. In both of these leachate dilutions, clumping took place by day 14, indicating maximum standing crops in excess of 1×10^6 cells/mℓ.

Maximum standing crops in the controls appeared to be only slightly higher than those of the leachate dilutions. Again, this suggested that the growth potential of higher leachate dilutions was as great as that of the controls.

Run #5

For Run #5, a sample of pure leachate was taken during the week of March 29, 1976. The constituent materials of the pure leachate for Run #5 appear in Table 5. Total and volatile solids concentrations, specific conductance, and COD were almost one and a half times as high as those of Run #3.

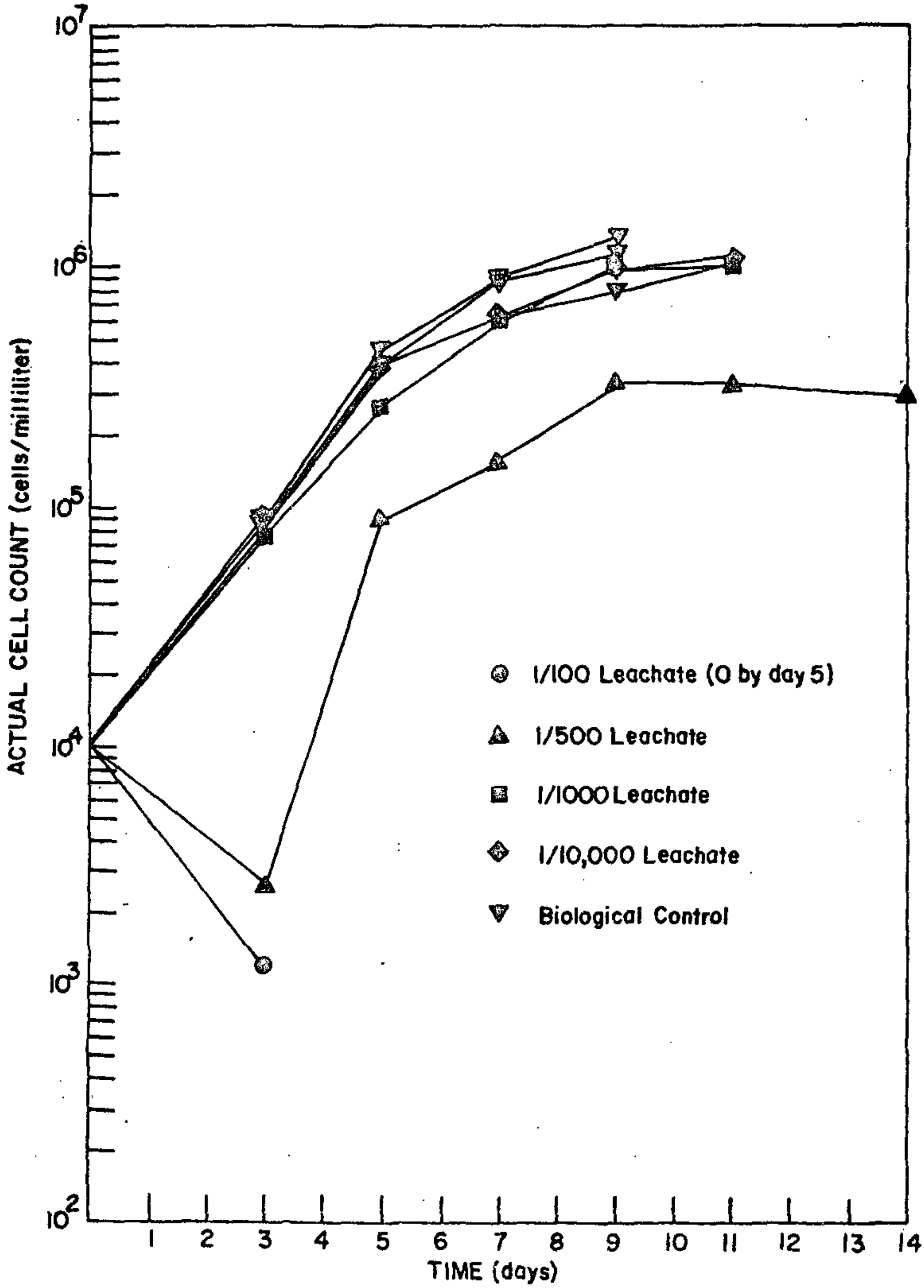

FIGURE 5. GROWTH CURVES FOR RUN NO. 4

Table 5. Constituent Materials of Leachate Dilutions for Run #5

Parameter	Concentration				
Dilution	1/1	1/100	1/500	1/1000	1/2000
Total Solids (mg/ℓ)	6190	143	71.0	56.0	58.0
Volatile Solids (mg/ℓ)	2250	41.0	18.0	11.5	15.0
COD (mg/ℓ)	8670	88.3	28.1	20.1	12.0
pH	5.45	5.85	6.40	6.60	6.70
Alkalinity (mg/ℓ $CaCO_3$)	2500	31.0	14.0	12.0	11.5
Chloride (mg/ℓ)	175	9.00	7.50	7.50	6.75
Phosphate Phosphorus (mg/ℓ)	0.380	0.300	0.230	0.210	0.340
Ammonia-Nitrogen (mg/ℓ)	128	1.66	0.530	0.290	0.230
Nitrate-Nitrogen (mg/ℓ)	4.29	2.03	2.18	2.30	2.03
Specific Conductance (μmho/cm)	12,000	300	150	150	130

The usual dilution factors of 1/100, 1/500, 1/1000, and 1/2000 were used to make up leachate dilutions, the constituent material concentrations of which are shown in Table 5.

Growth curves were plotted and appear in Figure 6. As can be seen, 1/100 leachate was inhibitory to algal growth as manifested by a slower growth rate. Maximum standing crop was only on the order of 200,000 cells/mℓ.

Unlike previous runs, leachate dilutions of 1/500 and greater seemed stimulatory to algae. Growth rate appeared to increase with an increase in leachate concentration. For example, maximum standing crop of 1/2000 leachate was in the neighborhood of the average maximum standing crop of the controls, while that of 1/1000 seemed to be higher. Because clumping took place in both 1/1000 leachate and 1/500 leachate, maximum standing crops could not be recorded.

Run #6

A sample of pure leachate was taken during the week of April 19, 1976, at the Barre landfill site. Constituent materials of pure leachate appear in Table 6. In terms of pollutional strength, this was the weakest of all samples tested. Total solids and volatile solids concentrations were extremely low. COD of the pure leachate for Run #6 (43.3 mg/ℓ) was so low that upon a 1:1000 dilution, no COD could be detected. The concentration of phosphate-phosphorus was the highest of all the samples of leachate. In contrast, concentrations of ammonia-nitrogen and nitrate-nitrogen were the lowest.

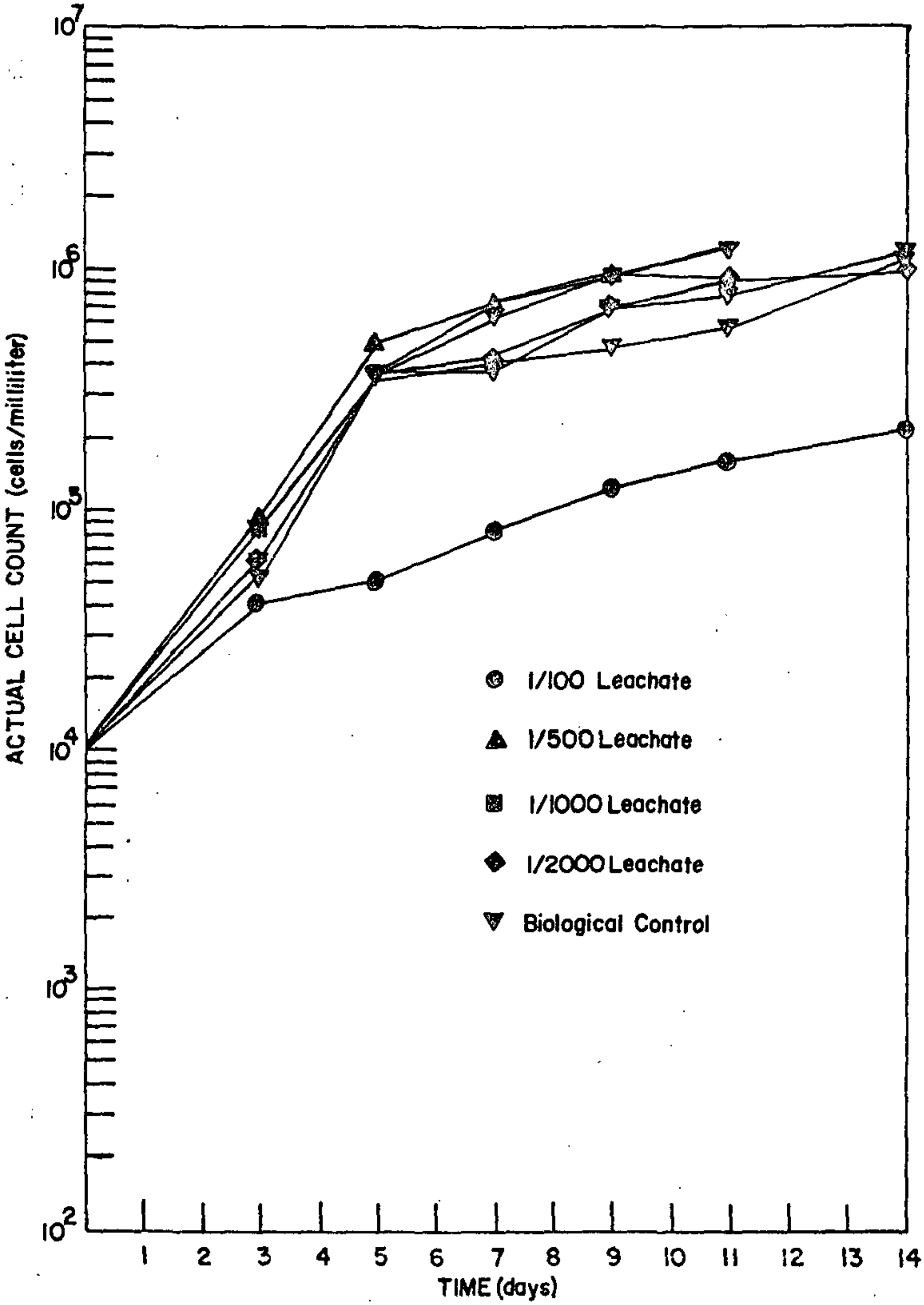

FIGURE 6. GROWTH CURVE FOR RUN NO. 5

Table 6. Constituent Materials of Leachate Dilutions for Run #6

Parameter	Concentration				
Dilution	1/1	1/100	1/500	1/1000	1/2000
Total Solids (mg/ℓ)	2780	92.0	82.0	74.00	88.0
Volatile Solids (mg/ℓ)	474	53.0	52.0	50.0	51.0
COD (mg/ℓ)	43.3	7.90	7.90	0.0	0.0
pH	6.10	6.65	6.70	6.80	6.20
Alkalinity (mg/ℓ $CaCO_3$)	1200	12.5	10.5	10.5	6.50
Chloride (mg/ℓ)	10.0	6.25	5.75	6.50	6.50
Phosphate-Phosphorus (mg/ℓ)	0.640	0.280	0.250	0.160	0.190
Ammonia-Nitrogen (mg/ℓ)	0.900	0.360	0.190	0.200	0.220
Nitrate-Nitrogen (mg/ℓ)	0.390	1.68	1.80	2.02	1.87
Specific Conductance (μmho/cm)	660	148	140	138	135

A closer look at the constituent materials of leachate dilutions for Run #6 (see Table 6) shows that total solids and volatile solids concentrations were similar. pH seemed to increase slightly with an increase in dilution factor. Alkalinity appeared to decrease with increasing dilution factor. Chloride concentration remained about the same.

Since the controls had followed regular growth patterns throughout the first five runs, two, rather than three, were prepared for Run #6. As shown in Figure 7, leachate seemed to be inhibitory to algae over the entire range of dilution factors as manifested by smaller maximum standing crops. Maximum standing crop was smallest in 1/100 leachate (some 600,000 cells/mℓ), and it appeared to be progressively bigger at higher leachate dilutions. Maximum standing crops in both of the controls, however, were higher.

Brief Summary of Runs 1 through 6

In summary, where leachate concentrations were greater than those that algae could tolerate, the effects were dramatic. Growth curves exhibited rapid decay, as in the cases of all the leachate dilutions for Run #1, 1/100 leachate in Run #2, and 1/100 leachate in Run #4. In two other instances, 1/100 leachate in Run #3 and 1/500 leachate in Run #4, leachate concentrations were obviously inhibitory to algal growth, as manifested by observable lag periods.

Little has been said, however, about the cases were leachate concentrations did not seem so obviously inhibitory, or indeed stimulatory, to algae. For these cases where algal growth could be described by an

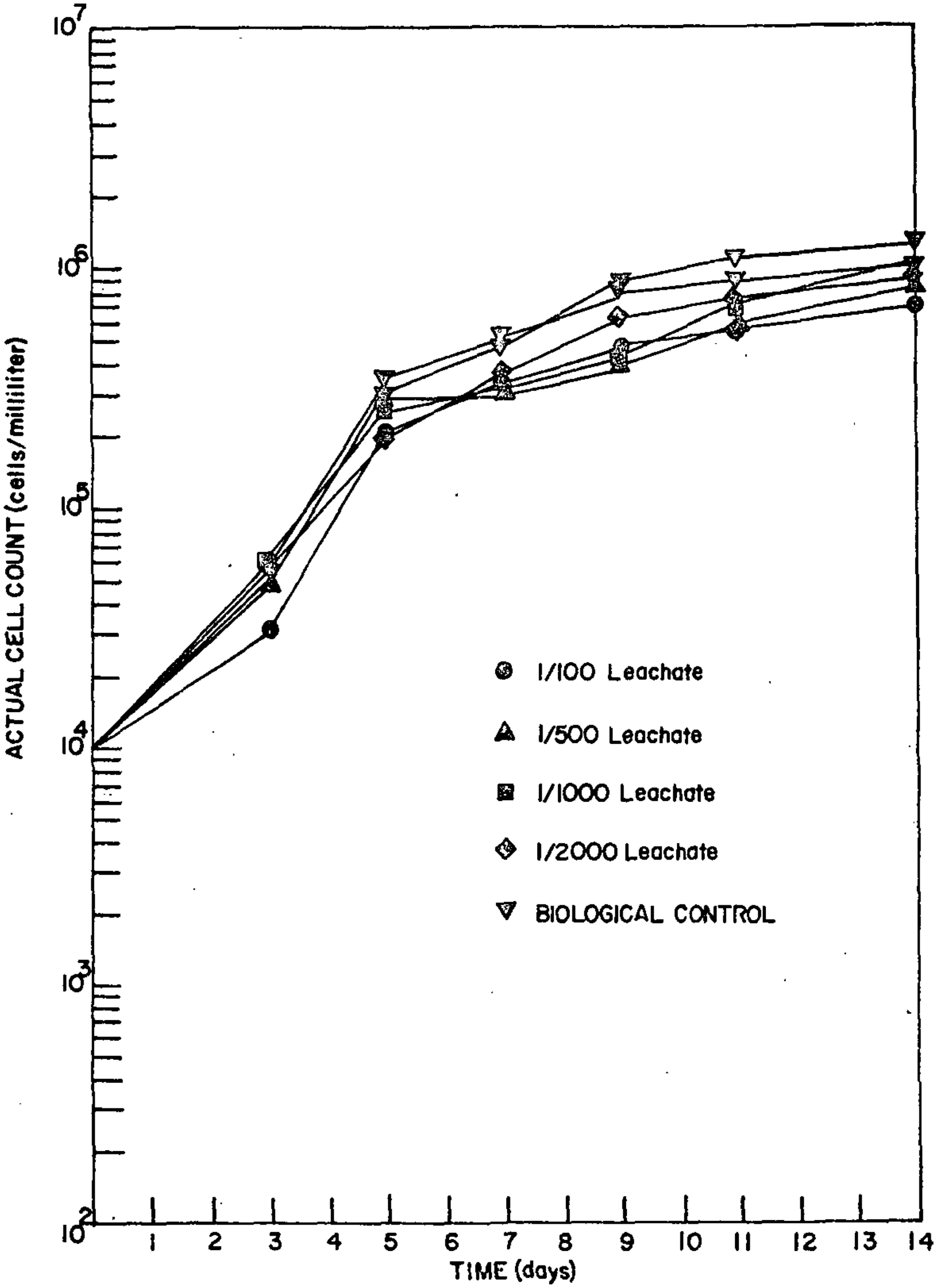

FIGURE 7. GROWTH CURVE FOR RUN NO. 6

asymptotic curve, a statistical approach was deemed necessary.

Statistical Analysis

A. Growth Curve

In the realm of bioassay work, the logistic function has been applied to experimental data to describe population growth. The form of the logistic curve from Stevens (11) is:

$$y = \frac{1}{\alpha+\beta\rho^{x}} \qquad (1)$$

where

y = dependent growth variable

x = independent growth variable

α,β,ρ = parameters.

For this study, a modified version of the logistic curve was developed and used to describe algal growth. The relationship between growth and time is given by:

$$y = \frac{1}{\alpha+\beta e^{-\gamma x^{\theta}}} \qquad (2)$$

where

y = log cell count

x = time, in days

$\alpha,\beta,\gamma,\theta$ = parameters

Taking the reciprocal of y, equation(2) becomes:

$$\frac{1}{y} = \alpha + \beta e^{-\gamma x^{\theta}} \qquad (3)$$

in which α represents the asymptotic value of $\frac{1}{y}$. β represents the change in $\frac{1}{y}$ as x goes from zero to infinity. The term $e^{-\gamma x^{\theta}}$ represents the factor by which the deviation of $\frac{1}{y}$ from its asymptote is changed as x changes, and γ represents the sign of the change. If $\gamma>0$, then the deviation of $\frac{1}{y}$ is reduced; if $\gamma<0$, then $\frac{1}{y}$ is increased. θ represents the rate at which $\frac{1}{y}$ approaches its asymptotic value. The greater the value of θ, the faster $\frac{1}{y}$ reaches it asymptotic value.

For purposes of statistical analyses, the relationship between log cell count and time, as defined in equation (2), was used. Since at time x = 0, y = 4.0 (i.e., the starting concentration of cells was 10,000 cells/mℓ), equation (2) reduces to:

$$y = \frac{1}{.250 + \beta(e^{-\gamma x^{\theta}}-1)} \qquad (4)$$

where $\alpha = .250-\beta$

In order to obtain a least-squares fit of the data to equation(4), a computer program entitled BMDP3R Nonlinear Regression (12) was employed. Output included parameter values, together with their standard deviations and the residual sum of squares. Values for α, β and θ were chosen from the iteration which had the smallest residual sum of squares. Those values and their respective standard deviations appear in Table 7.

B. Pooling Controls

To determine whether various dilutions of leachate were stimulatory or inhibitory to algae in those cases where growth was not obviously different from growth in the controls, the following procedure was used. First, the equality of the estimates for the three parameters in the growth curves of the controls were tested. Confidence intervals at

Table 7. Estimates of β, γ, and θ, Together With Their Standard Deviations.

Test Run	Culture Vessel (Leachate Dilution)	Degrees of Freedom	$\hat{\beta}$	$s(\hat{\beta})$	$\hat{\gamma}$	$s(\hat{\gamma})$	$\hat{\theta}$	$s(\hat{\theta})$
2	6(1/500)	57	0.06343	0.007418	0.01331	0.007765	3.447	0.4915
	7(1/1000)	32	0.08164	0.0006171	0.08140	0.01075	2.090	0.1067
	8(1/10,000)	42	0.08654	0.002037	0.2253	0.01833	1.100	0.07730
	9	52	0.07968	0.0005218	0.1772	0.02429	1.560	0.1062
	10 Biological	52	0.08192	0.001160	0.2786	0.03649	1.125	0.1030
	11 Controls	42	0.08453	0.0007972	0.3052	0.01939	1.102	0.0565
3	10(1/500)	57	0.07987	0.0003521	0.09400	0.01126	1.938	0.08948
	11(1/1000)	52	0.08069	0.0004278	0.06620	0.008546	2.045	0.09130
	12(1/2000)	38	0.3997	____*	0.07562	0.004827	0.4953	0.03140
	13	37	0.08352	0.0005036	0.08277	0.008023	1.944	0.07349
	14 Biological Controls	37	0.08415	0.006700	0.1079	0.01074	1.739	0.07716
	15	37	0.08420	0.0006425	0.1021	0.00374	1.710	0.06769
4	15(1/1000)	37	0.08486	0.0007174	0.1692	0.01091	1.371	0.05112
	16(1/10,000)	37	0.08374	0.0007512	0.1919	0.01772	1.407	0.07414
	17	32	0.08565	0.001041	0.1377	0.01837	1.631	0.1083
	18 Biological Controls	32	0.08632	0.0006775	0.1567	0.009180	1.500	0.05029
	19	47	0.08368	0.0006940	0.1760	0.01900	1.419	0.08046
5	17(1/100)	57	0.1184	0.05308	0.1626	0.06451	0.5826	0.1031
	18(1/500)	37	0.08153	0.0004094	0.1227	0.01118	1.829	0.07377
	19(1/1000)	47	0.08291	0.0002746	0.1360	0.007493	1.621	0.04116
	20(1/2000)	52	0.08155	0.0007547	0.1400	0.02062	1.543	0.1062
	21	52	0.07900	0.001198	0.2766	0.04125	1.143	0.1172
	22 Biological Controls	52	0.08267	0.001228	0.1984	0.02752	1.252	0.1031
	23	42	0.08306	0.0005752	0.07957	0.009454	1.906	0.08610
6	21(1/100)	57	0.7570	0.0006052	0.05389	0.01020	2.042	0.1261
	22(1/500)	57	0.07930	0.001394	0.1973	0.03042	1.203	0.1113
	23(1/1000)	52	0.08631	0.003237	0.2555	0.02703	0.9423	0.09847
	24(1/2000)	52	0.08184	0.0007339	0.1563	0.01158	1.308	0.05337
	25	47	0.08510	0.0008244	0.1444	0.01420	1.410	0.06990
	26 Biological Controls	52	0.08117	0.0005188	0.1083	0.01314	1.716	0.08660

*Since β was redundant (not pivoted on) or lay on a boundary, the computer assigned it a standard deviation of 0.0.

the 95 percent confidence limits were constructed. Since the variances of β, γ, and θ were known, the $1-\alpha$ level confidence interval from Dunn and Clark (13) is:

$$\hat{Y}_i - \hat{Y}_j + Z[1-\alpha/2m] \sqrt{s_i^{\,2} + s_j^{\,2}}$$

where $\hat{Y}_i$ = estimate of parameter in one growth curve

$\hat{Y}_j$ = estimate of same parameter in a second growth curve

m = number of linear combinations of estimates; in most cases, 9 so that joint probability = .95.

s^2 = variance of predicted value (standard deviation squared).

Accordingly, if the confidence interval, as defined above, contained a zero, then the null hypothesis ($\hat{Y}_i = \hat{Y}_j$) was accepted; estimates of β, γ, and θ in the growth curves of the controls were statistically the same. Thus, the growth curves were the same.

Second, for those controls whose growth curves were the same, predicted estimates of log cell count on day 14 were pooled, as were their variances. The pooled estimate is simply a weighted average with weights proportional to the degrees of freedom (14) and is given by:

$$\hat{Y}_p = \frac{(n_1-a)\ \hat{Y}_1 + (n_2-a)\ \hat{Y}_2 + \cdots (n_i-a)\hat{Y}_i}{(n_1-a) + (n_2-a) + \cdots (n_i-a)}$$

where $\hat{Y}_p$ = pooled estimate of log cell count on day 14

$\hat{Y}_i$ = estimate of log cell count on day 14 for growth curve

(n_i-a) = degrees of freedom, n being number of cases and a the number of parameters in growth curve, in this case, 3.

Similarly, the pooled variance is given by:

$$s^2(\hat{Y}_p) = \frac{(n_1-a)^2\ s^2(\hat{Y}_1) + (n_2-a)^2\ s^2(\hat{Y}_2) + \cdots (n_i-a)^2\ s^2(\hat{Y}_i)}{[(n_1-a) + (n_2-a) + \cdots (n_i-a)]^2}$$

Such a procedure for projecting log cell count on day 14 was repeated for test vessels.

C. Stimulatory vs. Inhibitory

Finally, for determining whether various dilutions of leachate were stimulatory or inhibitory to algal growth, equality of estimates of log cell count on day 14 for each test vessel and pooled estimates, where applicable, of log cell count on day 14 for the controls were tested. Again, confidence intervals at the 95 percent confidence limits were constructed. The 1-α level confidence interval is given below:

$$\hat{Y}_p = \hat{Y}_i \pm 1.96 \sqrt{s^2(\hat{Y}_p) + s^2(\hat{Y}_i)}$$

where $\hat{Y}_p$ = pooled estimate of log cell count on day 14 for controls

$\hat{Y}_i$ = estimate of log cell count on day 14 for test vessel

$s^2(\hat{Y}_p)$ = pooled variance for controls

$s^2(\hat{Y}_i)$ = variance of test vessel

For the purposes of this investigation, maximum standing crop was defined as log cell count on day 14. As shown in Table 8, maximum standing crops for 1/1000 leachate and 1/10,000 leachate and the controls were the same during Run #2. 1/1000 and 1/10,000 leachates yielded the same maximum standing crop as that of the AAPBT synthetic algal nutrient medium, which represented optimal growth. Lesser dilutions of leachate, however, were inhibitory or toxic to algal growth. Maximum standing crops were significantly smaller than that of the controls.

Table 8. Results of Testing for Equality of Log Cell Count on Day 14 for Controls and Test Vessels

Test Run	Test Vessel (Leachate Dilution)	$\hat{Y}_p - \hat{Y}_i$	$1.96\sqrt{s^2(\hat{Y}_p) + s^2(\hat{Y}_i)}$	Comments
2	6(1/500)	0.6210	0.05740	Inhibitory
	7(1/1000)	0.04100	0.05514	Same
	8(1/10,000)	-0.08300	0.08439	Same
3	10(1/500)	0.1420	0.03318	Inhibitory
	11(1/1000)	0.1140	0.03726	Inhibitory
	12(1/2000)	-0.5320	0.1553	Stimulatory
4	15(1/1000)	0.01000	0.05124	Same
	16(1/10,000)	0.04600	0.05603	Same
5	17(1/100)	0.5580	0.05826	Inhibitory
	18(1/500)	-0.03580	0.05437	Same
	19(1/1000)	-0.08500	0.05073	Stimulatory
	20(1/2000)	-0.03560	0.06947	Same
6	21(1/100)	0.3190	0.06517	Inhibitory
		0.1860	0.05274	Inhibitory
	22(1/500)	0.2220	0.08323	Inhibitory
		0.08900	0.07390	Inhibitory
	23(1/1000)	0.09300	0.09426	Same
		-0.04000	0.08613	Same
	24(1/2000)	0.1300	0.06452	Inhibitory
		-0.003000	0.05193	Same

For Run #3, leachate dilutions as great as 1/1000 proved inhibitory to algae. Maximum standing crops of 1/500 and 1/1000 leachate were statistically different than that of the controls. Differences seemed to decrease with increasing dilution to the point where 1/2000 leachate was actually stimulatory to algae.

In Run #4, maximum standing crops of 1/1000 and 1/10,000 leachate were statistically the same as that of the control. Again, dilution factors as low as 1/1000 resulted in "optimal growth".

For Run #5, 1/100 leachate proved inhibitory to algal growth as manifested by a smaller maximum standing crop. Maximum standing crop of 1/500 leachate was statistically the same as that of the controls, and 1/1000 leachate was actually stimulatory to algae. Following this trend, it would have been expected that 1/2000 leachate also to be stimulatory to algal growth. The reason for maximum crop having been the same as that of the controls is unclear.

In Run #6, because estimates of the maximum standing crop for the two controls (25,26) were not equal, equality of estimates for each test vessel and both controls, rather than a pooled control, were tested. Based upon the results of the tests with control (25), leachate seemed inhibitory to algae over the entire range of dilution factors. These results are in agreement with the plots of actual cell count vs. time depicted in Figure 7. And inhibitory effects appear to have decreased with increasing dilution factor. An apparent exception to this was test vessel 23, 1/1000 leachate, whose maximum standing crop was the same as that of the control.

Overall, dilution factors of 1/100 (or less) proved inhibitory and even toxic to algal growth, while leachate dilutions of 1/500 were inhibitory. One exception to this rule was Run #5 in which maximum standing crop of 1/500 leachate was statistically the same as that of the controls.

For most of the test runs, a dilution factor of 1/1000 resulted in the same maximum standing crop in both test vessels and controls. In Run #3, however, 1/1000 leachate was inhibitory to algae, while in Run #5, it was stimulatory.

Growth in greater dilutions of leachate (specifically, 1/2000 and 1/10,000 leachate) followed even more dissimilar trends. For example, in Runs #2 and 4, maximum standing crops of 1/10,000 leachate were statistically the same as those of the respective controls. But maximum standing crops of a lesser dilution, 1/2000 leachate, were stimulatory to algal growth, the same as the respective controls, or inhibitory to algae, depending on test run; i.e., the starting concentrations of the various constituent materials of leachate.

D. Regression Analysis

To provide some basis for design of an algal lagoon for leachate treatment, a dilution factor based upon one or more constituent materials of leachate would be extremely helpful. As a means to that end, a regression analysis was performed with 1/100 leachates, 1/500 leachates, 1/1000 leachates, 1/2000 leachates, and 1/10,000 leachates; i.e., those dilutions of leachate which had yielded the most meaningful results.

For determining the prediction equation for log maximum standing crop, a stepwise regression analysis was performed. The dependent variable, log maximum standing crop, was regressed on the starting concentrations of nine constituent materials of leachate analyzed (pH was not included in the regression analysis.) The summary table output is in Table 9. F-tests for multiple regression were performed at the 95 percent confidence limits. At these confidence limits, the best regression equation was:

$$\hat{Y} = 8.091 - .014\text{PARAM9}$$

where $\hat{Y}$ = estimate of log maximum standing crop

PARAM9 = starting value of specific conductance

with a multiple correlation coefficient R squared of 0.9259. According to this regression equation, the single variable which best predicts log maximum standing crop was specific conductance. Values of log maximum standing crop predicted from the regression equation are in Table 10.

In order to construct the regression line, the independent variable, specific conductance, was plotted against the dependent variable, log maximum standing crop predicted from the regression equation. For any point on the regression line, a 100 (1-α) percent confidence interval for the mean of Y given X, from Afifi and Azen (15), is:

$$\hat{Y} \pm s \cdot t_{1-\alpha/2,(n-2)} \left[\frac{1}{n} + \frac{(x-\bar{x})^2}{\sum_{i=1}^{n}(x_i-x)^2}\right]^{1/2}$$

where s = standard error in estimating dependent variable, log maximum standing crop

n-2 = degrees of freedom; in this case, 19.

n = number of cases; here, 21.

Table 9. Summary Table Output from Regressing Maximum Standing Crop on Constituent Materials of Leachate

Step No.	Variable Entered	Multiple R	RSQ	Increase in RSQ	F-to-Enter
1	PARAM9 (Specific Conductance)	.99622	.9259	.9259	237.2947
2	PARAM3 (COD)	.9649	.9311	.0053	1.3735
3	PARAM1 (Total Solids)	.9677	.9365	.0053	1.4281
4	PARAM2 (Volatile Solids)	.9737	.9481	.0116	3.5822
5	PARAM6 (Phosphate-Phosphorus)	.9788	.9581	.0100	3.5728
6	PARAM7 (Ammonia-Nitrogen)	.9795	.9594	.0013	.4435

Table 10. Values of Log Maximum Standing Crop Predicted from Regression Equation

Test Run	Test Vessel	Specific Conductance (μmho/cm)	Log Maximum Standing Crop
1	4	372	2.883
2	5	450	1.791
	6	200	5.291
	7	160	5.851
	8	140	6.131
3	9	230	4.871
	10	170	5.711
	11	150	5.991
	12	140	6.131
4	13	540	0.5310
	14	225	4.941
	15	185	5.501
	16	160	5.851
5	17	300	3.891
	18	150	5.991
	19	150	5.991
	20	130	6.271
6	21	148	6.019
	22	140	6.131
	23	138	6.159
	24	135	6.201

x = value of independent variable, specific conductance

$\bar{x}$ = mean of independent variable

$$\sum_{i=1}^{n} (x_i-\bar{x})^2 = (n-1)s_x^2$$

A plot of specific conductance vs. values of log maximum standing crops from both the growth curve and the regression equation is presented in Figure 8. For those cases where a modified version of the logistic curve was not used to describe algal growth (i.e., growth did not tend asymptotically toward a limit), actual observed values of log maximum standing crop were plotted. The regression line, together with 95 percent confidence intervals for points of interest, were labeled. The results show that the regression equation was a good fit to the observed values of log maximum standing crop.

The regression equation $\hat{Y}$ = 8.091 - .014PARAM9, with a multiple R squared of 0.9259, implies that a good overall measure of toxicity of leachate to algae is specific conductance. Analysts of leachate have expressed a similar viewpoint (16). Another way of stating this is that specific conductance is a strong indicator of the algal growth potential of leachate.

Results of this laboratory study may be applied to the preliminary design of leachate treatment facilities at a sanitary landfill site. It is likely that a treatment lagoon filled with a dilution of leachate whose specific conductance is greater than 375 μmho/cm will be toxic to algae. In Run #1, leachate dilutions with specific conductances

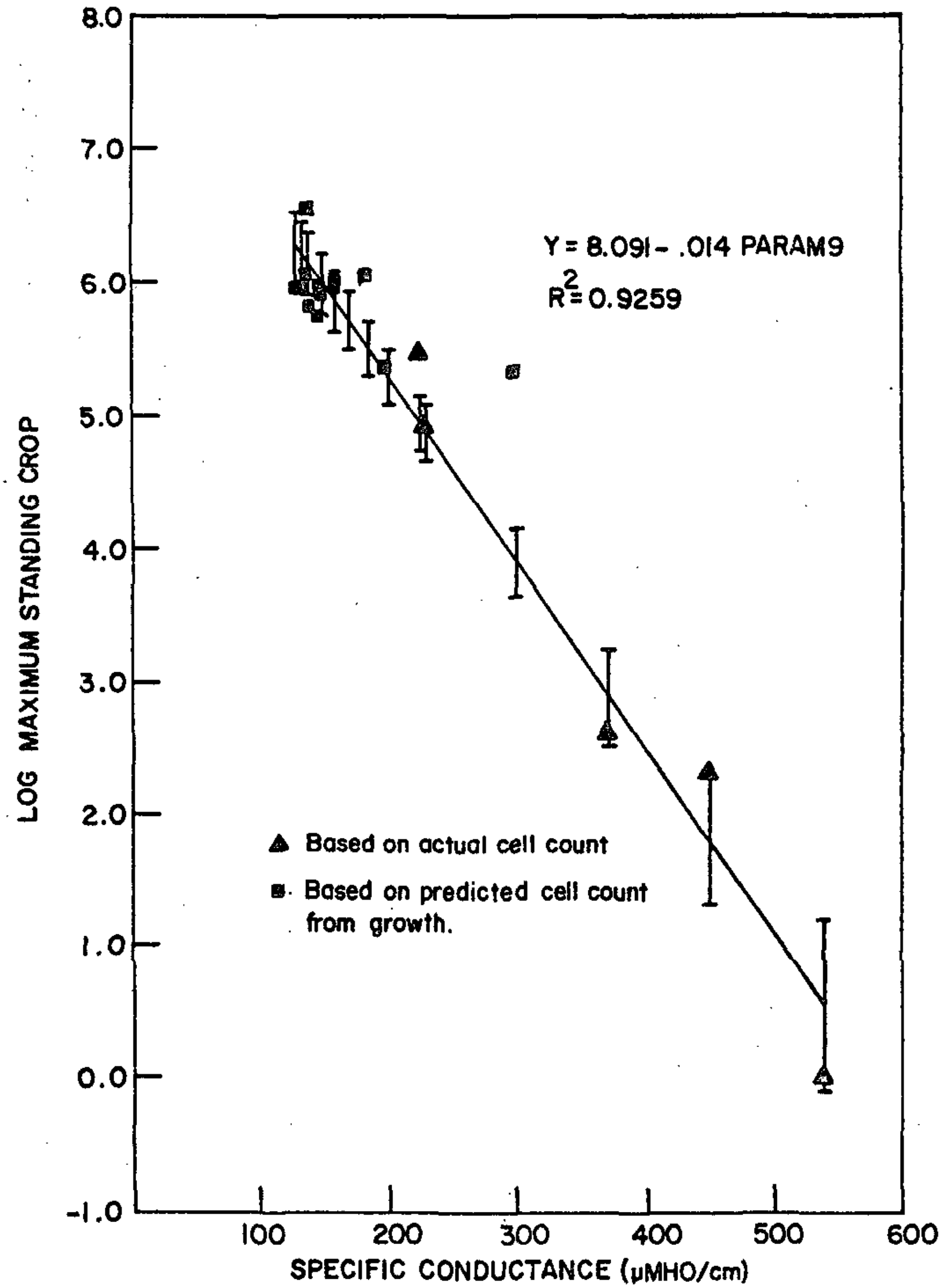

FIGURE 8. SPECIFIC CONDUCTANCE VS. LOG MAXIMUM STANDING CROP FROM GROWTH AND REGRESSION EQUATION

that ranged from 32,800 μmho/cm down to 372 μmho/cm resulted in die-offs of algal cells. For Run #2, a dilution of a leachate with a specific conductance of 450 mho/cm was also toxic to algae. And in Run #4, a leachate dilution with a specific conductance of 540 μmho/cm resulted in growth curve decay.

And it is also quite likely that a treatment lagoon filled with a dilution of leachate whose specific conductance is between 200 μmho/cm and 375 μmho/cm will be inhibitory to algal growth. Such were the cases for Runs #2, #3, #4, and #5.

In order, then to ensure that leachate does not enter treatment lagoons in concentrations that algae are unable to tolerate, the resulting specific conductance should be less than 200 μmho/cm. This translates to a dilution factor of greater than 1/100 for the pure leachate generated at the Barre landfill site. This has been substantiated by the preliminary field observations of Lavigne (17), who observed that algae flourished in pools of leachate which had undergone dilutions of 1/100 or greater.

Conclusions

1. Overall, dilution factors of 1/100 (or less) proved inhibitory or toxic.
2. Leachate dilutions of 1/500 were inhibitory to algal growth in most cases.
3. The single variable which best predicted the effects of leachate on algal growth was specific conductance.
4. In order to ensure that leachate does not enter a treatment lagoon in concentrations that algae are unable to tolerate, the resulting specific conductance should be less than 200 μmho/cm.

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