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THE INFLUENCE OF ORGANIC DECOMPOSITION ON CARBON DIOXIDE, HYDROGEN SULFIDE, DISSOLVED OXYGEN, AND ALGAE GROWTH IN THE DWORSHAK RESERVOIR

by

A. J. Lingg
Department of Bacteriology and Biochemistry
College of Agriculture

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John S. Gladwell, Director

FOREWORD

The Water Resources Research Institute has provided the administrative coordination for this study and organized the interdisciplinary team that conducted the investigation. It is the Institute's policy to make available the results of significant water related research conducted in Idaho's universities and colleges. The Institute neither endorses nor rejects the findings of the author. It does recommend careful consideration of the accumulated facts by those who are assuredly going to continue to investigate this important field.

ACKNOWLEDGMENTS

I wish to extend my gratitude to graduate students, Mr. Robert L. Platt and Mr. Stephen C. Hern, for their able assistance in this project. I also wish to thank Dr. C.M. Gilmour, Dr. C.M. Falter and Dr. F.W. Rabe for reviewing the manuscript.

ABSTRACT

In order to meet the increased demands on our domestic, industrial and recreational water supply, it is necessary to maintain, and in many cases to upgrade, the quality of our water resources. In order to accomplish these tasks it is paramount to fully understand those factors, natural and artificial, which lead to accelerated deterioration of our rivers, lakes and streams. Rampant eutrophication leading to massive algal blooms, anaerobic conditions and other undesirable qualities have been rapidly increasing for the last century.

This project was designed to explore the role played by carbon and its subsequent use by bacteria on various chemical and biological parameters in Dworshak reservoir. Carbon contribution to the reservoir is primarily from the existing organic material present on the preimpoundment floor. This study defines methodology enabling prediction of the levels of carbon in a reservoir prior to impoundment and explores some of the effects of that carbon. In addition, these methods can be used to determine the amount of leached carbon, readily usable, by heterotrophic bacteria.

The effect of carbon utilization by bacteria on algal growth is also explored. In the laboratory this symbiotic effect leads to significant increases in algal biomass even at carbon levels as low as 1.3 ppm. The

implication of this observation is that carbon can greatly stimulate algal growth rates and consequently algal blooms if made available by bacterial respiration.

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INTRODUCTION

Demands on the domestic, industrial and recreational water supply in the United States continue to increase. In order to meet the increased demand it is necessary to maintain the quality of our water supplies at the highest possible level. This can only be accomplished if we have a clear and precise understanding of the biological systems that operate in aquatic environments.

Water quality evaluation has been traditionally based on a variety of physical, chemical and biological parameters such as dissolved oxygen, hydrogen sulfide content, phosphorus and nitrogen concentrations, algal bloom incidence, ability to support aquatic animals and insects, etc. Each of these parameters is, however, related to and connected with others in that they do not operate independently, e.g. bacteria solubilize organic materials and minerals which are then utilized by algae. The algae are fed upon by zooplankton which in turn are eaten by fish and so on. It is, therefore, obvious that a break in this chain or an increased input will offset the stable relationship that is found in good quality water.

One of the parameters involved in this chain is carbon. Organic carbon leached from litter, shrubs, trees, etc., present on the impoundment floor and in drainage basin runoff is oxidized by bacteria. As a

result of oxidation, carbon dioxide will be produced and dissolved oxygen is utilized. The carbon dioxide can then stimulate algal growth, contributing to algal blooms which cause further oxygen depletion through decomposition. Depletion of oxygen below certain levels causes death of fish and other aquatic life. In addition, oxygen depletion enables other bacteria to produce hydrogen sulfide which exerts a direct toxicity on aquatic plants and animals. Initiation of this chain of events depends on sufficient organic input to the aquatic system.

This project was designed to determine the role played by usable carbon in the sequence of events as outlined and the effect of this carbon on the overall water quality of Dworshak reservoir.

Dworshak reservoir is a new reservoir created by a dam across the North Fork of the Clearwater River. The dam, 710 feet high, is located 1.9 river miles above the confluence of the North Fork and the mainstream of the Clearwater River in Clearwater County near Ahsahka, Idaho (Fig. 1).

The reservoir at full pool is 53.6 miles long, has a surface area of 17,090 acres and is fed by a drainage area of 2440 square miles. As can be seen from the above specifications the reservoir is long, narrow and deep. The North Fork drainage area as well as the reservoir itself lies in a sparsely populated area and, except for logging, is essentially free

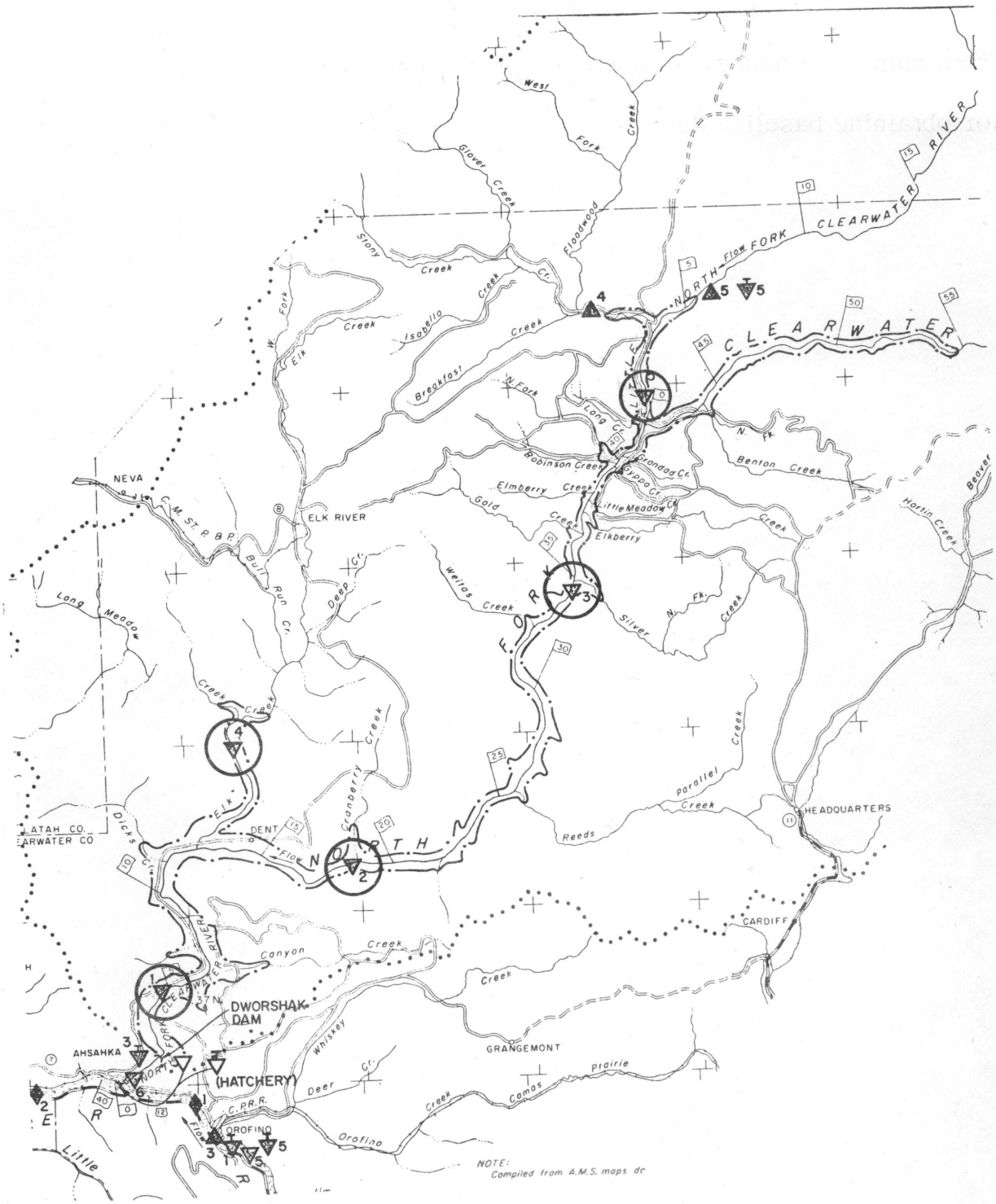

Fig. 1. Location of Dworshak reservoir and sampling sites. Sampling sites used are circled. River miles in flags.

from human influence. Consequently, it provides a unique study laboratory for obtaining baseline data in impoundments of this type.

OBJECTIVES

1. To quantitate the cyclic carbon turnover process in Dworshak reservoir.
   a. To determine water soluble carbon levels on reservoir floor prior to impoundment.
   b. To monitor the soluble carbon levels during filling and after peak reservoir levels have been attained.
2. To relate the accumulation of carbon to microbial activity.
3. To relate the obtained carbon values to potential algae bloom problems (feedback effect).
4. To evaluate the potential for hydrogen sulfide production and correlate that potential to available soluble carbon levels.

MATERIALS AND METHODS

Collection of Forest Litter Samples

Prior to impoundment in September of 1971 aerial photographs of the impoundment area were divided into 104 plots of 170 acres each using a polar planimeter. Three sampling sites in each plot were randomly selected and litter samples were collected as near to the selected sites as possible. Forest litter and existing shrubs, forbs, and grasses above the boundary of the mineral soil were collected from measured sample areas. Due to terrain some sample sites had to be adjusted and some samples were not taken because of inaccessibility. In these cases alternate samples were made as near as possible to the randomly selected site. Size of each sample in square feet was recorded.

Collection of Water Samples

Water samples were collected from 5 locations (Fig. 1) on the reservoir at the surface, mid-depth, and the bottom using a Kemmerer water sampler. Samples were taken at site 1 every 2 weeks from May through November. Other sites were sampled monthly during the same period.

Usable Carbon Determinations

Litter samples collected from the impounded floor were air dried and ground through a 20 mesh screen in a Wiley mill. Ten g portions of these samples were hydrated to 60% of moisture holding capacity and

placed in a respirometer cabinet (Fig. 2). The cabinet is a mechanism designed to allow collection of biological carbon dioxide (CO_2) from large numbers of samples under temperature controlled conditions. Basically, air scrubbed free of CO_2 by passage through alkali is passed through manifolds connected to the respiration flasks. The scrubbed air sweeps the CO_2 into individual tubes of 2N sodium hydroxide (NaOH) where it is trapped. Carbon dioxide in the base is then titrated between a pH of 8. 35 and 4. 6 by the classical double titration method using a Beckman automatic titrimator. Carbon dioxide produced from the decomposition of the samples was collected at various time intervals depending on the activity of the samples. Similar litter samples were extracted 5 times with cold water in a reciprocating shaker for 1 hour. The supernatant fluid from each extraction was collected by filtration through Whatman No. 1 filter paper and all were pooled after the extraction. The extracts as well as the solid litter samples were analyzed for usable carbon (persulfate oxidizable carbon - POC) by persulfate oxidation according to the procedure of Osborn and Werkman (1932) as modified by Katz et al. (1954) and Burgess et al. (1961). In this procedure, the samples (0. 1 g solids or the extract) are introduced into 250 ml flasks, diluted to 50 ml with CO_2 free water, and acidified with 1 ml of 5M H_2SO_4 to eliminate any inorganic carbon in the sample. The sample is then treated with 2 ml of 4% silver nitrate ($AgNO_3$) to precipitate any chlorides which interfere with the oxidation. Two g of potassium persulfate ($K_2S_2O_8$) are

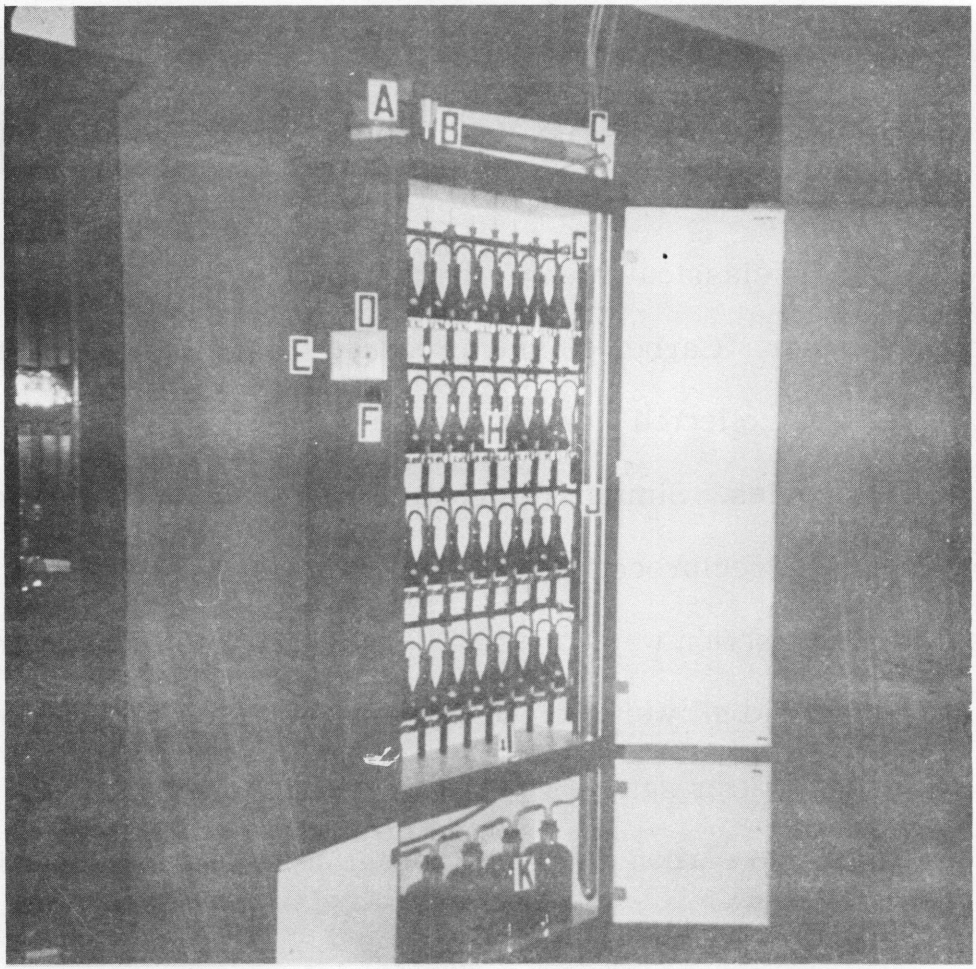

Fig. 2. Respiration cabinet. A - Fan, B - pilot light, C - cold water inlet, D, E, F - Thermostat and controls, G - manifold, H - Respiration flasks, I - CO_2 trap, J - Heating and cooling radiator, K - scrubbers.

added and the flask is tightly stoppered. Suspended from the stopper is a CO_2 trap consisting of a glass vial containing 4 ml of 5N NaOH (Fig. 3). Immediately after stoppering, the flask is evacuated by means of a water aspirator and hypodermic needle through a port in the stopper. The oxidation is completed at 70 C for 2 hours, cooled and CO_2 titrated as described. All titration values are expressed in mg carbon as CO_2.

Usable carbon (POC) in the water samples was determined by the method as described above except that 500 ml samples of water were used. Consequently, all other parameters in the test were expanded accordingly.

Bacterial Counts

Bacterial numbers in the reservoir were determined using the standard plate count on trypticase soy agar.

Algal Assay

Chlorella pyrenoidosa No. 26 was grown in 600 ml of carbon free synthetic algal medium (Sheridan, 1971) containing an excess of nutrients (Table 1), both in the presence of heterotrophic flora of Dworshak reservoir and in bacteria free cultures. Various amounts of sterile glucose (2, 4, 6, 8, 16, 32, 64 mg) were added to these cultures on a daily basis. Bacteria free cultures were grown in the same manner with carbon equivalent of bicarbonate added instead of glucose. Cultures were incubated under

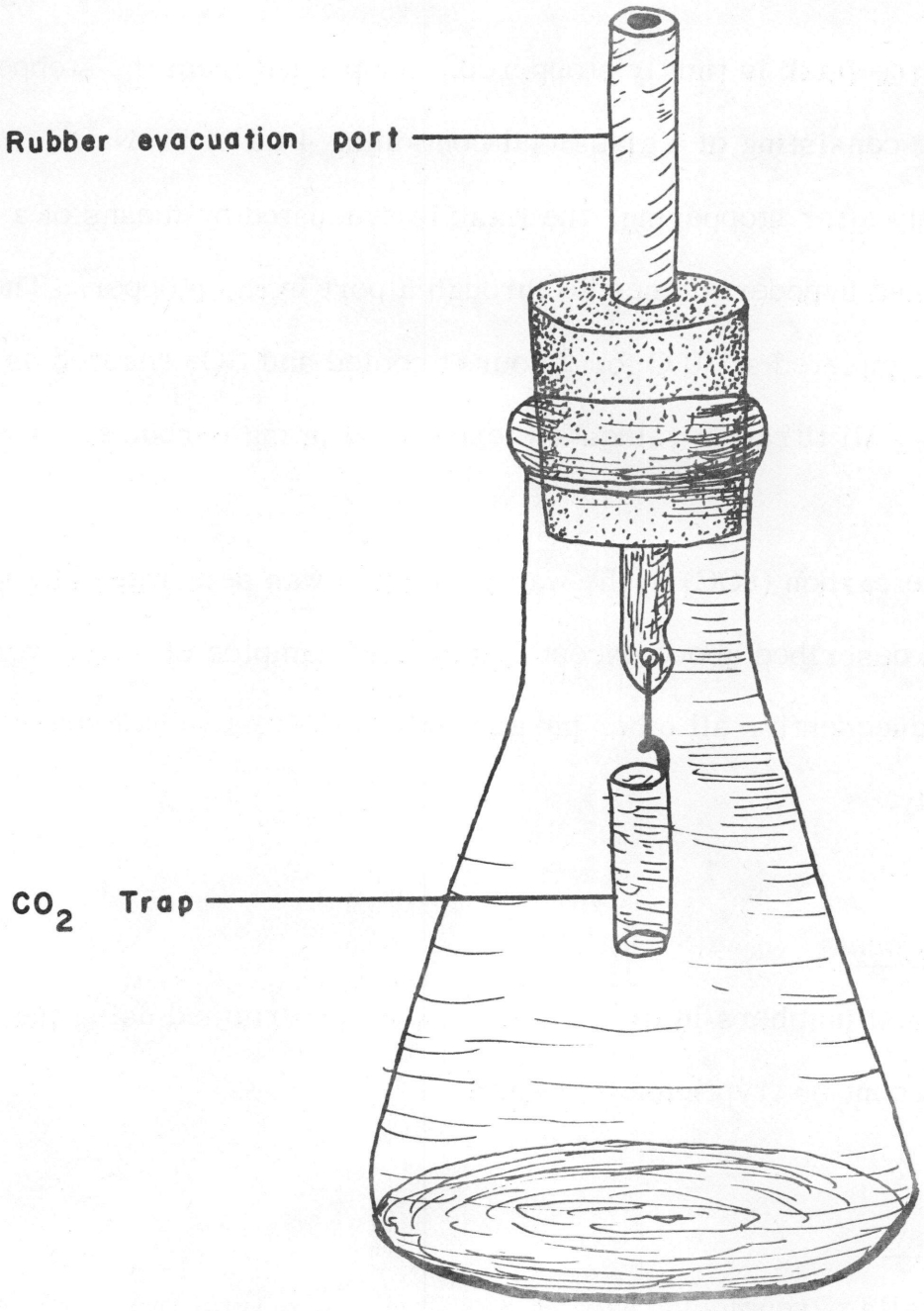

Fig. 3. POC Flask and trap. Evacuation is accomplished by insertion of a hypodermic needle into the rubber tube connected to the glass tube in the stopper. The end of the rubber tubing is sealed.

continuous flourescent lighting (approximately 1000 ft. candles) at 25 C for 10 days. Biomass was determined gravimetrically on 0. 45 um millipore filters and daily bacterial counts were made using trypticase soy agar. Both open and closed systems were used. Open systems consisted of 1 l cotton stoppered flasks. Closed systems were plugged with one-holed rubber stoppers containing an injection and sampling port consisting of a serum cap on a glass tube inserted through the rubber stopper. All flasks were vigorously agitated by magnetic stirrers.

Table 1. Composition of medium used for algal growth study.

Nitrilotriacetic Acid*	1. 000 g
Trace Element Solution**	5. 000 ml
$Fe\ Cl_3 \cdot 6H_2O$	1. 870 g
$CaSO_4 \cdot 2H_2O$	0. 600 g
$MgSO_4 \cdot 7H_2O$	0. 988 g
NaCl	0. 077 g
KNO_3	1. 032 g
$NaNO_3$	6. 888 g
$Na_2H\ PO_4$	1. 111 g
Distilled H_2O	10. 000 l

*Ingredients are added to water in order listed - each being allowed to dissolve prior to adding next. Medium was then autoclaved at 121 C for 15 minutes.

**Formula for trace element solution

Conc. HCl	3.000 ml
$MnCl_2 \cdot 4H_2O$	2.000 g
$Zn(NO_3)_2 \cdot 6H_2O$	0.500 g
H_3BO_3	0.025 g
$CuCl_2 \cdot 2H_2O$	0.025 g
$Na_2MoO_4 \cdot 2H_2O$	0.025 g
$CoCl_2 \cdot 6H_2O$	0.025 g
$V_2O_4(SO_4)_3 \cdot 16H_2O$	0.025 g
Distilled H_2O	1.000 l

RESULTS AND DISCUSSION

The role of soluble organic material in forest litter decomposition has long been recognized. In 1928 Waksman estimated that 3 to 22% of forest litter material could be removed by water extraction. Stoklass in 1912 was able to show that production of CO_2 was proportional to available carbon and not total carbon. Broadfoot and Pierre (1939) showed that a high correlation existed between water soluble organic matter and decomposition during the early stages of humification of forest litter. Other workers have since recognized the role of carbon solubilization in the role of decomposition since ultimately all high carbon moieties must be broken down to usable entities (solubilized) in order to provide energy for microorganisms. This is accomplished primarily through production of extracellular enzymes.

The role of extractable carbon can best be shown by typical respiration data (Fig. 4). Figure 4 is a graph of the CO_2 evolution of one of the litter samples used in this study. The sample was washed several times in cold water, placed in a respirometer cabinet and CO_2 evolution was compared to an unwashed portion of the same sample. Shown is both an interval plot and a cumulative plot. The initial flush of activity is obviously due to extractable carbon. Figure 5 is a plot of a different sample which has been treated in the same manner.

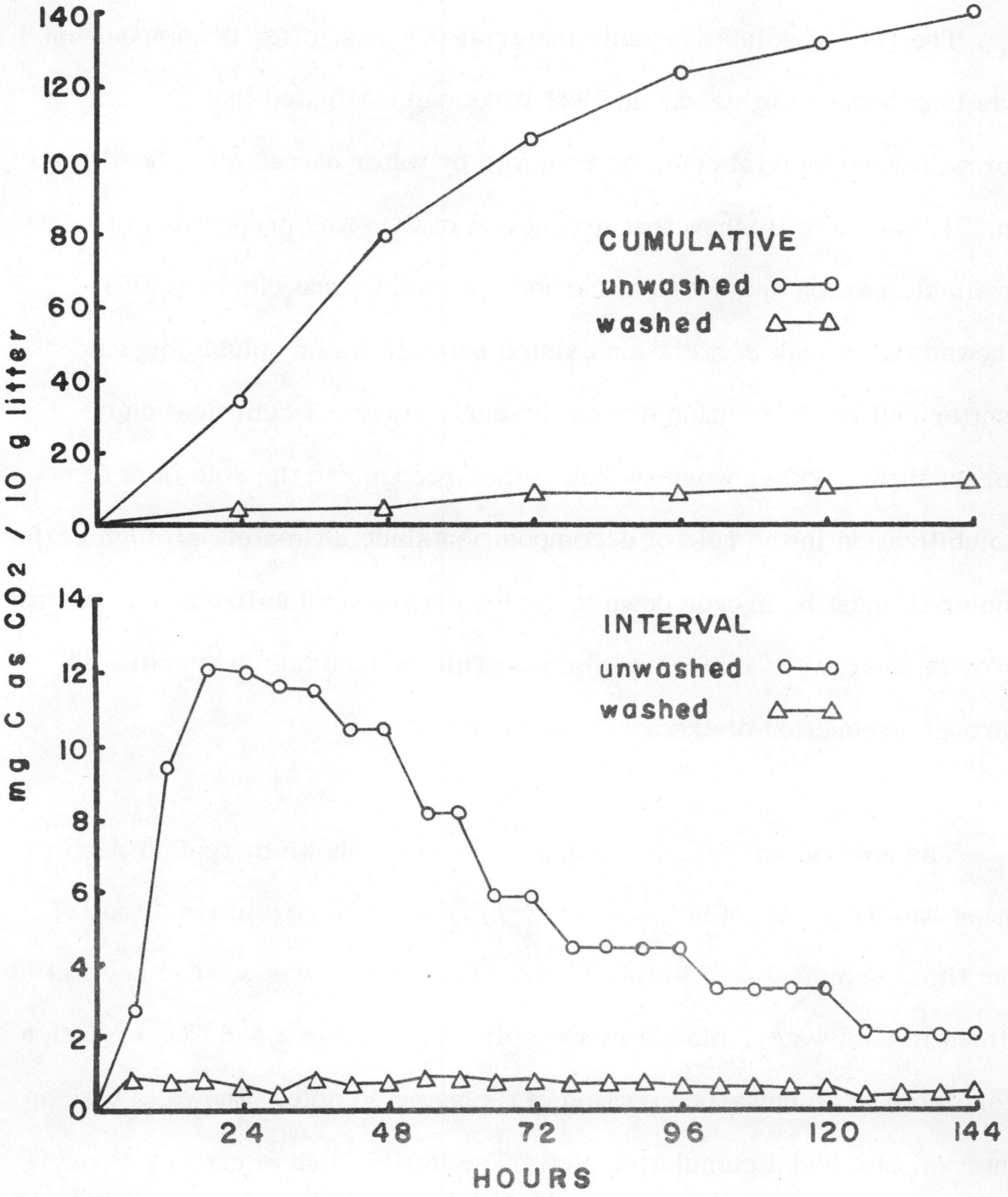

Fig. 4. Typical respiration curves from Dworshak litter having a high usable carbon content and respiration after 5 extractions.

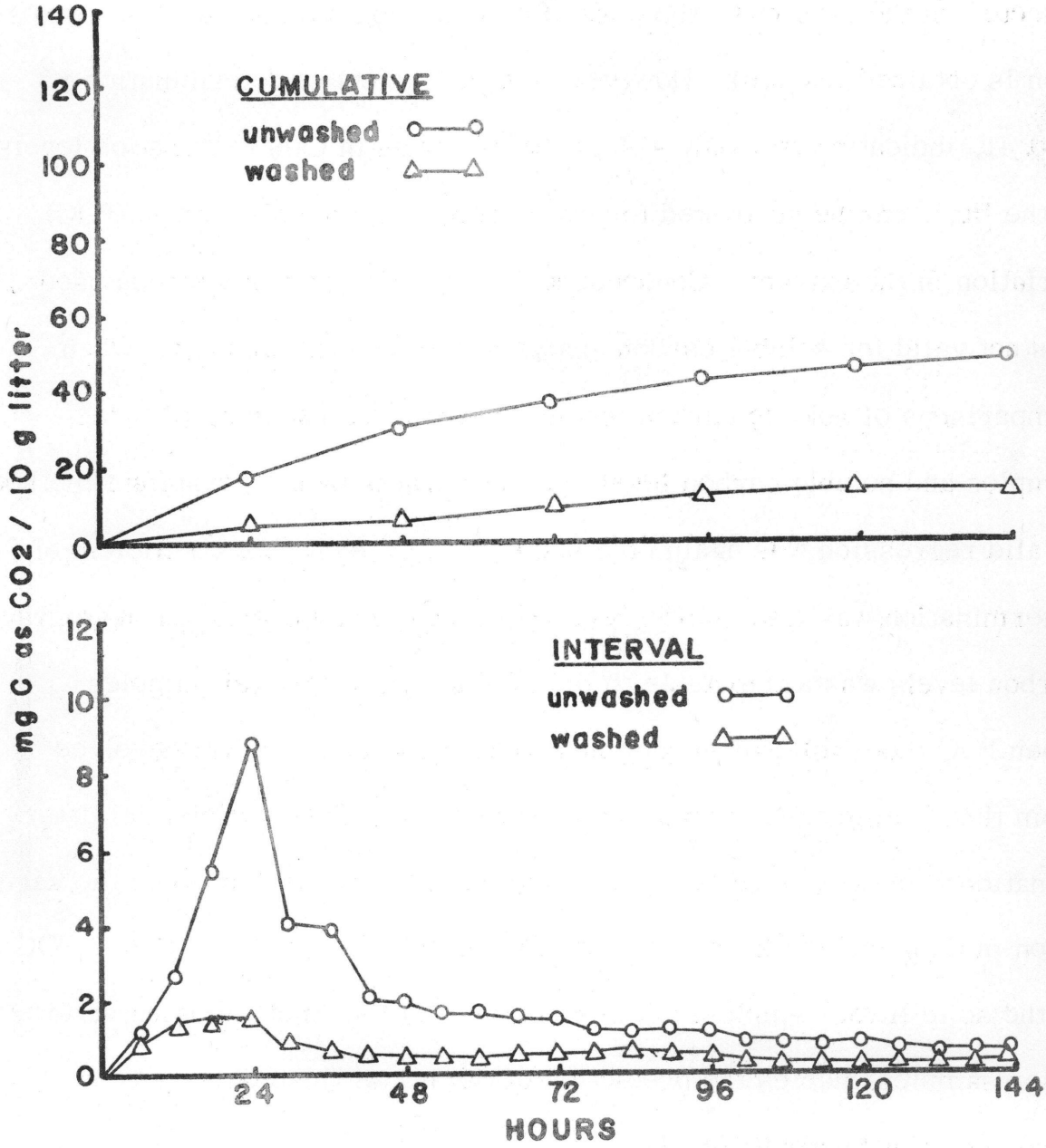

Fig. 5. Typical respiration curves from Dworshak litter having a low usable carbon content and respiration after 5 extractions.

Initial extraction experiments indicated a great deal of variation in extractibility of samples. In addition, repeatability of extraction on similar samples was erratic. When CO_2 evolution is compared to carbon detected on the extract by the persulfate oxidation method, a valid regression is obtained (Fig. 6). However, the coefficient of determination r^2 is 0.41, indicating that only 41% of the variation in CO_2 respiration levels of the litter can be accounted for by persulfate oxidizable carbon (POC) variation in the extract. Consequently, extraction by the methods used was not valid for soluble carbon analysis in the litter samples. When comparisons of soluble carbon levels were made between solid litter samples and soluble carbon levels in the extracts by the persulfate method, a valid regression was again obtained (Fig. 7). Again the coefficient of determination was low. Fifty-seven percent of the variation in the extract carbon levels was attributable to the POC in the solid litter samples. When POC from solid litter samples was compared to the CO_2 evolved from those samples in the respirometer cabinet, the coefficient of determination r^2 was 0.78 (Fig. 8). This value indicates that most of the variation in CO_2 evolved in the cabinets can be explained by variation in POC in the solid litter samples. Consequently, the POC method using solid litter samples enables the prediction of the initial flush of activity in respiration of forest litter.

An analysis of all the samples gave a total POC concentration in the Dworshak impoundment area of 7.255×10^9 grams of usable carbon or

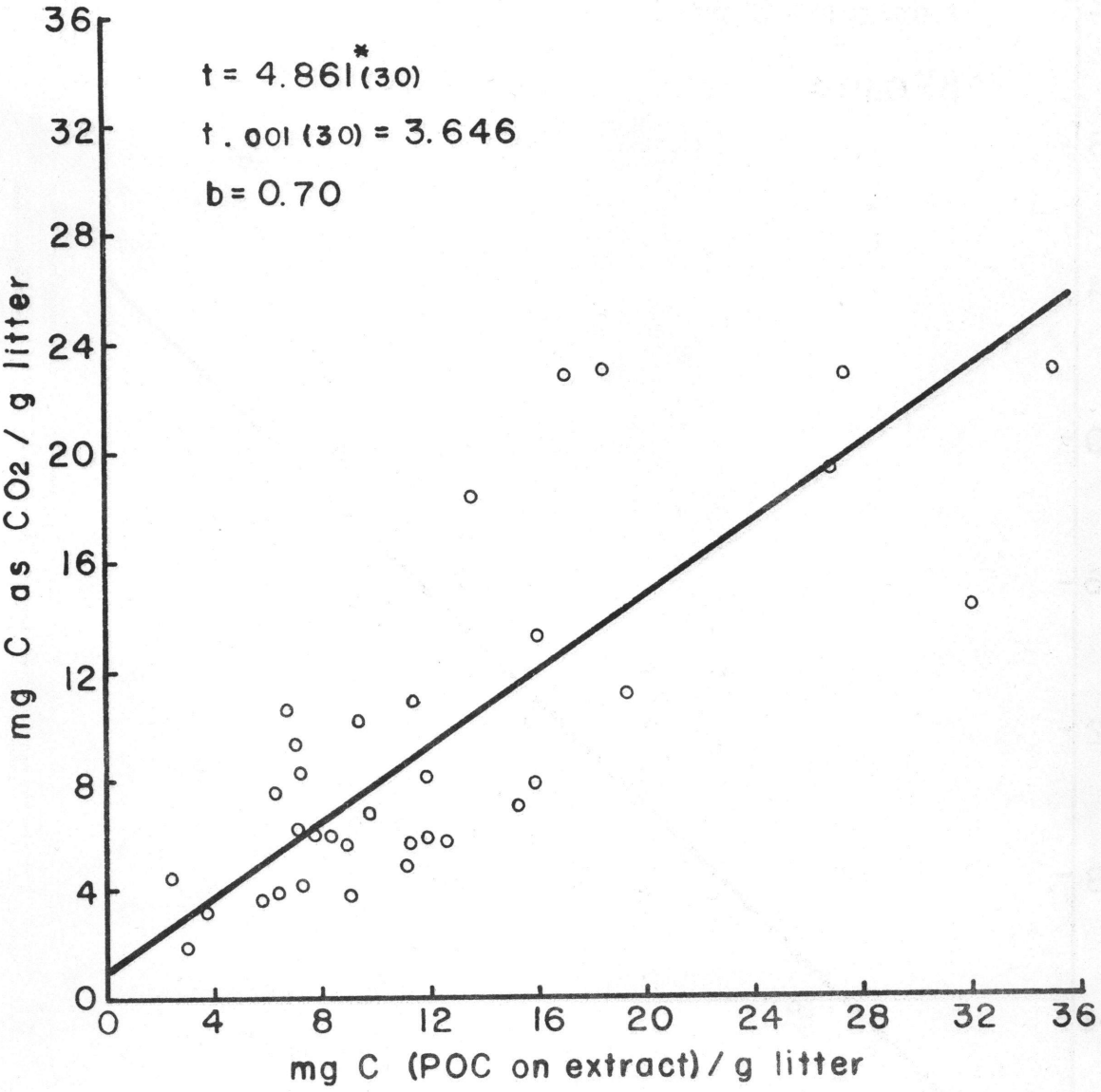

Fig. 6. Comparison of carbon as CO_2 evolved from litter to usable carbon in extract determined by the POC method. $r^2 = 0.41$.

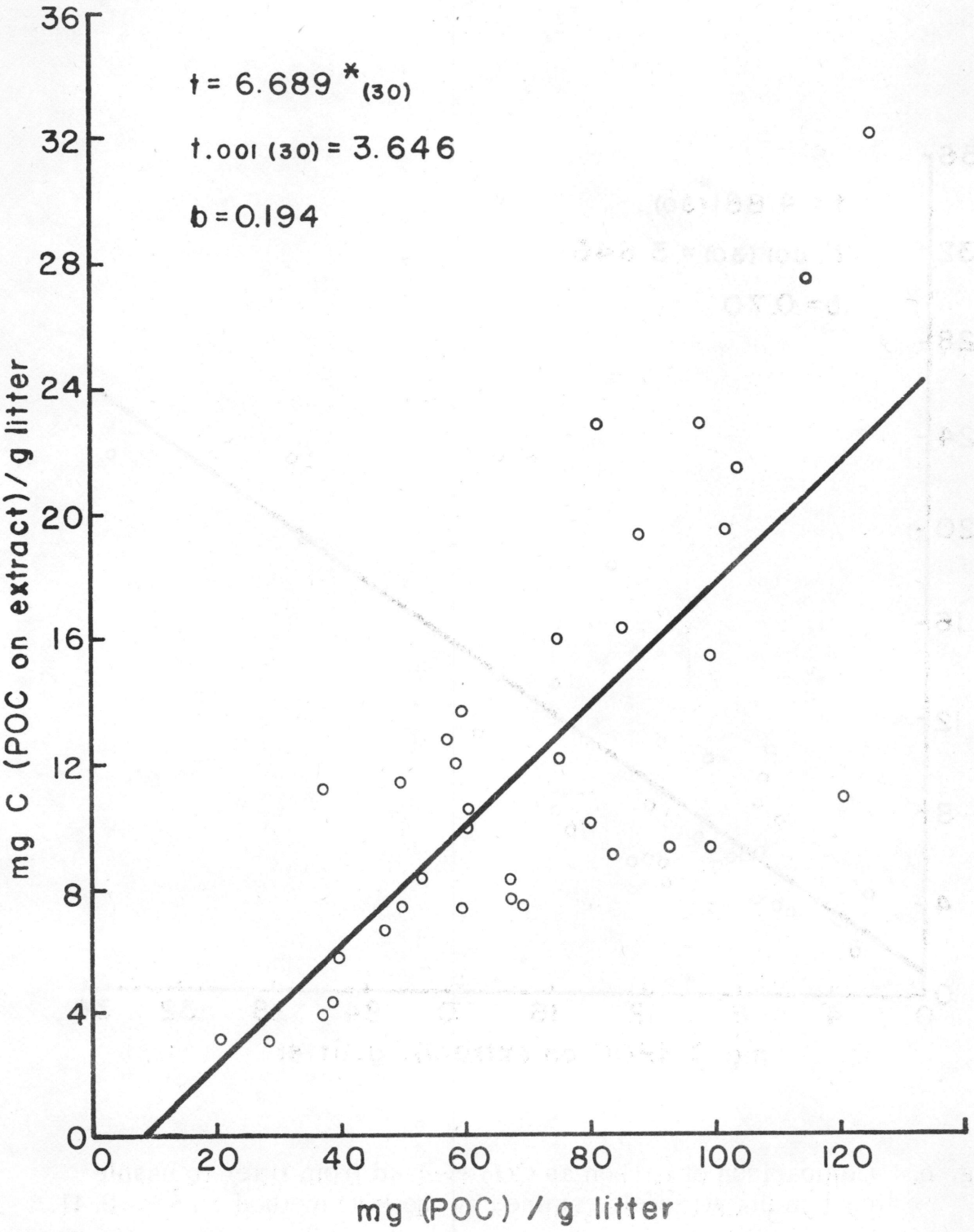

Fig. 7. Comparison of usable carbon detected in extract by POC method compared to usable carbon in the unextracted solid litter. $r^2 = 0.57$.

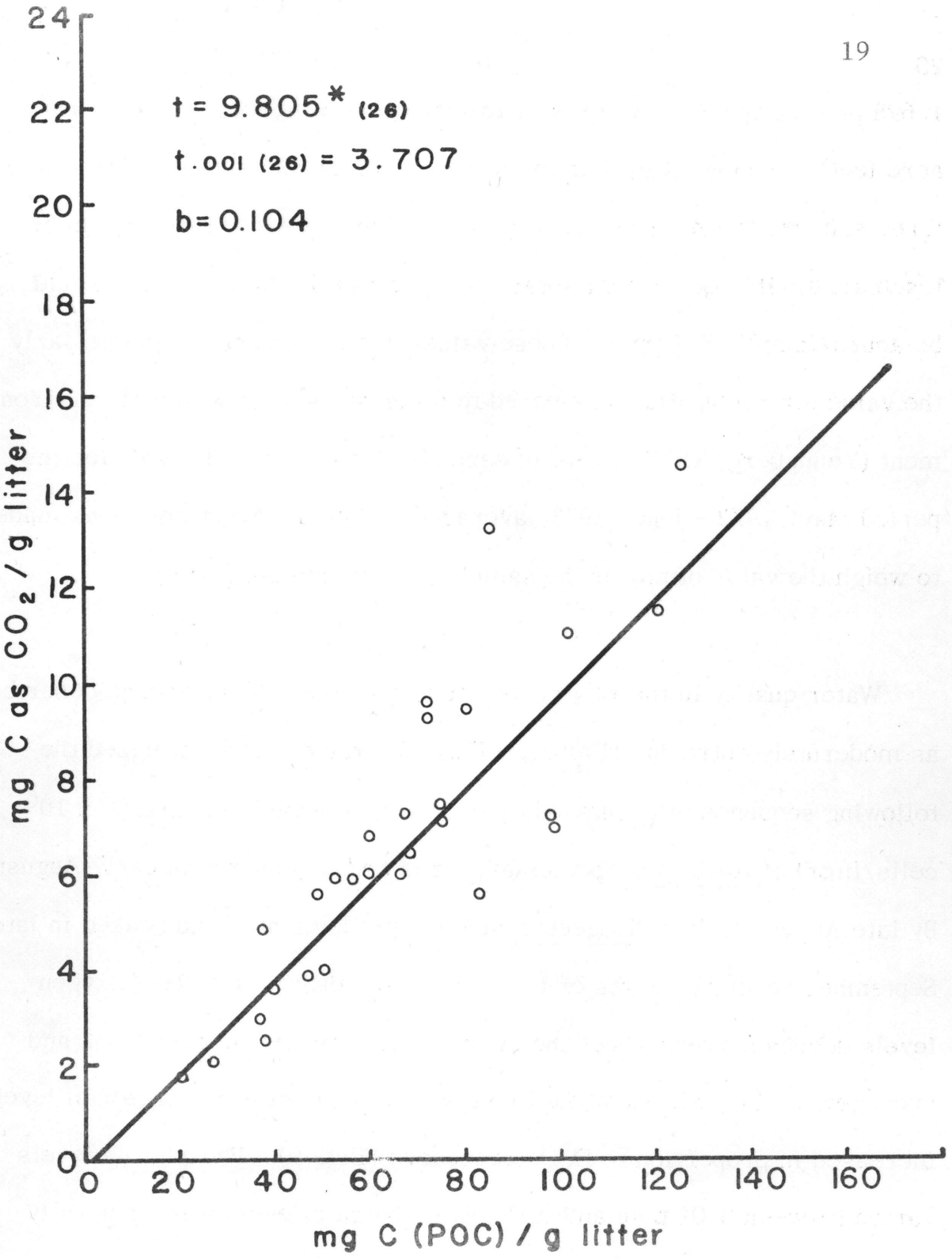

Fig. 8. Comparison of respiration carbon as CO_2 from solid unextracted litter to usable carbon elected by the POC method in solid unextracted litter. $r^2 = 0.78$.

1. 698 ppm on the basis of gross capacity of the reservoir (3. 468 x 10^6 acre feet). If input of carbon in the reservoir is considered to be from three sources (floor litter, canopy needles and trees, and runoff input) (each arbitarily equal) then total usable carbon in the reservoir would be approximately 5. 1 ppm. These values are conservative, particularly the value for runoff when compared to other streams in a similar environment (Youngberg, 1972). Usable carbon values in the reservoir for the period May, 1972 - June, 1973, averaged 5. 2 ppm. No attempt was made to weigh the value of any of the samples or the sampling site.

Water quality in the reservoir during this period can best be described as moderately eutrophic (Falter, 1972). Falter has characterized the following sequence of events. In July, 1972, a heavy bloom (2. 09 x 10^6 cells/liter) of Anabaena species appeared and terminated in early August. By late August, 1972, Mougeotia species predominated and peaked in late September reaching counts of 1. 5 x 10^6 cells/liter. Dissolved oxygen levels decreased throughout the summer until anaerobic conditions and hydrogen sulfide existed at the lower levels of reservoir. Bacterial levels increased in proportion to D. O. decreases (Fig. 9). Phosphorus levels varied between 0. 01 ppm and 0. 07 ppm. Nitrate levels were generally low except for peaks that coincided with bloom peaks.

In the laboratory studies pure cultures of Chlorella pyrenoidosa responded to glucose additions only if grown in the presence of bacteria.

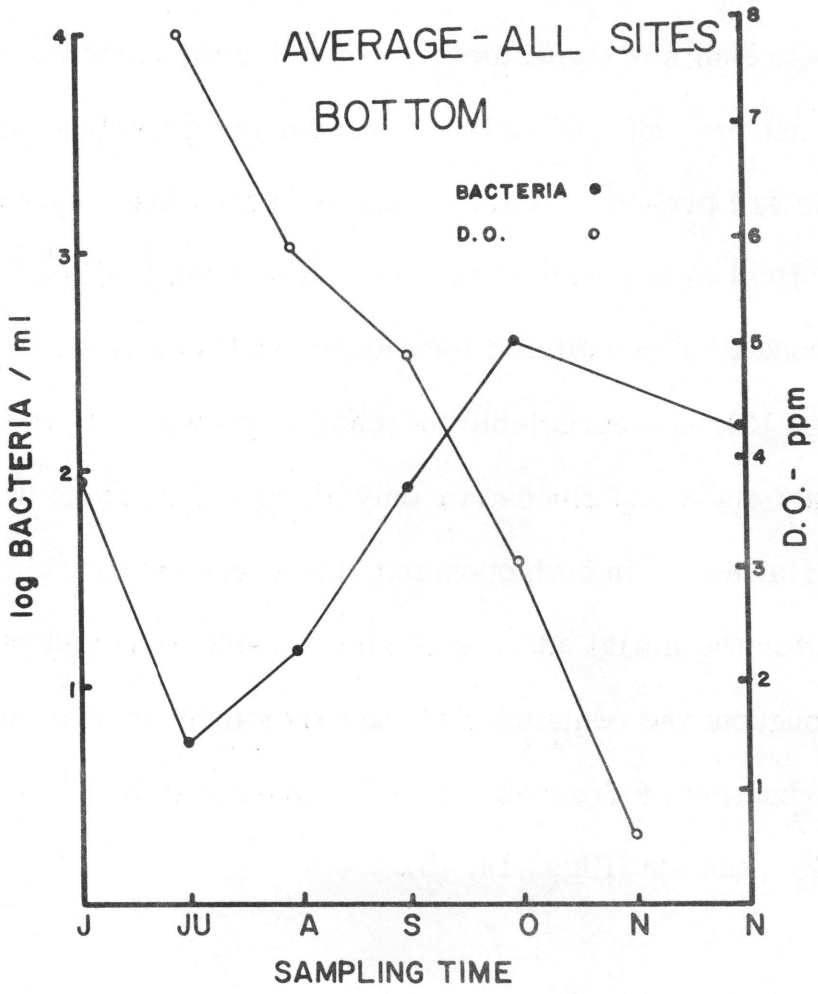

Fig. 9. Relationship of average number of bacteria at all bottom sampling sites to average dissolved oxygen concentration at bottom sites during 1972 sampling season in Dworshak reservoir.

The response to glucose at the different levels in an open system is shown in Fig. 10. Algal biomass is proportional to the amount of carbon added. Fig. 11 shows results of the same system under the same conditions when bacteria are not present. Clearly, stimulation of growth is undeniable when bacteria are present. Algal response in the closed systems is virtually identical to the open system as can be seen in Figs. 11 and 12. When bicarbonate is substituted for glucose in bacteria free Chlorella cultures (Fig. 13), no appreciable increase in growth over the controls containing only algae and glucose or only algae and bacteria was noted. Viable bacterial levels in both open and closed systems reached a steady state level after the initial addition of glucose and remained relatively constant throughout the remainder of the experiment indicating that large and constant bacteria increases are not necessary to promote algal growth in this situation (Figs. 14, 15).

The role of nutrients which may limit algal growth in hypertrophic waters has been the subject of much controversy in recent years. Increases in phosphorus, nitrogen, carbon, various trace elements, etc., have all been cited as responsible for nuisance blooms in one situation or another by various individuals. Kuentzel (1969), Legge and Dingledein (1970), Kerr (1970), Lange (1967) and others have suggested that carbon availability rather than phosporus may act in some situations to limit growth. Many others have countered this thesis by citing numerous

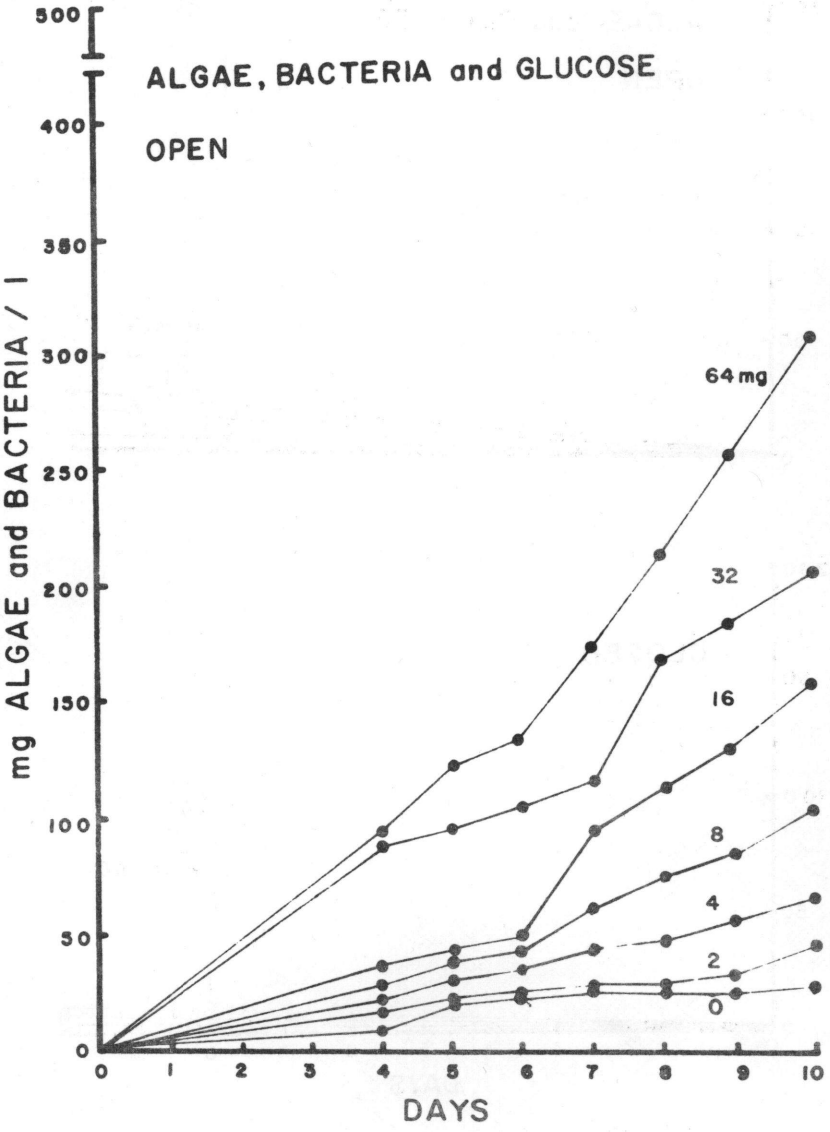

Fig. 10. Biomass levels obtained when Chlorella pyrenoidosa and bacteria are grown together in an open system and glucose is added daily. The number on each curve indicates the amount of glucose added daily.

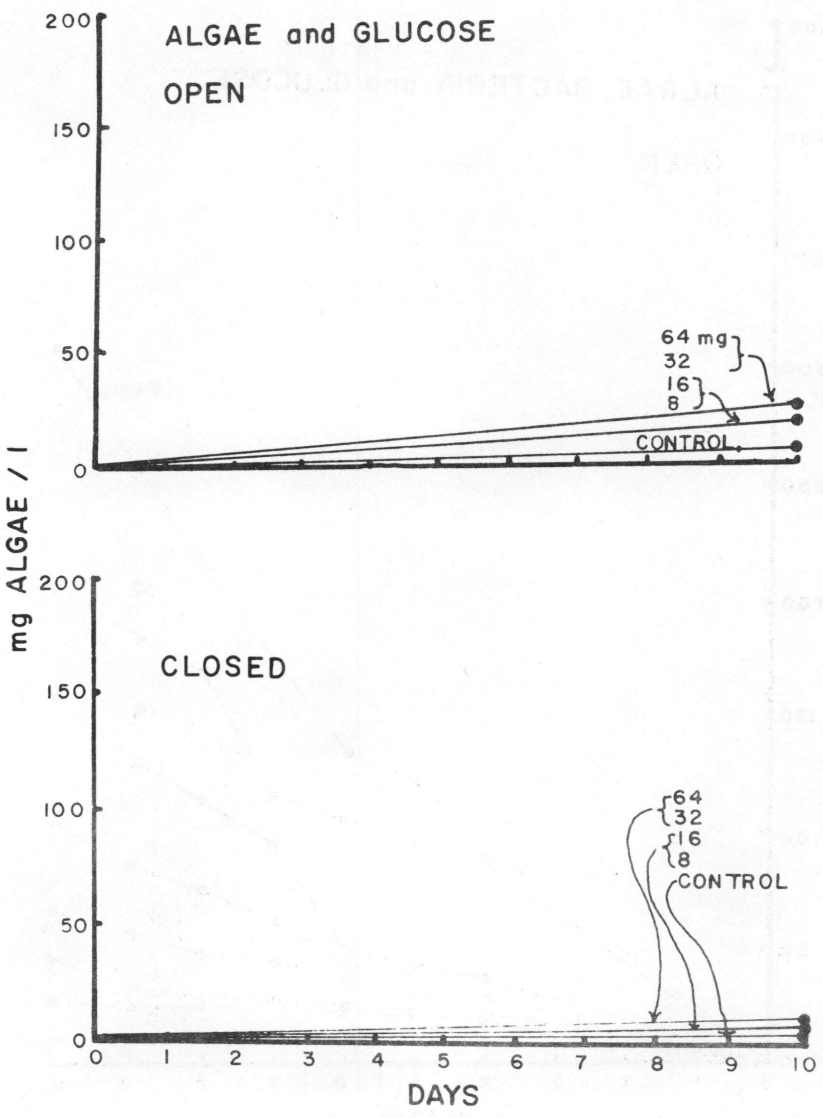

Fig. 11. Biomass levels obtained when Chlorella pyrenoidosa is grown in both open and closed systems in the absence of bacteria. The number on each curve indicates the amount of glucose added daily.

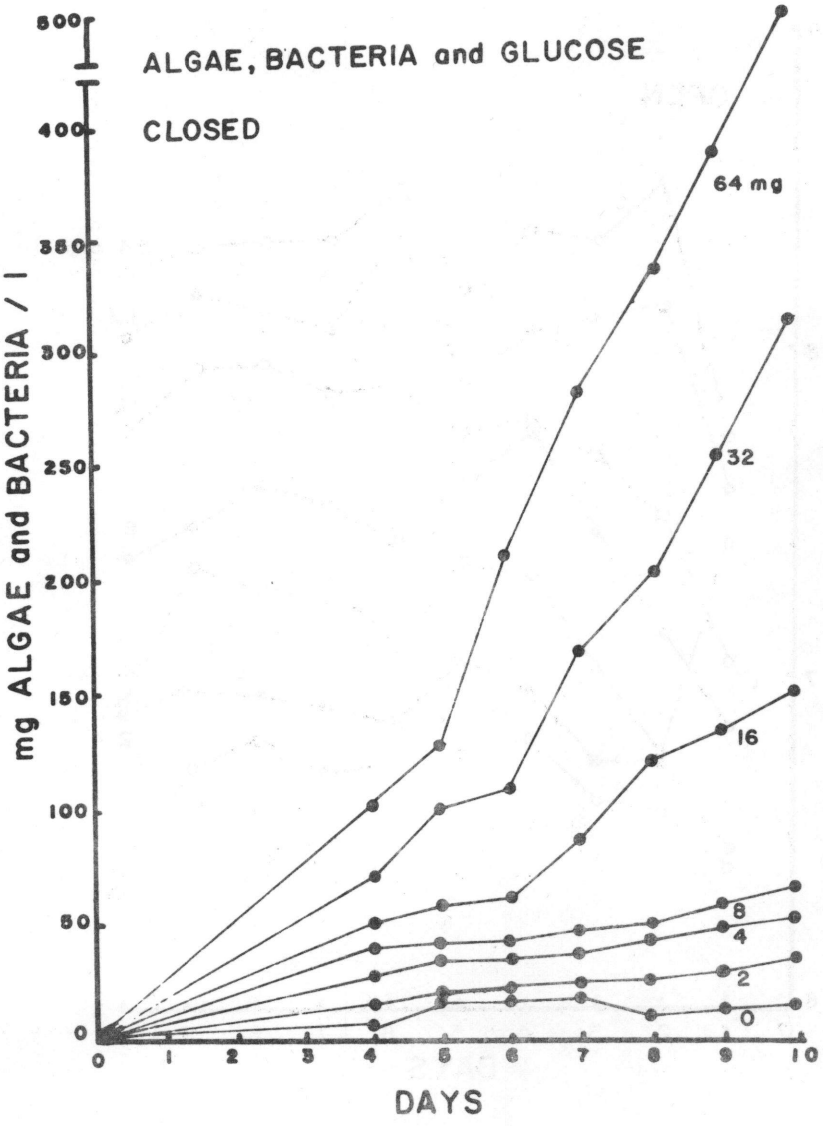

Fig. 12. Biomass levels obtained when Chlorella pyrenoidosa and bacteria are grown together in a closed system and glucose is added daily. The number on each curve indicates the amount glucose added daily.

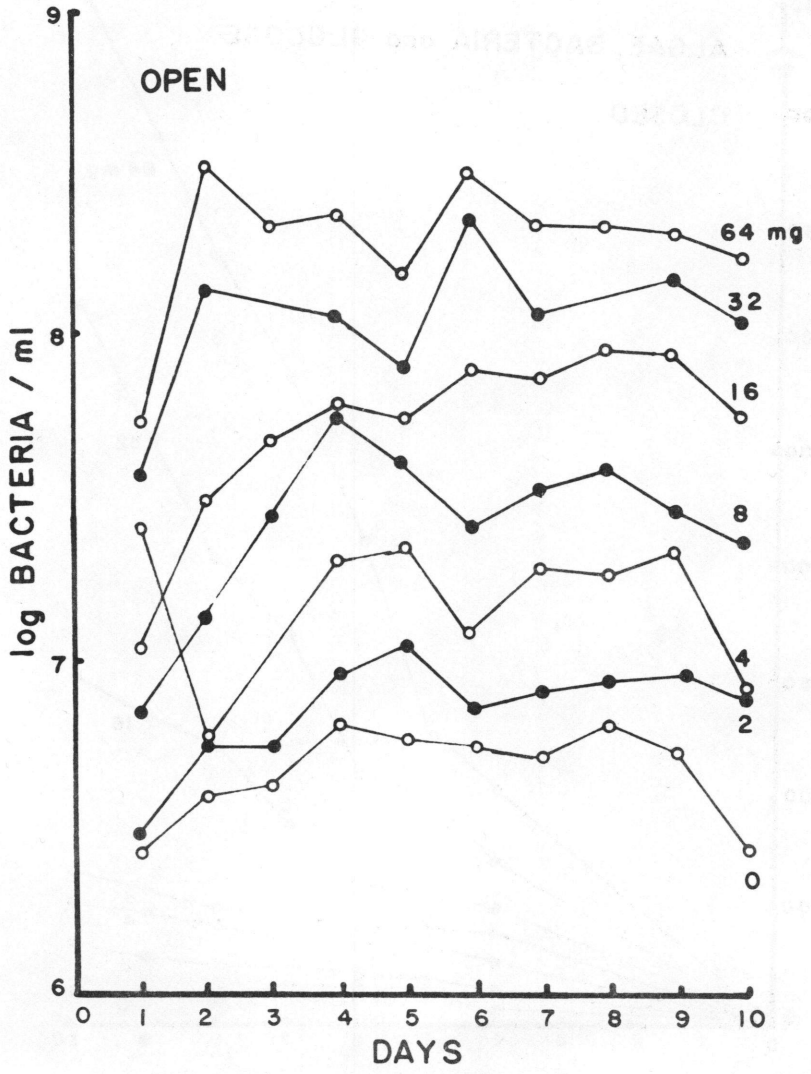

Fig. 13. Bacteria present in open system at different glucose levels.

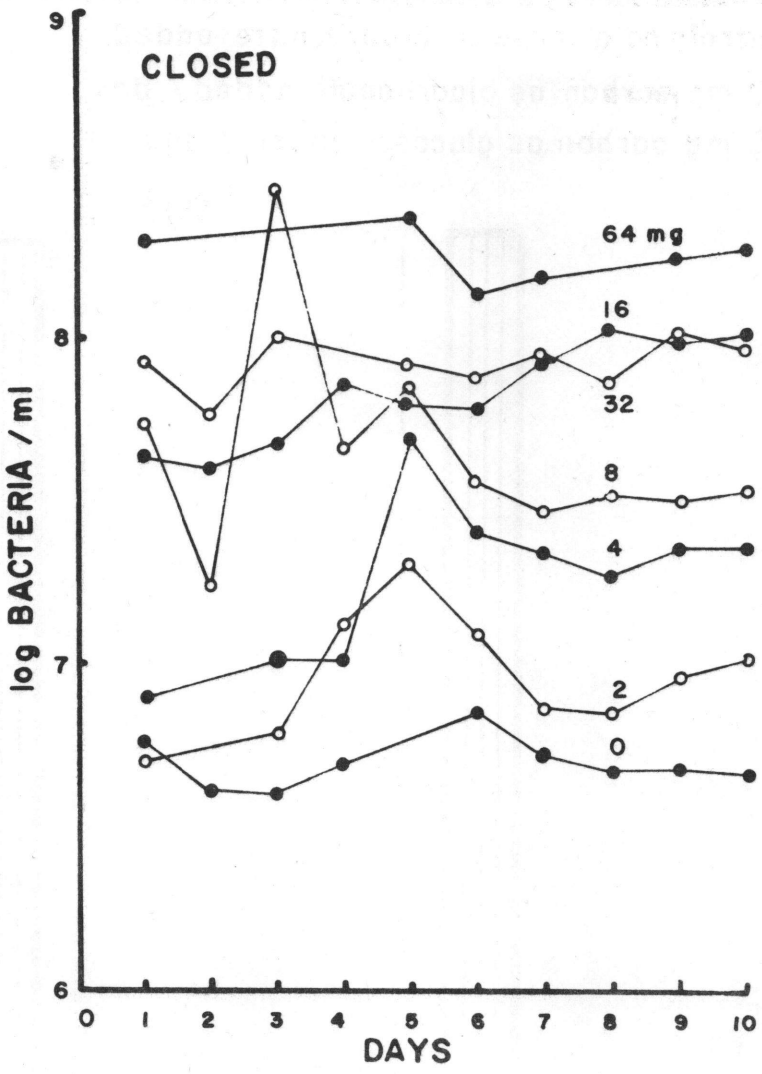

Fig. 14. Bacteria present in closed system at different glucose levels.

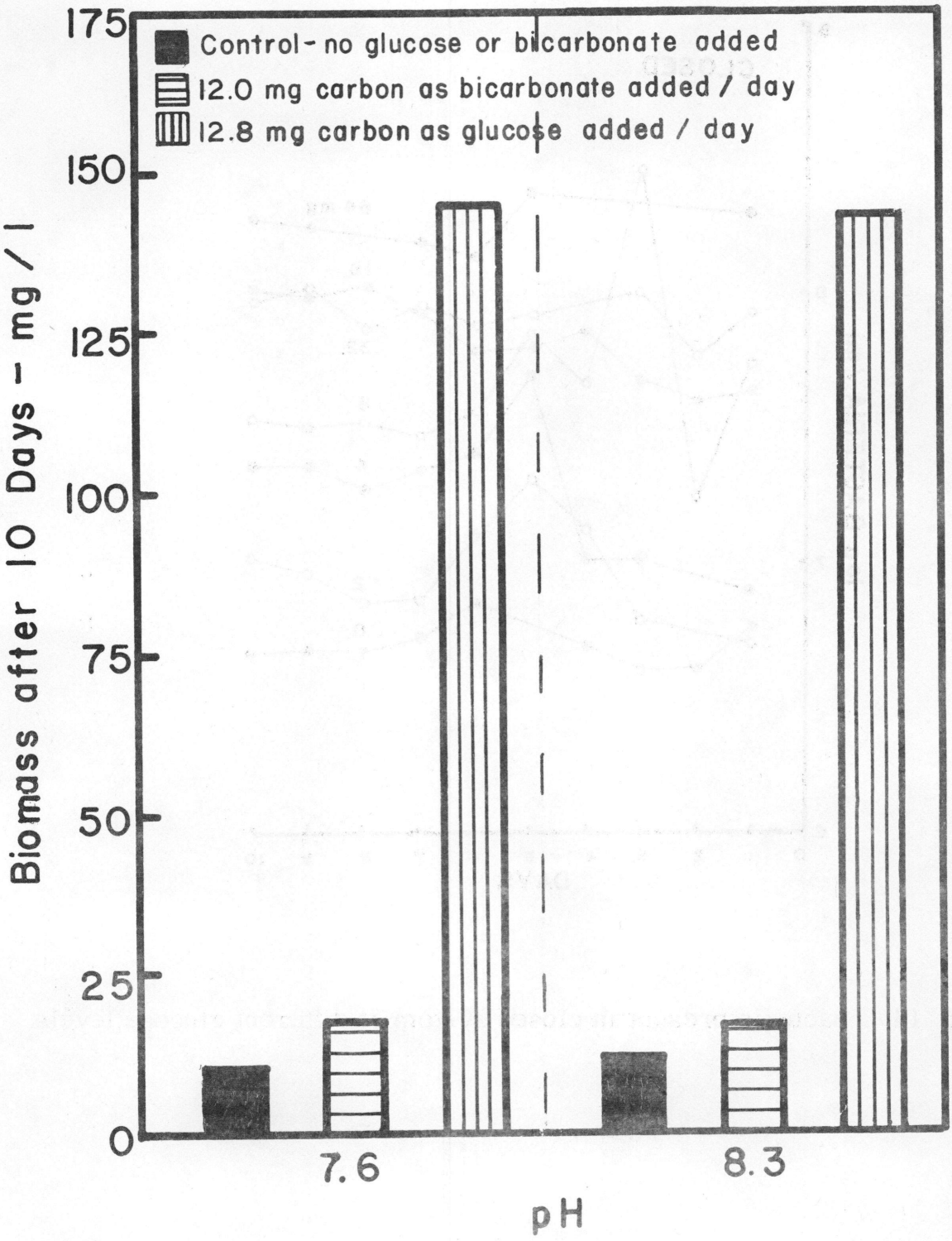

Figure 15. Effect of bicarbonate addition in Chlorella pyrenoidosa bacterial systems.

reasons why carbon cannot be limiting. Among these are the availability of CO_2 from the air, the ability of algal cells to use carbonates and bicarbonates, etc. Since this is not a review, no attempt will be made to give the various arguments for or against the limiting nutrient debate. Let it suffice to say that all essential nutrients are necessary for algal growth and in that respect any nutrient can be limited provided all other nutrients are in excess.

Algal growth in this laboratory study must have been limited by carbon. In the absence of bacteria and glucose, very little difference in the amount of growth was noted between open and closed systems although the open systems were slightly higher than the closed indicating a slight carbon contribution from air. The Chlorella did not respond to bicarbonate addition either in open or closed systems nor did it respond to glucose in the absence of bacteria. In the presence of bacteria and glucose, a 40 fold increase of biomass was demonstrated. Obviously the combination of bacteria and glucose was contributing a limiting nutrient or providing a stimulatory substance to promote algal growth. If a trace element, vitamin, etc., was limiting growth in this study, it would be difficult to explain the proportionality of growth to glucose added since micronutrients are usually needed only in extremely small quantities. No threshold values indicative of micronutrient stimulation were noted. In related studies in our laboratory, bacterial extract simulated growth

of bacteria-Chlorella cultures in proportion to the carbon levels of the extract added.

The significant difference in biomass between cultures in which low levels of glucose were added (2 mg and 4 mg) and controls (no glucose addition) is of particular interest. Two mg of glucose per 600 ml is equivalent to approximately 1.3 ppm carbon. This is a carbon level much lower than any found in Dworshak reservoir during the sampling period. Considering the level of biological activity that existed in the reservoir last year, it is conceivable that much the same type of symbiosis could have occurred in the reservoir as in the laboratory study. In the reservoir an adequate supply of usable carbon was available from the litter, foliage, trees, etc. Carbon was also in a form usable by bacteria as shown by the laboratory study. Bacterial activity was high as indicated by increased counts and anaerobic conditions which progressed during the sampling period. Two separate algal blooms of massive levels occurred during the period. Preimpoundment investigations (U. S. C. of E. Supplement No. 1, Design Memorandum 19, Dworshak, 1969) concluded that the reservoir would be an oligtrophic (deficient of plant nutrients) body of water. Obviously this is not the case.

SUMMARY AND CONCLUSIONS

Evidence presented in the study can be summarized as follows:

1. The contributions of carbon from forest litter or other types of organic material to an aquatic environment such as Dworshak reservoir can be predicted prior to impoundment. In addition, the amount of carbon readily oxidized by bacteria from these sources can be accurately determined by the persulfate oxidation method.

2. The amount of carbon contributed from the litter is sufficient to initiate a high level of bacterial activity in a reservoir such as Dworshak. As little as 1.3 ppm carbon as glucose stimulated algal growth when bacteria were present in laboratory studies.

3. The algal growth studies in the laboratory indicate that carbon mineralization by heterotrophic bacteria can cause massive increases in algal biomass production. Neither glucose by itself nor bacteria in the absence of a carbon substrate was capable of stimulating the growth of Chlorella pyrenoidosa. Bicarbonate additions had no appreciable effect on Chlorella growth.

4. Evidence presented here indicates that carbon level should be included in any algal assay which makes no attempt to exclude the effect of bacteria on the assay results.

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