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ENVIRONMENTAL ENGINEERING PROGRAM
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Massachusetts Water Resources Commission

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Environmental Engineering Program
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PREFACE

This publication is a reproduction of a portion of the Master's Project Report prepared by Ms. Elizabeth Johnson as part of the requirements for the MS Degree she received in the Environmental Engineering Program at the University of Massachusetts. The research project which supported her investigation was directed by Dr. Donald Dean Adrian.

Dr. Enrique J. La Motta helped to guide part of the research studies. The cooperation of Mr. Leonard Martone of Martone Trucking Company in Barre, Massachusetts is acknowledged with thanks. The contributions of Mrs. Dorothy Pascoe and Ms. Christina Moore in the final preparation of the report are also greatly appreciated.

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ABSTRACT

During the summer of 1979, the sanitary landfill leachate lagoons at Barre, Massachusetts were used to study the role of indigenous algae in the biological treatment process. Investigations included systematic lagoon monitoring and controlled laboratory experiments in order to quantify the photosynthetic and heterotrophic capabilities of phytoflagellates of the genera Euglena and Pyrobotrys.

The parameters measured in the monitoring program include: numbers and species of algae, chlorophyll, carotenoids, mixed liquor suspended and volatile solids, and total carbon. Laboratory experiments were designed to determine the following: (1) the importance of photosynthesis to the algae and its effects on the uptake of dissolved carbon with nutrient additions; (2) the degree of competition between algal and bacterial populations for the organic carbon substrate in the absence of nutrients, and, (3) the rate of carbon removal by algae alone in the presence of various nutrients.

Experiments provided evidence that the indigenous algae in the leachate lagoons studied are effective competitors against the bacteria for soluble organics, and that these algae performed either auto- or heterotrophically depending upon the nutrient additions.

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Readers wishing to examine the raw data tables for the experiments described herein should consult APPENDIX B of Ms. Johnson's Masters Thesis.

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LIST OF ABBREVIATIONS USED IN THE TEXT

Abbreviation	Meaning
N	number of observations on a sample from a population
SD	standard deviation of observations on a sample from a population
X	mean of observations on a sample from a population
Sp	pooled standard deviation
df	degrees of freedom
t	[illegible] statistic
BOD	biochemical oxygen demand
MW	molecular weight
MLSS	mixed liquor suspended solids
MLVSS	mixed liquor volatile suspended solids
DO	dissolved oxygen
COD	chemical oxygen demand

I. INTRODUCTION

The leachate produced by the percolation of rain through a sanitary landfill has been proved to be a genuine threat to existing ground water.[1] Not only is this leachate stronger than raw sewage in typical pollutional parameters, but ground water flow regimes and velocities are such that the contamination problem will remain for extended periods of time, perhaps for hundreds of years.[2] Because of the severity of this problem, many recent research efforts have concentrated on the problem of collection and treatment of this potentially toxic waste. Since landfill leachates typically have high concentrations of organic matter, the possibility of effective biological treatment has not been overlooked. Cook and Foree[3] studied aerobic biostabilization of leachate with no-recycle completely mixed laboratory-scale units. With the addition of lime and nutrients, they obtained 99.7% removal of BOD_5 with a detention time of ten days. Boyle and Ham[4] report promising removals of organics in raw leachate by an anaerobic method, followed by an aerobic polishing of the effluent. Their experiment with an aerobic method displayed more problems: namely, foaming and poor solids-liquid separation. Palit and Qasim[5] performed a bench-scale activated sludge study, and concluded that the leachate could be treated biologically at a conventional sewage treatment plant, but admitted that nutrient addition may be necessary, and that sludge bulking would be a problem. Uloth and Mavinic[6] studied high strength leachate treatment with nutrient addition in aerobic biostabilization digesters. They recommend a hydraulic detention time of twenty days. They found that a stable biological community would develop

even with the high concentrations of heavy metals, and that a high percentage of these metals was removed with the settled sludge.

In contrast to these previous studies, this report will focus on an oxidation pond method of treatment. The Martone Trucking Company, in conjunction with the Environmental Engineering Program at the University of Massachusetts, and supported in part by a grant from the Massachusetts Division of Water Pollution Control, has devised a collection and treatment system that is perhaps economically more suitable for small communities. A clay lining under the landfill collects the leachate, whereupon it is processed through a series of shallow lagoons. An oxidation pond method of treatment has been used in the past very frequently in the Midwest and West for sewage and other organic industrial wastes. Shallow lagoons of the non-toxic aqueous waste of concern are naturally colonized by many forms of life, including bacteria and algae, which use the organic molecules as food.

Classical oxidation pond theory, since its inception in the early 1900's, envisioned a mutually beneficial relationship between the indigenous bacteria and algae. The theory maintains that the bacterial populations consume the organics, thereby reducing the BOD, but soon oxygen becomes limiting, as it is required for their respiration. Herein lies the usefulness of the algae, because the photosynthetic process produces oxygen. Therefore, the ponds are generally shallow so that light may penetrate to all depths, enabling photosynthesis to occur. A large surface area to volume ratio also encourages oxygen transfer by purely physical means. The relationship between organic waste,

bacteria, and algae is shown in Figure 1. Note that this schematic does not represent any symbiosis between the bacteria and algae, rather, it demonstrates the general reciprocity of respiration and photosynthesis. In almost all cases of a true symbiosis, there is a physical unity between the two species of the partnership. Thus, the algal-bacterial relationship within the oxidation pond environment is correctly labeled a plant-animal relationship typical of many ecosystems, and the term symbiosis should not be used in this context.

Heretofore in the field of oxidation pond engineering and biological treatment in general, algae were delegated the very important role of oxygen production as their single effort. Recent literature has proved the existence of some situations in which the classical oxidation pond theory does not correctly describe the mechanics of the biological treatment. Abeliovich and Weisman[7] studied high-rate sewage oxidation ponds and found that the process of BOD reduction of raw wastewater in these ponds was carried out primarily by algae, while the bacteria played a minor role.

Pivotal in this discussion is the understanding of the variation in algal modes of metabolism. Many algae can be forced into a heterotrophic mode of nutrition by keeping them in the dark with an organic carbon source; such a method was employed by Abeliovich and Weisman.[7] Other algal species occur naturally as intermediate plant-animals, particularly those unicellular motile algae, the phytoflagellates. These organisms are essentially protozoa with chlorophyll and are able to obtain the major portion of their metabolic energy from either photosynthesis or

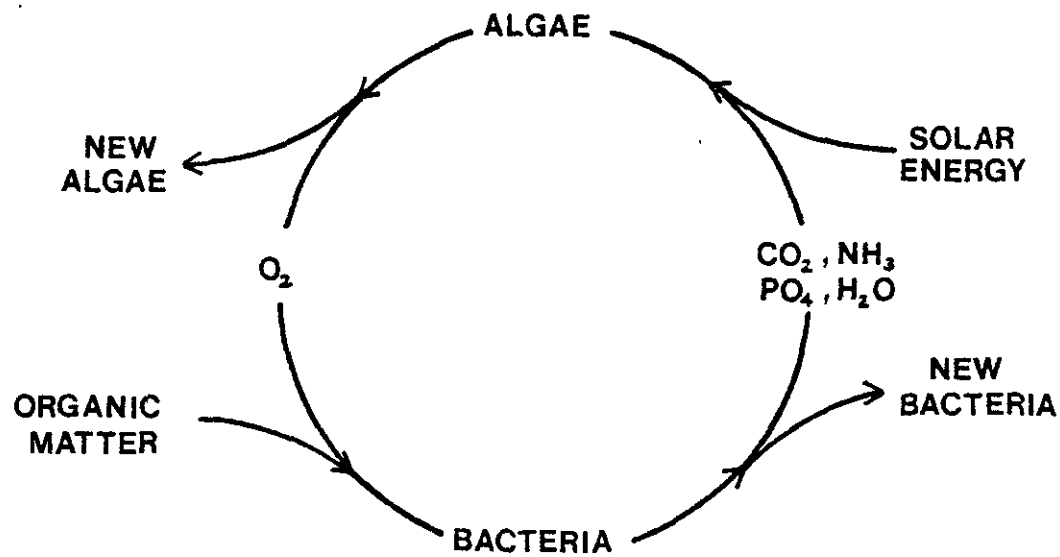

Figure 1. Schematic Representation of the Relationship between Algae and Bacteria.

heterotrophic respiration of the organic carbon, depending upon the environmental conditions. Indeed, considerable energy is required for motility, necessitating the ingestion of organic carbon at a very high rate. Thus there is a complete spectrum of variation in the unicellular eucaryotes, ranging from complete autotrophism, as in true plants, to complete heterotrophism, as in true animals.

Basic textbooks in the field[8,9] contend that these protozoan-like algae are not effective competitors against bacteria in the consumption of organic matter. This is undoubtedly quite correct, because the design of activated sludge units usually includes detention times which preclude the luxuriant growth of eucaryotic organisms. Eucaryotes have maximum growth rates that average about one tenth of that typical of bacteria. However, in another recent investigation by Bhatla and Gaudy,[10] they discovered that protozoa are indeed important in the activated sludge process, being responsible for about 30% of the BOD_5 exerted.

The phytoflagellates of concern here are included in the Class Euglenophyta, rather large green cells with one flagellum and a red eye-spot. Because preliminary research has revealed that these algal flagellates are present in the sanitary landfill leachate lagoons at Barre, MA, in what would appear inordinately high numbers (millions of cells per milliliter), further research into the exact role of these algae was undertaken. These algae are highly motile, do not have the rigid cell wall typical of true plants, and are known to be alternately photosynthetic and heterotrophic, depending upon light and substrate

conditions. Some of them are known as acetate flagellates, because they prefer organic acids and alcohols over sugars.

Their preference for organic acids leads them to find the organic composition of sanitary landfill leachate attractive. Research by Chian[11] revealed that 49% of the initial total organic carbon of a leachate from a young landfill was in the form of acetic, propionic, isobutyric, butyric, isovaleric, valeric and hexanoic acids. Further studies on the volatiles and organics of leachate by Khare and Dondero[12] identify many alcohols and acids, although no quantitative data were presented. Leachate would be a favorable environment for those organisms preferring organic acids and alcohols.

The suggestion that these phytoflagellates are capable of a dual role in the biological treatment process is not unusual. Recently, other researchers have proved that the true algae can adapt to, and require, an organic carbon substrate. Abeliovich and Weisman[7] forced the immotile, hard cell-walled Scenedesmus to a heterotrophic mode of nutrition. Sachdev and Clesceri[13] experimented with various molecular weight fractions of the organics from secondary effluent. They found that the fractions of MW 700 and above were particularly stimulatory to the growth of Selenastrum capricornutum (Kutz). Pipes[14] performed a pure culture study with Chlorella in various fractions of domestic sewage and found that some organics enhanced the growth rate of this alga.

This study will attempt to determine the role of the indigenous algae in the sanitary landfill leachate lagoons at Barre, MA, in the biological treatment process. Systematic lagoon monitoring and labora-

tory experiments under various controlled parameters will elucidate the physiological capabilities of these microorganisms, upon which the success of the biological treatment depends.

II. EXPERIMENTAL DESIGN

The proposed experimental method for the quantification of both the photosynthetic and the heterotrophic capabilities of the algae in the leachate lagoons at Barre, MA, involves both a lagoon monitoring program and controlled laboratory studies.

The lagoon monitoring program involves the measurement of chlorophyll, carotenoids, numbers and species of algae, mixed liquor suspended and volatile suspended solids, and total carbon. The chlorophyll measurement will be indicative of the photosynthetic capabilities of the lagoon algae at the sampling time. Carotenoids are accessory pigments in photosynthesis, and research of Stern[15] has shown that the molar ratio of chlorophyll to carotenoid must be greater than unity for a significant amount of oxygen to be photosynthetically evolved in the alga Euglena gracilis. This phytoflagellate is typical of the algae observed in the lagoons. Optimum photosynthetic performance occurred at a chlorophyll to carotenoid ratio of 2.5 to 1.[15] Mixed liquor suspended solids will predict turbidity. Mixed liquor volatile suspended solids was used as the parameter indicative of biomass by Palit and Qasim.[5] Total carbon will measure available substrate. Grab samples were collected from the surface pools, with no preservation techniques being employed. Any alteration of pH or addition of poisons would disrupt, or even destroy, the indigenous microbial populations. Analysis of the sample was performed on the date of collection.

Since the leachate at Barre is treated in a batch mode, all experiments will attempt to simulate this type of operation. All laboratory

experiments were conducted in beakers and flasks, and the contents were stirred prior to analysis. The Barre lagoons received no such mechanical aeration such as this, but these lagoons had a larger surface area to volume ratio. The mixing was deemed necessary for representative results. The details of each experiment will be discussed individually.

III. MATERIALS AND METHODS

All carbon analyses were performed with the Beckman 915 Total Organic Carbon Analyzer, with the Beckman 215A Infrared Analyzer and a ten-inch recorder. Spectrophotometric determinations were done with the Spectronic Spec 20 and the Perkin-Elmer Model 202 ultraviolet-visible spectrophotometer.

Chlorophyll determinations were performed by filtering an appropriate volume of sample with a Gelman type A-E fiber filter to which a pinch of $MgCO_3$ was added. The magnesium carbonate prevents any active chlorophyll from degrading to pheophytin during the extraction procedure. The filter was then macerated with a mortar and pestle in a solution of 80% spectral grade acetone. This solution was allowed to remain overnight in the dark to complete the extraction of the chlorophyllous pigments. The next day the total volume of extract was measured and noted, then filtered to remove suspended matter. The filtrate was analyzed with the spectrophotometers. Following this reading, the filtrate was acidified with 1 to 2 drops of 1 N HCl, and the absorbance of this acidified solution was determined. The spectrophotometric equations of Lorenzen[16] were employed. The equation for the carotenoids was obtained from Stern.[17] All of these equations are listed in the Appendix.

An improved method for measuring active chlorophyll in the presence of degradation products has been suggested by Lorenzen.[16] The extraction with 80% acetone and the absorbance at 663 millimicrons wavelength will measure all chlorophylls and their breakdown products, pheophytins. Pheophytins are chlorophyll molecules that have lost the integral

magnesium ion, and also absorb at 663 nm. Upon acidification, all chlorophyllous compounds lose the magnesium ion to become pheophytins. Lorenzen[16] isolated pure chlorophyll and pure pheophytin, and combined them in various proportions. He found that a solution of pure chlorophyll showed a ratio of 1.7 between the absorbance before and after acidification, while for pure pheophytin, the ratio was unity. This ratio of absorbances before and after acidification was called the "acid factor." Acid factors for solutions intermediate between zero and 100 percent chlorophyll show a linear relationship. The acid factor will be used in this study to indicate the photosynthetic activity of the lagoon algae.

Cell counts were made with a Spencer Bright-Line Haemocytometer at 10x with a Leitz Wetzlar phase contrast microscope. Dissolved oxygen was measured with a Yellow Springs instrument #57. MLSS and MLVSS were measured according to the procedures defined in Standard Methods for the Examination of Water and Wastewater.[18]

IV. LAGOON MONITORING STUDY

The Barre leachate treatment system is composed of a series of shallow lagoons, with the leachate proceeding through the series in a stepwise manner. From the catch basin, the leachate flows to the primary oxidation ponds. These lagoons generally contain very strong leachate, with total carbon ranging from 1000 to 2000 mg/l. As a rule, no algae are present in leachate of this strength, using total carbon as a general measure of pollutant level. The second pair of lagoons receive the partially oxidized leachate from the first pair, and the carbon levels in these secondary ponds is usually less than 1000 mg/l. The secondary lagoons typically contain the phytoflagellates aforementioned in large numbers. These lagoons were monitored during the summer of 1979. See Table 1 for the data.

The lagoon system operated in a batch mode, with detention times varying according to the amount of rainfall, and therefore the amount of leachate generated by the landfill. At the beginning of the summer, considerable rainfall produced a large amount of leachate, necessitating short detention times, and the secondary lagoons became quite strong. As the summer wore on, the rainfall decreased, and detention times lengthened. By the month of August, the leachatesin the secondary lagoons were very low in pollutional parameters, with carbon values less than 200 mg/l. The phytoflagellate population that previously had flourished in these lagoons disappeared, with sparse numbers of the tiny, sessile forms being the only algae present. It was at this time that the primary lagoon became the site of the phytoflagellate population.

This leachate had a carbon value of 500 mg/l. There was 100 times more chlorophyll a and active chlorophyll in the primary pond than in the secondary at this time.

The principal phytoflagellates present in the lagoons were of the genera Euglena and Pyrobotrys. The Euglenoid type are large, single, green, highly motile cells, and the Pyrobotrys are the same size, but colonial in units of 4 to 10 cells, highly motile, and very green. Comparing the data of 5-30 and 7-6, when the major species is Pyrobotrys the active chlorophyll content is 25 times that when the Euglenoids are the major species, even though cell counts indicated 10 times as many Euglenoid cells. The Pyrobotrys undoubtedly photosynthesize to a much greater degree than the Euglenas. Probably the energy requirements for motility of colonial algae are not proportionately as great as that for a single cell. Therefore these algae would not be so dependent upon an outside organic carbon source. The data of 7-27 reveal a large Pyrobotrys population in a leachate with only 175 mg/l carbon. Euglenas disappear when the carbon levels get below 300 mg/l, on the average. The parameters used to measure photosynthetic efficiency of the algal population, the acid factor and the molar ratio of chlorophyll to carotenoids, show that when the Pyrobotrys are present, the physiological state is always excellent, but when the Euglenas predominate, the photosynthetic capabilities are sometimes poor. See the data for 5-30.

TABLE 1. LAGOON MONITORING DATA, SUMMER 1979

Date	5-30	7-6	7-13
Cells/ml	1.65×10^6	1.5×10^5	4.3×10^4
Total carbon	965	850	560
Inorganic carbon	53	200	160
MLSS	190	585	230
MLVSS	110	470	60
Acid factor	1.4	1.7	1.7
DO	0.75	0.5	0.5
Chlorophyll a	0.08	2.4	0.26
Active chlorophyll	0.17	4.2	0.39
Ratio of chlorophyll to carotenoids	1.82	2.5	1.86
Carotenoids	0.054	0.98	0.12
Species	Euglena	Pyrobotrys	Euglena & Pyrobotrys
pH	7.4	7.1	7.4

All data are presented in units of ppm or mg/l, except the ratios and the acid factor, pH, and cells/ml.

TABLE 1. (Continued) LAGOON MONITORING DATA, SUMMER 1979

Date	7-20	7-27	8-3
Cells/ml	3.2×10^4	2.7×10^5	3.9×10^3
Total carbon	300	175	175
Inorganic carbon	120	ND	ND
MLSS	223	38	40
MLVSS	83	22	24
Acid factor	1.7	1.6	1.3
DO	0.8	ND	ND
Chlorophyll a	0.17	0.86	0.04
Active chlorophyll	0.25	1.19	0.07
Ratio of chlorophyll to carotenoids	1.82	1.58	0.87
Carotenoids	0.08	0.44	0.047
Species	*Euglena*	*Pyrobotrys*	*Chlorella* & *Scenedesmus*
pH	ND	ND	ND

All data are presented in units of ppm or mg/l, except the ratios and the acid factor, pH, and cells/ml.

TABLE 1. (Continued) LAGOON MONITORING DATA, SUMMER 1979

Date	Primary Lagoon 8-16	Secondary Lagoon 8-16
Cells/ml	7.1×10^5	5.2×10^4
Total carbon	450	187
MLSS	300	26
MLVSS	250	15
Acid factor	1.58	1.13
DO	0.4	12
Chlorophyll a	1.2	0.015
Active chlorophyll	1.65	0.017
Ratio of chlorophyll to carotenoids	1.65	0.24
Carotenoids	0.58	0.04
Species	Pyrobotrys & Euglena	Chlorella

All data are presented in units of ppm or mg/l.

TABLE 1. (Continued) LAGOON MONITORING DATA, SUMMER 1979

Date	Primary Lagoon 9-7	Secondary Lagoon 9-7
Cells/ml		
Pyrobotrys	2.5×10^3_2	2.2×10^3_3
Euglena	5.6×10^2_4	1.7×10^3_3
Chlamydomonas	1.7×10^4	1.7×10^3
Total carbon	120	100
MLSS	70	27.5
MLVSS	55	27.5
DO	10	13

All data are presented in units of ppm or mg/l.

V. LABORATORY EXPERIMENT #1

In this experiment, the leachate sample with an indigenous phytoflagellate population was split into light and dark runs to ascertain the importance of photosynthesis to these algae, and, to determine the difference, if any, in dissolved carbon uptake. Because preliminary experiments had revealed that studies without nutrient addition have very slow and erratic carbon removal rates, the necessary nutrients were added in order to accelerate and to reduce the scatter in measured removals. Therefore, if the algae present are surviving in the leachate by photosynthetic means or by organic carbon consumption, this will be evident in the difference between light and dark runs.

A grab sample of leachate was collected from a secondary Barre lagoon and split into two 250-ml aliquots in 1000-ml erlenmeyers. One flask was maintained at 25°C in the dark and the other under two 46" cool-white fluorescent bulbs. The following nutrients were added to both flasks: 500 mg/l NH_4Cl, 100 mg/l KH_2PO_4, and 250 mg/l $CaCO_3$. The flasks were monitored daily for dissolved organic and inorganic carbon, MLSS, MLVSS, and phytoflagellate cells. See Table 2 for the initial characteristics of the leachate and Figures 2, 3, and 4 for graphical results.

The initial leachate had a carbon concentration of 1000 mg/l, but a small biomass. Upon nutrient addition, the biomass level increased approximately 500%. See Figure 2. There was a severe nutrient limitation in the initial leachate, so with nutrient addition, the biomass

TABLE 2. CHARACTERISTICS OF LEACHATE USED IN EXPERIMENT #1

(Collected July 20, 1978, from Secondary Lagoon)

Parameter	Concentration in mg/l
Total dissolved carbon	1000
Inorganic dissolved carbon	11
MLSS	75
MLVSS	65
BOD_5	1590
COD	2899
pH	7.7
Alkalinity	1340
Ammonia nitrogen	112
Iron	6.8

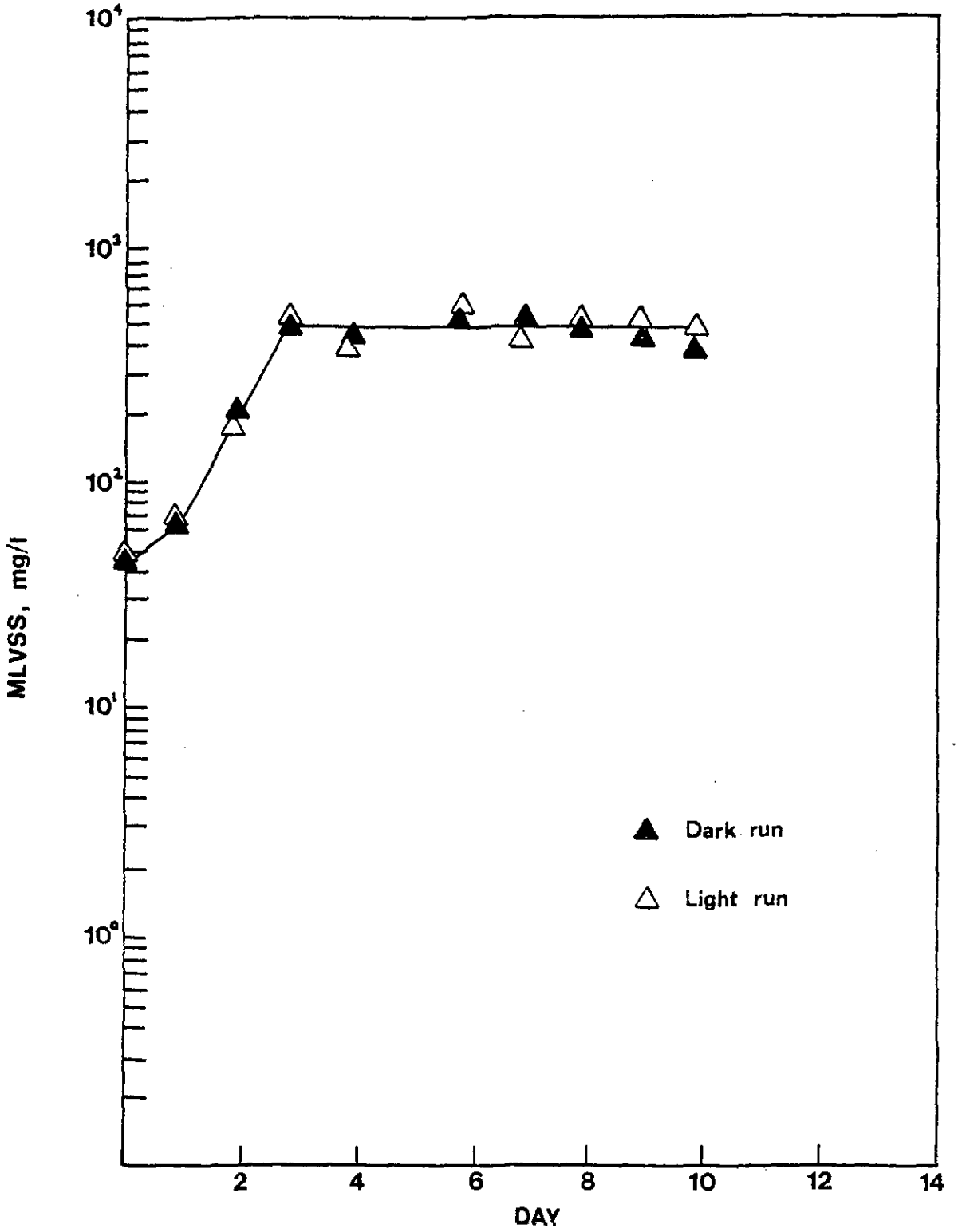

Figure 2. Biomass Curve for Experiment #1

level was limited only by the substrate concentration. The acclimation phase, from day 0 to day 1, did not obey the linear kinetics predicted by current theories regarding microbial growth curves. From day 1 to day 3, however, the logarithmic growth period ensued. From day 3 onward, the maximum standing crop had been attained, and values hovered at 500 mg/l MLVSS. This phase has been called the endogenous, or declining, growth phase. The phytoflagellate population increased from approximately 3×10 cells/ml to approximately 1×10 cells/ml by day 2.

Upon nutrient addition, carbon removal rates proceeded according to first-order kinetics. Given the necessary nutrients, the only limiting parameter was the substrate concentration, organic carbon. See Figure 3. Note that with nutrient addition, both light and dark flasks performed essentially identically. Both attained algal cell concentrations of approximately one million cells per milliliter. These algae were large and extremely motile. If the indigenous algae were photosynthetic to some degree, they reverted to a heterotrophic mode of nutrition with the nutrient addition. The only other explanation for the identical carbon removals in both light and dark runs would be that the algae were obligately photoautotrophic. If they were, then they would consume no organic matter while surviving in the light, and they would completely die off in the dark. Since algae were present in both flasks, this cannot be the explanation. The only conclusion to be made is that with complete nutrient addition, the phytoflagellate cells perform completely heterotrophically. On a practical note, the addition

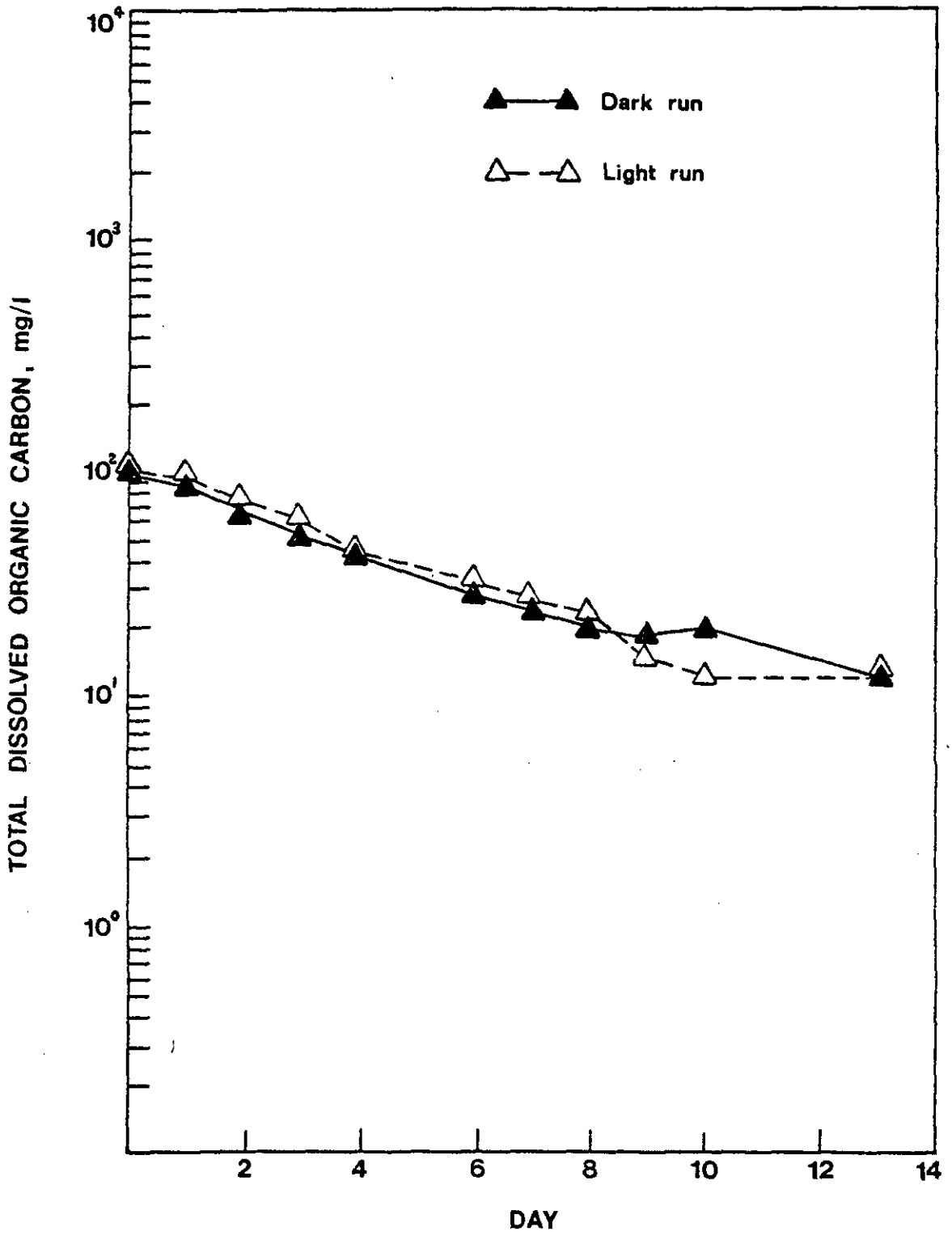

Figure 3. Substrate Removal Curve for Experiment #1.

of nutrients resulted in an extremely turbid leachate, as signified by the mixed liquor suspended solids. This is indicative of what would surely be a problem in clarification of the effluent. See Figure 4. A summary of the growth rate and substrate removal kinetics is presented in Table 3.

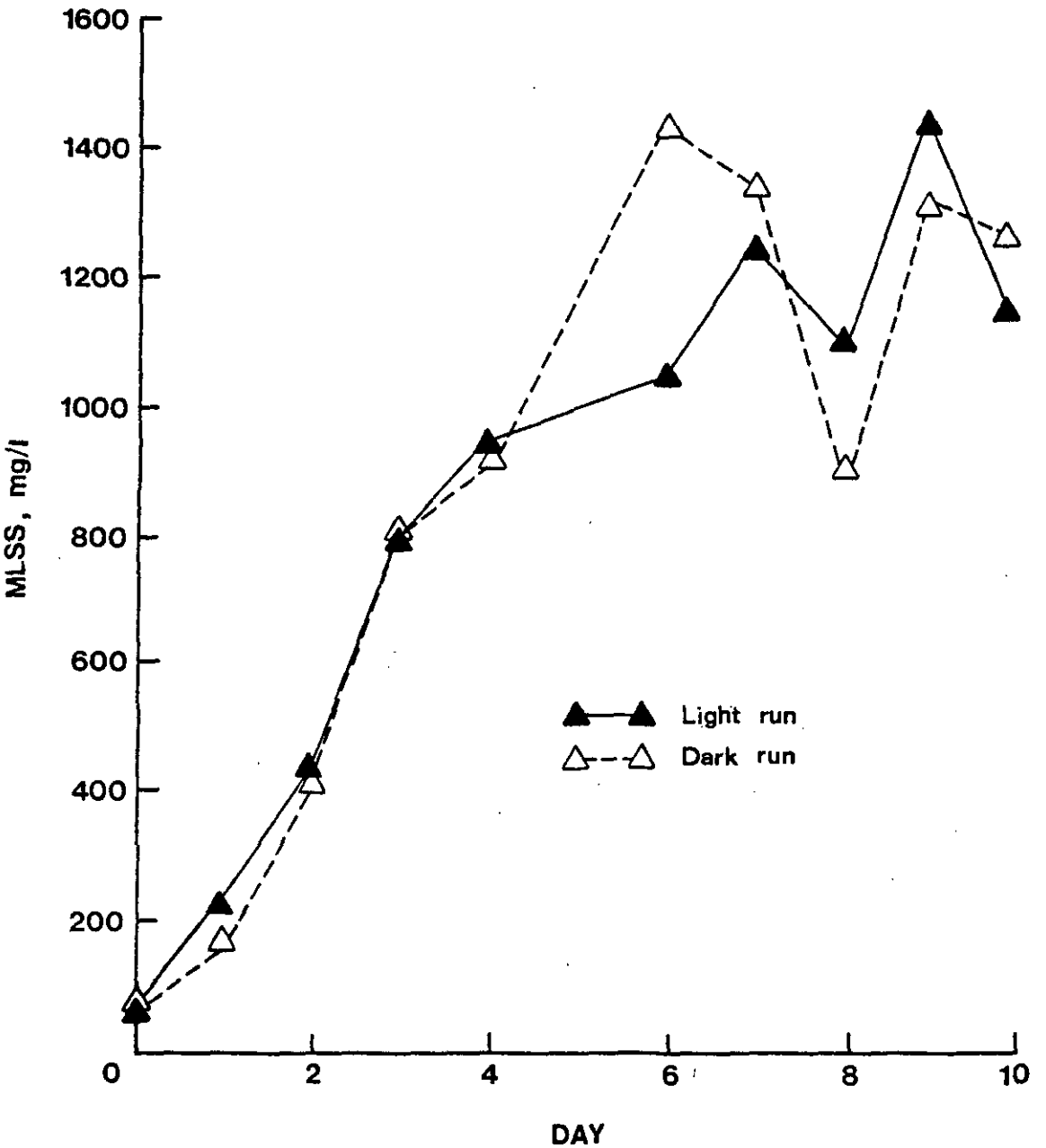

Figure 4. Suspended Solids Curve for Experiment #1.

TABLE 3. SUMMARY OF GROWTH RATE AND SUBSTRATE REMOVAL KINETICS AND CONSTANTS

Equation used to describe the first-order kinetics:

$$\frac{dX}{dt} = kX$$

Parameter X, in mg/l	Experimental k value
Total dissolved organic carbon	-0.2 per day
MLVSS	+1.0 per day

VI. LABORATORY EXPERIMENT #2

This experiment employed a method used by Bhatla and Gaudy[10] in their study of the importance of protozoa in BOD exertion. The broad-spectrum antibiotic chloramphenicol is a specific inhibitor of procaryotic protein synthesis. This acts as a bacteriostatic agent, preventing further growth of all bacteria. The die-off kinetics would depend upon the age distribution of the bacterial population, since it does not affect the fermentation or the respiration of glucose, but precludes replication.[10] Therefore, the chloramphenicol effect should have a lag time of a couple days, but would become more pronounced with time. A concentration of 10 mg/l chloramphenicol effectively prevents growth in many bacterial species, while this affects eucaryotic species only minimally.[10] Euglena is resistant to chloramphenicol at this level.[19] If these phytoflagellates are consuming organic matter, then they must be in competition with the indigenous bacteria for the available carbon. A comparison of algal population and carbon consumption will reveal if indeed there is a competition for the organic carbon substrate.

Another metabolic inhibitor used in this experiment prevents eucaryotic photosynthesis. Diuron, or DCMU, at an amount of 10^{-6}M was added to the leachate to determine the effect on the algal population, and subsequent performance in terms of carbon removals and biomass levels. This study did not use nutrient additions.

A grab sample of leachate was collected from a secondary Barre lagoon, and split into six 600-ml aliquots in 1000-ml beakers. Chloramphenicol was added to make 10 mg/l and DCMU to make 10^{-6}M. These runs

were monitored every other day for total organic carbon, MLSS, MLVSS, and phytoflagellate cells. See Table 4 for the initial characteristics of the leachate, Table 5 for an outline of experimental conditions and additives, and Figure 5 for the graphical results.

This leachate had an initial carbon concentration of 850 mg/l, and a large biomass and algal cell population. In the first seven days of this run, the algae demonstrated an adverse reaction to the laboratory environment. All dark flasks showed a pronounced dip in algal population. See Figure 5. The only one of these to recover to the initial algal population was the flask with the chloramphenicol addition. Of the light runs, the control and the DCMU addition showed a definite leap in the algal population, followed by a dramatic fall. The chloramphenicol addition to the light run showed a slight dip in algal population. The reason for the dramatic shifts in population densities can be explained by considering the species involved. The initial algal population consisted mainly of the Pyrobotrys. By the second day, all Pyrobotrys had disappeared from the dark flasks, but took longer to die off in the light flask. As a result of the death and lysis of the Pyrobotrys, an immense number of Vorticellids appeared. These stalked protozoans found a new ecological niche with the formation of the Pyrobotrys cell debris, but once this was consumed, they too disappeared.

Because the algal cell count data is amenable to statistical analysis, it can be proven that the algal population in the beakers with chloramphenicol addition was significantly higher than the algal population in

TABLE 4. CHARACTERISTICS OF LEACHATE USED IN EXPERIMENT #2

(Collected July 6, 1979, from Secondary Lagoon)

Parameter	Concentration in mg/l
Total dissolved carbon	850
Inorganic dissolved carbon	200
MLSS	585
MLVSS	470
pH	7.1
Ammonia nitrogen	127

(See also the data in Table 1, Lagoon monitoring data, under leachate of 7-6)

TABLE 5. SUMMARY OF EXPERIMENT #2 ADDITIONS AND CONDITIONS

Beaker	Lighting Conditions	Metabolic Inhibitor
1	light	chloramphenicol
2	dark	chloramphenicol
3	light	DCMU
4	dark	DCMU
5	light	none
6	dark	none

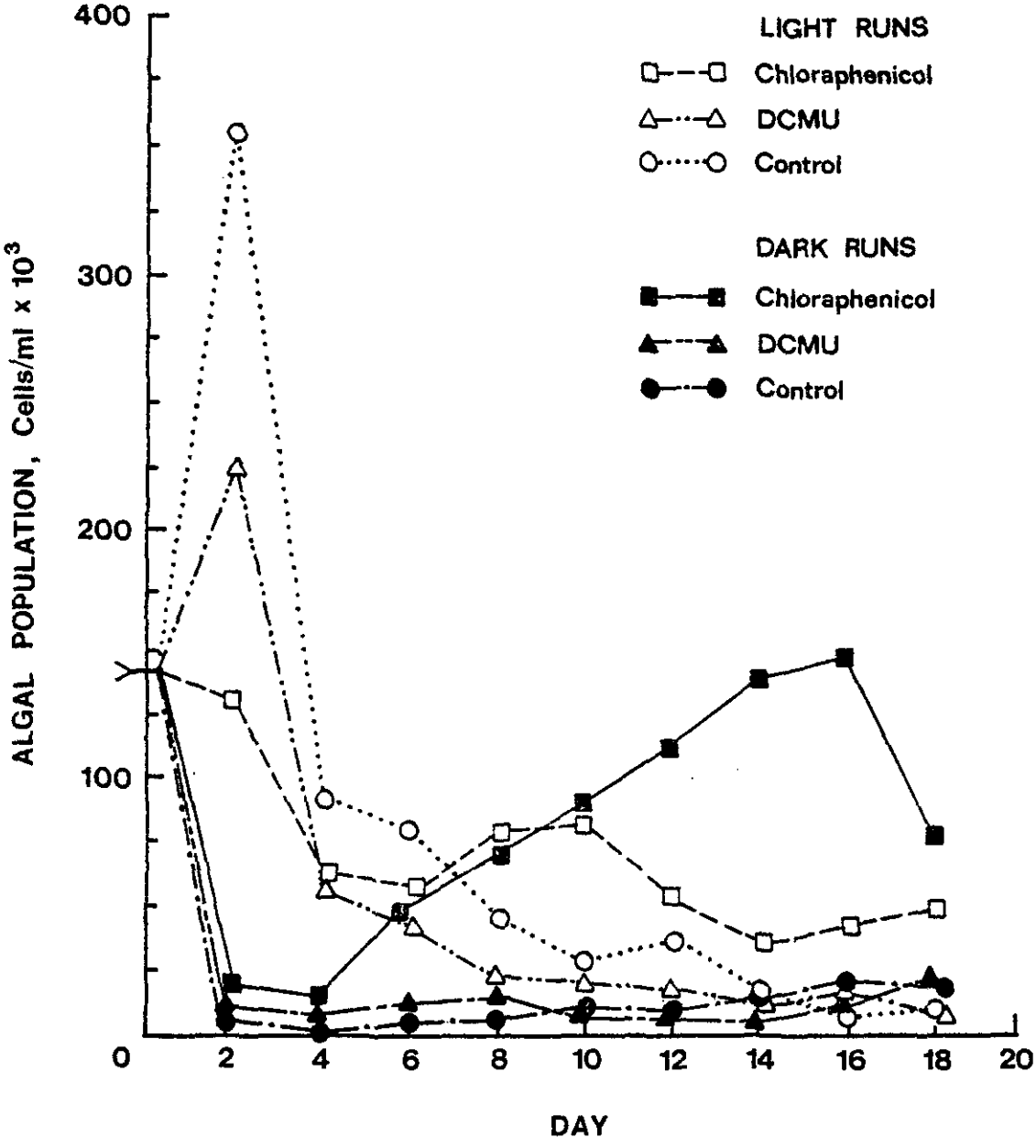

Figure 5. Algal Cell Counts for Experiment #2.

the controls. See Table 6. Classical oxidation pond theory is unable to explain this phenomenon, and, in fact, would predict the opposite. As the algal population flourished when the bacterial growth was prevented, there must exist a competition between the two populations for some nutrient or substrate. Note that this effect becomes more pronounced with time. During the first seven days, the dramatic population shifts and the mode of chloramphenicol action combined to obscure this phenomenon. Consistent with the theory of the chloramphenicol effect, the difference in algal population between the control and chloramphenicol flasks became more pronounced with time.

It can also be statistically proven that the beaker with a DCMU addition in the light maintained a significantly lower population of algae than did its respective control. See Table 7. The degree of inhibition was approximately 60%; the algal population in the light/DCMU flask was about 40% less than the light/control. Without supplemental nutrient addition, the indigenous phytoflagellate population did photosynthesize to some degree, but inhibiting the photosynthetic capabilities of the algae did not decimate the population. Extrapolating this data, the algae of this experiment appeared to obtain more than half of their nutrition from heterotrophic metabolism. In the dark, the DCMU and the control populations maintained essentially the same values. This is the logical expected result, as photosynthesis is impossible without light at any rate.

An interesting and unexpected result of this experiment was that, although the algal populations differed widely from flask to flask, the

TABLE 6. STATISTICAL ANALYSIS OF ALGAL CELL COUNTS: COMPARE POPULATIONS OF CHLORAMPHENICOL AND CONTROL FLASKS MAINTAINED IN THE LIGHT, EXPERIMENT #2

(Data in ten thousands of cells/milliliter)

	Beaker #1		Beaker #5			
	Chloramphenicol		Control			
Day	Mean	st.dev.	Mean	st.dev.	Sp	t
2	13.5	4.2	36.2	6.0	5.2	+13.1
4	6.5	3.3	9.3	5.1	4.3	+ 1.9
6	5.7	2.3	7.8	3.1	2.7	+ 2.3
8	8.2	2.4	4.5	1.6	2.0	- 5.6*
10	9.8	2.8	2.7	1.6	2.2	- 9.7*
12	5.7	1.4	4.1	1.9	1.6	- 2.9*
14	3.9	1.4	1.3	1.2	1.3	- 6.0*
16	3.9	1.6	0.5	0.6	1.1	- 9.3*
18	4.9	1.4	0.9	0.8	1.1	-10.9*

Null hypothesis: mean of chloramphenicol algal population is less than or equal to mean of control algal population.
Alternative: mean of chloramphenicol run is greater than that of the control.
Number of samples counted each time = 18 = N_1 = N_5
Degrees of freedom = df = $N_1 + N_5 - 2 = 34$

For 34 degrees of freedom, the t statistic for the 95% confidence level is ±1.697. Therefore, reject the null hypothesis if t less than -1.697.

*Indicates value is significant at the 99.5% level of confidence for rejection of the null hypothesis.

From day 8 and thereafter, reject the null hypothesis and conclude that there are significantly more algae in the chloramphenicol flask than in the control flask.

TABLE 7. STATISTICAL ANALYSIS OF ALGAL CELL COUNTS: COMPARE POPULATIONS OF DCMU AND CONTROL FLASKS MAINTAINED IN THE LIGHT, EXPERIMENT #2

(Data in ten thousands of cells/milliliter)

	Beaker #3		Beaker #5			
Day	DCMU Mean	st.dev.	Control Mean	st.dev.	Sp	t
4	6.3	2.5	9.3	5.1	3.8	-2.4*
6	4.3	1.9	7.8	3.1	2.5	-4.2*
8	2.7	1.6	4.5	1.6	1.6	-3.4*
10	2.2	0.7	2.7	1.6	1.2	-1.4
12	1.2	1.3	4.1	1.9	1.6	-5.4*
14	0.9	0.9	1.3	1.2	1.0	-1.1
16	1.1	1.0	0.5	0.6	0.8	+2.3
18	0.6	0.6	0.9	0.8	0.7	-1.2

*Indicates value is significant at the 97.5% level of confidence for rejection of the null hypothesis.
Null hypothesis: mean of the control algal population is less than or equal to the DCMU algal population.
Alternative: mean of control population is greater than the DCMU run.

On every day except day 16, the null hypothesis can be rejected with at least 80% confidence. From the beginning up until day 10, the control flask has significantly more algae than the DCMU flask.

biomass (as MLVSS) was relatively constant. This leads to the conclusion that though the biomass may be composed of various organisms in varying proportions, the absolute value of the MLVSS is dependent only upon the given leachate. A recommended future study would be to investigate the concentrations of carbon, phosphorus, nitrogen, and possibly other nutrients contained in various leachates, and correlate these to the existing biomass level of the leachate. With this information, one could predict the limiting nutrient of a particular leachate and recommend a given treatment process.

As stated before, the carbon removal kinetics are obscure and erratic in a non-nutrient study. Growth proceeds until the limiting nutrient or substrate is consumed, upon which the cells die and lyse, releasing the nutrients or substrates again, then growth ensues again, ad infinitum. Very slow and essentially identical carbon removal rates were observed.

VII. LABORATORY EXPERIMENT #3

This experiment combined the conditions of experiments #1 and #2. The bacteriostatic agent chloramphenicol was used in conjunction with various nutrients, in order to measure the effect on carbon removal rates. It was proven, in experiment #1, that the indigenous phytoflagellate population would behave in a completely heterotrophic manner if nutrients were supplied. In experiment #2, it was proven that the algae were indeed in competition with the bacterial population. This experiment will ascertain whether carbon removal rates by algae alone can approach those rates with significant bacterial populations. Another goal of this experiment is to determine if any one nutrient is more important than the others.

A grab sample of leachate was collected from a primary lagoon and split into nine 200-ml aliquots in 250-ml beakers. Nutrient additions of 250 mg/l $Ca(OH)_2$, 500 mg/l NH_4Cl, and 100 mg/l KH_2PO_4 were used. Chloramphenicol was added at 0.01 g/l. The analysis of this leachate is presented in Table 1, in the Lagoon Monitoring Data of the primary lagoon on 8-16-79. The experimental conditions are outlined in Table 8.

This leachate initially had a vigorous algal population with a moderate level of dissolved carbon. The addition of various nutrients did not cause a dramatic increase in biomass levels (see Table 9) in contrast to the results of experiment #1. Adding the nutrients resulted in a decrease in the number of algae that the leachate would support. See Figure 6. This substantiates the results of experiment #1 in the

TABLE 8. OUTLINE OF CONDITIONS AND ADDITIONS TO THE LEACHATE SAMPLES IN EXPERIMENT #3

Beaker	Conditions and Additions
#1	lime, phosphorus, nitrogen, light
#2	lime, phosphorus, nitrogen, light, chloramphenicol
#3	light control
#4	lime, phosphorus, nitrogen, dark
#5	lime, phosphorus, nitrogen, dark, chloramphenicol
#6	dark control
#7	nitrogen, light
#8	phosphorus, light
#9	lime, light

TABLE 9. BIOMASS LEVELS MAINTAINED IN LEACHATE SAMPLES, EXPERIMENT #3

	Biomass as MLVSS, mg/l	
Day	Beaker #1	Beaker #3
0	250	250
1	240	220
2	320	300
3	380	260
4	360	220
5	280	260
6	360	320
7	360	420
8	260	240
9	320	400
10	260	260

Beaker #1 had all nutrients added.
Beaker #3 had no nutrients added.

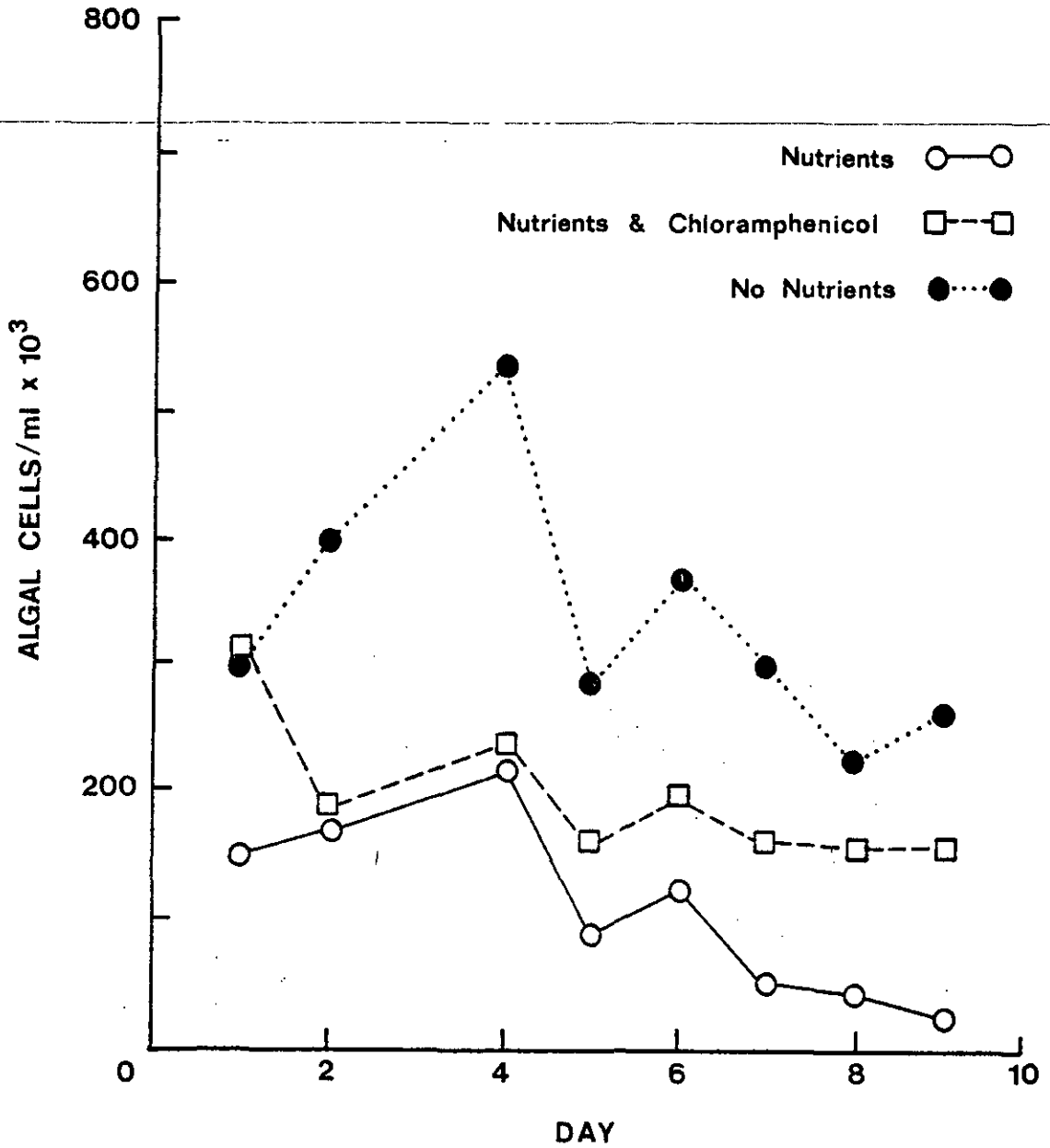

Figure 6. Algal Populations Maintained in Leachate Samples in the Light. Experiment #3.

conclusion that, given the necessary nutrients, the indigenous algae would revert to a heterotrophic mode of nutrition. A given leachate could logically support a larger autotrophic biomass than it could a heterotrophic one. Completely independent support of the results of experiment #2 is also depicted in Figure 6. In experiment #2, while nutrients were not added to any sample, algal populations flourished to a greater degree with the addition of chloramphenicol than without chloramphenicol. Now in experiment #3, the same phenomenon has repeated itself, when samples had the nutrient additions. See Table 10 for the statistical proof of this. Notice, however, in Figure 7, the carbon uptake rates are the same for both samples with and without chloramphenicol. In conclusion, with nutrient addition, the indigenous algae will revert to a heterotrophic mode of nutrition, to consume carbon at a rate identical to those populations with a significant percentage of bacteria present.

The effect of the various nutrients on carbon removal rates is presented in Figure 8. This figure shows that no nutrients represented the slowest removal, while all three nutrients considerably improved carbon uptake. The various nutrients added singly provide data intermediate to the two extremes. Although these data alone are not conclusive, it appears that phosphorus alone improved carbon removal more than nitrogen alone; and, in turn, nitrogen had more of an effect than did lime. Algal cell count data support this very contention. See Table 11 for statistical proof that the phosphorus beaker maintained a

TABLE 10. STATISTICAL ANALYSIS OF ALGAL CELL COUNTS: COMPARE POPULATIONS OF ALGAE IN FLASKS WITH AND WITHOUT CHLORAMPHENICOL, EXPERIMENT #3

(Data in ten thousands of cells/milliliter)

	Beaker #2			Beaker #1						
Day	X	SD	N	X	SD	N	Sp	df	t	**
1	21.5	8.7	6	14.7	4.1	15	5.6	19	- 2.5	97.5
2	18.4	9.3	11	15.0	4.1	16	6.7	25	- 1.3	90.0
4	25.0	4.2	2	10.8	3.1	18	3.2	18	- 5.9	99.5
5	16.4	4.1	18	8.9	2.4	18	3.4	34	- 6.6	99.5
6	20.1	5.2	18	12.1	1.7	18	3.8	34	- 6.3	99.5
7	17.3	4.4	18	5.3	1.0	18	3.2	34	-11.3	99.5
8	16.5	4.1	18	4.6	2.0	18	3.2	34	-11.2	99.5
9	16.7	5.5	18	2.1	1.3	18	4.0	34	-11.0	99.6

**Last column indicates the level of confidence for rejecting the null hypothesis.

Null hypothesis: mean of algal population in chloramphenicol is less than or equal to mean of population without chloramphenicol.
Alternative hypothesis: mean of algal population in chloramphenicol is greater than mean of algal population without chloramphenicol.

Conclusion: reject the null hypothesis. The number of algae maintained in the chloramphenicol flask was significantly greater than the number of algae without the chloramphenicol addition.

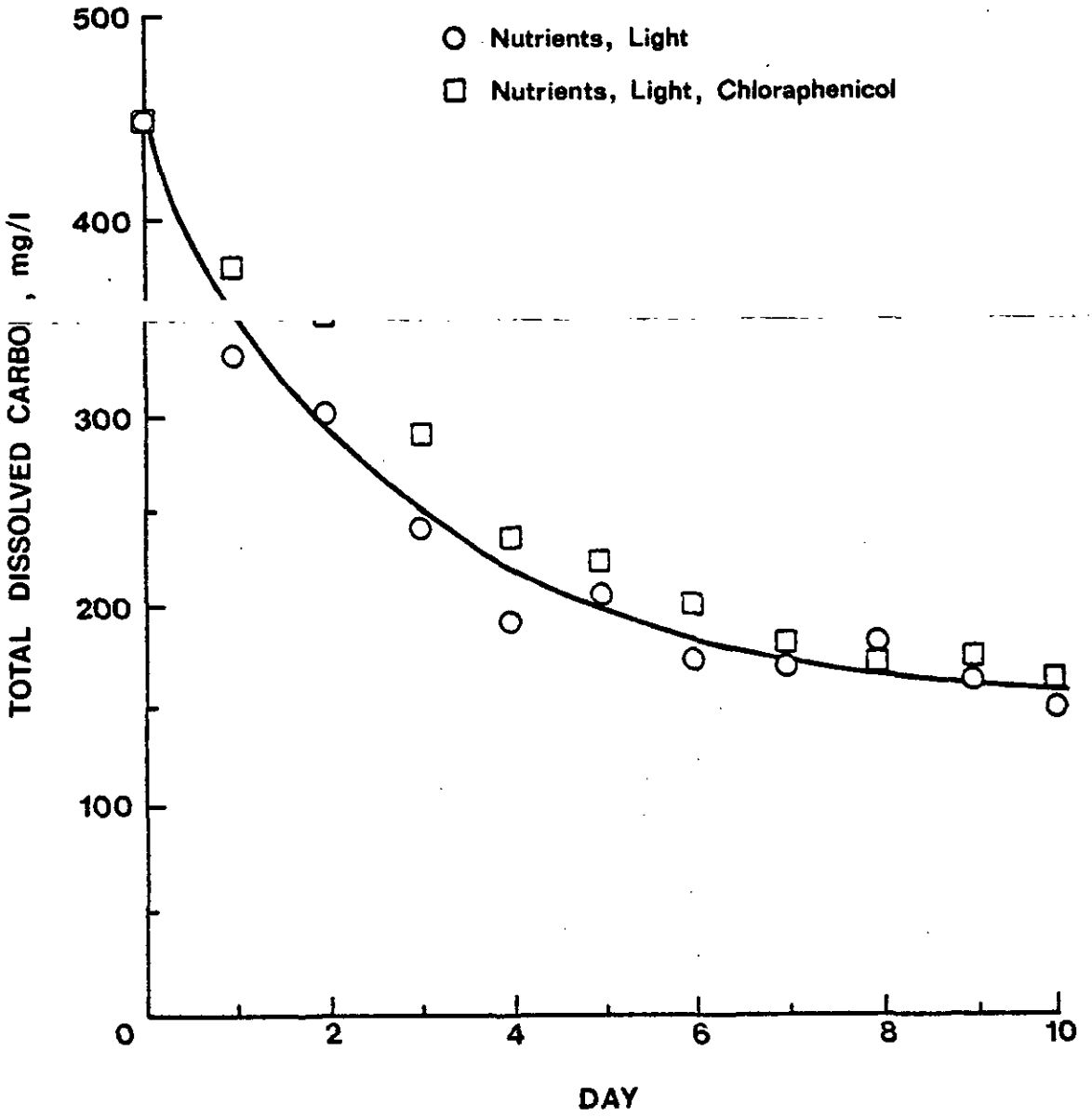

Figure 7. The Effect of Chloramphenicol on Carbon Removal Rates in Samples with Nutrient Addition.

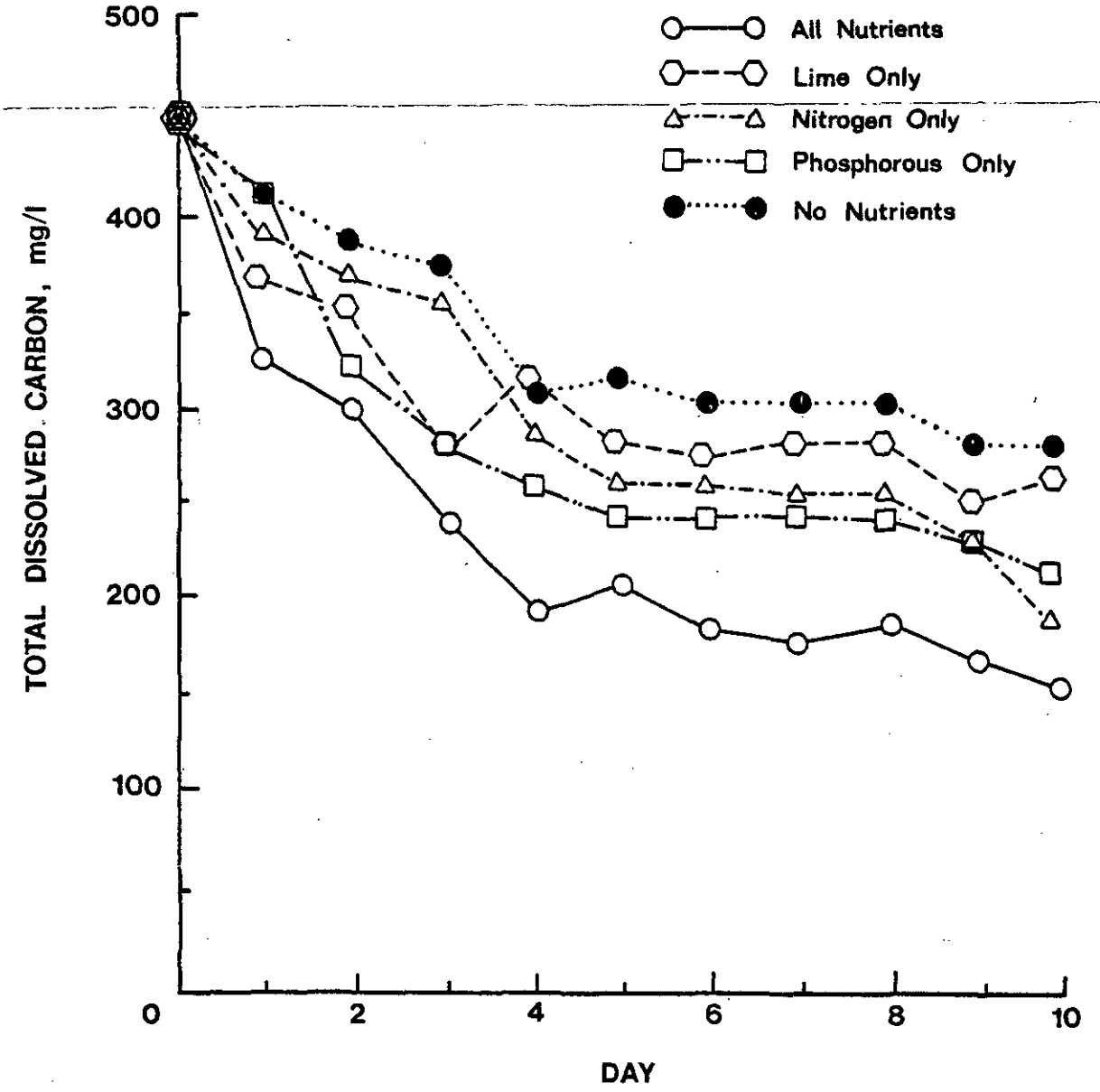

Figure 8. Effect of Various Nutrients on Carbon Removals of Samples Maintained in the Light.

TABLE 11. STATISTICAL ANALYSIS OF ALGAL CELL COUNTS: COMPARE EFFECT OF VARIOUS NUTRIENTS ON THE NUMBER OF ALGAE MAINTAINED IN LEACHATE SAMPLES, EXPERIMENT #3

(Data in ten thousands of cells/milliliter)

PART A. COMPARE NITROGEN AND LIME

	Nitrogen Beaker #7			Lime Beaker #9						
Day	$\bar{x}$	SD	N	$\bar{x}$	SD	N	Sp	df	t	**
1	36.0	7.1	2	13.7	8.0	14	7.9	14	- 3.7	99.5
2	17.8	5.0	9	13.8	3.0	8	4.2	15	- 1.9	95.0
4	14.6	3.4	18	13.7	3.3	18	3.4	34	- 0.8	70.0
5	12.0	3.8	18	13.4	2.8	18	3.3	34	+ 1.3	0.0
6	9.6	4.5	18	8.4	3.6	18	4.1	34	- 0.9	80.0
7	9.7	3.0	18	4.8	1.3	18	2.3	34	- 6.4	99.5
8	9.7	1.7	18	1.7	1.2	18	1.5	34	-16.0	99.5
9	5.8	1.6	18	0.5	0.6	18	1.2	34	-13.3	99.5

**Last column indicates the level of confidence for rejecting the null hypothesis.

Null hypothesis: mean of algal population in the nitrogen flask is less than or equal to the mean of the algal population in the lime flask. Alternative hypothesis: mean of algal population in the nitrogen flask is greater than that in the lime flask.

Conclusion: for the majority of the time, the null hypothesis can be rejected. The mean of the algal population in the nitrogen flask is significantly greater than the mean of the algal population in the lime flask.

TABLE 11. STATISTICAL ANALYSIS OF ALGAL CELL COUNTS: COMPARE EFFECT OF VARIOUS NUTRIENTS ON THE NUMBER OF ALGAE MAINTAINED IN LEACHATE SAMPLES, EXPERIMENT #3

(Data in ten thousands of cells/milliliter)

PART B. COMPARE PHOSPHORUS AND NITROGEN

	Nitrogen Beaker #7			Phosphorus Beaker #8						
Day	X	SD	N	X	SD	N	Sp	df	t	**
1	36.0	7.1	2	51.7	25.5	3	21.2	3	-0.8	70.0
2	17.8	5.0	9	48.0	4.2	2	4.9	9	-7.9	99.5
4	14.6	3.4	18	23.9	5.2	18	4.4	34	-6.3	99.5
5	12.0	3.8	18	30.2	15.3	18	11.0	34	-5.0	99.5
6	9.6	4.5	18	32.0	8.5	2	4.8	18	-6.3	99.5
7	9.7	3.0	18	17.1	4.5	9	3.5	25	-5.2	99.5
8	9.7	1.7	18	13.6	4.1	18	3.1	34	-3.8	99.5
9	5.8	1.6	18	13.3	3.4	18	2.7	34	-8.3	99.5

**Last column indicates the level of confidence for rejecting the null hypothesis.

Null hypothesis: mean of algal population in the phosphorus flask is less than or equal to the mean of the algal population in the nitrogen flask. Alternative hypothesis: mean of algal population in the phosphorus flask is greater than the mean in the nitrogen flask.

Conclusion: reject the null hypothesis. The number of algae maintained in the phosphorus flask was significantly greater than the number of algae maintained in the nitrogen flask.

larger algal population than did the nitrogen, and likewise the nitrogen beaker maintained a larger algal population than did the lime.

Presented in Figure 9 is unequivocal proof that the very high suspended solids values that occur with nutrient addition are caused by the lime.

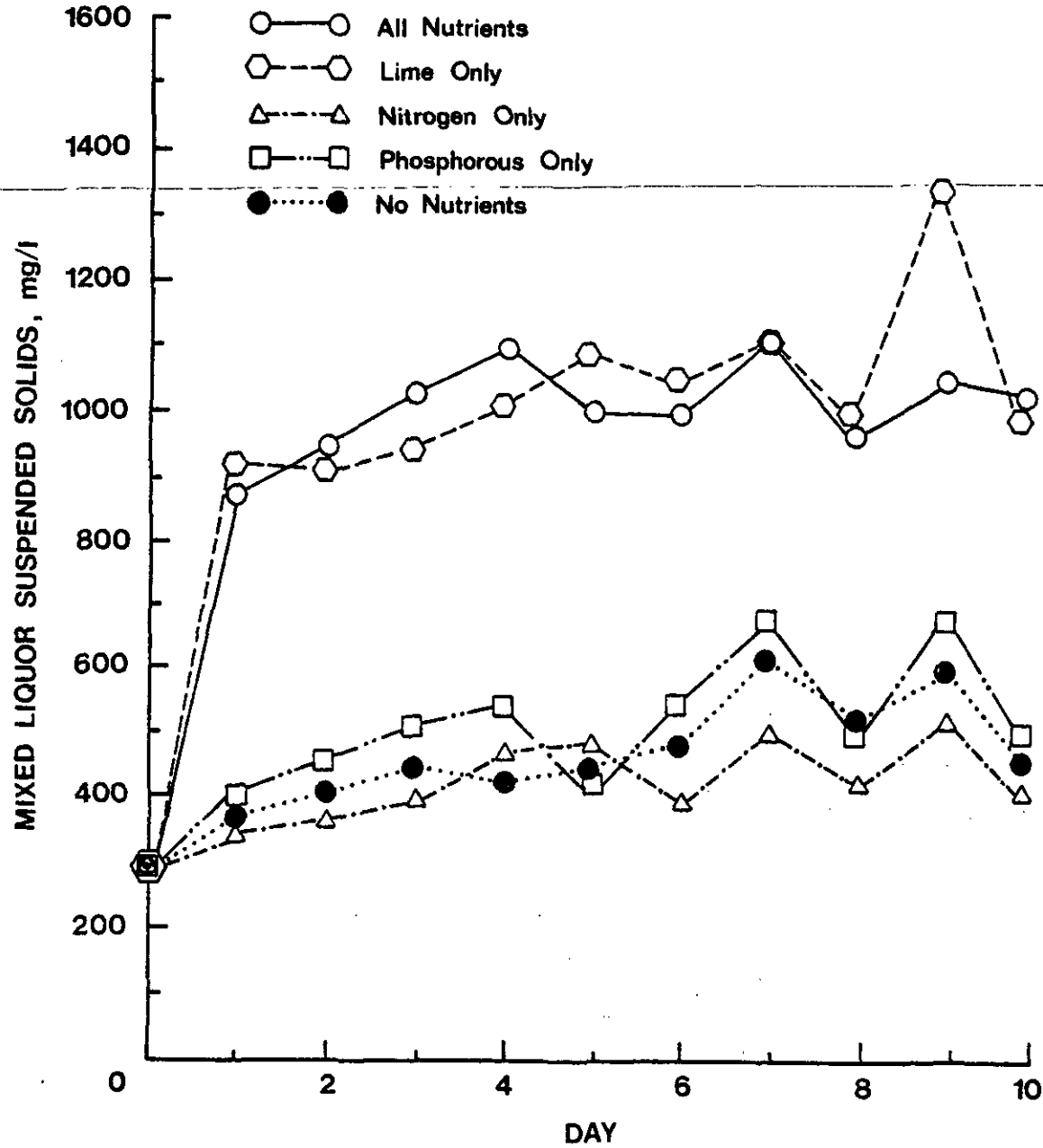

Figure 9. Effect of Various Nutrients on Suspended Solids Values of Samples Maintained in the Light.

VIII. SUMMARY

Experiment #1 revealed that the addition of nutrients resulted in a significant increase in biomass and dissolved carbon removal proceeded rapidly. This approach resulted in very high suspended solids values, however; and this would surely be a major problem at the treatment plant level. The indigenous phytoflagellate population assumes a completely heterotrophic mode of nutrition with the addition of these nutrients.

Experiment #2 was a non-nutrient study, with biomass levels maintaining approximately the initial values and dissolved carbon removal rates proceeding slowly in comparison to the nutrient studies. Because the phytoflagellate populations increased with the addition of chloramphenicol to numbers even greater than the control, it is apparent that the algal and bacterial populations are in competition for some substrate. This phenomenon cannot be explained by classical oxidation pond theory. Another result that is indicative of the heterotrophic nature of the algae are the biomass levels that the leachate maintains. All MLVSS's hover around the same value, indicating that the leachate will support a given heterotrophic biomass, regardless of whether the biomass is mostly algal or mostly bacterial. The inhibitor of photosynthesis, DCMU, caused the phytoflagellate population to decrease to approximately 60% of the control population. This data can be extrapolated to indicate that, without nutrient addition, the algal flagellates photosynthesize to such a degree that they obtain 40% of their nutrition from that process. Thus, protozoan-like algae that survive in the leachate in such large numbers are able to do so because they have available to them

two modes of nutrition. Lack of nutrients in the leachate lagoons forces them to the auxiliary metabolism of photosynthesis.

Experiment #3 studied the effect of both nutrients and chloramphenicol. Experiment #2 proved that the addition of chloramphenicol caused a comparative increase in the algal population relative to a control. However, in a non-nutrient study, carbon removal rates are slow and erratic. In this next study, with nutrient addition, the carbon uptake rates were identical from chloramphenicol flask to the control. Therefore, the indigenous phytoflagellate population of the leachate lagoons is indeed an effective competitor for the carbon. Another result of this study which was not shown in either experiment #1 or #2 is that, in some situations, the nutrient addition will cause the algae to revert to a heterotrophic mode, and therefore, decrease the algal population. When a healthy autotrophic population is well established, the addition of nutrients may only cause a decrease in absolute numbers of the population. It is possible that adding the nutrients is a chemical signal to the biomass to shift to a heterotrophic mode; and while the autotrophic biomass that a given leachate could support is virtually limitless, the heterotrophic biomass that a leachate can support is fixed and bounded. Therefore, in experiment #3 the sample with no nutrients added maintained a larger algal population than those samples with nutrients added.

Another facet of the third experiment is the comparative nutrient study. It was found that lime, nitrogen, and phosphorus, in that order, had increasing effects on both carbon removal rates and the number of

algal cells maintained in the leachate. Although none of the nutrients singly had as much effect as all of them added in conjunction, it seems reasonable that phosphorus is the most limiting nutrient in this leachate. Nitrogen, however, is important also, although it is probably present in the leachate in greater amounts than is phosphorus. Unless it can be proven otherwise for another situation, lime is not recommended as a treatment additive. The disadvantage of the excessive alkalinity far outweighs the little effect it has on carbon removals and algal populations.

This work has attempted to prove that the algae indigenous to sanitary landfill leachate lagoons at Barre, MA, are indeed effective competitors against the bacteria for the soluble organics. These algae will perform either auto- or heterotrophically depending on nutrient conditions. In future considerations of biological treatment of organic wastes that are non-conventional, the role of algae should not be assumed to be completely autotrophic in nature.

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X. APPENDIX

A. Equations that disregard the possible presence of breakdown products.

1. Chlorophyll a concentration in micrograms/milliliter =

$$(12.7(A_{663}) - 2.7(A_{645}))\left(\frac{\text{volume extract}}{\text{volume sample}}\right)$$

2. Chlorophyll b concentration in micrograms/milliliter =

$$(22.9(A_{645}) - 4.7(A_{663}))\left(\frac{\text{volume extract}}{\text{volume sample}}\right)$$

*3. Chlorophylls a and b, in micrograms/milliliter =

$$(20.2(A_{645}) + 8.02(A_{663}))\left(\frac{\text{volume extract}}{\text{volume sample}}\right)$$

4. Carotenoids, in micrograms/milliliter =

$$(4(A_{475}))\left(\frac{\text{volume extract}}{\text{volume sample}}\right)$$

B. Equations that consider the presence of breakdown products.
**(Subscript b is before acidification, a is after.)

1. Active chlorophyll a concentration, micrograms/milliliter =

$$(26.73(A_{663b} - A_{663a}))\left(\frac{\text{volume extract}}{\text{volume sample}}\right)^{**}$$

2. Acid factor, unitless ratio

$$(A_{663b})/(A_{663a})$$

3. Equation from regression analysis of data of Lorenzen[16]

Percent active chlorophyll = 148(acid factor) - 150.3

4. Amount of active chlorophyll =

(percent active chlorophyll) x (amount of chlorophylls a and b*) from equation A-3 above.

5. Molar ratio of active chlorophyll to carotenoids =

$$\left(\frac{\text{amount of active chlorophyll/liter}}{\text{amount of carotenoids/liter}} \right) \times (0.5834)$$